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## Additive effects of developmental acclimation and physiological syndromes on lifetime metabolic and water loss rates of a dry- skinned ectotherm

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### Abstract :

Developmental plasticity and thermal acclimation can contribute to adaptive responses to climate change by altering functional traits related to energy and water balance regulation. How plasticity interacts with physiological syndromes through lifetime in long-lived species is currently unknown.

Here, we examined the impacts of long term thermal acclimation in a long-lived temperate ectotherm (*Vipera aspis*) and its potential flexibility at adulthood for two related functional traits: standard metabolic rate (SMR) and total evaporative water loss (TEWL).

We used climatic chambers to simulate three contrasted daily thermal cycles (warm, medium and cold) differing in mean temperatures (28, 24, and 20°C respectively) and amplitudes (5, 10 and 13°C respectively) during immature life (0 to 4 years of age). Individuals were then maintained under common garden conditions (medium cycle) for an additional 3-years period (4 to 7 years of age). SMR and TEWL were repeatedly measured in the same individuals throughout life during and after the climate manipulation.

Individuals reduced their SMR (negative compensation) when experiencing the warm cycle but flexibly adjusted their SMR to common garden conditions at adulthood. In addition, thermal conditions during the juvenile life stage led to changes in TEWL persisting until adulthood.

We further found consistent intra-individual variation for SMR and TEWL and a positive intra-individual and inter-individual covariation between them throughout life. Thus, plastic responses were combined with a physiological syndrome linking SMR and TEWL.

Our study demonstrates the capacity of long-lived organisms to flexibly shift their SMR to reduce daily maintenance costs in warmer and less variable thermal environments, which might be beneficial for low

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energy specialist organisms such as vipers. It further suggests that thermal conditions provide cues for developmental changes in TEWL. Beside plasticity, contrasted individual physiological syndromes could be selected for and contribute to the response to climate change.

**Keywords** : metabolism, physiology, plasticity, temperature, water loss

## Introduction

Physiological plasticity refers to the ability of an organism to change its physiological state in response to external cues and is an important process for coping with thermal variations (Bonamour et al., 2019; Fuller et al., 2010). In particular, physiological plasticity in response to changes in thermal conditions might determine ‘winner or losers’ in the context of global warming (Seebacher et al., 2015; Somero, 2010). Thermal plasticity involves a range of distinct but interconnected mechanisms including flexible acclimation responses (shift in physiological routines throughout life) and developmental plasticity (persistent effects induced during development; da Silva et al., 2019; Healy et al., 2019; Sultan, 2017). Recent attention has focused on physiological acclimation capacity to a persistent increase in environmental temperatures (Angilletta, 2009; Huey et al., 2012). Thermal acclimation response for ectotherms involves shifts in physiological responses, which thereby changes the optimal temperature for biological process, and the temperature tolerance limits (Angilletta Jr., 2009; Seebacher, 2005). Such responses are critical to cope with global warming for long-lived ectotherms because of their presumed limited potential for rapid genetic evolution (Chevin et al., 2010; Seebacher et al., 2015; Urban et al., 2014). Yet, uncertainty surrounds the speed and breadth of thermal acclimation responses and their efficiency as a means to buffer ectotherms from global warming (Gunderson & Stillman, 2015; Havird et al., 2020; Morley et al., 2019; Rohr et al., 2018; Seebacher et al., 2015).

There are several reasons why we might underestimate the true acclimation capacity of long-lived terrestrial ectotherms such as some species of amphibians and reptiles. First, most experiments on these organisms do not last long enough to ensure that individuals are fully acclimated (Rohr et al., 2018; Seebacher et al., 2015). Time to acclimate is typically longer for large-bodied species, based on energetic models and empirical evidence (Kingsolver & Huey, 2008; Pörtner et al., 2017) and recent comparative analyses across a diversity of ectothermic organisms (Rohr et al., 2018). Long-term (> 1 year) acclimation studies are crucial to investigate the plastic responses of long-lived ectotherm but are very rare. Second, the flexibility of phenotypic responses to acclimation temperatures is rarely tested for, and how persistent are the effects of developmental plasticity remains unknown for most organisms (Beaman et al., 2016; Ligon et al., 2012). Irreversible acclimation responses to developmental conditions might lead to maladaptive mismatches between the phenotype and ecological conditions in highly variable and unpredictable environments (Gluckman et al., 2007; Piersma and Gils, 2011). Therefore, the ability of organisms to flexibly adjust their phenotype to environmental variations throughout their lifetime may determine their capacity

to cope with climate change (Morley et al., 2019; Pallarés et al., 2020). Similarly, whether plastic responses supersede fixed inter-individual differences in physiology has been poorly investigated so far, despite evidence of consistent physiological syndromes in some ectotherms (Goulet et al., 2017a; Mell et al., 2016).

In ectotherms, SMR represents the minimal energy requirements to fuel maintenance energy demands, and variation in SMR within and between species may faithfully reflect life history strategies (Clarke, 2006; Seebacher et al., 2015). Considerable variation in MR exists within and between species (Careau et al., 2019), potentially reflecting thermal adaptations (Addo-Bediako et al., 2002) and additive constraints from habitat aridity (Dupoué et al., 2017). Within species, individuals can respond to thermal fluctuations through MR modulation (Noble et al., 2018; While et al., 2018). For instance, after exposure to warm temperatures, MR may decrease (“negative compensation”) to limit the energy costs of self-maintenance and minimize oxidative stress (see Costantini, 2008, Angilletta, 2009; Norin et al., 2014). Temperature is also a proximate cue for water constraints inducing adjustment of evaporative water loss (TEWL see Riddell et al., 2019). In terrestrial species, a universal cost of metabolic gas exchange is the loss of water vapor such that there could be a functional trade-off between SMR and TEWL (Woods & Smith, 2010). Because climate change will affect both energy and water balance, considering jointly SMR, TEWL and associated tradeoffs between both is required (Riddell & Sears, 2020; Riddell et al., 2018b). Individual variation in metabolism can co-vary with variation in other physiological or in personality traits through consistent pace-of-life syndromes, where “fast” phenotypes are characterized by a higher SMR (Biro & Stamps, 2010; Careau et al., 2019, 2014). Thus, phenotypic correlations may also exist between SMR and TEWL and could influence individual sensitivity to temperature and water constraints. Despite its relevance, the long-term developmental effect on variation and co-variation in SMR and TEWL has not been addressed to date, and the pace-of-life syndrome framework has not been extended yet to these functionally related traits.

