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

Fonti Kar1, Shinichi Nakagawa1,2, Christopher Friesen3, Daniel Noble1,2,4

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

2 *Diabetes and Metabolism Division, Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst, Sydney, NSW 2010, Australia*

3

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

Correspondence author: Fonti Kar

Correspondence email: fonti.kar@gmail.com

# Abstract

Metabolic rate, an intrinsic property of an individual, can limit many biological processes from individual to ecosystems. An individual’s energetic expenditure is sensitive to changes in the internal (e.g. body mass) and external environment (e.g temperature), but whether this metabolic plasticity is consistently expressed or not is well understood. Biological variation in metabolic rate and body mass is hierarchically organised, if this data structure is not properly modelled, energetic scaling relationships at individual level may be problematic when generalised to higher taxonomic levels. We repeatedly sampled metabolic reaction norms in an ectotherm model (*Lampropholis delicata* – the delicate skink) to characterise the repeatability of metabolic thermal plasticity and to identify the patterns of cross-temperature correlations of the reaction norm. We also tested whether mass-scaling exponents change with temperature and whether within-individual changes in body mass can affect among-individual mass-scaling exponents. Making use of both function-valued and character state approaches, we found that the slope of the metabolic reaction norm is significant repeatable. We 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) as temperatures got hotter. Cross-temperature correlations in metabolic rate differed between the character-state and function-valued approach demonstrating that the evolutionary inferences we draw on how the shape of the reaction norm can evolve can depend on what statistical approaches researchers employ. Finally, we show that mass-scaling exponents at both among- and within- individual levels are temperature dependent and differ substantially. The proximate causes and implications of individual variation in metabolic plasticity are discussed in the context of the evolution of phenotypic plasticity and energetic scaling.

# Keywords

Phenotypic plasticity, reaction norm, gene-by-environment interaction, thermal sensitivity, repeatability, pace-of-life

# Introduction

Animals live in a multifaceted world where their external and internal environment is often a state of flux. Changes in rainfall, density of conspecifics, age and body condition can all simultaneously affect an individual. In order to cope with environmental heterogeneity, animals respond 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). Metabolic rate, in particular, determines an individual’s energetic capacity for competing processes such as growth, reproduction and somatic maintenance, making it a fundamental measure in ecology and evolution (De Jong & Van Noordwijk 1992; Brown *et al.* 2004). Metabolic rate is likely a target for selection because it may be the casual mechanism for driving variation in suites of correlated traits, known as ‘paces-of-life’ (Réale *et al.* 2010; Ricklefs & Wikelski 2002; Malishev, Bull & Kearney 2017; Biro & Stamps 2010). For example, tropical birds tend to have low basal metabolic rate, produce fewer slow-growing offspring and longer longevity relative to their tropical counterparts (Wiersma *et al.* 2007; Williams *et al.* 2010). Furthermore, an individual’s metabolism contributes to the flow of energy, biomass and nutrients in the population, which can have cascading impacts at the community and ecosystem level (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 (Wikelski et al., 2003, 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 such as species diversification (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 the first principles of biology, chemistry and physics to explain how energetic demands changes 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 changes with temperature following a logarithmic function (Gillooly *et al.* 2001; Brown *et al.* 2004). This generalisation across all 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 of the universal mass-scaling exponent are based on the assumption that supply of resources such as macronutrients to the metabolic machinery is constant, but this is known to vary between individuals (Reviewed in Glazier 2005, 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, in addition to biotic factors such as endo-/ectothermy, can interact to influence how species respond to the environment which drives variation in metabolic scaling among species (White & Kearney 2012; Barneche, White & Marshall 2016, Uyeda *et al.* 2017, Clarke 2004, Killen, Atkinson & Glazier 2010, Glanville & Seebacher 2006). Interspecific variation in scaling exponents may have true biological meaning, but it may also be due to an ‘exception fallacy’ where the energetic scaling relationships described at the intraspecific level is incorrectly attributed to broader taxonomic levels (Van de pol, 2009). In other words, scaling relationships within an individual can impact among-individual relationships and which could cascade up 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. 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 body mass of a given species. The small dots represent the mean log metabolic rate and mean 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 one 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.

