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

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# Abstract

1. Physiological processes of individuals can be highly variable and accumulating evidence shows that individuals differ in their response to environmental change. Repeatability, or lack thereof, in metabolic rate across temperatures (i.e., metabolic thermal plasticity) may affect mass-scaling at the population level and has important consequences for understanding the evolution of reaction norms. Nonetheless, only a small number of studies have explicitly quantified repeatability in metabolic plasticity, and fewer have explored how it can impact mass-scaling.
2. We repeatedly measured standard metabolic rate of forty-two delicate skinks (*Lampropholis delicata*) at six temperatures over the course of three months (*N*[observations] = 5040). Using hierarchical statistical techniques, we accounted for multi-level variation and measurement error in our data in order to quantify more precise estimates of reaction norm repeatability and mass-scaling exponents at different acute temperatures
3. Our results show that individual differences in metabolic thermal plasticity was consistent over time, albeit repeatability estimates were weak. After accounting for measurement error which increased steadily with temperature, we show that among individual variance remained consistent across all temperatures. Congruently, temperature specific repeatability of average metabolic rate was stable across temperatures. Cross-temperature correlations were positive but were not uniform across the reaction norm.
4. After taking into account multiple sources of variation, our estimates for mass-scaling did not change with temperature and were in line with published values for snakes and lizards. This implies that repeatable plastic responses may contribute to thermal stability of scaling exponents.
5. Our work contributes to our understanding of how energy expenditure scales with abiotic and biotic factors and the capacity for reaction norms to respond to selection. This is pertinent for ectotherms coping with rapid environmental change within their lifetime.

# Keywords

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

# Introduction

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

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

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

Here we examine how individuals differ in energy expenditure in relation to body size and acute temperature changes and how it may impact mass-scaling exponents in male delicate skinks (*Lampropholis delicata*). We repeatedly measured routine metabolic rate over four months address three key questions. (1) Does metabolic thermal plasticity consistently differ among individuals? (2) How does repeatability of MR change at a given temperature? (3) Do population mass-scaling exponents change with temperature when accounting for among- and within-individual variation in MR? Unravelling the complexities of individual physiological processes will have important consequences for understanding how populations respond in warming environments.

# Materials and Methods

## Lizard collection and husbandry

*Lampropholis delicata* is a small oviparous, skink found in throughout Eastern Australia (Chapple et al., 2011). They have a short lifespan (2 – 4 years in the wild) and their reproductive season is from September – February (Chapple et al., 2014). Between 28 August and 8 September 2015, forty-two male *L. delicata* were collected from two sites near Sydney, Australia. Lizards were caught by hand or by mealworm fishing and were transported individually in calico bags in an ice-cooler to Macquarie University. Lizards were housed in a temperature-controlled room set at 26ºC and were provided with a thermal gradient to allow for thermoregulation. Each lizard was kept individually in an opaque plastic enclosure measuring 35cm x 25cm x 15cm (L x W x H). Each enclosure was lined with newspaper and lizards were given access to a water bowl and tree bark as a refuge. Enclosures were placed under UV light (11L:13D). Lizards were fed three to four small crickets (*Acheta domestica*) dusted with calcium powder and multi-vitamin every two days when metabolism measurements were not taking place. Animal collection was approved by the New South Wales National Parks and Wildlife Service (SL101549) and procedures were approved by the Macquarie University Ethics committee (ARA 2015/015) and University of New South Wales Animal Care and Ethics committee (ACEC 15/51A).

## Measuring metabolic rate at different temperatures

Given the scale of our experiment, we used closed-system respirometry instead of intermittent-flow through respirometry. We measured routine metabolic rate (hereafter referred to as metabolic rate [MR]) as our measurements also included the energetic costs of random activity that we were not able to completely control for (Withers 1992; Mathot & Dingemanse 2015). MR was measured as the volume of CO2 production per unit time ( mL min-1) for animals in a post-absorptive state because CO2 production is more sensitive to change in smaller organisms, and is less susceptible to fluctuations in water vapour. Our data showed that CO2 production was strongly correlated with O2 consumption nonetheless (*r* =0.94, p = <0.05]). Measurements took place between 26 December 2016 - 19 March 2017. Lizards were randomly assigned to one of two blocks for MR measurements (block 1: n = 23, block 2: n = 22). We used two incubators (LabWit, ZXSD-R1090) to precisely control the acute temperature at which measurements were taken (+/- 1ºC). Measurements were taken in a random order at 22ºC, 24ºC, 26ºC , 28ºC , 30ºC and 32ºC over three days (measurements at two temperatures per day). Each animal was repeatedly measured across these temperatures every 10 days (10 sampling sessions in total). We also statistically accounted for the order of temperatures animals experienced in our analyses to control for any possible carry over effects that higher temperatures may have on individuals in subsequent MR measurements (see below).

