# 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 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 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. 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 providing 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 and future studies that utilise the predictive power of energetic scaling relationships should bear in mind the different levels of aggregation.

# 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 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 cost’(Biro & Stamps 2008) and restricts how much energy is allocated to competing processes such as growth, reproduction and maintenance (De Jong & Van Noordwijk 1992). Variation in metabolic rate is a likely a target for selection because it thought to be 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). Furthermore, an individual’s metabolism contributes to the flow of energy, biomass and nutrients in the population, which can have 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 will help elucidate eco-evolutionary dynamics (Brown *et al.* 2004; Glazier 2015).

Metabolic theories attempt to unify ecological processes based on physiological rates at the individual level (Gillooly *et al.* 2001; Brown *et al.* 2004). These theories rely on first principles from biology, chemistry and physics to explain how energetic demands change with body mass. 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). However, this may be overly simplistic view given that physiological mechanisms at the individual level are highly complex and variable (Glazier 2005; White & Kearney 2012). For example, many explanations for a ‘universal’ mass-scaling exponent are based on the assumption that supply of macronutrients to metabolic machinery is constant, but this is known to vary between individuals (Speakman *et al.* 2004; Steyermark 2005; Metcalfe 2005). Moreover, species differences in mass scaling exponents have been linked to factors such as temperature and endo-/ectothermy, further challenging this ‘one-size-fits-all’ line of thinking (Barneche, White & Marshall 2016, Killen, Atkinson & Glazier 2010). While variation in scaling exponents may have true biological meaning, it may also represent a misleading generalisation where energetic scaling relationships described at the lower levels are incorrectly attributed to higher taxonomic levels (van de Pol & Wright 2009). Processes governing shifts in metabolic rate within individuals can thus impact among-individual relationships that ultimately carry over to species level relationships (Fig. 1). While the mechanisms driving variability in energetic scaling remains contentious, individual level metabolic responses may provide important insights 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 represents the species average of log metabolic rate and log body mass. The small dots represent the individual averages of log metabolic rate and log body mass 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, after which, among species metabolic scaling relationship is estimated (in set).

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

(Somero 1978) and mitochondrial capacity (Salin *et al.* 2012) are known to respond to fluctuations in body condition, as well as changes in the thermal environment (reviewed in Seebacher 2005). Individual differences in metabolic plasticity may explain why there is no agreed upon, 'universal’ metabolic scaling relationship (Salin *et al.* 2015; 2016). Given that physiological machinery adjusts to oscillations in temperature and body mass within the lifetime of an individual (e.g. seasonal changes), the mass scaling relationship with metabolic rate 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), 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 fundamental in understanding the capacity for plasticity itself to evolve. Repeated sampling of reaction norms is logistically tedious but important for metabolic scaling studies as body mass and metabolic rate tend to be measured only once. This makes metabolic scaling studies strongly susceptible to sampling variability if scaling relationships exhibit low repeatability.

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, function-valued approaches (also known as ‘polynomial’ approach, Via *et al.* 1995), describe phenotypic changes 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 individual level variation for metabolic scaling. 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) Does the relationship between metabolism and body size differ at the among- and within- individual level?

# 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.

## 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).

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 enticed into an 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 chambers. The chambers were left in the incubator for animals to respire. After 90 minutes, two 3mL air samples were taken 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):

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). For more details on data cleaning see electronic supplementary materials (ESM). In all our models, is log-transformed. Initial analyses show that there were no differences in logbetween 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 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 (wAIC (Full model – reduced model) = -4.73 and loo = -4.73) compared to a model without it, we therefore included ‘previous temperature experience’ in all subsequent analyses (Table S1. see ESM).

We made use of Bayesian generalised linear mixed models (LMM) from either the R package ‘brms’ (Bürkner 2017) or ‘MCMCglmm' (Hadfield 2010). For details on model set up and diagnostics see ESM. We present pooled posterior means from all chains and their 95% credible intervals. We presented our model structures following in the ESM.