Individuals are physiologically diverse which results in individual differences in metabolic plasticity. The mechanisms that underpin how energetic demands respond to the changes in the internal environment such as body mass, are also undoubtedly sensitive to external environmental conditions. Membrane composition (Hulbert *et al.* 2007), enzyme structure and function

(Somero 1978) and mitochondrial capacity (Salin *et al.* 2012) are known to remodel in response to fluctuations in body condition, as well as, changes in the thermal environment (reviewed in Seebacher 2005). Individual variation in physiological structures that determine metabolic plasticity to internal and external environments may be a promising explanation of why there is no universal metabolic scaling relationship. Given that physiological machinery will adjust to oscillations in temperature and body mass within a lifetime of an individual (e.g. seasonal changes), the mass scaling relationship with metabolic rate will likely shift as well.

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 natural selection acts on. 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’) and has only been reported in only a few species (Briga & Verhulst 2017, Careau, Gifford & Biro 2014b). Characterising repeatability in metabolic reaction norms is one of the first steps in understanding the capacity for it to evolve. However, it is logistically tedious and requires repeated measures of the same individuals over an ecologically relevant duration. This is rarely performed in metabolic scaling studies where body mass and metabolic rate tend to be measured once, which is likely to be capturing a lot of sampling variability if mass-scaling exhibits low repeatability.

Part of the challenge in quantifying the repeatability of metabolic plasticity is that there are multiple ways in which plasticity itself can be modelled. Broadly speaking, two approaches in modelling plasticity are prevalent and debated in evolutionary biology (Via *et al.* 1995, Hunt 2014). The character-state approach models phenotypic change in a set of environments as discrete ‘states’. Under this method, selection pressures can vary between environments and the mean phenotypic values in each environment are subjected to selection. Whereas in the function-valued approach (also known as ‘polynomial’ approach, Via *et al.* 1995), changes in a trait across an environmental range is described by 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 metabolic rate in relation to body size and thermal plasticity 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 about individual variation of reaction norms . (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) Do these mass scaling exponents change with temperature? (4) Does mass scaling exponents 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 was provided with a thermal gradient. 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 license was approved by the New South Wales National Parks and Wildelife Service (SL101549). All procedures were approved by the Macquarie University Ethics committee (ARA 2015/015) and University of New South Wales Animal Care and Ethics committee (ACEC 15/51A).

## Quantifying metabolic reaction norms

Metabolism assays were conducted between 26 December 2016 - 19 March 2017. We measured metabolic rate as CO2 production per unit time (VCO2 ml min-1). Animals were measured at a random temperature in an inactive, post-absorptive state (Withers 1992). Overall, measurements were taken at temperatures between 22ºC and 32ºC at 2ºC increments 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). 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.

Lizards were fasted for at least 24 hours prior to metabolic measurements, during which they had access to water in their enclosure between each sampling day. Within each sampling session, lizards were randomly assigned to opaque cylindrical metabolism chambers (volume = 146ml). On the day of measurements, body temperature of each individual was taken using an infrared laser gun) inside their enclosure (Stanley stht0-77365. Lizards were then gently encouraged into their assigned chambers and then weighed using a digital scale to the nearest 0.01g (Ohaus SP-202). After assignment, opened chambers were maintained in a dark environment inside the incubators for 30 minutes for animals to settle down. After 30 minutes, each chamber was flushed with fresh air and then sealed. 3 mL of air was then immediately removed from each chamber via a two-way valve to account for any residual CO2 that was not flushed out from the chambers. The chamber was then left in the incubator for an additional 90 minutes at the set temperature to measure CO2 production. At the end of the 90 min, two air samples were taken from each chamber and chambers were reopened and placed back into the incubator again for the next measurement temperature following the same procedure.