After a 24-hour fasting period, the body temperature of each individual inside their enclosure was taken using an infrared laser gun (Stanley stht0-77365) in the morning (~06:00). Each lizard was gently encouraged into their 146mL opaque chamber and then weighed using a digital scale to the nearest 0.01g (Ohaus SP-202). Chambers were placed inside the incubators in the dark at a randomised temperature for 30 minutes. The lids of the chambers were left ajar during this time to minimise CO2 build up. After 30 minutes, each chamber was flushed with fresh air and sealed. A 3 mL ‘control/baseline’ air sample was immediately taken via a two-way valve to account for any residual CO2 that was not flushed from the chambers. The chambers were left in the incubator at the set temperature for lizards to respire for 90 minutes. After this time, two 3mL air samples were taken from each chamber. Chambers were then reopened and flushed with fresh air before placed back into the incubator for the second measurement temperature (2 temperatures / day) 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 and *.* Water vapour was scrubbed from the inlet air with Drierite. Output peaks were processed using the R package ‘metabR’ (<https://github.com/daniel1noble/metabR>). The rate of CO2 produced by an individual was calculated following equation 4.21 in Lighton 2008:

Equation: 1

where %CO2 is the maximum percentage of CO2 in air sample above baseline, 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 (146 mL); 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 analyses

All statistical analyses were conducted using the R environment, version 3.6.1 (Core Team 2013). Details on data cleaning are presented in the electronic supplementary materials (ESM). Initial analyses showed that there were no differences in logbetween statistical blocks of lizards therefore ‘block ID’ was not included in our final models (ESM). Although lizards were kept in a temperature-controlled room, there may still have been temperature differences between enclosures that had carry-over effects on metabolic rate. We tested whether the body temperature measured in the home enclosure before the first measurement or the previous measurement temperature (if MR measurements were underway) influenced log at subsequent temperatures. We found that a model containing ‘previous temperature experience’ as a covariate was better supported compared to a model without it (WAIC (Full model – reduced model = -8.39), we therefore included ‘previous temperature experience’ in all subsequent analyses (Table S1). Collinearity between our predictor variables was checked using a scatterplot matrix (Fig. S1) and Pearson correlation coefficients are presented in Table S2. All data and code with which to generate our results are openly available via the Open Science Framework (see Data Accessibility).

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

Measurement error and repeatability of metabolic thermal plasticity

Repeatability is a ratio of among-individual and residual variance components (R = σA / (σA + σR) and represents the proportion of phenotypic variance attributed to among-individual differences (Nakagawa & Schielzeth 2010). The relative contribution to each variance component can shed light on the processes that promote repeatable traits (Dingemanse & Dochtermann, 2013). Measurement error, however, can bias the estimation of variance components and affect repeatability estimates (Ponzi et al., 2018). Given that we took two air samples for each MR measurement, we are able to partition measurement error among the two samples by including a nested random effect of individual ID, sampling session and temperature (Individual\_ID:Session\_ID:Temp, hereafter referred to as measurement error) in our models. This term partitions out variance attributed to measurement error among replicates so that the residual variance represents within individual variance. We also wanted to take into consideration that metabolic rate could change over time our study spanned over four months. We therefore fitted a nested random effect of individual ID and sampling session (ID:sampling session, hereafter referred to as series) in our models to decompose among sampling session within individual variance (see Araya-Ajoy et al., 2015 for further explanation)

We fitted the following random slope model in 'MCMCglmm’ (nobs = 4952) in order to quantify the repeatability of metabolic thermal plasticity (i.e. slopes for each individual).

log ~ Temp + zlogBodyMass + PriorTemp + (1+ Temp| Individual\_ID) + (1+ Temp| Individual\_ID:Session\_ID) + (1| ID:Session\_ID:Temp)

where: log is log-transformed ; Temp is the temperature in degrees Celsius; zlogBodyMass is log-transformed body mass that is then subsequently z-transformed; PriorTemp is previous temperature experienced by the lizard (enclosure temperature or the previous treatment temperature). Individual ID and series and measurement error was included as a random intercept. Temperature was included as a random slope for both individual ID and series to estimate individual slopes and among-sampling session, within individual slopes. The repeatability of the slope is calculated following equation 1 in the ESM (see also Araya-Ajoy et al., 2015).