We used a 7-years long experiment to characterize the long-lasting consequences of thermal conditions on the asp viper (*Vipera aspis*), a temperate ectotherm with a late sexual maturation (age at maturation between 3 to 4 years depending on growth; Bonnet et al., 1999) and a relatively long generation time (maximum lifespan >10 years) compared to other squamate reptiles. We hypothesized that thermal conditions should shape both SMR and TEWL, and that chronic exposure would result in persistent effects. We applied three contrasted daily thermal cycles (warm, medium, and cold) with contrasted temperature and

thermal amplitude from birth to sexual maturity (4 years). Individuals were then placed in a common garden treatment (medium thermal cycle) during their adult life (from 4-7 years old). Building up from previous findings in ectotherms, we tested the following three predictions. First, individuals raised in the warmest thermal cycle should exhibit the lowest SMR due to a negative compensation (Bruton et al., 2012; Clarke, 2006; Zari, 1996). Second, chronic exposure to the thermal treatments during growth should lead to persistent acclimation responses in both SMR and TEWL (Nettle & Bateson, 2015; Refsnider et al., 2019). Third, SMR should correlate with TEWL among and within individuals because of a functional relationship between the two traits (e.g., water loss caused by ventilation) and/or to some persistent early environment or genetic effects on an underlying trait influencing both SMR and TEWL (Riddell et al., 2018a; Rozen-Rechels et al., 2019).

## Material and Methods

### Thermal treatment and experimental design

The use of realistic thermal treatment mimicking natural condition is of critical importance to address the impact of climatic changes on physiological responses (Morash et al., 2018; Potter et al., 2013). The asp viper is a diurnal species that behaviorally thermoregulates to reach preferred temperature close to 30°C (Lorioux et al., 2013). At night this species will usually hide in a shelter and body temperature will fluctuate with the temperature of the resting habitat. This implies that warmer climate conditions within the thermal critical limits will lead to an extended time spent at the basking temperature, and exposure to warmer nighttime temperatures in vipers (Davy et al., 2017), as predicted by mechanistic models and as seen in empirical studies of heliothermic squamate reptiles (Blouin-Demers & Weatherhead, 2001). This implies higher mean body temperature and to lower thermal variability in a warmer climate.

Therefore, we simulated three realistic daily thermal cycles (Fig. 1A, here after designated as “warm”, “medium” and “cold” cycles) that differed in mean temperature (28, 24 & 20°C respectively) but also in the time at preferred temperature and daily amplitude. Preferred temperature (30°C) was simulated in all of our treatments but with different durations (14, 9 and 5 hours in warm, medium and cold cycles). We also simulated different night-time temperatures (25, 20°C and 17°C respectively in warm, medium and cold cycles; see Fig. 1A). Thus, daily thermal amplitude differed between groups (5, 10, and 13°C respectively). For all treatments, relative humidity was set at a constant value of 50% and water vapor pressures deficit were respectively, 1.91 kPa (warm cycle), 1.55 kPa (medium

cycle) and 1.24 kPa (cold cycle). We simulated these cycles using three climatic chambers (Vötsch VP 600, Balingen, Germany) with the SIMPATI software (ver.2.06 Vötsch).

We used 49 captive-born snakes from a colony of aspic vipers maintained in the Centre d'Etudes Biologiques de Chizé, France. Mothers were collected in west-central France from neighboring populations and were reproduced in captivity where they were maintained in the same standard conditions during pregnancy (see Lориoux et al., 2013 for details on maintenance conditions and thermal cycles). From birth (September-October 2009) to maturity (October 2013), individuals were randomly assigned within families to one of three treatment (n=16, 16 and 17 respectively for warm, medium and cold cycle; see Fig. 1B). In November 2013, we transferred all adult individuals to a common garden at the medium cycle for an additional 3-year period (Fig. 1A). Individuals exposed to the medium cycle during development were thus kept in the same conditions across the experimental timeframe. Individuals from hot and cold cycles were exposed to new thermal conditions when transferred to the common garden, allowing us to test for the flexibility of acclimation responses in these treatment groups.

Snakes were housed individually in plastic boxes (35 x 25 x 13 cm) with ad-libitum access to drinking water in a glass bowl. In all experimental treatments, photoperiod followed that of natural day light cycles. We fed the vipers with thawed laboratory mice (*Mus musculus*). The size of the mice was scaled at 20% ( $\pm 10$ ) of the snake mass. Digestion rate, feeding intake and feeding performance increases with temperature in vipers (Naulleau, 1983; Vincent & Mori, 2007), and the maximum feeding rate is therefore strongly constrained by environmental temperatures. In order to avoid providing food that cannot be processed and digested at low temperatures, we adjusted the feeding regimes to each thermal treatment in order to allow *ad libitum* feeding rates (every 7, 10 and 15 days for warm, medium and cold cycles respectively).

