# Individual variation in thermal plasticity and its impact on energetic scaling across different hierarchical levels

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# Abstract

1. Metabolic rate limits many biological processes and is organised in a hierarchical manner. The relationship between metabolic rate and body mass can therefore vary drastically across individuals, species and populations.
2. Energetic scaling at higher taxonomic levels may be confounded with among- and within-individual effects if variation in metabolic rate and body mass at these lower levels is not properly modelled. This is because an individual’s energetic expenditure is sensitive to changes in the internal (e.g., body mass) and external environment (e.g., temperature) which can alter the scaling relationship. However, whether individuals consistently differ in their sensitivity to the environment (metabolic reaction norms) is not well characterised or understood.
3. We repeatedly measured individual’s metabolic rate across a temperature gradient in an ectotherm model (*Lampropholis delicata* – the delicate skink) to characterise the repeatability of metabolic thermal plasticity and to identify the patterns of phenotypic correlations of metabolic rate at different temperatures (cross-temperature correlations). We also tested whether the relationship between body mass and metabolic rate changed with temperature and differed at the among- and within-individual level.
4. Making use of two statistical frameworks, we found that the thermal metabolic reaction norms are significantly repeatable. We also show that repeatability of 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. Cross-temperature correlations in metabolic rate differed between the two modelling approaches and provide different evolutionary insights into the how the shape of the reaction norm can be shaped by natural selection.
5. Finally, we show that mass-scaling exponents did not change with temperature but differ substantially at among- and within- individual level.
6. Our work suggests that metabolic plasticity has the capacity to respond to selection, which has crucial implications for animals coping with rapid temperature changes. Moreover, we show that the relationship between body mass and metabolic rate is scale-dependent. Studies that utilise the predictive power of energetic scaling relationships should bear in mind the different levels of variation.

# Keywords

Phenotypic plasticity, reaction norm, thermal sensitivity, repeatability

# Introduction

Animals live in a multifaceted world where their external and internal environment is often in a state of flux. Changes in rainfall, density of conspecifics, age and body condition can all simultaneously affect an individual. Animals respond to environmental heterogeneity by adjusting many aspects of their phenotype including life history (Westneat, Stewart & Hatch 2009), locomotor performance (Careau *et al.* 2014a), behaviour (reviewed in(Dingemanse *et al.* 2010) and physiology (Boratyński, Jefimow & Wojciechowski 2017). Resting metabolic rate, in particular, represents an individual’s ‘energetic overhead’ (Biro & Stamps 2008) and restricts how much energy is allocated to competing processes such as growth, reproduction and maintenance (De Jong & Van Noordwijk 1992; Brown *et al.* 2004). Variation in metabolic rate is a likely a target for selection because it has been posited as the casual mechanism for promoting suites of correlated traits that are important to fitness, known as ‘paces-of-life’ (Réale *et al.* 2010; Ricklefs & Wikelski 2002; Friesen, Johansson & Olsson 2017; Malishev, Bull & Kearney 2017). For example, tropical birds tend to have low basal metabolic rate, produce fewer slow-growing offspring and longer longevity relative to their temperate-region counterparts (Wiersma *et al.* 2007; Williams *et al.* 2010). Furthermore, an individual’s metabolic rate contributes to the flow of energy, biomass and nutrients in the population, which can have an impact on communities and ecosystems (Allen, Gillooly & Brown 2005; Barneche *et al.* 2014). Given the ecological and evolutionary significance of metabolism, there is a growing interest in the proximate and ultimate causes of variation in metabolic rate, as well as its sensitivity to biotic and abiotic factors (Norin, Malte & Clark 2016; Pettersen, White & Marshall 2016). The degree to which metabolic rate changes with body mass and temperature, can be highly variable among vertebrate taxa (Uyeda *et al.* 2017), populations of the same species (Burton *et al.* 2011) as well as individuals within the same population (Norin & Gamperl 2018). Understanding the link between the environment and metabolic rate across different hierarchical levels of biological variation can help elucidate eco-evolutionary dynamics (Brown *et al.* 2004; Glazier 2015).

Metabolic theories attempt to unify ecological processes across populations, communities and ecosystems based on physiological rates at the individual level (Gillooly *et al.* 2001; Brown *et al.* 2004). These theories rely on first principles from chemistry and physics to explain how energetic demands change with body mass. Generally, metabolism is thought to exhibit a fixed scaling relationship with body mass (i.e., 3/4 or 2/3 power law) and change with temperature following a logarithmic function (Gillooly *et al.* 2001; Brown *et al.* 2004). Generalisation of these scaling relationships across multiple levels of biological organisation may be overly simplistic given that physiological mechanisms that determine how metabolic rate changes with body mass at the individual level are highly complex and variable (Glazier 2005; White & Kearney 2012). For example, many explanations for ‘universal’ mass-scaling exponents are based on the assumption that supply of macronutrients to the metabolic machinery is constant, but this is known to vary between individuals (Speakman *et al.* 2004; Steyermark 2005; Metcalfe 2005).

Mass scaling exponents are influenced by numerous factors, challenging the ‘one-size-fits-all’ line of thinking. Abiotic factors such as temperature, and biotic factors such as endo-/ectothermy, can interact to influence how species respond to the environment driving variation in metabolic scaling among species (Barneche, White & Marshall 2016, Uyeda *et al.* 2017, Killen, Atkinson & Glazier 2010). Interspecific variation in scaling exponents may have true biological meaning, but it may be a misleading generalisation where the energetic scaling relationships described at lower levels are incorrectly attributed to broader taxonomic levels (van de Pol, 2009). In other words, processes governing shifts in metabolic rate within individuals can impact among-individual relationships that can ultimately carry-over to relationships observed at the species level. This is potentially problematic in typical metabolic scaling studies where metabolic rate and body mass are averaged across a sample of unique individuals to estimate mass-scaling relationship for an entire species (Fig. 1). If the goal of metabolic theories is to use among-species energetic scaling as a means to explain large scale ecological processes (Glazier 2015), it is in our best interest to correctly model hierarchical variation in metabolic rate to better elucidate the processes driving variation in scaling at higher levels. While the mechanisms driving interspecific variability in energetic scaling remains contentious, variation in metabolic plasticity across individuals may provide important insight to our understanding.

**Figure 1** – Diagram representing the hierarchical organisation of log metabolic rate and log body mass data of three hypothetical species. The large dots represent the mean log metabolic rate and mean log body mass of a given species. The small dots represent the mean log metabolic rate and mean log body mass for one individual in the sample of each species. The black line represents the metabolic scaling relationship across the three species, the coloured lines represent the scaling relationship within each species. The ‘magnified’ box shows the within-individual scaling relationship in log metabolic rate and log body mass. Typically, in metabolic scaling studies, individuals are rarely measured more than once, and averages of log metabolic rate and log body mass are taken across individuals of the sample to represent the species estimate, after which, across species metabolic scaling relationship is estimated.

Individual physiological processes respond to the environment in diverse ways which is expected to lead to individual differences in metabolic plasticity and scaling relationships. Membrane composition (Hulbert *et al.* 2007), enzyme structure and function

(Somero 1978) and mitochondrial capacity (Salin *et al.* 2012) are known to change in response to fluctuations in body condition, as well as changes in the thermal environment (reviewed in Seebacher 2005). Individual variation in physiological processes that determine metabolic plasticity to internal and external environments may be a promising explanation for why there is no agreed upon, 'universal’ mass-scaling exponent (Salin *et al.* 2015; 2016) Given that physiological machinery adjusts to oscillations in temperature and body mass within a lifetime of an individual (e.g. seasonal changes), the mass scaling relationship will likely shift as well, however this has rarely been considered in empirical studies (Uyeda *et al.* 2017).