## 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, temperature and ‘previous temperature experience’ were log-transformed, body mass was first log-transformed and then z-transformed (mean of 0 and sd of 1, hereafter referred to as ‘zlog bodymass’). All equations for calculating repeatability of various reaction norm attributes can be found in the ESM. 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 reaction norms within an individual among sampling sessions (Araya-Ajoy, Mathot & Dingemanse 2015). 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

Repeatable intercepts describe whether individuals’ average metabolic rate vary consistently at a particular temperature. We compared three methods of calculating repeatability of the intercept. The first function-valued method involves setting the intercept at a relevant temperature by mean-centering (Araya-Ajoy, Mathot & Dingemanse 2015). We fitted six models, each centered on a different measurement temperature. In these models, log was the response and log centered temperature, zlog body mass and log ‘previous temperature experience’ were included as fixed predictors. We fitted individual ID and series ID as random intercepts and centered log temperature as random slopes (ESM model 1).We calculated adjusted repeatability of the intercept at each measurement temperature using ESM equation 1. To assess how the repeatability of the intercept changed over the course of our study, we also calculated ‘short term’ and ‘long term’ adjusted repeatability (ESM equation 2, 3, Araya-Ajoy, Mathot & Dingemanse 2015).

The second function-valued method derives ‘conditional’ repeatability for intercept values at each temperature following Singer & Willett (2003) (ESM equation 4, see also Briffa, Bridger & Biro 2013). To the best of our knowledge, the method to derive ‘conditional’ repeatability has not been extended to multiple random effects. As a consequence, we fitted a model which has the same predictors as the previous function-valued models, except that session number was included as an additional fixed predictor, as opposed to using seriesID as a random effect (ESM model 2).

The final method to calculate temperature-specific adjusted 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 response variables as a 6 x 6 response matrix with zlog body mass and log previous temperature experience as fixed predictors. Note that temperature is no longer a predictor as this is now part of the response matrix. Individual ID and sampling session were included as random intercepts (ESM model 3). This process requires a substantial amount of data because the model estimates (co)variances at each temperature at the among-individual and within-individual level. Using the variance-covariance matrix of this model, we calculated temperature specific adjusted repeatability following ESM Equation 5.

## 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. One can use any of the temperature-centered function-valued models to calculate the repeatability of the slope using ESM equation 6. However, we chose to use a function-valued model where log temperature is not mean-centered as this model was also used to derive cross-temperature correlations (see below).While the character state approach does not estimate a slope as it assumes that login each environment are “distinct” traits, one can still assess the thermal plasticity of a trait by identifying changes in repeatability across temperatures using ESM equation 5. This is conceptually the same as quantifying whether slopes are repeatable or not.

## 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 can be estimated directly from the character-state model at both the among- and within individual level, although these are not directly estimated from function-valued models as they are part of the general slope describing the reaction norm. However, correlations can be derived from function-valued models using matrix algebra and the variance-covariance matrix of the intercept and slope following Brommer 2013 (For more details and equations see ESM). We derived correlations at the among individual and within-individual-among-sampling-session level (seriesID) from the function-valued model that was used to calculate the repeatability of the slope (see above).

## Hierarchical mass scaling exponents at different temperatures

Using ‘brms’, we set out to test whether mass-scaling exponents were temperature dependent and whether mass-scaling relationships differed at the among and within-individual level. Exploratory statistics show that the mean percentage change in mass across the experiment within an individual (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). First, we calculated mean mass across all sampling sessions for each individual (among-individual effect). Second, we obtained a within-individual effect by subtracting an individual’s mass from its own mean (i.e. within-individual centering, van de Pol & Wright 2009).

In the first model, we fitted logas the response and included the among-individual and within-individual mass effect as well as, their interactions with temperature as fixed predictors (ESM model 4). The second model has the same structure but did not include the interactions with temperature (ESM model 5). In both models, we included individual ID as a random intercept and the within individual effect as a random slope since exploratory graphs show that individuals mass change at different rates through the study (See Fig 5B).