#### Laboratory measurements

To investigate how experimental treatments affected growth rate, we weighed ( $\pm 0.01$ g) and measured (snout-vent-length, SVL  $\pm 0.5$ cm) all individuals throughout their life. Body mass (BM) was measured at birth and then at the same time points as the respirometry exchanges measurements (see below), while we measured SVL at birth, and when snakes were 30, 49 and 85 month-old. To assess both short-term and long-term phenotypic responses, we quantified SMR and TEWL during four successive measurement sessions (Fig. 1B). We measured respiratory exchanges when individuals were 19 and 47 month-old in order to

examine to pace of the acclimation response during the development period exposed to contrasted thermal treatments. We conducted measurements 3 months after the beginning of the common garden situation (at 49 month-old) in order to detect any quick changes in SMR or TEWL that may occur following the change in thermal conditions. Later, we realized a last measurement session 3 years later (at 85 month-old) in order to test for long-term persistent effects of developmental plasticity.

#### Respiratory exchanges

We measured the rate of oxygen consumption ( $\dot{V}O_2$  proxy for SMR;  $\text{ml}\cdot\text{h}^{-1}$ ) and the total evaporative water loss (TEWL;  $\text{mg}\cdot\text{h}^{-1}$ ) on resting individuals at two test temperatures (20°C and 30°C), to calculate Q10 and acclimation response (Einum et al., 2019; Havird et al., 2020). We randomized temperature trials for each individual. To measure  $\dot{V}O_2$ , we used a closed-system respirometry at 19 months post-birth measurements because of the small size of the snakes, and an open-flow respirometry system at 47, 49 and 85 months post-birth measurements (see detailed methods regarding respirometry systems on Appendix S1 in Supporting Information). We measured TEWL together with  $\dot{V}O_2$  using the open flow-system (Fig. 1A). We measured gas exchanges at night on post-absorptive snakes after a minimum of 7 days of fasting. To stabilize the test temperatures and avoid exploratory behaviors, we placed each individual in the temperature-controlled chamber to acclimate for 3 h prior to each measurement.

#### Statistical analyses

All analyses were performed in the R environment, version 3.6.3 (Holding the windsock, (R Core Team, 2020))

#### *Linear model to estimate treatment effects on growth rate*

Growth rate for SVL was computed for each individual during three successive life stages (see Figure 1 and Table 1) and analyzed with a linear model including effects of initial SVL, sex, treatment groups and first order interaction.

#### *Bayesian models to estimate treatment effects on BM, SMR and TEWL*

We fitted linear mixed models using a Bayesian inference approach with Markov Chain Monte Carlo (MCMC) techniques from the *MCMCglmm* library (Hadfield, 2009). Results from Bayesian models are further presented as medium estimates  $\pm$  95% highest posterior

density intervals (HPDI). To investigate independently the effects of experimental conditions on body mass and physiological trait, we first ran univariate mixed effects models with individual identity and mother identity included as random intercepts. In these univariate models, the variance attributed to individual identity represents the intra-individual variance  $V_I$ , whereas the variance attributed to maternal identity represents the intra-litters variance  $V_m$ . For each univariate model, treatment group (warm, medium, or cold cycle), effects of session (see Figure 1), and their interaction were included as fixed factors. The SMR, TEWL, BM trait values were treated as the dependent variable in each univariate model. In univariate models for BM, we included sex as a factor to account for sexual dimorphism. In univariate models for SMR and TEWL, we tested the effect of the ambient temperature during the measurement by including trial temperature (20 or 30°C), along with its interactions with treatment and session, as factors. Because BM can strongly influence SMR and TEWL, individual mass at the time of measurement was also included as a covariate in these univariate models but we did not include sex based on preliminary analyses indicating no sexual differences in metabolism and water loss rates.

We ran the full model including all variables and interactions following standard guidelines of the *MCMCglmm* package (Hadfield, 2009). The analysis was performed on raw variables, assuming a Gaussian distribution of the residuals, and poorly informative priors for fixed effects (Gaussian distributed with zero mean and large variance), and uncorrelated poorly informative priors for variance components (inverse Wishart distribution with  $nu = 0.004$  and  $V$  set at 1). The MCMC algorithm was set to 120,000 iterations after a minimum burn-in phase of 10,000 iterations to allow convergence and using a thinning interval of 50 to avoid autocorrelation issues. We examined the posterior distribution of model estimates, calculated autocorrelation terms to evaluate independence of the samples in the posterior distributions, and performed half-width tests of convergence, which showed satisfactory properties of the sampling distribution. Using the full model, we then tested for the significance of random effects using model comparison with the deviance information criterion (DIC).

We next performed a model selection procedure of the fixed effects based on DIC and the posterior probability associated to the null hypothesis or pMCMC. To ease interpretation of the results, we further conducted Tukey post-hoc comparisons using the posterior chains of parameter estimates converted into posterior samples of contrasts with the *emmeans* package (Lenth et al., 2020). Note that the results of the *MCMCglmm* model selection for univariate



models were similar to those of classical, maximum likelihood based approaches fitted with the *lme* procedure (Pinheiro & Bates, 2006).

#### *Bivariate mixed model to estimate phenotypic correlation between SMR and TEWL*

We estimated the phenotypic correlation between SMR and TEWL by including the two as dependent variables in a bivariate mixed model and allowing a heterogeneous residual variance structure. The bivariate model included a regression with body mass, an additive temperature effect, a two-way interaction between treatment and session, residual variance components  $V_e$  for SMR and TEWL and a covariance between the trait-specific  $V_e$  ( $COV_e$ ), random intercept variance of individual identity  $V_I$  for SMR and TEWL and a covariance between trait-specific  $V_I$  ( $COV_I$ ). We performed this analysis on centered and scaled variables, assumed a Gaussian distribution of the residuals, and used poorly informative priors for fixed effects (Gaussian distributed with zero mean and large variance) and uncorrelated poorly informative priors for variance components (inverse Wishart distribution with  $nu = 2$  and  $V$  set at 0.5). The MCMC algorithm was run for 60,000 iterations with a burn-in phase of 10,000 iterations and a thinning interval of 25. We used the DIC to test the significance of the covariance terms and calculated intra-individual ( $r_e$ ), inter-individual ( $r_I$ ) and total ( $r_T$ ) phenotypic correlations from the sampled distributions.