Currently it is unclear whether metabolic plasticity consistently differs among individuals. Consistent inter-individual variation is typically represented as repeatability (the proportion of phenotypic variation that is attributed to individual differences, (Nakagawa & Schielzeth 2010) and has important evolutionary consequences because it is the raw material on which natural selection acts. While it is established that metabolic rate is a heritable and repeatable trait (reviewed in Nespolo & Franco 2007; White, Schimpf & Cassey 2013; Auer *et al.* 2016), consistent variation in metabolic plasticity (i.e. metabolic ‘norm of reaction’) has only been reported in only a few species (Briga & Verhulst 2017; Careau, Gifford & Biro 2014b). Characterising repeatability in metabolic reaction norms is logistically tedious but fundamental in understanding the capacity for plasticity itself to evolve. Repeated measures of reaction norms over an ecologically relevant duration may be important for metabolic scaling studies as body mass and metabolic rate tend to be measured only once making these studies susceptible to sampling variability,

An additional challenge in quantifying the repeatability of metabolic plasticity is that there are multiple ways in which plasticity itself can be statistically modelled. Broadly speaking, two approaches to modelling plasticity are prevalent and debated in evolutionary biology (Via *et al.* 1995; Hunt 2014). Character-state approach models phenotypic change in a set of environments as discrete ‘states’ or categories. In contrast, the function-valued approach (also known as ‘polynomial’ approach, Via *et al.* 1995), describes changes in a trait across an environmental gradient using parameters of a mathematical function. In this circumstance, the model parameters (e.g. intercept and slope) are the main targets of selection. Granted that the same phenotypic trait measured in multiple environments are inherently correlated, this may give rise to evolutionary constraints on how the reaction norm can be moulded (Falconer 1952). Non-zero correlations between ‘states’ in different environments, and between the intercept and slope can dictate the extent to which these must evolve in tandem (Hunt 2014). While the conceptual differences between the modelling approaches have sparked debates, both approaches can contribute to our understanding on how the shape of reaction norms can evolve. To the best of our knowledge, no study has assessed the merits of both approaches in understanding individual variation in reaction norms.

Here we examine how individuals vary in their resting metabolic rate (RMR) in relation to body size and temperature using an ectotherm model, the delicate skink (*Lampropholis delicata*). We take advantage of both function-valued and character state approaches to answer the following four questions to better understand whether metabolic plasticity has the capacity to undergo selection and the consequences of mass-scaling exponents when hierarchical variation is accounted for. More specifically, we ask (1) Do individuals consistently differ in their plastic responses to temperature (i.e. thermal reaction norms) over time? (2) What are the cross-environment correlations of metabolic rates between different temperatures? (3) Does the relationship between metabolism and body size change with temperature? (4) How does the relationship between metabolism and body size change when variation at the among- and within- individual level is properly partitioned?

# Materials and Methods

## Lizard collection and husbandry

Forty-two male *L. delicata* were collected across two sites between 28 August and 8 September 2015, across the Sydney region. 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 and were provided with a thermal gradient to allow 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. An animal collection was approved by the New South Wales National Parks and Wildelife 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).

## Quantifying metabolic reaction norms

Closed-system respirometry assays were conducted between 26 December 2016 - 19 March 2017. We measured resting metabolic rate (RMR) as CO2 production per unit time ( mL min-1). RMR is the metabolic rate of animals at a given temperature (usually its preferred body 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). Measurements were taken at temperatures between 22ºC and 32ºC at 2ºC increments in a random order over a three-day period (measurements at two temperatures per day) and were repeated every 10 days (10 sampling sessions in total). Due to logistical constraints, lizards were randomly assigned to one of two blocks for metabolism measurements (block 1: n = 23, block 2: n = 22). Given the large sample of animals, and the number of repeated measurements, it was not feasible to use intermittent, flow-through respirometry. We used two incubators (LabWit, ZXSD-R1090) to precisely control the ambient temperature at which measurements were taken (+/- 1ºC). In order to account for the carry over effects of extreme temperatures experienced by an individual on subsequent metabolic measurements at other temperature, the temperature order was randomly allocated to the incubators across the three days, within each sampling session.

After a minimum of 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 CO2 build up. After 30 minutes, each chamber was flushed with fresh air and sealed. A 3 mL air sample was taken via a two-way valve to account for any residual CO2 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 *.* Water vapour was scrubbed from the inlet air with Drierite. Output peaks were integrated using WartHog Systems LabAnalyst software to calculate the percentage of CO2 (Mark Chappell, Regents of University of California). The rate of CO2 produced by an individual was calculated as following Lighton (2008):

Eqn: 1

where %CO2 is the percentage of CO2 in air sample, which was corrected by subtracting any ‘residual’ CO2 from the initial flush from the larger of the two air samples, Vchamber is the volume of the chamber (146mL), Vlizard 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 analysis

All statistical analyses were conducted using ‘R’ (Core Team 2013). We used a complete dataset of 2418 observations. For more 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).

In all our models, is log-transformed. Collinearity between our predictor variables were checked using a scatterplot matrix (Fig. S1) and Pearson correlation coefficients are presented in Table S2. Initial analyses show that there were no differences in log2 between blocks of lizards or incubators therefore these parameters were not included in our final models (see ESM for these analysis). Although lizards were kept in temperature-controlled room, there may still be differences in temperature of each enclosure which could have carry-over effects on metabolic rate due to acclimation. We therefore tested whether the previous measurement temperature or body temperature measured in home enclosure influenced log at subsequent temperatures. We found that the model containing ‘previous temperature experience’ as a covariate was better supported compared to a model without it (wAIC (Full model – reduced model) = -4.73 and loo = -4.73), we therefore included ‘previous temperature experience’ in all subsequent analyses (Table S1).

We made use of Bayesian generalised linear mixed models (LMM) from either the R package ‘brms’ (Bürkner 2017) or ‘MCMCglmm' (Hadfield 2010). For every model, we pooled the posterior estimates for multiple chains. For ‘brms’ models, we used default priors and ran 4 chains of 2000 iterations with a burn in of 1000 and a thinning interval of 1. For ‘MCMCglmm’ models, we used uninformative, parameter expanded priors to assist with model convergence (Hadfield 2010). We ran 3 chains of 7510000 iterations with a burn in of 10000 and a thinning interval of 5000 for our ‘MCMCglmm’ models. All models were checked for proper mixing and convergence by visually inspecting trace plots and ensuring scale reduction factors are smaller than 1.1. We also checked whether our chains were not autocorrelated. We present pooled posterior means and their 95% credible intervals. Below, we present our model structures following O'Hara (2009).

## Repeatability of thermal reaction norms

We used function-valued and character state approaches to quantify the repeatability of thermal reaction norms in metabolism to determine whether they differed consistently among individuals. All function-valued models were run using ‘MCMCglmm’, while the character-state model were run using ‘brms’. In all our repeatability models, metabolic rate and temperature were log-transformed, body mass was first log-transformed and then z-transformed (mean of 0 and sd of 1). Given that the reaction norm of the same individual can change throughout the ten sampling sessions of the experiment, we coded a ‘series ID’ random effect in our function-valued models to account for variation in individual reaction norms within-individual and among sampling sessions. 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, ID001 was measured at six temperatures in session five, its six measurements will be assigned the series ID of ID005\_session5. See provided code and Araya-Ajoy, Mathot & Dingemanse 2015 for in depth explanation)

## Reaction norm intercept – average metabolic response at each temperature

Repeatable reaction norm intercepts indicate that individuals’ average metabolic rate vary consistently at a particular temperature. Typically, the intercept represents the average trait value when x = 0 (i.e. log temperature = 0). We compared three methods of calculating adjusted repeatability of the intercept (hereafter referred to as ‘repeatability’, Nakagawa & Schielzeth 2010).

The first function-valued method involves setting the intercept at a relevant temperature by mean-centering. We fitted six models, each centered on a different measurement temperature with the following structure,

log ~ logTempcen + zlogBodyMass + logPriorTemp + (1+ logTempcen | ID) + (1+ logTempcen | seriesID)

where log log-transformed , logTempcen is the mean-centered log temperature in degrees Celsius at a given measurement temperature so that the intercept represents the average response at given log measurement temperature. For example, if a model is centered at log(22ºC) the logTempcen value= 0, and value for log(24ºC) centered at 22ºC = 0.09 (see data for more details), zlogBodyMass is log-transformed body mass that is then subsequently z-transformed, logPriorTemp is log-transformed previous temperature. Individual ID and series ID were included as random intercepts and logTempcen as random slopes. Repeatability (equation 2) of the intercept at each measurement temperature following Araya-Ajoy *et al.* 2015.

Equation 2:

where is the repeatability estimate for logat a particular temperature; *Vind0* is the individual intercept and *Vseries0* is the series intercept. To assess how repeatability of the intercept changed over the course of our study, we also calculated ‘short term’ and ‘long term’ adjusted repeatability according to Araya-Ajoy *et al.* 2015.

Equation 4:

Equation 5:

where *Ve0* is the residual variance. ‘Short-term’ repeatability can be interpreted as among-individual variation that includes both intrinsic differences between individuals as well as the effects of the sampling session. In contrast, ‘long-term’ repeatability is a more conservative measure and represents repeatability in the classical sense, where phenotypic variation due sampling session is a part of the total pool of variation in the data (i.e. in the denominator of the calculation, Nakagawa & Schielzeth 2010).