After air sampling, each 3 mL air sample was injected into the inlet line of an open-flow respirometer system (Field Metabolic System Sables System, Las Vegas NV, USA) to measure VCO2*.* The percentage of CO2 in our samples was measured using a flow rate set to 200 ml min-1. Water vapour was scrubbed from the inlet air with Drierite prior to the measurement of CO2. The area of output peaks was integrated using WartHog Systems LabAnalyst software to calculate the percentage of CO2 (Mark Chappell, Regents of University of California). The total volume (ml) of CO2 produced by an individual was calculated as: n

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 the data cleaning process see ESM). Data and code with which to generate our results are openly available via the Open Science Framework (see Data Accessibility). In all our models, VCO2 is log-transformed. Collinearity between our predictor variables were checked using a scatterplot matrix (Fig. S1). Pearson correlation coefficients of these are presented in Table S2. Initial analyses show that there were no differences in logVCO2 between blocks of lizards or incubators therefore these parameters were not included in our final models (see ESM for these analysis). We tested whether the previous measurement temperature or body temperature measured in home enclosure influenced logVCO2 at subsequent temperatures due to acclimation. We found that a model containing ‘previous temperature experience’ as a covariate was better supported by using information criterions (wAIC and loo) compared to a model with it excluded, we therefore included ‘previous temperature experience’ in all subsequent analyses (Table S1. see ESM for more details).

We made used 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 for 4 chains of 2000 iterations with a burn in of 1000 and a thinning interval of 1. For ‘MCMCglmm’ models, we used uninformative, expanded-parameter 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 (i.e. Rhat values) are smaller than 1.1. We also checked whether our chains were not autocorrelated. We present posterior means and their 95% credible intervals. Below, we present our model structures in a simply ‘primer’ format following (O'Hara 2009 and Wilkinson & Rogers 1973 so that readers are clear on the final models we ran (see code for further details).

### Repeatability of thermal reaction norms

We used function-valued and character state approaches to quantify the repeatability of thermal reaction norms to determine whether they differed consistently among individuals. In all our repeatability models, metabolic rate and temperature was log-transformed, body mass was first log-transformed and then z-transformed to account for the allometric scaling relationship between metabolic rate and body mass (Nakagawa *et al.* 2017). In order to account for variation in individual reaction norms between sampling sessions in our function-valued models, we coded ‘series ID’ - a categorical variable which denotes a unique combination of individual IDs and the sampling session IDs (e.g. ID001\_Session5, see provided code and Araya-Ajoy, Mathot & Dingemanse 2015 for more details)

#### Reaction norm intercept – average response

Repeatable intercepts tell us whether individuals’ average metabolic rate vary consistently at a particular temperature. First, we used function-valued models to estimate the repeatability of the intercept of individual reaction norms. This class of models assumes that the intercept covaries with the slope of the linear reaction norm. The intercept represents the average trait value when log temperature = 0 (i.e. 1ºC), however this can be set at a biologically relevant temperature by mean-centering. Using ‘MCMCglmm’, we fitted six models to estimate the repeatability of the intercept at each measurement temperature where,

logVCO2 ~ logTempcen + zlogBodyMass + logPriorTemp + (1+ logTempcen | ID) + (1+ logTempcen | seriesID)

where logVCO2 log-transformed VCO2, 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 (i.e. log(22ºC) = 3.09, log(22ºC)centered at 22ºC = 0, log(24ºC)centered at 22ºC = 0.09, log(26ºC)centered at 22ºC = 0.17, etc), zlogBodyMass is log-transformed body mass that is then subsequently z-transformed (mean of 0 and sd of 1), logPriorTemp is log-transformed previous temperature. We fitted individual ID and series ID as random intercepts and logTempcen as random slopes (i.e. (1+ logTempcen | ID) and (Series + logTempcen)). For each of these models, we calculated adjusted repeatability (eqn 2) of the intercept VCO2 at each measurement temperature following Araya-Ajoy *et al.* (2015), using the entire posterior distributions of the relevant variance components:

Eqn 2:

where is the repeatability estimate for logVCO2 at 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).