#### Quantification of the compensation

We used a  $Q_{10}$  formulation of the Arrhenius equation relating metabolic and water loss rates with temperature to calculate the predicted energy budget during inactivity and total evaporative loss during the day for each viper from each treatment at each measurement session. The  $Q_{10}$  formulation of a process rate  $R$  can be written as:

$$R(T) = R(20^\circ\text{C}) \times Q_{10}^{(T-20)/10} \quad (1)$$

where  $R(T)$  is the predicted process rate at temperature  $T$ ,  $R(20^\circ\text{C})$  is our reference process rate at  $20^\circ\text{C}$  and  $Q_{10}$  is the ratio of the process rate at  $30^\circ\text{C}$  and  $20^\circ\text{C}$ . We calculated  $Q_{10}$  for each viper at each measurement session. We then used equation (1) to calculate the predicted SMR and TEWL over each 30 minutes' time bin from the daily nycthemeral cycle of ambient temperatures for each treatment group of the viper. Daily energy expenditure during inactivity (DEE<sub>i</sub>; cal.day<sup>-1</sup>) and daily total evaporative water loss (DEWL; mg.day<sup>-1</sup>) were calculated by summing the predicted SMR and TEWL over each 30-minute time bin through a day. This calculation therefore accounts for both acute effects of temperature (through

individual  $Q_{10}$  and the nycthemeral cycle) and acclimation effects of temperature (through changes in  $R(20^{\circ}\text{C})$  and  $Q_{10}$  among treatments. To quantify the degree of metabolic and water loss compensation induced by the observed acclimation response, we compared the DEEi and DEWL of each treatment group at each session with those predicted by a null model. Individuals had lower SMR in response to warmer and less variable temperatures (see results below). Thus, in order to quantify how much individuals saved energy through acclimation to warmer and less variable temperatures, the null model for DEEi assumed no warm acclimation response. We therefore used the mean  $R(20^{\circ}\text{C})$  and  $Q_{10}$  of vipers from the cold cycle to predict the DEEi from the warm and medium cycle treatment in the absence of metabolic acclimation. We estimated the degree of metabolic compensation only at 19 and 47 months post-birth because the acclimation response in SMR did not persist at adulthood in the common garden (49 and 85 months post-birth, see Fig. 2A). In addition, individuals had lower TEWL in both cold and warm cycle treatment than in medium treatment (see results below). In order to quantify the compensation in DEWL induced by lower TEWL, the null model for DEWL therefore used the mean  $R(20^{\circ}\text{C})$  and  $Q_{10}$  from vipers from the medium cycle treatment as a baseline reference for the two other groups.

## Results

### Morphological differences between treatments

There was strong inter-individual variation in body mass but a negligible variation among littermates in the full model (DIC, full model: -929.76, model without  $V_i$ : -644.09, model without  $V_m$ : -929.57). The change in body mass through time was weakly influenced by a sex-specific effect of treatment groups (DIC, full model: -929.76; full model without three-way interaction: -927.7). In females, body mass did not change between treatments but increased through time faster than in males, especially between session 2 and session 3 (analysis on subset of females data; DIC, model with Treatment  $\times$  Time: -453.2, model without Treatment  $\times$  Time: -457.7). In males, body mass was weakly influenced by treatments (analysis on subset of males data; DIC, model with Treatment  $\times$  Time: -475.02, model without Treatment  $\times$  Time: -473.98) because males from the cold cycle were slightly bigger at 19 months post-birth than males from the two other treatments (Tukey post-hoc contrast between cold and warm cycle: 0.104 [-0.034, 0.244] 95% HPDI, warm and medium cycle: 0.099 [-0.034, 0.237] 95% HPDI see Table S1). After accounting for these fixed effects, the inter-individual variation in body mass was highly significant ( $R_I = 0.660$  [0.523, 0.743] 95% HPDI).

Growth rate measured in each life stage differed between sexes consistently (faster growth in females, all  $P < 0.001$ ) and also between treatments from birth to 30 months post-birth ( $F_{2,44} = 6.69, P = 0.003$ ) and from 49 months post-birth to 85 months post-birth ( $F_{2,44} = 3.75, P = 0.03$ ). During the acclimation phase, vipers grew longer in the warm cycle than in the medium and cold cycle. During the common garden phase at adulthood, there was a compensatory growth response in vipers from the medium and cold cycle, which reached slightly higher SVL than vipers from the warm cycle group at 85 months post-birth (see Table 1).

#### Univariate models for SMR and TEWL

According to our model selection procedure, there was strong inter-individual variation in SMR and comparatively little variation among littermates in the full model (DIC, full model: -186.69, model without  $V_I$ : -171.7, model without  $V_m$ : -186.03). Regarding the fixed effects (see Table S2), SMR increased with body mass (pMCMC  $> 0.001$ ) and was higher at 30°C than at 20°C (pMCMC  $> 0.001$ ), but neither the treatment group nor the trial temperature influenced the allometric relationship with body mass (pMCMC  $> 0.10$ ). Independently from body mass, treatment groups influenced SMR differently through time and trial temperature levels (DIC, model without  $V_m$  and without Treatment  $\times$  Time  $\times$  Temperature: -182.3). At 20°C, vipers raised in the warm cycle treatment had slightly lower SMR than vipers raised in the medium and cold cycles during the acclimation phase (19 and 47 months post-birth) and this difference was significant three months after the end of the manipulation (49 months post-birth; post-hoc contrasts of the median posterior distribution, see Fig. 2A). Two years later, at 85 months post-birth, SMR values were not different between treatments irrespective of body temperatures. At 30°C, vipers raised in the warm cycle treatment had significantly lower SMR than vipers raised in the medium and cold cycles during the acclimation phase (19 and 47 months post-birth), but this difference vanished right after the end of the manipulation (49 and 85 months post-birth, see Fig. 2A). After accounting for these fixed effects, the inter-individual variation in SMR was significant but relatively small ( $R_I = 0.129$  [0.049, 0.231] 95% HPDI).