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). To the best of our knowledge, the method to derive ‘conditional’ repeatability has not been extended to multiple random effects. We therefore, fitted a model that has the following structure,

log ~ logTemp + zlogBodyMass + logPriorTemp + sessionID +(1+ logTemp| ID)

where sessionID is the sampling session when measurements took place. Note that session is included as a fixed predictor, as opposed to using seriesID as a random effect. Temperature-specific repeatability estimates were derived using equation 6,

Equation 6:

where *Covind0,ind1* is the covariance of the individual intercepts and individual slopes and *T* is the measurement temperature at which repeatability is estimated.

The final method to calculate temperature-specific repeatability uses the character-state method described by Hunt 2014 (see also Houslay 2017). This approach assumes variation of log at each temperature are ‘distinct’ categorical traits that are correlated. We treated log measured at each temperature as a 6 x 6 response matrix.

~ zlogBodyMass + logPriorTemp + (1|ID) + (1|sessionID)

where, is the metabolic rate for individual 1 in sampling session 1 at 22ºC and so forth. Note that temperature is no longer a predictor as this is now part of the response. We also included individual ID and sampling session as random intercepts.

This process requires a substantial amount of data because the model estimates variance, as well as their covariances with every other temperature at the among-individual and within-individual level (Hunt 2014; Houslay 2017). Using the variance-covariance matrix, we calculated temperature specific repeatability () following Equation 8. Indeed, this equation is equivalent to Equation 4, except that it is using temperature-specific variance components (Nakagawa & Schielzeth 2010).

Equation 8:

where*Vind0,T* represents the individual intercept, *Vseries0,T*  is the series intercept and *Ve0,T* is the residual variance component at a given temperature.

## Slope – plasticity

Function-valued approaches use the slope of a linear thermal reaction norm to represent the plasticity of a trait over a temperature gradient. If slopes are repeatable, this would indicate that individuals differ consistently in how they respond to temperature over time. We used the following function-valued model that has the same structure as the models except that logTemp is not mean-centered. This is because we also used this model to derive cross-temperature correlations (see below).

log ~ logTemp + zlogBodyMass + logPriorTemp + (1+ logTemp| ID) + (1+ logTemp| seriesID)

The above model was used to calculate the repeatability of the slope following,

Equation 9:

where *Vind1* and *Vseries1* is the individual slope and series slope, respectively. The character state approach does not estimate a slope because it assumes that login each environment are “distinct” traits, However, one can assess the thermal plasticity of a trait by identifying changes in repeatability across temperatures using RT (equation 8). å

## Cross-temperature correlations in metabolic rate

*A priori*, we expect metabolic rate measured at one temperature to be correlated with metabolic rate measured at another temperature. We estimated these cross-environment correlations using both statistical approaches. Correlations are conveniently estimated by character-state models at both the among- and within individual level. Whereas in function-valued models, within-individual-among-sampling-session level correlations need to be derived using matrix algebra as they are part of the slope describing the reaction norm. We derived correlations following Brommer (2013) using the model that we used to calculate the repeatability of the slope (see above). The variance-covariance (*K*) from this model is denoted as:

Equation 10:

where or is the covariance of the slope and intercept. The six measurement temperatures can be represented as a 2 x 6 matrix,

Equation 11:

where the first column contains ones and the second column is the six unique measurement temperatures on the same scale as the predictor used to estimate *K* (e.g. logTemp). The among-individual phenotypic variance-covariance matrix, *P,* for the six temperatures can then be derived by multiplying K with and its transpose in equation 12.

Equation 12:.

which results in a 6 x 6 variance-covariance matrix as follows,

where the diagonal , , are the among-individual variances in log metabolic rate at all six temperatures and the off-diagonals represent the covariances of log metabolic between all six temperatures. The same calculation is repeated using the seriesID variance-covariance matrix to obtain within-individual-among-sampling-sessions phenotypic variance-covariance matrix. Cross-temperature covariances can then be scaled to correlations by dividing the covariance between two temperatures by the square-root product of the variance in each of the two temperatures (i.e. the standard deviation at each temperature).

Equation 13:

## Hierarchical mass scaling exponents at different temperatures

Mass-scaling exponents describes the relationship between metabolic rate and body mass across a sample of individuals. However, sample population estimates of scaling exponents may be a result of (1) an among-individual effect whereby individuals of different weight have different metabolic rates, or (2) a within-individual effect in which an individual’s metabolic rate can vary as its weight changes, or (3) a composite of both within- and among-individual effects. Exploratory statistics show that the mean percentage change in mass within an individual, across the experiment (mass at the first sampling session – the last sampling session) was 9.13% (n = 42, SE = 0.32, range in percentage change= -5.15, 27.53) which could influence sample population estimates. We therefore wanted to explore whether accounting for among- and within-individual effects would affect the overall estimates of mass-scaling exponents as this is rarely considered in typical metabolic scaling studies and whether mass-scaling exponents changed with temperature.

We partitioned out the among- and within-individual variation in body mass, by first calculating the mean mass across all sampling sessions for each individual (among-individual effect). Then, we calculated a within-individual effect by subtracting an individual’s mass from its own mean (i.e. within-individual centering, see van de Pol & Wright 2009).

Using ‘brms’, we fitted two models. Model 1 had the following structure,

log~ Temp \* AmongIDMass + Temp \* WithinIDMass + (1 + WithinIDMass|ID)

where logas a response and Temp \* AmongIDMass is the interaction term between the among individual mass effect with temperature and Temp \* WithinIDMass is the interaction term between the within individual mass effect with temperature. We also included individual ID as a random intercept and the within subject effect as a random slope given that individuals mass change at different rates through the study (See Fig 5B). Model two did not include the temperature interactions and had the following structure,

log~ Temp + AmongIDMass + WithinIDMass + (1 + WithinIDMass|ID)

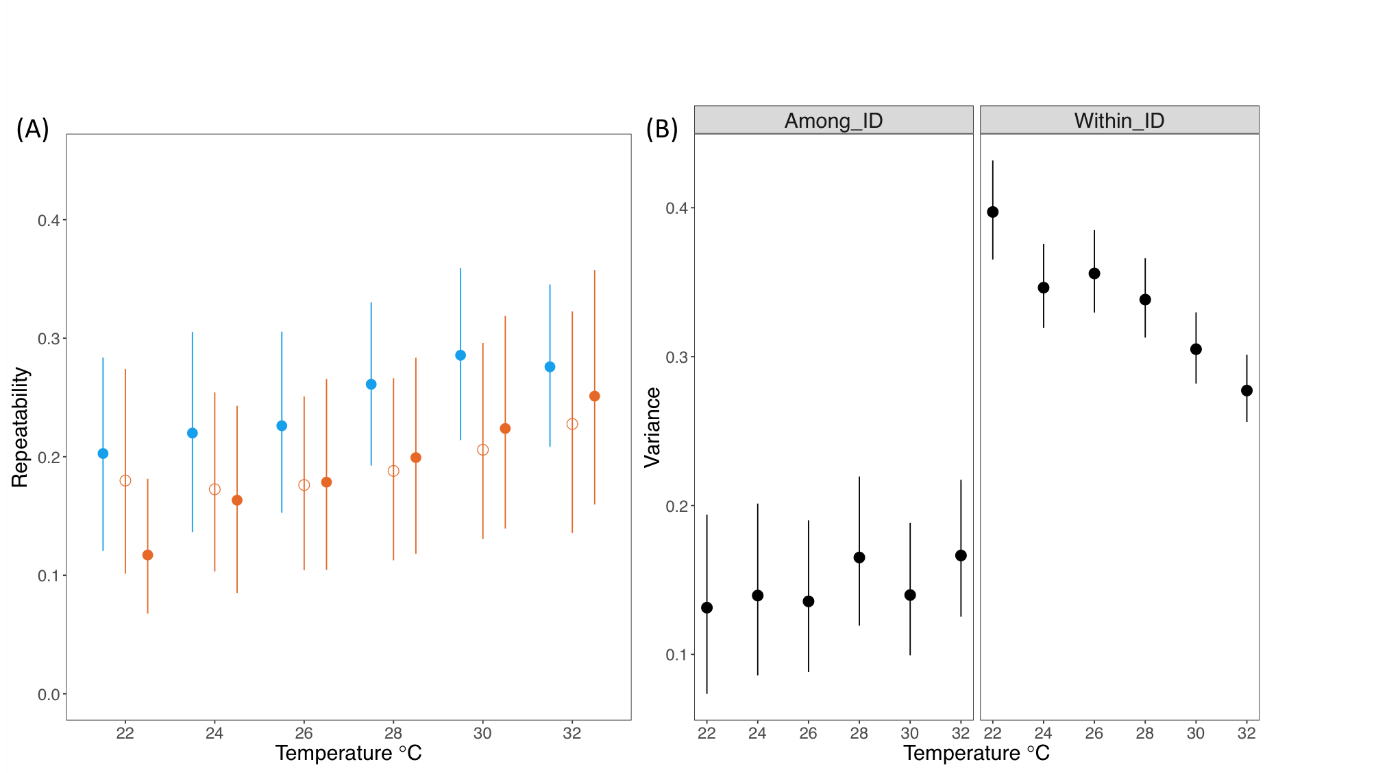
In order to test for temperature dependence, we compared information criterions (wAIC and loo values) between model one and two to see which model was a better supported.