Repeatability of the average MR at each temperature and cross-temperature correlations of metabolic rate

After assessing whether individuals differ in their metabolic thermal plasticity, we were interested in knowing whether consistent among-individual differences in average MR change across temperatures. To achieve this, we fitted a multivariate response model by treating MR measurements for each of the six temperatures as separate traits (nobs = 802) in a 6 x 6 response matrix:

~ zlogBodyMass + PriorTemp + (1|ID) + (1| Individual\_ID:Session\_ID)

where, is the metabolic rate for individual 1 in sampling session 1 at 22ºC and is the metabolic rate for individual 1 in sampling session 10 at 22ºC and so forth. Similar to the random slope models, we included zlogBodyMass and PriorTemp as fixed effects. Note that temperature is no longer a predictor or a random slope term as temperature is now part of the response matrix. In some instances, mechanical errors occurred during air collection. Given that ‘brms’ requires complete data in the response matrix, we used the ‘mi’ function to impute the missing samples at each temperature as this prevented us to exclude 607 rows of data. We included individual ID and series were as random intercepts. In this model, series is responsible for partitioning out measurement error from the residuals. We calculated temperature specific repeatability following Equation 2 in the ESM.

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

Mass-scaling exponents at different temperatures

Population estimates of scaling exponents can be affected by within and amongindividual variation (van de Pol & Wright 2009). We therefore wanted to partition out within individual effects in order to obtain more precise estimates of mass-scaling across temperatures. To achieve this, we calculated the mean mass across all sampling sessions for each individual (among individual effect), and subtracted an individual’s mass from its own mean to account for within individual effects (also known as within-individual centering, see van de Pol & Wright 2009). These mass effects were log-transformed and included in two models fitted in ‘brms’ (nobs = 3933). The first model (interaction model) had the following structure,

log~ Temp \* logAmongIDMass + Temp \* logWithinIDMass + (1 + logWithinIDMass|ID) + (1| ID:sampling session :Temp)

where: Temp \* logAmongIDMass is the interaction term between temperature and the log transformed among individual mass effect; Temp \* logWithinIDMass is the interaction term between temperature and the log transformed within individual mass effect. Individual ID was fitted a random intercept with logWithinIDMass as a random slope as individuals masses changed at different rates through the study (see Fig S3). We also included the measurement error term. The second model (main effects model) only had the main effects of temperature, the among individual mass effect and the within-individual mass effect and the same random effects structure as the interaction model. We tested whether population mass-scaling exponents (i.e. the among individual mass effects) changed with temperature by comparing different information criterion (wAIC and loo values) between model one and two. We also present in the ESM (Fig. S4, Table S5) an analysis that compared the mass scaling exponents with estimates from a model that represents the typical analysis of a metabolic scaling study from a model that did not account for the multi-level variation in the data.

# Results

## Repeatability of metabolic thermal plasticity

Individual slopes describing the effect of temperature on MR were weakly repeatable (Rslope = 0.23, Lower CI = 1.52 10-3, Upper CI = 0.84), suggesting individuals consistently varied in how their metabolic rate changed with temperature (Fig. 1).



**Figure 1** – Predicted individual reaction norms for forty-two individuals of log metabolic rate (mL /min) at six measurement temperatures at an average log mass and for sampling session one (left panel), five (middle panel) and ten (right panel) reaction norms were estimated using

## Repeatability of metabolic rate at each temperature

We found that the repeatability of MR (i.e. individual intercepts) was stable across acute temperatures (Fig. 2). Temperature-specific repeatability was greatest at 24ºC, however credible intervals overlapped with estimates at other temperatures (Fig. 2 ,Table S3). Upon closer inspection of the variance components at each temperature, we show that measurement error decreased steadily with increasing temperature, whereas among individual variation remained relatively consistent with temperature (Fig 2B). In contrast, within individual variance showed no consistent pattern with temperature, however it was highest in 32ºC. In other words, individuals were responding more variably as 32ºC while differences among individual maintained relatively stable (Fig 2).



**Figure 2** – A) Posterior mean of repeatability and variance components of log metabolic rate (mL min-1) at six measurement temperatures estimated over four-month period across n = 42 individuals. Error bars represent 95% credible intervals.