Eqn 4:

Eqn 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 on individuals. 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).

We also used another method to derive ‘conditional’ repeatability (eqn 6) for intercept values at each temperature following Singer & Willett (2003) and Briffa, Bridger & Biro (2013). We fitted the following ‘MCMCglmm’ model which is similar to previous function-valued models, except that session number of when data was sampled is included as a fixed predictor (as opposed to seriesID),

logVCO2 ~ logTemp + zlogBodyMass + logPriorTemp + session +(1+ logTemp| ID)

This is because, so far, the method to derive ‘conditional’ repeatability has not be extended to multiple random effects. This allowed us to derive temperature-specific repeatability estimates using the covariance of the intercept and slope at each temperature using

Eqn 6:

where *Covind0,ind1* is the covariance of the individual intercept and individual slope and *T* is the measurement temperature at which repeatability is estimated. Note that *T* variable must be on the same scale as the temperature variable with which the intercept and slopes variance components were estimated (i.e. logTemp).

The character-state approach can also be used to calculate temperature-specific adjusted repeatability. This approach estimates variation of logVCO2 at each temperature and assumes they are ‘distinct’ traits that are correlated. Using ‘brms’, we treated logVCO2 measured at each temperature were treated as response variables and like previous models included the same as predictors. Note that temperature is no longer a predictor as this is now part of the response. We also included individual ID and sampling session number as random intercepts.

logVCO2 at six temperatures ~ zlogBodyMass + logPriorTemp + (1|ID) + (1|session)

This process requires a substantial amount of data points because the model estimates ‘intercepts’ at each temperature and their variance-covariance 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 adjusted repeatability following Equation 8. Indeed, this equation is equivalent to Equation 4, except that it is using temperature-specific variance components (Nakagawa & Schielzeth 2010).

Eqn 8:

where refers to the adjust repeatability at a given temperature; *Vind0,T* , *Vseries0,T*  and *Ve0,T*

represents the individual intercept, series intercept and residual variance component at a given temperature, respectively.

#### Slope – plasticity

Function-valued approaches uses 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 a function-valued model with the following structure,

logVCO2 ~ logTemp + zlogBodyMass + logPriorTemp + (1+ logTemp| ID) + (1+ logTemp| seriesID)

where logTemp, in this case is not mean-centered like the previous models but has the same fixed predictors and random effects as the models. This model was used to calculate the repeatability of the slope following,

Eqn 9:

where *Vind1* and *Vseries1* is the individual slope and series slope, respectively. The character state approach does not estimate a slope because assumes that logVCO2 in each environment is distinct traits, However, one can assess the thermal plasticity of a trait by identifying changes in repeatability across temperatures using RT (eqn 8). This is conceptually the same as quantifying whether slopes are repeatable or not as in the function-valued approach.

### Cross-temperature correlations in metabolic rate

Metabolic rate measured at one temperature will be undoubtedly correlated with metabolic rate measured at another temperature. We estimated these cross-environment correlations using both statistical approaches. While correlations can be estimated directly from the character-state model at both the among- and within individual level (by setting rescor = T in ‘brms’ or rcov = ~us(trait):units in ‘MCMCglmm’), 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). We derived correlations from the function-valued model that we used to calculate the repeatability of the slope (see above). This enabled us to calculate correlations at the among and within-individual-among-sampling-session level. The variance-covariance (*K*) from this model is denoted as:

Eqn 10:

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

Eqn 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 eqn 12. The same calculation is repeated using the ‘series’ variance-covariance matrix to obtain within-individual-among-sampling-sessions phenotypic variance-covariance matrix.

Eqn 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. These 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).

Eqn 13:

### Hierarchical mass scaling exponents at different temperatures

We set out to test whether mass-scaling exponents were temperature dependent and whether among and within-individual mass-scaling relationships differed. Exploratory statistics show that the mean change in mass within an individual (mass at the first sampling session – the last sampling session) was 0.12g (n = 42, SD = 0.09, range in mass differences = -0.04, 0.32).