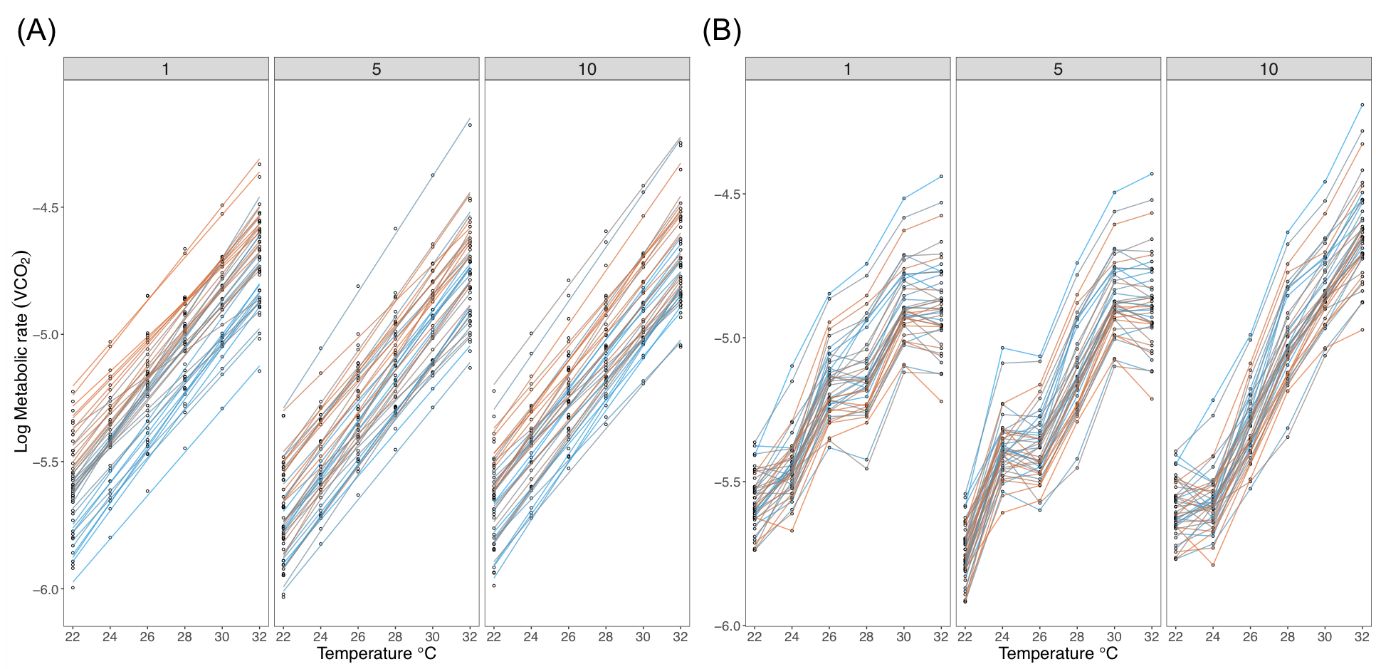
We also compared the within- and among individual scaling exponents with exponents from a model that represents the typical analysis of a metabolic scaling study model (i.e. does not account for the hierarchal variation in the data). This analysis is presented in the ESM.

# Results

## Repeatability of thermal reaction norms

Overall, repeatability in metabolic rate increased with temperature (Fig. 2A). Although there was a trend for metabolic rate and body mass to decrease across the sampling sessions (Fig. S4), there was significant among-individual variation in intercepts (average metabolic rate) at all temperatures over short and long temporal scales (Table 1a). Both function-valued methods 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 there was a very slight increase in the between individual variation, explaining the higher repeatability. Congruent with the change in repeatability with temperature, individual slopes were significantly repeatable (Rslope = 0.48, Lower C.I = 0.06, Upper C.I = 0.91) indicating a significant individual by environment interaction (I x E) that was consistent over time (Fig. 3).



**Figure 2** – A) Posterior mean of repeatability of log (mL) at six measurement temperatures. Orange circles represent function-valued approaches in modelling thermal plasticity, orange open circles represent the function-valued ‘conditional’ repeatability method (equation 6), orange filled circles represent the function-valued ‘long’ term repeatability method (equation 5). Blue filled circles represent the character state approach in estimating repeatability. See Statistical analyses for more details. Error bars represent 95% credible intervals. B) Posterior mean of variance of log (mL) at the among (right panel) and within (left panel) individual level across six measurement temperatures. Error bars represent 95% credible intervals. 

**Figure 3** – Predicted individual reaction norms for forty-two individuals of log (mL) across 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) function-valued approaches and (B) character-state approaches. Points represent predicted trait values. Each line represents a unique individual (n = 42)

## 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 3). However 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. S3). This creates a positive relationship between metabolic rate at the among individual-level. Metabolic rate measured at neighbouring temperatures (e.g. 22ºC and 24ºC) are strongly correlated, but the strength of this correlation decreased with increasing differences between the two temperatures (Fig. 4).

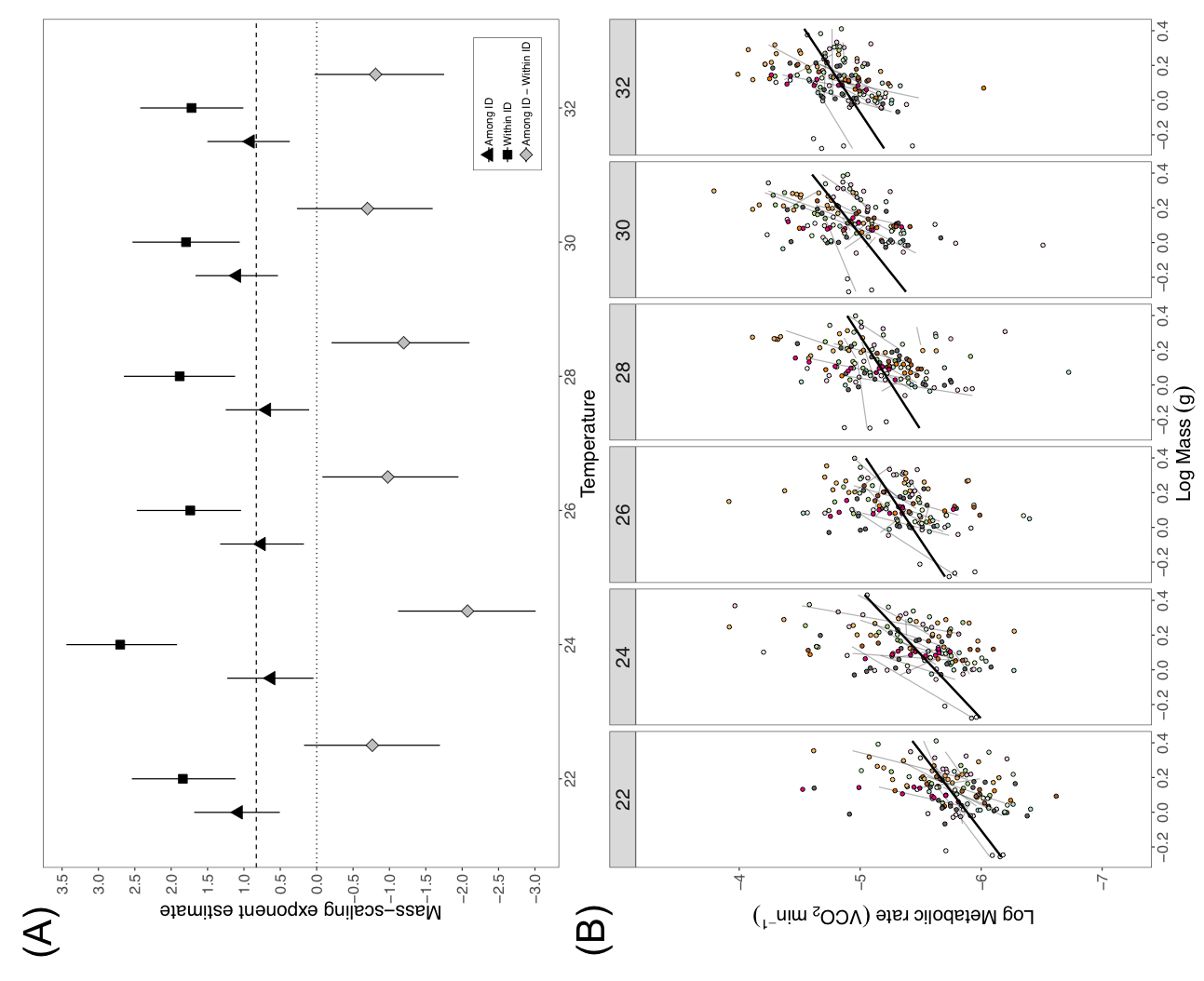
Overall, cross-temperature correlations estimated using the function-valued approaches were a lot higher and the credible intervals were very narrow. The correlations estimated using function-valued approaches are congruent with the character-state approach. The function-valued estimates were generally larger (i.e. stronger correlation) however there were estimation issues with the credible intervals as some *R*2 exceeded 1.