Similarly, there was inter-individual variation in TEWL and comparatively little variation among littermates in the full model (DIC, full model: -97.7, model without  $V_I$ : -66.2, model

without  $V_m$ : -97.9). The TEWL increased with body mass similarly between treatment and temperature groups (pMCMC > 0.05) and was higher at 30°C than at 20°C (pMCMC > 0.001). Contrary to SMR, treatment groups significantly influenced TEWL independently from time and trial temperature (see Table S2). On average, vipers raised in the medium cycle treatment displayed consistently higher TEWL rates through lifetime relative to the warm treatment (Tukey post-hoc contrast: 0.1203 [0.0281, 0.2141] 95% HPDI) and also slightly higher TEWL than in the cold cycle treatment (Tukey post-hoc contrast: 0.092 [-0.0035, 0.1827] 95% HPDI) irrespective of trial temperature (see Fig. 2B). After accounting for these fixed effects, the inter-individual variation in TEWL was significant and twice than the one for SMR ( $R_I = 0.237$  [0.115, 0.373] 95% HPDI).

#### Bivariate models for SMR and TEWL

The bivariate mixed models indicated that both the intra-individual and inter-individual covariance between SMR and TEWL were positive and significantly different from zero as shown by the DIC (full model: DIC = 1173.91,  $COV_e$  set to zero: DIC = 1268.6;  $COV_I$  set to zero: DIC = 1198.21; see Table 2). The inter-individual correlation was slightly larger ( $r_I = 0.69$  [0.46, 0.88] 95% CI) than the intra-individual correlation ( $r_e = 0.60$  [0.51, 0.68] 95% CI) and the total phenotypic correlation ( $r_p = 0.44$  [0.15, 0.75] 95% CI, see Fig. 3A and 3B).

#### Metabolic and water loss compensation

Individuals from warm and medium cycle treatment groups had lower predicted DEE<sub>i</sub> than expected in the null model without an acclimation response. Individuals from the warm cycle treatment compensated for approximately 40% of their DEE<sub>i</sub> at both 19 and 45 months post-birth. In the medium cycle treatment group, individuals compensated for 28.5 % of their DEE<sub>i</sub> at 45 months post-birth (Table 3, Fig. 4A). Similarly, DEWL was lower than expected in the null model without an acclimation response for individuals from the warm and cold cycle treatment groups. At 47 months post-birth, DEWL was reduced by 36.4 % for individuals acclimated to the warm cycle treatment, and by 24.2 % for individuals acclimated to the cold cycle treatment. At 49 months post-birth individuals acclimated to the cold cycle treatment compensated for 32.7 % of their DEWL, and individuals acclimated to the warm cycle treatment compensated for 49.5 % of their DEWL at 85 months post-birth (Table 3, Fig. 4B).

## Discussion

Thermal conditions simulated in three different daily thermal cycles led to developmental changes in TEWL that persisted at adulthood but individuals flexibly adjusted their SMR to thermal conditions experienced *in situ*. Further, we found that inter-individual variation of these two functional traits was consistent and positively correlated throughout life. This persistent phenotypic (co)variation at the individual level likely reflects a physiological syndrome linking metabolism and water loss.

#### *Negative compensation and flexible acclimation in SMR*

During the time period of exposure to contrasted treatments (until the age of 4 years old), snakes exposed to the warmest and less variable thermal conditions adjusted their SMR with a negative compensatory response (Clarke, 2006; Dupoué et al., 2017) when compared to snakes maintained at lower and more variable temperatures (medium and cold cycle). This physiological response to thermal conditions did not persist in a common garden, providing unambiguous evidence that acclimation in SMR was flexible. The compensatory response was quantified and allowed vipers to save as much as 40% of their DEEi relative to a null model hypothetical estimate simulating no acclimation response. The asp viper is a typical capital breeder with a low feeding frequency and a slow pace of life strategy. Therefore, lower SMR is likely to be advantageous according to the “allocation model” of energy metabolism where individuals with lower SMR benefit from more energy to spend in maintenance (e.g., survival), activity and/or reproduction (Careau et al., 2008; Mathot & Dingemans, 2015). In support of this scenario, we found that warm-acclimated vipers had both the lowest SMR and the highest growth rate. Thus, warm-acclimated snakes were able to advantageously invest more energy into growth, given their lower energetic demands. Even though the relationship between SMR and fitness can be quite variable across organisms (Arnold et al., 2021), these results confirm earlier findings where MR reduction in warm-acclimated ectotherms minimized the daily energetic costs of self-maintenance and limited oxidative stress by decreasing respiratory activity (Norin et al., 2014; Seebacher et al., 2010). In addition, the covariation between SMR and TEWL suggests that lower SMR is likely to positively impact water balance through reduced respiratory water loss (Dupoué et al., 2015). In colder conditions, MR may remain high to support performance, activity, and physiological functions despite lower temperatures (Berg et al., 2017). We further found that treatment-induced changes in SMR differed at body temperatures of 20 and 30°C, suggesting that thermal acclimation changed also the acute effects of temperature on metabolism (Xie et al., 2017).