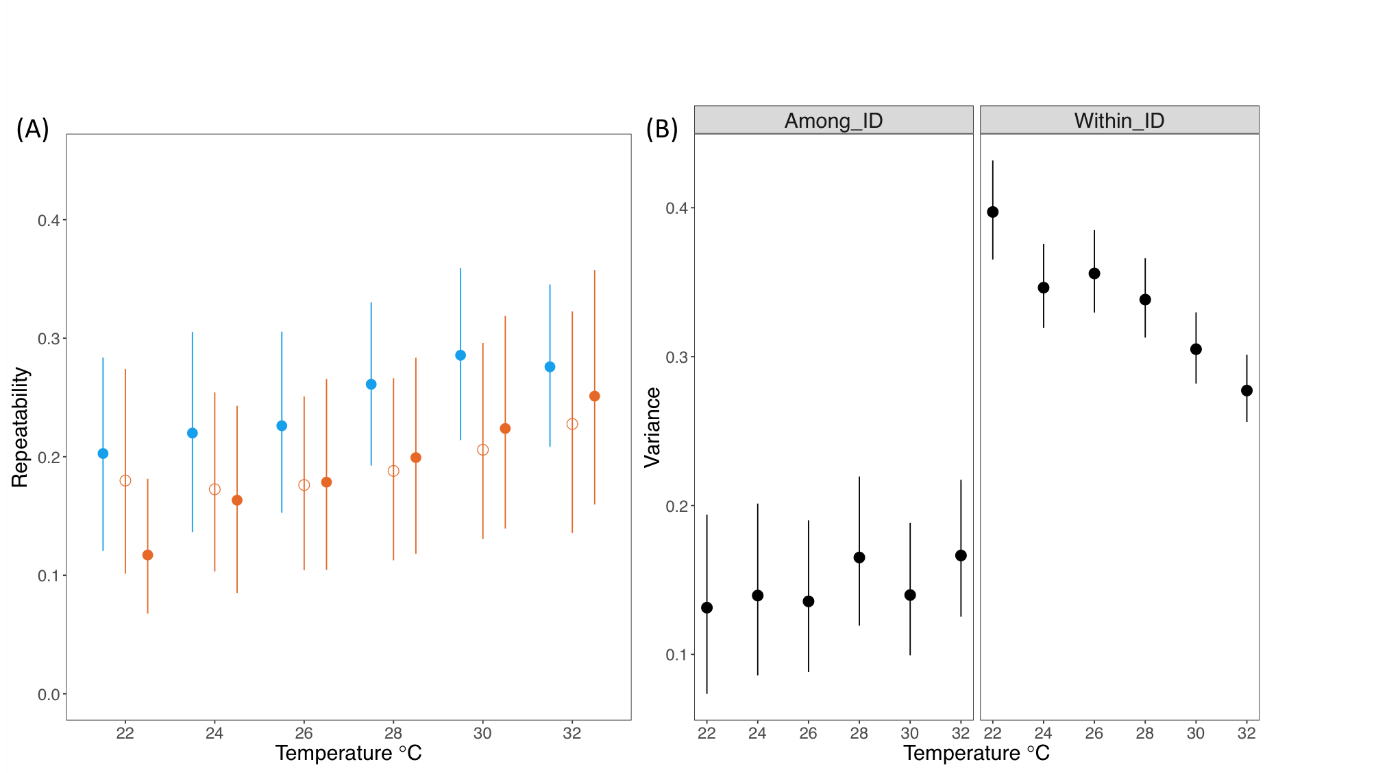
In order to test for temperature dependence, we assessed whether the first model or second model was better supported or not using wAIC and loo. To test whether the contrast between among and within individual mass scaling significantly different from zero at each temperature, we subtracted the coefficient for within-individual interaction from the coefficient of among-individual interaction.