## Cross-temperature correlations in metabolic rate

Metabolic rate across temperatures were positively correlated at among-individuals (Fig. 3, Table S4). Positive correlations indicate that some individuals maintained a consistently high metabolic rate relative to other individuals, while others had a relatively low metabolic rate across all temperatures. Metabolic rate measured at neighbouring temperatures (e.g. 22ºC and 24ºC) were strongly correlated, but the strength of this correlation decreased with increasing differences between the two temperatures (Fig. 3).

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

## Temperature dependence of population mass-scaling exponents

The model containing only the main effects of temperature was better supported than a model that included the interaction terms (Main effects model: WAIC = 2133.9, loo = 2322.2, Interaction model: WAIC = 2124.90, loo = 2358.5), suggesting a lack of temperature dependence in mass scaling (Fig. 4). Across all temperatures, the average mass-scaling exponent was 0.96 (Lower CI = 0.39, Upper CI = 1.52) which is in line with values reported for Squamates (Uyeda et al., 2017). Mass-scaling exponents tended to be spurious and estimated with a larger degree of error when the within individual effects and measurement error were not statistically accounted for (Fig. S4, Table S5).

**Figure 4** – (Top) Posterior mean estimates of population mass scaling exponents (i.e. among individuals) of log metabolic rate (mL /min) across six measurement temperatures when within individual variation in mass and measurement error in metabolic rate has been statistically accounted for. The dashed line represents the mass-scaling exponent of 0.83 estimated for squamates from Uyeda (2017). Error bars represent 95% credible intervals. (Bottom) Raw log metabolic rate plotted against log body mass for a random subset of n = 20 individuals at six measurement temperatures. Each uniquely coloured point represents one individual. Thick bold line represents the change in log metabolic rate over log body mass across all individuals (among-individual mass-scaling slope). Thin lines represent the change in log MR over log body mass within an individual (within-individual mass-scaling slopes)

# Discussion

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

## Consistent variation in metabolic thermal plasticity

Natural selection acts on phenotypic variation among individuals. Consistent among-individual variation is therefore a key prerequisite for any trait to evolve and sets the ‘upper limit of heritability’ (Falconer 1952, c.f. Dohm 2002). Our findings show that individuals differ consistently in how their MR responds to acute temperature changes over an ecologically relevant time period. Assuming that individual differences have a genetic basis and are therefore heritable, our results suggests that metabolic thermal plasticity may be capable of evolutionary change allowing shifts in population-level metabolic reaction norms (Ghalambor *et al.* 2007). Average metabolic rate was also repeatable and stable across temperatures and suggests that the operable range of temperature in *L.delicata* promotes consistency in physiological traits (Goulet et al., 2017; Matthews et al., 2016). To our surprise, measurement error declined with increasing temperature presumably because individuals were respiring at a higher rate maybe it easier to detect changes in CO2 production. Measurement error can inflate repeatability estimates if it is not accounted for statistically (Ponzi et al., 2018). Indeed, we found a significant increase in repeatability and among individual variance when we took averages between the two replicate air samples (Fig. S5). Consequently, one would mistakenly conclude that the capacity to selection to act on MR would increase at hotter temperatures. stress the importance of accounting for confounding sources of variance. We stress the importance of considering confounding sources of variances such as measurement error or shared environmental effects among individuals to ascertain the potential for repeatable physiological traits to undergo selection.

## Cross-temperature correlations

Metabolic rate was positively correlated across all temperatures at the among-individual level. This suggests that individuals with high MR at one temperature also tend to exhibit high MR at other temperatures (and vice versa for individuals with low MR). Individuals could vary in their acquisition or allocation of resources to their physiological system which enables certain individuals to maintain a consistently high MR across all temperatures (Angilletta Jr et al., 2003; De Jong & Van Noordwijk, 1992). Moreover, consistent individual differences in MR, irrespective of the thermal environment, may be functionally linked with other aspects of the phenotype (Biro & Stamps, 2010). Our results give precedence to ‘pace-of-life’ theory where individual differences in energetic expenditure may promote consistent differences in behaviour and life-history within the same population (Biro & Stamps, 2010; Careau et al., 2008).