Both the mean temperature and the thermal variability from each daily cycle could drive these acclimation responses (Kern et al., 2015). At a given mean temperature, it has been suggested that a higher daily thermal fluctuation can lead to an increased tolerance to extreme temperature (Verheyen & Stoks, 2019). However, the effects of temporal variation of temperatures on acclimation responses of metabolism and other functional processes are often species dependent in ectotherms (Niehaus et al., 2011; Noer et al., 2020), and can also interact with changes in mean temperature (Bozinovic et al., 2011; Colinet et al., 2015). Further studies are required to elucidate whether acclimation responses of SMR and other functional traits in asp vipers are causally linked with changes in mean temperature, changes in the thermal variability or both. Our results however likely illustrate more realistic responses to climate change compared to findings from studies focusing on acclimation responses at a constant daily temperature because animals are exposed to climate variability in their environment and predictions from laboratory studies in complex thermal environments are more likely to predict future changes in natural populations (Morash et al., 2018).

Some SMR differences persisted among groups for a month after the beginning of the common garden (49 months post-birth, at 20°C), but no group difference in SMR was found 3 years later (85 months post-birth). This indicates that SMR responds dynamically to the thermal environment to optimize performance and satisfy daily energetic costs (Berg et al., 2017). Such reversibility is adaptive in seasonal, variable, or unpredictable environments (Piersma & Gils, 2011), and thus was likely selected for in temperate ectotherms such as the asp viper (Little & Seebacher, 2016). In turn, the inherent capacity of these organisms to reversibly shift their SMR in response to fluctuating temperatures significantly buffers their energetic sensitivity to climate change and to extreme weather events by allowing them to compensate for the energy costs of life at warmer temperatures (Pallarés et al., 2020; Seebacher et al., 2015). Flexible metabolic shifts may be achieved by a range of short-term proximal mechanisms, such as changes in the structure (membrane fluidity, protein activity) and density of mitochondria (Chung et al., 2017; Norin & Metcalfe, 2019; Seebacher et al., 2010). Exploring the mechanisms underlying thermal acclimation of SMR in vipers would require further functional studies of their energy metabolism, especially detailed analyses of the thermal plasticity of mitochondrial activity. Additional experimental design could also help to fully disentangle between developmental plasticity and thermal acclimation *sensu stricto* by investigating how flexible is SMR plasticity to temperature at different stages through life (Beaman et al., 2016).

### *Developmental plasticity in TEWL*

Thermal conditions during development induced long-lasting effects on TEWL across the experimental timeframe. Thus, our study provides a clear demonstration that, at a given relative humidity, mean temperature and thermal variability can lead to developmental plasticity in a key functional trait related to water balance. Individuals raised in both the warmest and the coldest conditions (warm and cold cycles) had lower TEWL rates on average than those raised in the intermediate conditions (medium cycle treatment). Metabolic compensation associated with the warm cycle treatment may have contributed to the decrease in TEWL that we observed in this group as lower metabolic gas exchanges are associated with reduced loss of water vapor (Dupoué et al., 2015; Riddell et al., 2018a). However, individuals raised in the cold treatment also had low evaporative water loss, despite their high oxygen consumption rates. We calculated the daily evaporative water loss (DEWL) taking into account solely the thermal dependence of TEWL. This showed that individuals from warm and cold cycles were able to reduce their DEWL by approximately 30 %, and even up to 50 % for individuals from the warm cycle treatment group at 85 months post-birth. The decrease of TEWL for snakes from the warm cycle treatment may thus be adaptive in anticipation of hotter and drier conditions by reducing DEWL and thus reducing the risks of dehydration. The fact that TEWL also decreased under the cold cycle treatment remains intriguing because the water vapor deficit was minimal and vipers had low predicted DEWL in this treatment group. This reduction in cold conditions could reflect a plastic response of TEWL to the lower absolute air humidity in this treatment or to some behavioral constraints (less frequent drinking behavior) imposed by activity restriction in cold environments. Our results suggest that mean and variance of temperature can provide a relevant cue to regulate water balance (Riddell et al., 2019) probably because temperature, water vapor pressure deficit and water constraints are correlated in natural habitats and organisms cue on temperature to assess dehydration risks (Dai, 2013). Manipulating both temperature and humidity levels will be required to elucidate how temperature and humidity levels jointly influence TEWL plasticity (Rozen- Rechels et al., 2019). Heretofore, the pace of TEWL plasticity and the period of sensitivity during development also remain unknown, as well as the proximal mechanisms involved.

### *Persistent individual differences and covariation between SMR and TEWL*

Phenotypic (Baškiera & Gvoždík, 2020; Careau et al., 2019; Nilsson et al., 2009) and quantitative genetic (Pettersen et al., 2018) studies of energy metabolism have uncovered that MR and its thermal sensitivity are often consistent over time and variable among individuals within the same species (Nespolo et al., 2003; Nespolo & Franco, 2007; Réveillon et al., 2019). In line with these results, our study shows consistent inter-individual variation in SMR throughout 7 years of life and, for the first time, also in TEWL. In addition, SMR and TEWL were strongly positively correlated both within and among individuals and so independently of developmental treatments. The quite remarkable age span over which we examined these individual differences suggests very strong and persistent effects of pre-natal factors on these two functional traits regardless of post-natal conditions and growth trajectories. Our dataset does not allow to elucidate the origin of these physiological syndromes that possibly include long-lasting effects of early environmental conditions and/or genetic factors (White et al., 2019).

The pace-of-life syndrome describes covariation between MR and individual personality, behavior, and life history traits. It is an useful framework to understand how variation in SMR may correlate with maximum MR, behavioral activity and risk-prone behaviors (Careau et al., 2019, 2014; Mell et al., 2016). This theoretical framework has recently been extended to thermal physiology where inter-individual in MR and behavior coincide with variation along the cold-hot axis of thermal physiology (Goulet et al., 2017a; Goulet et al., 2017b). Our results suggest that such a physiological syndrome can also integrate functional traits related to water and energy balance. Although this is the first quantitative analysis of this physiological syndrome, the shared proximal mechanisms, joint plasticity and potential co-evolution between functional traits related to energy balance, respiration, thermal physiology and water biology have already been emphasized (Riddell et al., 2019, 2018a; Rozen- Rechels et al., 2019).