First, we calculated mean mass across all sampling sessions for each individual (among-individual effect). In order to obtain a within individual effect, we calculated for each individual, for each measurement day, deviations of mass on given day, in a given sampling session from the individuals mean (i.e. within-individual effect, see van de Pol & Wright 2009 for more details). In order to test for temperature dependence, we fitted two models using ‘brms’ and assessed whether the model containing an interaction with temperature and the within- and among-individual mass effects was better supported by information criterions (wAIC and loo). The first model with the interaction had the following structure,

logVCO2 ~ Temp \* AmongIDMass + Temp \* WithinIDMass + (1 + WithinIDMass|ID)

where logVCO2 as a response and included an interaction term between the among individual mass effect with temperature and another 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 since exploratory graphs show that individuals mass change at different rates through the study (See Fig 5B). The second model was fitted without the interaction as following,

logVCO2 ~ Temp + AmongIDMass + WithinIDMass + (1 + WithinIDMass|ID)

To test whether the contrast between among and within individual mass scaling significantly different from zero or not at each temperature, we subtracted the coefficient for Temp \* WithinIDMass from the coefficient of AmongIDMass\*Temp.

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 log VCO2 increased with temperature (Fig. 2A). The intercept at all temperatures were repeatable over short and long temporal scales (Table 1a). The function-valued ‘conditional’ method showed highest repeatability at 22ºC, while the function-valued – ‘long term’ method found repeatability was highest at 30ºC. In contrast, 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 VCO2 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 VCO2 at the among (right panel) and within (left panel) individual level across six measurement temperatures. Error bars represent 95% credible intervals.

**Figure 3** – A) Predicted individual reaction norms for forty-two individuals of log VCO2 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 function-valued approaches (left) and character-state approaches (right). 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 the among individual level (Fig. 4, Table 3). Certain individuals maintained a high metabolic rate, 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 across different temperatures. 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).

We detected a similar positive correlation pattern at the within individual level (Fig 4, Table 3), although the magnitude was weaker. 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 however, estimates differ in magnitude and credible intervals may not be estimated accurately.



**Figure 4** – Cross-temperature correlations of metabolic rate 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). This indicates that mass-scaling exponents are not temperature dependent. 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 were 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 across six measurement temperatures. Within individual scaling exponents (Black square ) describes the change in log VCO2 as an individual’s mass changes on the logarithmic scale. Between individual scaling exponents (Black triangle) describes the change in log VCO2  across individuals as mass changes on the logarithmic scale after accounting for within individual effects. Similarly, the ‘average’ individual mass scaling exponents (White circles) represents the change log VCO2  across individuals as mass changes on the logarithmic scale, however it *does not account* for within individual variation in mass changes. The grey diamonds ♦ represent the difference between the between 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 VCO2 plotted against log body mass for a random subset of 20 individuals across six measurement temperatures. Each uniquely coloured point represents one individual. Thick bold line represents the change in log VCO2  over log body mass across all individuals. Faint grey lines represent the change in log VCO2  over log body mass within an individual.