The strength of cross-temperature correlations can help identify trade-offs in physiological processes across environments. Such trade-offs have been hypothesised to be important mechanisms in shaping reaction norms (Angilletta Jr et al., 2003). Generalist-specialist trade-offs occur when some individuals have enhanced physiological function in one environment but diminished function in another environment, manifesting as a negative cross environment correlation (Berger et al., 2014). We show that across different temperatures, correlations were all positive, providing no support for trade-offs between temperatures in energy expenditure. While our temperatures fell within the normal temperature range experienced by animals in the wild, trade-offs may exist in other parts of the thermal performance curve (Angilletta Jr et al., 2003). Assuming phenotypic cross-temperature correlations reflect the underlying genetic architecture of metabolic rate (Roff 1995), the strength of correlation can dictate how strongly selection acting on one component of the reaction norm will result in indirect selection on another (Via et al., 1995). This implies that response to selection would be stronger between neighbouring temperatures (e.g., 28°C vs. 32°C) compared to more distant temperatures (e.g., 22°C vs. 32°C) which might be important to give rise to non-linear reaction norms (Berger et al., 2013).

## Population mass scaling across different temperatures

Mass-scaling exponents were robust to acute temperature changes, which is in disagreement with a growing number of studies that show temperature dependence of mass scaling exponents (Glazier 2005; Killen *et al.* 2010; Price *et al.* 2012; Glazier 2015; Barneche *et al.* 2016). Discrepancies may be due to the method with which we quantified mass scaling exponents. In our study, we sampled sexual mature adults repeatedly over four months in order to estimate a static mass scaling relationship, while other studies tend to measure ontogenetic allometry (change in body mass and metabolic rate throughout development, Glazier 2009). The energetic demands of growth during ontogeny may be more sensitive to temperature change and therefore result in temperature-dependence in ontogenetic mass scaling exponents (Hirst, Glazier & Atkinson 2014; Barneche & Allen 2018). In support of this, a recent comparative analysis has shown that development (passing through life stages) shows stronger temperature dependence than increases in mass (Forster, Hirst & Woodward 2011).

The magnitude and precision of mass scaling exponents may be affected by processes occurring at different hierarchical levels. Genetic and developmental differences that impact the physiological system can maintain permanent differences among individuals (Dingemanse & Wolf 2013). While fluctuations in the internal environment, such as circulating hormones and body composition can affect the within individual responses (Dupoué et al., 2013; McCue, 2010; Scott et al., 1996). After accounting for within individual effects and measurement error, our mass-scaling exponent estimates were in line with values reported from a phylogenetically informed analyses in squamates (Uyeda et al., 2017). This result may have important implications for current designs of metabolic scaling studies as MR and body mass tend to only be measured once, making them sensitive to sampling error and within-individual ‘noise’. Theoretical studies that make use of predictive relationship between body mass and metabolism should be more aware of the different sources of variation when trying to extrapolate individual level processes to higher levels of biological organisation. Future work is warranted to investigate the degree to which intra-individual variance in MR and body mass impact scaling exponents as this has largely been neglected and yet may help elucidate why mass scaling exponents are variable at higher levels of biological organisation (Glazier, 2005; Maxwell et al., 2003; McLean & Speakman, 2007).

# Conclusion

In this study, we found support that individual consistency of thermal plasticity promotes stability in mass-scaling. Our work implies that selective processes has the opportunity to shape reaction norms of metabolic rate. This may ultimately how populations respond to temperatures and allow them to persist in under warming climate. Quantitative genetic and experimental evolution studies are necessary to truly understand the evolutionary potential of metabolic thermal plasticity. Our work emphasises important methodological considerations that are often overlooked in evolutionary physiological studies. Confounding sources of variance can misconstrue our evolutionary relevance of phenotypic variability in physiological traits (Ponzi et al., 2018). Neglecting to consider individual variation, even in theoretical research may misguide predictions about ecological processes across levels of biological organisation (Botero et al., 2015).

# Author contributions

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

# Acknowledgements

This study would not have been possible without the support of the Australian Research Council (ARC) Discovery Early Career Research Award to D. W. A. N (DE150101774); also, S.N. was supported by an ARC Future Fellowship (FT13010026). 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 the construction of our metabolic chambers. We would also like to acknowledge Martin Thompson at the Division of Research, University of New South Wales for his technical aid with our models. Finally, we thank David Mitchell and Tobias Uller insightful discussions and Rose O’Dea for her comments on an earlier draft of this manuscript. All authors declare no conflict of interest.

# Data accessibility

Datasets and code used to generate results of this study is accessible via Open Science Framework (DOI 10.17605/OSF.IO/TZ2H5).

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