**Figure 4** – Cross-temperature correlations of log (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.

## Multilevel mass scaling exponents and temperature dependence

The model containing no interactions between temperature and our among- and within-individual mass effects was better supported than a model that included the interaction (Model with no interaction: wAIC = 1868.53, loo = 1869.02, Model with interaction: wAIC = 1876.58, loo = 1877.34) suggesting a lack of temperature dependence in coefficients. However, there was a trend for within-individual exponents to be larger than among-individual exponents (Fig. 5A, Table 4). Among- and within- individual mass scaling exponents were significantly different from each other at 24ºC and 28ºC (Fig. 5A, Table 4). The largest difference between within and among individual exponents was at 24ºC (Difference =-2.09, lower = -3.05, upper =-1.14, Fig. 5A). When the within- and among-individual effects were not statistically accounted for, mass-scaling exponents tended to be quite spurious and estimated with a large degree of error (Table S3).



**Figure 5** – (A) Posterior mean estimates of three types of mass-scaling exponents of (mL) across six measurement temperatures. Within individual scaling exponents (Black square) describes the change in log as an individual’s mass changes on the logarithmic scale. Between individual scaling exponents (Black triangle) describes the change in log across individuals as mass changes on the logarithmic scale after accounting for within individual effects. The grey diamonds represent the difference between the among- and within-individual scaling exponents. The dashed line represents the exponent of 0.83 estimated for squamates from Uyeda (2017). The dotted line represents 0. Error bars represent 95% credible intervals. (B) Raw log log 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 over log body mass across all individuals. Faint grey lines represent the change in log over log body mass within an individual.

# Discussion

We take advantage of both function-valued and character-state approaches to investigate the repeatability of thermal plasticity in metabolic rate. In addition, we also explored the patterns of cross-temperature correlations in these thermal reaction norms at the among- and within-individual level and tested whether mass-scaling exponents changed with temperature and differed at the among- and within-individual level. Our results show consistent among-individual differences in how metabolic rate plastically responds to the environment, over both short and long-time scales. Overall, cross-temperature correlations of metabolic rate were positive at the among- and within-individual level. However, the strength of these cross-temperature correlations was not uniform across all temperatures and differed between the character-state and function-valued approach. We demonstrate mass-scaling exponents did not change with temperature, but within-individual exponents can be considerably larger than among-individual exponents. Mass-scaling exponents, therefore, may confound among- and within- individual effects if the hierarchical structure of population-level data is not correctly modelled. Below we discuss the details of our main results, and their implications on understanding how plasticity may evolve and how metabolic rate scales at different hierarchical levels.

## Consistent variation in thermal reaction norms

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 that metabolic plasticity (i.e., the slope of metabolic reaction norms) was significantly repeatable over time, in other words, there was significant consistent among-individual differences in plasticity. Repeatability of metabolic rate increased as a function of temperature owing to the changes in the relative contributions of among- and within- individual variance components. Within-individual variation, also referred to as ‘predictability’, constitutes as the deviation of an individual relative it its own mean (Westneat, Wright & Dingemanse 2014; Cleasby, Nakagawa & Schielzeth 2014). Interestingly, while the variation among individuals were relatively consistent across temperatures, within individual variance decreased with increasing temperature. In other words, an individual’s metabolic rate had greater predictability as temperatures became hotter. It is unlikely that individuals reached a physiological ‘ceiling’ given that we were not measuring maximal metabolic rate (Biro et al 2018). Alternatively, the breakdown of macronutrients and the production of ATP may be at a homestatic balance at warmer temperatures which could promote consistency within individuals

(Somero 1978). Indeed, 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 compounded effect of high among-individual and low within-individual variation in warmer environments may mean that, not only is there a greater opportunity for selection in hot thermal environments, but selection can operate more effectively (Cleasby *et al.* 2014; Nakagawa *et al.* 2015). This may facilitate adaptive evolutionary changes in the population metabolic reaction norm, particularly in thermal environments that are novel to the population (Ghalambor *et al.* 2007).

## Cross-temperature correlations of metabolic rate: 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 metabolic rate is maintained across different thermal environments. This result is contrary to the idea that individuals can trade-off between better performance at one temperature, at a cost of function at another temperature as seen in killifish (*Fundulus heteroclitus*) where there are hot and cold temperature specialists for swimming endurance within the same population (Powers & Schulte 1998; Angilletta *et al.* 2003). It is important to note that consistent individual differences in metabolic rate, irrespective of the thermal environment, may be functionally linked with consistent differences in behaviour and life-history. Our results supports that notion that individual differences in energetic expenditure could drive different ‘paces-of-life’ or correlated suites of traits within the same population (Biro & Stamps 2008; Careau *et al.* 2009). For example, trade-offs between energy availability, reproduction and longevity can favour ‘proactive’ individuals with a high metabolic rate, active and bold personalities, that reproduce earlier at the cost of a shorter lifespan (Réale *et al.* 2010).

Assuming phenotypic correlations are congruent with underlying genetic correlations (Roff 1995), cross-temperature correlations may have important implications in understanding the constraints on the evolution of metabolic plasticity. The strength of correlations 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. 32C) were stronger compared to correlations at more distinct temperatures (e.g., 22C vs. 32C) when modelling with the character-state approach. Greater measurement error at cooler temperatures could explain this change in correlation strength as lizards were not respiring as much as they did in 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. This may be due to the important assumption of function-valued approaches whereby phenotypic values are strongly dependent on the covariance between the intercept and slope. When modelling under the assumptions of the character-state approach, the shape of thermal reaction norms may evolve with weaker constraints and greater malleability. Although both approaches are equivalent when modelling phenotypic change in two environments (Via *et al.* 1995; Hunt 2014), one advantage of function-valued approaches is the feasibility to describe more complex reaction norm shapes by fitting higher order polynomials using fewer parameters. 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).

## Hierarchical differences in metabolic scaling at different temperatures

Our results are inconsistent with the growing number of interspecific 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 within-individual metabolic allometry. In our study, we sampled sexual mature adults repeatedly in order to estimate the intra-individual mass-scaling relationship, while other studies tend to measure ontogenetic allometry (i.e. measure body mass and metabolic rate throughout development, e.g. 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 (the rate at which individuals passes through life-stages) shows stronger temperature dependence than growth (the increase in mass, Forster, Hirst & Woodward 2011). Alternatively, compensatory adjustments involving changes in membrane composition or enzyme efficiency can alter how individuals of different masses to respire at different rates across at different temperatures, thereby impacting mass scaling exponents (Reviewed in Seebacher *et al.* 2010, Glanville & Seebacher 2006).