# Discussion

We take advantage of both function-valued and character-state approaches to investigate how repeatable is thermal plasticity of 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. We also tested whether mass-scaling exponents changed with temperature and whether mass-scaling relationship differed at the among- and within-individual level. Our results show that metabolic rate and how it plastically responds to the environment, consistently differs across individuals over 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 values can be considerably larger than among-individual exponents. Mass-scaling exponents may be poorly estimated when the hierarchical structure of population-level data (i.e., disassociating within and between-individual variance) is not correctly modelled. Below we discuss the details of our main results, and also 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. M R 2002). Our findings show that metabolic plasticity (i.e., the slope of metabolic reaction norms) was significantly repeatable 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. In other words, an individual’s metabolic rate had greater predictability as temperatures became hotter. Individuals may have reached their physiological ‘ceiling’ at high temperatures and was therefore respiring more predictably. However, this is unlikely 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 of 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; 2017), 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. 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. On the other hand, the character-state approach requires a lot more data points to estimate means and covariances for each environment to uncover non-linear patterns. 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 for ~four months 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). Interestingly, we found that among-and within-individual exponents were most different at 24ºC. We hypothesise that thermal acclimation may this pattern. Individuals of different masses can remodel their physiology in order to optimise their energy expenditure to common housing conditions (~25ºC) (Chevin, Lande & Mace 2010; Seebacher, White & Franklin 2014). These compensatory adjustments involve changes in membrane composition or enzyme efficiency, which 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. This means that when the hierarchical structure of metabolic scaling data is not correctly modelled, as in that in typical metabolic scaling studies, population or species mass-scaling exponents 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 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 although the range in body mass in our sample population is small (range in mass = 0.75g – 1.637g), which could give rise to large values of within-individual mass-scaling exponents. Nonetheless, it is still intriguing that our estimates were in line of 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 energetic scaling (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 inGlazier 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 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 utilises 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 the population reaction norm in the face of warming climate. Given that within-individual variance declined with increasing temperatures, this may allow selection to operate more efficiently 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 evolutionary inferences we draw from them. We provide our dataset and code to show how all these techniques can be implemented, with the hope to encourage researchers to use both approaches in order to gain a holistic view of their “reaction-norm” data. Molecular studies elucidating the mechanisms of metabolic plasticity would be invaluable to better explain deviations from the ¾ power law 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

# Acknowledgements

This study would not have been possible without the funding of XXXX. We recognise The Office of Environment and Heritable, New South Wales for our wildlife collection permit and the animal ethics committee from University of New South Wales and Macquarie University for our animal ethics permit. We express gratitude for all the members of the Lizard Lab at Macquarie University for assistance and support throughout this study. Especially, A/Prof. Martin Whiting for the use of his facilities. We are in debt to Christine Wilson for her assistance with animal husbandry. We really appreciated the help of Stephan Klopper in helping the design and construction of our metabolic chambers. We would also like to acknowledge Martin Thompson at the Division of Research, University of New South Wales for his technical aid with our models. Finally, we thank David Mitchell, James Van Dyke and Tobias Uller their insightful discussions.

# 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 (ed RB O'Hara). **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 (ed P Mumby). *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 (ed B Worm). *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*, jeb.160069.

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.

Chevin, L.-M., Lande, R. & Mace, G.M. (2010) Adaptation, Plasticity, and Extinction in a Changing Environment: Towards a Predictive Theory (ed JG Kingsolver). *PLoS Biology*, **8**, e1000357.

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 (ed J Hadfield). *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.

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. 1–373.

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*, 1–9.

M R, D. (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 (ed L Börger). *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*, 1–7.

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

Nakagawa, S., Kar, F., O'dea, R.E., Pick, J.L. & Lagisz, M. (2017) Divide and conquer? Size adjustment with allometry and intermediate outcomes. *BMC Biology*, **15**, 379.

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 (ed RB O'Hara). *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 (ed S Killen). *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*.

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 (ed J Chave). *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.

Roff, D.A. (2017) THE EVOLUTION OF GENETIC CORRELATIONS: AN ANALYSIS OF PATTERNS. *Evolution*, **50**, 1392–1403.

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 biology*, **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.

Seebacher, F., White, C.R. & Franklin, C.E. (2014) Physiological plasticity increases resilience of ectothermic animals to climate change. *Nature Climate Change*, **5**, 61–66.

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 (eds DC Collar and AA Winn). *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.

Wilkinson, G.N. & Rogers, C.E. (1973) Symbolic Description of Factorial Models for Analysis of Variance. *Applied Statistics*, **22**, 392.

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

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Table 1.** Adjusted repeatability and their 95% credible intervals, across six measurement temperatured 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 |

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Table 3.** Cross-temperature correlations (*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 |

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **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 |