### *Conclusion*

Vipers exhibited two distinct plastic responses to thermal treatment: a phenotypic flexibility of SMR and persistent developmental plasticity in TEWL. In addition, SMR and TEWL strongly co-varied both within and among individuals over their lifespan. Therefore, terrestrial ectotherms can simultaneously exhibit strong individual variation, reflecting physiological syndromes and likely genetic variation, and plastic responses in two functional traits related to thermal and water balance. These two sources of variability may allow these organisms to cope with climate change by combining plastic, adaptive responses to droughts



and heatwaves (Gunderson et al., 2017; Seebacher et al., 2015) and responses to selection on physiological syndromes (Žagar et al., 2018).

### Data accessibility

Data are available at Zenodo with DOI: <https://doi.org/10.5281/zenodo.5561142> (Dezetter et al., 2021)

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### **Supporting information**

Additional supporting information may be found in the online version of this article.

Appendix S1 [Detailed methods regarding respirometry systems]

Table S1 [Parameter estimates from the univariate linear mixed model for body mass]

Table S2 [Parameter estimates from the univariate linear mixed models for SMR and TEWL]



**Table 1.** Descriptive statistics (mean and SE) for body mass and body size (SVL) of aspik vipers maintained in three different thermal conditions during immature life stage (birth to 47 months post-birth) and kept in common environmental conditions during adult life stage (49 and 85 months post-birth).

		Treatment group (sample size)		
		Warm (16)	Medium (16)	Cold (17)
Body mass (g)	Time of measurement			
Females (24)	Birth	6.51 ± 0.35	7.12 ± 0.38	7.32 ± 0.41
	19 months post-birth	17.86 ± 2.05	22.97 ± 1.7	21.18 ± 2.08
	47 months post-birth	117.31 ± 4.94	125.89 ± 4.66	139.29 ± 5.55
	49 months post-birth	112.39 ± 4.31	126.40 ± 4.74	137.61 ± 4.42
	85 months post-birth	156.50 ± 8.84	172.41 ± 7.02	187.79 ± 4.57
Males (25)	Birth	7.23 ± 0.55	7.12 ± 0.24	7.12 ± 0.37
	19 months post-birth	17.89 ± 1.97	17.62 ± 1.54	23.13 ± 2.57
	47 months post-birth	89.80 ± 7.78	77.89 ± 3.93	79.72 ± 4.07
	49 months post-birth	83.65 ± 7.07	76.26 ± 3.85	80.19 ± 4.22
	85 months post-birth	88.93 ± 7.11	79.62 ± 4.69	86.03 ± 4.32
Body size (cm)	Time of measurement			
Females (24)	Birth	17.91 ± 0.49	18.27 ± 0.38	18.2 ± 0.49
	30 months post-birth	41.28 ± 1.46	38.12 ± 1.82	36.22 ± 2.42
	49 months post-birth	52.36 ± 1.18	50.86 ± 2.25	50.5 ± 3.02
	85 months post-birth	55.22 ± 1.2	56.93 ± 1.55	58.44 ± 1.23
Males (25)	Birth	18.09 ± 0.46	18.47 ± 0.46	18.7.12 ± 0.27
	30 months post-birth	39.22 ± 2.11	36.5 ± 2.11	37.39 ± 1.56
	49 months post-birth	46.54 ± 2.18	45.48 ± 2.18	46.92 ± 1.28
	85 months post-birth	47.36 ± 2.25	46.89 ± 2.25	47.83 ± 1.19

**Table 2.** Variance and covariance components for residual ( $V_e$  and  $COV_e$ ) and individual ( $V_I$  and  $COV_I$ ) intercept of centered and scaled standard metabolic rate (SMR) and total evaporative water loss (TEWL) rates in aspik vipers after controlling for trait-specific allometric relationships with body mass and treatment-specific changes through time. Estimates are from the posterior-distribution of (co)variance matrices.

Trait	Variance component	Estimate $\pm$ 95% CI
SMR	$V_e$	0.400 [0.33, 0.482]
	$V_I$	0.252 [0.107, 0.415]
TEWL	$V_e$	0.569 [0.463, 0.671]
	$V_I$	0.217 [0.08, 0.367]
SMR-TEWL	$COV_e$	0.286 [0.217, 0.363]
	$COV_I$	0.168 [0.048, 0.324]

**Table 3.** Degree of metabolic and water loss compensation induced by acclimation response (% differences between values predicted by a null model and calculated DEEi and DEWL values) of each treatment group at each session. P values are from t-test comparing DEEi and DEWL predicted by a null model with calculated DEEi and DEWL. Because the acclimation response in SMR did not persist at adulthood in the common garden (49 and 85 months post-birth, see Fig. 2A), we estimated the degree of metabolic compensation only at 19 and 47 months post-birth.