Body mass is a key driver of metabolic processes and varies within the life time of an individual. We found significant differences in the mass-scaling relationship at the within-individual level and the among-individual level. When the levels of aggregation of metabolic scaling data is not correctly modelled, as is typical in metabolic scaling studies, species estimates represent as a composite for among- and within-individual effects. This has important implications for predictive models that make use of interspecific energetic scaling to extrapolate individual level processes to ecosystems. Notably, our within-individual estimates were at least three times greater than the widely adopted 3/4 power law (1.73 – 2.71), which is substantially higher than the within-individual scaling exponent of 0.79 reported in another reptile species, the green iguanas (Maxwell, Jacobson & McNab 2003). It is important to note that the range in body mass in our study is small (range in mass = 0.75g – 1.637g). This could give rise to large values of within-individual mass-scaling exponents due to sampling error. Nonetheless, it is still intriguing that our estimates were in line with studies of endotherms such as bats and birds (McLean & Speakman 2007, Kvist & Lindstrom 2001), possibly indicating that even the slightest changes body composition within an individual’s life can impact metabolism (Scott, Mitchell & Evans 1996). In support of this, changes in fat mass strongly predicted within-individual variation in basal metabolic rate in Redshanks, a species of migratory bird (Scott *et al.* 1996). Food limitation and metabolising different energy stores could help explain these within individual effects given animals were intermittently fasted prior to measurements and were measured over a long period of time (4 months) (McCue 2010). Animals are known to adjust their physiological systems by shifting from carbohydrate-based energy reserves to more lipid- or protein-based reserves during periods of intermittent fasting (McCue 2010). Utilising different types of energy sources that require different amounts of ATP to catabolise can in turn could impact metabolic rate. Catabolism of different energy fuels may help explain the diversity of intra-individual scaling exponents observed in vertebrate empirical studies (Reviewed in Glazier 2005, 0.77–1.88). For example, an impressive intra-individual scaling exponent of 1.82 was observed in long-distance migratory waders that have specifically evolved to mobilise, transport and utilise a range of energy reserves to travel long distances with limited opportunities to feed (Kvist & Lindstrom 2001). Future work is needed to investigate the physiological mechanisms and interactions of food limitation, fuel supply on scaling of metabolic rate.

# Conclusion

Our study emphasises the importance of considering individual variation in metabolic plasticity in response to changes in temperature and body mass. Individual variation in metabolic rate and mass is organised hierarchically and if the hierarchical nature of individual data is not correctly modelled, population level mass-scaling relationships may be mix of among-and within individual (or population) effects. This can lead to ‘hasty generalisations’, particularly in theoretical models that utilise energetic scaling across taxa to predict ecological system dynamics. From our study, it is apparent that thermal plasticity of metabolic rate is indeed repeatable and could be subjected to natural selection to shape population reaction norms in the face of 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, however this was dependent on what statistical approach we used. While we do not advocate the use of any single approach in modelling plasticity, our goal is to illustrate how differences in assumptions between the function-valued and character-state approach can influence the inferences we draw from them. Molecular studies elucidating the mechanisms of metabolic plasticity in response to the internal and external environment would be invaluable to better explain deviations from the ¾ power law and ‘universal temperature dependence’ as well as, the capacity for metabolic reaction norms to evolve.

# Author contributions

FK, DN and SN conceived the ideas and designed the study; FK and CF collected the data; FK, DN, SN analysed the data; All authors contributed to writing 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

# References

Allen, A.P., Gillooly, J.F. & Brown, J.H. (2005) Linking the global carbon cycle to individual metabolism. *Functional Ecology*, **19**, 202–213.

Angilletta, M.J., Jr, Wilson, R.S., Navas, C.A. & James, R.S. (2003) Tradeoffs and the evolution of thermal reaction norms. *Trends Ecol Evol,* **18**, 234–240.

Araya-Ajoy, Y.G., Mathot, K.J. & Dingemanse, N.J. (2015) An approach to estimate short-term, long-term and reaction norm repeatability. *Journal of Animal Ecology,* **6**, 1462–1473.

Auer, S.K., Bassar, R.D., Salin, K. & Metcalfe, N.B. (2016) Repeatability of metabolic rate is lower for animals living under field versus laboratory conditions. *The Journal of Experimental biology*, **219**, 631–634.

Barneche, D.R. & 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., Kulbicki, M., Floeter, S.R., Friedlander, A.M., Maina, J. & Allen, A.P. (2014) Scaling metabolism from individuals to reef-fish communities at broad spatial scales. *Ecol Lett*, **17**, 1067–1076.

Barneche, D.R., White, C.R. & Marshall, D.J. (2016) Temperature effects on mass-scaling exponents in colonial animals: a manipulative test. *Ecology*, **98**, 103–111.

Biro, P.A. & Stamps, J.A. (2008) Are animal personality traits linked to life-history productivity? *Trends Ecol Evol*, **23**, 361–368.

Biro, P.A. & Stamps, J.A. (2010) Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? *Trends Ecol Evol*, **25**, 653–659.

Boratyński, J.S., Jefimow, M. & Wojciechowski, M.S. (2017) Individual differences in the phenotypic flexibility of basal metabolic rate in Siberian Hamsters are consistent on short- and long-term timescales. *Physiological and Biochemical Zoology*, **90**, 139–152.

Briffa, M., Bridger, D. & Biro, P.A. (2013) How does temperature affect behaviour? Multilevel analysis of plasticity, personality and predictability in hermit crabs. *Animal Behaviour*, **86**, 47–54.

Briga, M. & Verhulst, S. (2017) Individual variation in metabolic reaction norms over ambient temperature causes low correlation between basal and standard metabolic rate. *The Journal of Experimental biology*, **220**, 3280–3289.

Brommer, J.E. (2013) Variation in plasticity of personality traits implies that the ranking of personality measures changes between environmental contexts: calculating the cross-environmental correlation. *Behavioral Ecology and Sociobiology*, **67**, 1709–1718.

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M. & West, G.B. (2004) Toward a Metabolic Theory of Ecology. *Ecology*, **85**, 1771–1789.

Burton, T., Killen, S.S., Armstrong, J.D. & Metcalfe, N.B. (2011) What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? *Proceedings of the Royal Society of London B: Biological Sciences*, **278**, 3465–3473.

Bürkner, P.C. (2017) brms: An R package for Bayesian multilevel models using Stan. *Journal of Statistical Software*, **80**.

Careau, V., Bininda-Emonds, O.R.P., Thomas, D.W., Réale, D. & Humphries, M.M. (2009) Exploration strategies map along fast–slow metabolic and life-history continua in muroid rodents. *Functional Ecology*, **23**, 150–156.

Careau, V., Biro, P.A., Bonneaud, C., Fokam, E.B. & Herrel, A. (2014a) Individual variation in thermal performance curves: swimming burst speed and jumping endurance in wild-caught tropical clawed frogs. *Oecologia*, **175**, 471–480.

Careau, V., Gifford, M.E. & Biro, P.A. (2014b) Individual (co)variation in thermal reaction norms of standard and maximal metabolic rates in wild-caught slimy salamanders (ed M Konarzewski). *Functional Ecology*, **28**, 1175–1186.

Clarke, A. (2004) Is there a Universal Temperature Dependence of metabolism? *Functional Ecology*, **18**, 252–256.

Cleasby, I.R., Nakagawa, S. & Schielzeth, H. (2014) Quantifying the predictability of behaviour: statistical approaches for the study of between-individual variation in the within-individual variance. *Methods in Ecology*, **6**, 27–37.

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

De Jong, G. & Van Noordwijk, A.J. (1992) Acquisition and Allocation of Resources: Genetic (Co)variances, Selection, and Life Histories. *The American Naturalist*, **139**, 749–770.

Dingemanse, N.J., Kazem, A.J.N., Réale, D. & Wright, J. (2010) Behavioural reaction norms: animal personality meets individual plasticity. *Trends Ecol Evol*, **25**, 81–89.

Doody, J.S. (2009) Superficial lizards in cold climates: Nest site choice along an elevational gradient. *Austral Ecology*, **34**, 773–779.

Falconer, D.S. (1952) The Problem of Environment and Selection. *The American Naturalist*, **86**, 293–298.

Forster, J., Hirst, A.G. & Woodward, G. (2011) Growth and Development Rates Have Different Thermal Responses. *The American Naturalist*, **178**, 668–678.

Friesen, C.R., Johansson, R. & Olsson, M. (2017) Morph-specific metabolic rate and the timing of reproductive senescence in a color polymorphic dragon. *Journal of Experimental Zoology Part a-Ecological and Integrative Physiology*, **327**, 433–443.