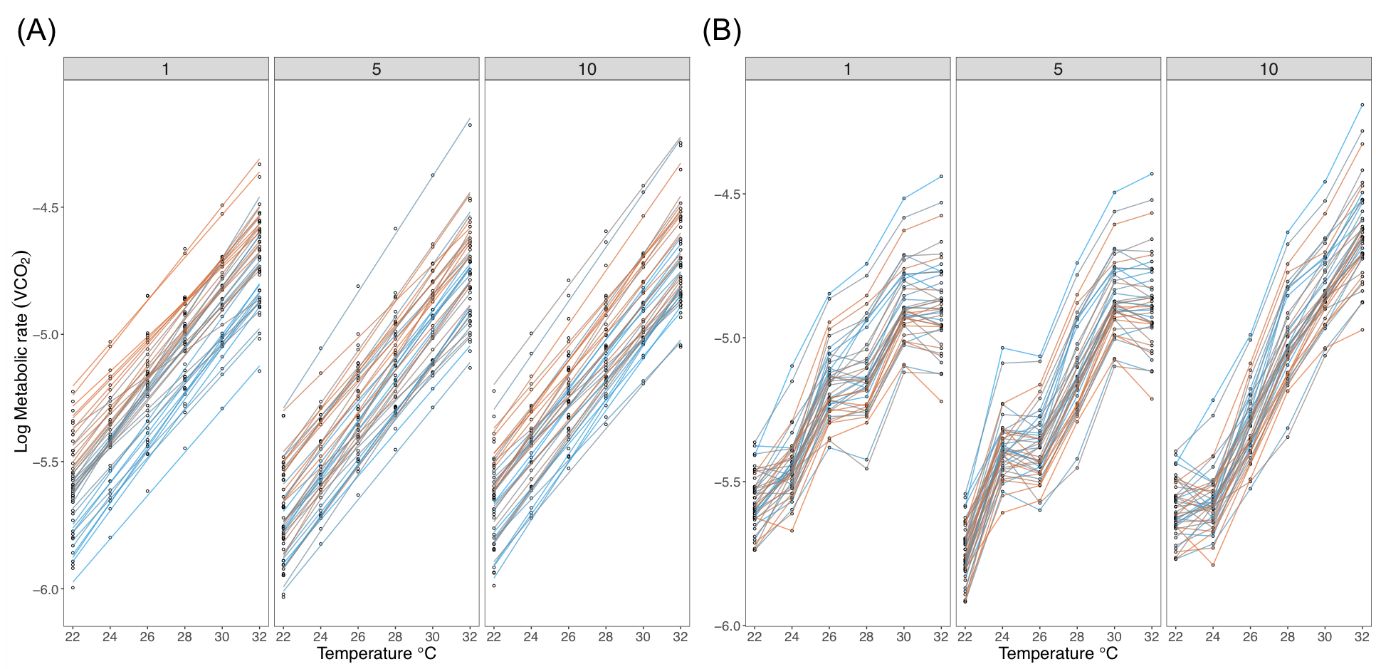
We also compared the within- and among individual scaling exponents with exponents from a model that represents the typical analysis of a metabolic scaling study model (i.e. does not account for the hierarchal structure in the data). This analysis is presented in the ESM.

# 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). 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, orange filled circles represent the function-valued ‘long’ term repeatability method. Blue filled circles represent the character state approach in estimating repeatability. See Statistical analyses for more details. B) Posterior mean of variance of log 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).

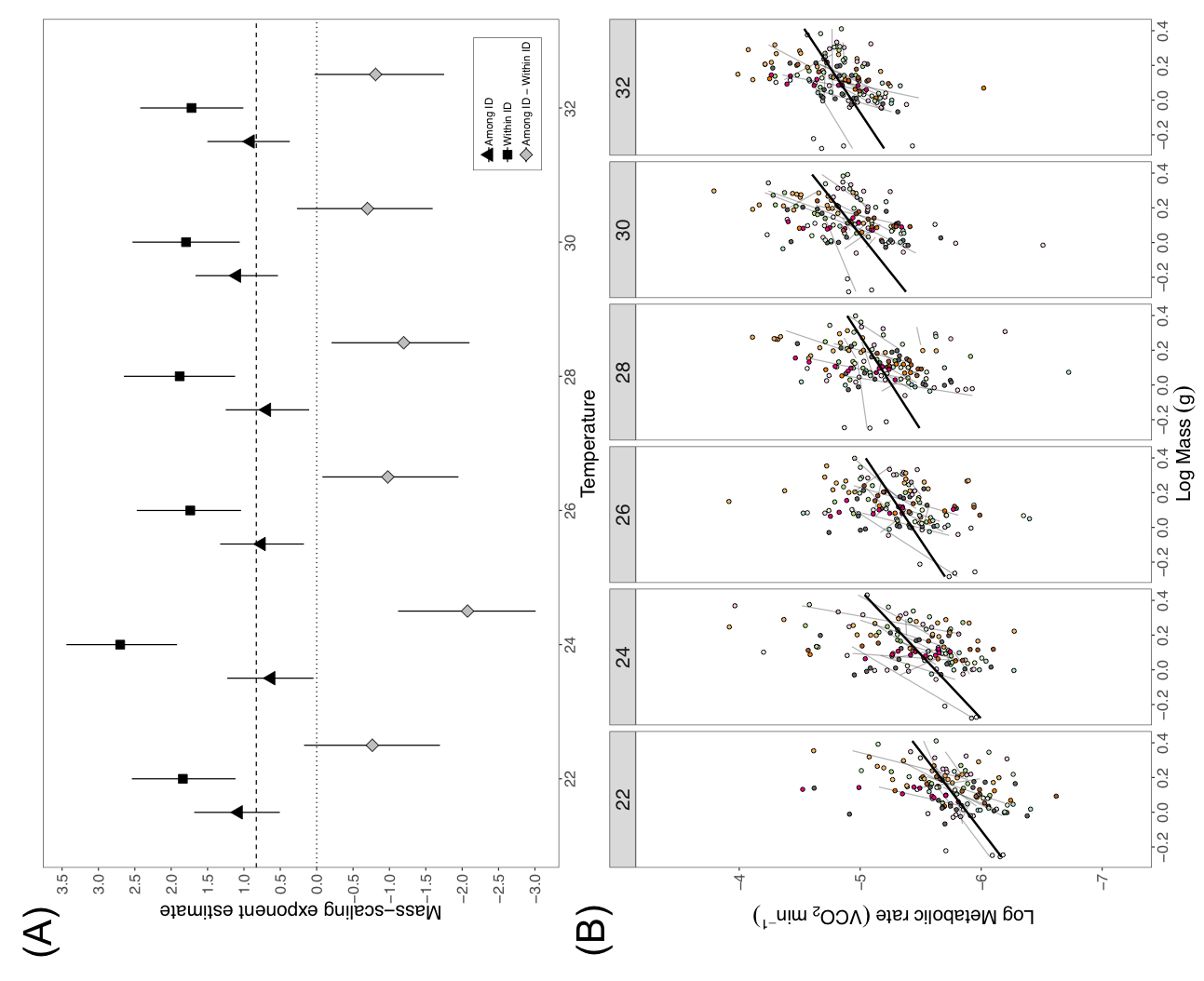
Overall, cross-temperature correlations estimated using the function-valued approaches were a lot higher and the credible intervals were very narrow. The correlations estimated using function-valued approaches are congruent with the character-state approach. The function-valued estimates were generally 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 no temperature dependence in coefficients. 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 log mass changes. Between individual scaling exponents (Black triangle) describes the change in log across individuals as log mass changes on the 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 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 among-individual scaling relationship. Faint grey lines represent the within-individual scaling relationship