Ghalambor, C.K., McKay, J.K., Carroll, S.P. & Reznick, D.N. (2007) Adaptive versus non‐adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology*, **21**, 394–407.

Gillooly, J.F., Brown, J.H., West, G.B., Van M Savage & Charnov, E.L. (2001) Effects of Size and Temperature on Metabolic Rate. *Science*, **293**, 2248–2251.

Glanville, E.J. & Seebacher, F. (2006) Compensation for environmental change by complementary shifts of thermal sensitivity and thermoregulatory behaviour in an ectotherm. *The Journal of experimental biology*, **209**, 4869–4877.

Glazier, D.S. (2005) Beyond the ‘3/4-power law’: variation in the intra- and interspecific scaling of metabolic rate in animals. *Biological Reviews*, **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. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, **153**, 403–407.

Glazier, D.S. (2015) Is metabolic rate a universal ‘pacemaker’ for biological processes? *Biological Reviews*, **90**, 377–407.

Goulet, C.T., Thompson, M.B. & Chapple, D.G. (2016) Repeatability and correlation of physiological traits: Do ectotherms have a ‘thermal type’? *Ecology and evolution*, **7**, 710–719.

Hadfield, J.D. (2010) MCMC methods for multi-response generalized linear mixed models: The MCMCglmm R package. *Journal of Statistical Software*, **33**, 1–22.

Hirst, A.G., Glazier, D.S. & Atkinson, D. (2014) Body shape shifting during growth permits tests that distinguish between competing geometric theories of metabolic scaling (ed D Marshall). *Ecol Lett*, **17**, 1274–1281.

Hulbert, A.J., Pamplona, R., Buffenstein, R. & Buttemer, W.A. (2007) Life and Death: Metabolic Rate, Membrane Composition, and Life Span of Animals. *Physiological Reviews*, **87**, 1175–1213.

Hunt, J. (2014) *Genotype-by-Environment Interactions and Sexual Selection*. Wiley Blackwell. West Sussex, UK.

Killen, S.S., Atkinson, D. & Glazier, D.S. (2010) The intraspecific scaling of metabolic rate with body mass in fishes depends on lifestyle and temperature. *Ecol Lett*, **13**, 184–193.

Kvist, A. & Lindstrom, Å. (2001) Basal metabolic rate in migratory waders: intra-individual, intraspecific, interspecific and seasonal variation. *Functional Ecology*, **15,** 465–473

Lighton, J.R.B. (2008) *Measuring Metabolic Rates*. Oxford University Press, New York.

Dohm, M.R. (2002) Repeatability estimates do not always set an upper limit to heritability. *Functional Ecology*, **16**, 273–280.

Malishev, M., Bull, C.M. & Kearney, M.R. (2017) An individual-based model of ectotherm movement integrating metabolic and microclimatic constraints. *Methods in Ecology*, **9**, 472–489.

Maxwell, L.K., Jacobson, E.R. & McNab, B.K. (2003) Intraspecific allometry of standard metabolic rate in green iguanas, *Iguana iguana*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, **136**, 301–310.

McCue, M.D. (2010) Starvation physiology: Reviewing the different strategies animals use to survive a common challenge. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, **156**, 1–18.

McLean, J.A. & Speakman, J.R. (2007) Effects of Body Mass and Reproduction on the Basal Metabolic Rate of Brown Long-Eared Bats (*Plecotus auritus*). *Physiological and Biochemical Zoology*, 1–10.

Merritt, L., Matthews, P.G.D. & White, C.R. (2013) Performance correlates of resting metabolic rate in garden skinks *Lampropholis delicata*. *Journal of Comparative Physiology B*, **183**, 663–673.

Metcalfe, N.B. (2005) Intraspecific variation in competitive ability and food intake in salmonids: consequences for energy budgets and growth rates. *Journal of Fish Biology*, **28,** 525–531.

Nakagawa, S. & Schielzeth, H. (2010) Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. *Biological Reviews*, **85**, 935–956.

Nakagawa, S., Poulin, R., Mengersen, K., Reinhold, K., Engqvist, L., Lagisz, M. & Senior, A.M. (2015) Meta-analysis of variation: ecological and evolutionary applications and beyond. *Methods in Ecology*, **6**, 143–152.

Nespolo, R.F. & Franco, M. (2007) Whole-animal metabolic rate is a repeatable trait: a meta-analysis. *The Journal of Experimental Biology*, **210**, 3877–3878.

Norin, T. & Gamperl, A.K. (2018) Metabolic scaling of individuals vs. populations: Evidence for variation in scaling exponents at different hierarchical levels. *Functional Ecology*, **32**, 379–388.

Norin, T., Malte, H. & Clark, T.D. (2016) Differential plasticity of metabolic rate phenotypes in a tropical fish facing environmental change. *Functional Ecology*. **3,** 369–378.

O'Hara, R.B. (2009) How to make models add up — a primer on GLMMs. *Annales Zoologic Fennici*, **46**, 124–137.

Pettersen, A.K., White, C.R. & Marshall, D.J. (2016) Metabolic rate covaries with fitness and the pace of the life history in the field. *Proceedings of the Royal Society of London B: Biological Sciences*, **283**, 20160323

Powers, D.A. & Schulte, P.M. (1998) Evolutionary adaptations of gene structure and expression in natural populations in relation to a changing environment: A multidisciplinary approach to address the million‐year saga of a small fish. *Journal of Experimental Zoology*, **282**, 71–94.

Price, C.A., Weitz, J.S., Savage, V.M., Stegen, J., Clarke, A., Coomes, D.A., Dodds, P.S., Etienne, R.S., Kerkhoff, A.J., McCulloh, K., Niklas, K.J., Olff, H. & Swenson, N.G. (2012) Testing the metabolic theory of ecology. *Ecol Lett*, **15**, 1465–1474.

Réale, D., Garant, D., Humphries, M.M., Bergeron, P., Careau, V. & Montiglio, P.-O. (2010) Personality and the emergence of the pace-of-life syndrome concept at the population level. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, **365**, 4051–4063.

Ricklefs, R.E. & Wikelski, M. (2002) The physiology/life- history nexus. *Trends Ecol Evol*, 1–7.

Roff, D.A. (1995) The estimation of genetic correlations from phenotypic correlations: a test of Cheverud's conjecture. *Heredity*, **74**, 481–490.

Salin, K., Auer, S.K., Rey, B., Selman, C. & Metcalfe, N.B. (2015) Variation in the link between oxygen consumption and ATP production, and its relevance for animal performance: Table 1. *Proceedings of the Royal Society of London B: Biological Sciences*, **282**, 20151028–9.

Salin, K., Auer, S.K., Rudolf, A.M., Anderson, G.J., Selman, C. & Metcalfe, N.B. (2016) Variation in Metabolic Rate among Individuals Is Related to Tissue-Specific Differences in Mitochondrial Leak Respiration. *Physiological and Biochemical Zoology*, **89**, 511–523.

Salin, K., Luquet, E., Rey, B., Roussel, D. & Voituron, Y. (2012) Alteration of mitochondrial efficiency affects oxidative balance, development and growth in frog (Rana temporaria*) tadpoles. The Journal of Experimental Eiology,* ***215****, 863–869.*

Scott, I., Mitchell, P.I. & Evans, P.R. (1996) How does Variation Body Composition Affect the Basal Metabolic Rates of Birds of Birds? *Functional Ecology*, **10**, 307.

Seebacher, F. (2005) A review of thermoregulation and physiological performance in reptiles: what is the role of phenotypic flexibility? *Journal of Comparative Physiology B*, **175**, 453–461.

Seebacher, F., Brand, M.D., Else, P.L., Guderley, H., Hulbert, A.J. & Moyes, C.D. (2010) Plasticity of Oxidative Metabolism in Variable Climates: Molecular Mechanisms. *Physiological and Biochemical Zoology*, **83**, 721–732.

Singer, J.D. & Willett, J.B. (2003) *Applied Longitudinal Data Analysis: Modeling Change and Event Occurrence*. Oxford University Press, New York.

Somero, G.N. (1978) Temperature Adaptation of Enzymes: Biological Optimization Through Structure-Function Compromises. *Annual Review of Ecology and Systematics*, 1–30.

Speakman, J.R., Talbot, D.A., Selman, C., Snart, S., McLaren, J.S., Redman, P., Krol, E., Jackson, D.M., Johnson, M.S. & Brand, M.D. (2004) Uncoupled and surviving: individual mice with high metabolism have greater mitochondrial uncoupling and live longer. *Aging Cell*, **3**, 87–95.

Steyermark, A.C. (2005) Physiological and morphological correlates of among-individual variation in standard metabolic rate in the leopard frog *Rana pipiens*. *The Journal of Experimental Biology*, **208**, 1201–1208.

Uyeda, J.C., Pennell, M.W., Miller, E.T., Maia, R. & McClain, C.R. (2017) The Evolution of Energetic Scaling across the Vertebrate Tree of Life. *The American Naturalist*, 000–000.

van de Pol, M. & Wright, J. (2009) A simple method for distinguishing within- versus between-subject effects using mixed models. *Animal Behaviour*, **77**, 753–758.

Via, S., Gomulkiewicz, R., De Jong, G., Scheiner, S.M., Schlichting, C.D. & Van Tienderen, P.H. (1995) Adaptive phenotypic plasticity: consensus and controversy. *Trends Ecol Evol*, **10**, 212–217.

Westneat, D.F., Stewart, I.R.K. & Hatch, M.I. (2009) Complex interactions among temporal variables affect the plasticity of clutch size in a multi-brooded bird. *Ecology*, **90**, 1162–1174.

Westneat, D.F., Wright, J. & Dingemanse, N.J. (2014) The biology hidden inside residual within-individual phenotypic variation. *Biological Reviews*, **90**, 729–743.

White, C.R. & Kearney, M.R. (2012) Determinants of inter-specific variation in basal metabolic rate. *Journal of Comparative Physiology B*, **183**, 1–26.

White, C.R., Schimpf, N.G. & Cassey, P. (2013) The repeatability of metabolic rate declines with time. *The Journal of experimental biology*, **216**, 1763–1765.

Wiersma, P., Munoz-Garcia, A., Walker, A. & Williams, J.B. (2007) Tropical birds have a slow pace of life. *Proceedings of the National Academy of Sciences*, **104**, 9340–9345.

Williams, J.B., Miller, R.A., Harper, J.M. & Wiersma, P. (2010) Functional Linkages for the Pace of Life, Life-history, and Environment in Birds. *Integrative and comparative biology*, **50**, 855–868.

Withers, P.C. (1992) *Comparative Animal Physiology*. Philadelphia: Saunders College Pub.

# Tables

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Table 1.** Adjusted repeatability of log metabolic rate and their 95% credible intervals, across six measurement temperature estimated by function-valued models. Rintercept is the repeatability at the intercept, Rshort is 'short-term' repeatability, Rlong is 'long-term' repeatability. See statistical analyses for more details on the technical differences of three estimates. N = 42, nobs = 2410. All estimates are significantly different from zero. | | | | | | | | | |
|  | Rintercept | | | Rshort | | | Rlong | | |
| Temperature | Estimate | Lower | Upper | Estimate | Lower | Upper | Estimate | Lower | Upper |
| 22 | 0.76 | 0.59 | 1 | 0.15 | 0.1 | 0.22 | 0.12 | 0.07 | 0.18 |
| 24 | 0.46 | 0.3 | 0.62 | 0.36 | 0.28 | 0.43 | 0.16 | 0.09 | 0.24 |
| 26 | 0.53 | 0.38 | 0.69 | 0.33 | 0.26 | 0.41 | 0.18 | 0.1 | 0.27 |
| 28 | 0.61 | 0.47 | 0.75 | 0.32 | 0.25 | 0.4 | 0.2 | 0.12 | 0.28 |
| 30 | 0.68 | 0.53 | 0.82 | 0.33 | 0.24 | 0.42 | 0.22 | 0.14 | 0.32 |
| 32 | 0.72 | 0.55 | 0.89 | 0.35 | 0.25 | 0.45 | 0.25 | 0.16 | 0.36 |

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| **Table 3.** Cross-temperature correlations of log metabolic rate (*R*2)and their 95% credible intervals, estimated using the **a)** function-valued approach, **b)** character-state approach at the among-individual and within-individual level. N = 42, nobs = 2410. Bolded estimates are signfiicantly different from zero. \* indicates that correlation estimate has been set to 1 because there were estimation issues in the function-value model where *R*2 exceeded 1. | | | | | | | | | | | | | |
|  | **a)** Function-valued approach | | | | | | **b)** Character-state approach | | | | | | |
|  | Among-individual | | | Within-individual | | | Among-individual | | | | Within-individual | | |
| Pairwise comparison (*R*2) | Estimate | Lower | Upper | Estimate | Lower | Upper | Estimate | Lower | Upper | Estimate | | Lower | Upper |
| 22ºC – 24ºC | **0.98** | 0.95 | 1 | **0.99** | 0.98 | 1 | 0.43 | -0.04 | 0.80 | **0.23** | | 0.12 | 0.34 |
| 22ºC – 26ºC | **0.93** | 0.83 | 0.99 | **0.96** | 0.9 | 1 | 0.44 | -0.03 | 0.81 | 0.06 | | -0.06 | 0.17 |
| 22ºC – 28ºC | **0.86** | 0.67 | 0.98 | **0.9** | 0.72 | 1 | **0.56** | 0.13 | 0.87 | **0.2** | | 0.09 | 0.31 |
| 22ºC – 30ºC | **0.78** | 0.52 | 0.97 | **0.8** | 0.46 | 1 | **0.59** | 0.23 | 0.87 | 0.12 | | 0 | 0.23 |
| 22ºC – 32ºC | **0.71** | 0.39 | 0.97 | **0.67** | 0.21 | 1 | 0.37 | -0.04 | 0.72 | **0.19** | | 0.07 | 0.29 |
| 24ºC – 26ºC | 0.98 | 0.96 | 1 | **0.99** | 0.97 | 1 | **0.72** | 0.40 | 0.92 | **0.2** | | 0.09 | 0.31 |
| 24ºC – 28 | 0.93 | 0.79 | 1\* | 1\* | 1\* | 1\* | 0.38 | -0.05 | 0.76 | 0.02 | | -0.09 | 0.13 |
| 24ºC – 30 | 0.88 | 0.71 | 1\* | 1\* | 1\* | 1\* | 0.42 | 0.00 | 0.75 | **0.26** | | 0.14 | 0.36 |
| 24ºC – 32 | 0.82 | 0.63 | 1\* | 1\* | 1\* | 1\* | **0.66** | 0.30 | 0.91 | **0.29** | | 0.19 | 0.39 |
| 26ºC – 28 | **0.99** | 0.97 | 1 | **0.98** | 0.95 | 1 | **0.80** | 0.55 | 0.95 | **0.14** | | 0.03 | 0.25 |
| 26ºC – 30 | **0.96** | 0.9 | 1 | **0.93** | 0.8 | 1 | 0.24 | -0.18 | 0.65 | 0.04 | | -0.07 | 0.15 |
| 26ºC – 32 | **0.92** | 0.82 | 0.99 | **0.84** | 0.59 | 1 | **0.46** | 0.07 | 0.78 | **0.14** | | 0.03 | 0.25 |
| 28ºC – 30 | **0.99** | 0.98 | 1 | **0.98** | 0.94 | 1 | **0.72** | 0.41 | 0.93 | 0.08 | | -0.03 | 0.2 |
| 28ºC – 32 | **0.97** | 0.93 | 1 | **0.92** | 0.8 | 1 | **0.72** | 0.45 | 0.91 | **0.21** | | 0.1 | 0.31 |
| 30ºC – 32 | **0.94** | 0.89 | 0.99 | 1\* | 0.91 | 1\* | **0.81** | 0.55 | 0.95 | **0.21** | | 0.09 | 0.31 |

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| **Table 4.** Multilevel mass-scaling exponents and their 95% credible intervals estimated at the among-individual and the within-individual level across six measurement temperatures. N = 42, nobs = 2410. Bolded estimates are significantly different from zero | | | | | | |
|  | Among-individual | | | Within-individual | | |
| Temperature | Estimate | Lower | Upper | Estimate | Lower | Upper |
| 22 | **1.09** | 0.49 | 1.68 | **1.86** | 1.13 | 2.62 |
| 24 | **0.62** | 0.04 | 1.2 | **2.71** | 1.93 | 3.46 |
| 26 | **0.76** | 0.15 | 1.31 | **1.74** | 1.06 | 2.53 |
| 28 | **0.69** | 0.08 | 1.26 | **1.89** | 1.16 | 2.68 |
| 30 | **1.1** | 0.5 | 1.67 | **1.8** | 1.08 | 2.52 |
| 32 | **0.91** | 0.25 | 1.44 | **1.73** | 1.06 | 2.47 |