# Discussion

We took advantage of both function-valued and character-state approaches to investigate the repeatability of thermal plasticity in metabolic rate. 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. Cross-temperature correlations of metabolic rate were all 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’ (Falconer 1952, c.f. Dohm 2002). Our findings show that metabolic plasticity consistently differed among-individuals over time. 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. 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 (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 the correlations from the function-valued approach are in agreement with the character-state approach, the magnitude of correlations across all temperatures remained strong. 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 (Killen *et al.* 2010; Price *et al.* 2012; 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).

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- and 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. It is important to note that the range in body mass in our study is small (0.75g – 1.637g) which could give rise to large within-individual mass-scaling exponents due to sampling error. Nonetheless, our within-individual estimates were in line with studies of bats and birds (McLean & Speakman 2007, Kvist & Lindstrom 2001). Large within-individual mass scaling exponents may indicate that slight changes body composition 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 for measurements throughout the course of the study. 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 and 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 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

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# 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 has been set to one because there were estimation issues in the function-value model. | | | | | | | | | | | | | |
|  | **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 | 1.09 | 1.28\* | 1.08 | 1.49 | 0.38 | -0.05 | 0.76 | 0.02 | | -0.09 | 0.13 |
| 24ºC – 30 | 0.88\* | 0.71 | 1.08 | 1.39\* | 1.08 | 1.78 | 0.42 | 0.00 | 0.75 | **0.26** | | 0.14 | 0.36 |
| 24ºC – 32 | 0.82\* | 0.63 | 1.05 | 1.46\* | 1.03 | 2.17 | **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 | 1.04\* | 0.91 | 1.23 | **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 | | | Among – Within | | |
| Temperature | Estimate | Lower | Upper | Estimate | Lower | Upper | Estimate | Lower | Upper |
| 22 | **1.09** | 0.49 | 1.68 | **1.86** | 1.13 | 2.62 | -0.77 | -1.68 | 0.14 |
| 24 | **0.62** | 0.04 | 1.2 | **2.71** | 1.93 | 3.46 | **-2.09** | -3.05 | -1.14 |
| 26 | **0.76** | 0.15 | 1.31 | **1.74** | 1.06 | 2.53 | -0.97 | -1.94 | -0.09 |
| 28 | **0.69** | 0.08 | 1.26 | **1.89** | 1.16 | 2.68 | **-1.2** | -2.15 | -0.25 |
| 30 | **1.1** | 0.5 | 1.67 | **1.8** | 1.08 | 2.52 | -0.7 | -1.62 | 0.23 |
| 32 | **0.91** | 0.25 | 1.44 | **1.73** | 1.06 | 2.47 | -0.82 | -1.79 | 0.06 |