<b>Time of measurement</b>	<b>Treatment group</b>	<b>Degree of metabolic compensation</b>	
19 months post-birth	Warm	38.36 %	P < 0.001
	Medium	9.34 %	P = 0.26
	Cold	0.21 %	P = 0.98
47 months post-birth	Warm	42.21 %	P < 0.001
	Medium	28.50 %	P < 0.001
	Cold	5.42 %	P = 0.61
<b>Time of measurement</b>	<b>Treatment group</b>	<b>Degree of water loss compensation</b>	
47 months post-birth	Warm	36.36 %	P < 0.001
	Medium	13.46 %	P = 0.15
	Cold	24.24 %	P < 0.001
49 months post-birth	Warm	27.67 %	P = 0.05
	Medium	14.92 %	P = 0.14
	Cold	32.72 %	P < 0.001
85 months post-birth	Warm	49.48 %	P < 0.001
	Medium	16.26 %	P = 0.22
	Cold	23.97 %	P = 0.08

## Figure captions

**Figure 1.** A) Daily temperature ( $^{\circ}\text{C}$ ) cycle applied for each thermal treatment (warm, medium, cold). B) Experimental design and variables collected through time. 19 and 47 months post-birth are during the time in which vipers were exposed to thermal treatments, while sessions 49 and 85 months post-birth are during the time in which vipers were in a common garden.  $\dot{V}\text{O}_2$  is the oxygen consumption (standard metabolic rate); TEWL is Total Evaporative Water Loss; SVL is Snout-Vent Length; BM is Body Mass.

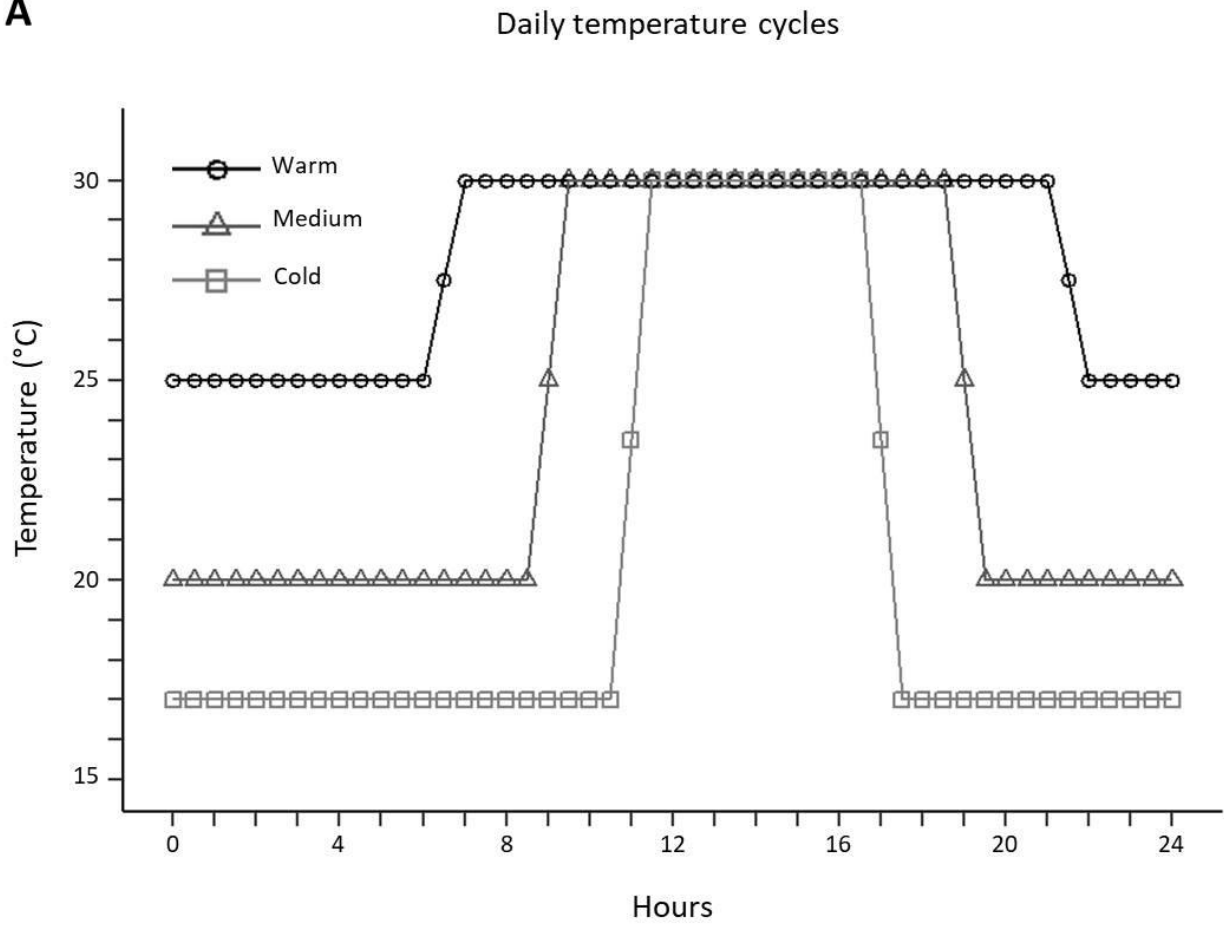
**Figure 2.** Effect of the thermal treatments on the residual standard metabolic rate (log transformed  $\dot{V}\text{O}_2$  provided in mL per hour, A) and residual total evaporative water loss (log transformed TEWL provided in mg per hour, B) measured at different time points through lifetime (see Figure 1) at  $20^{\circ}\text{C}$  and  $30^{\circ}\text{C}$  body temperatures. Residual SMR and TEWL scores were calculated from a log-log regression of individual raw values with body mass and thus accounts for ontogenetic and inter-individual variation in body mass. Error bars represent SE. The \* sign and different letters indicate significant differences among treatment groups based on Tukey post-hoc tests of contrasts performed A) in each treatment and temperature group, B) on average for all treatment and temperature group. ns = not significant

**Figure 3.** A) Residual total evaporative water loss (log transformed TEWL provided in mg per hour) against residual standard metabolic rate (log transformed  $\dot{V}\text{O}_2$  provided in mL per hour) for all aspik vipers across all lifetime stages, treatments and temperatures. B) Best linear unbiased predictors (BLUP) of residual  $\dot{V}\text{O}_2$  for the random effect of identity as function of BLUP for TEWL for all aspik vipers with concurrent measurements of  $\dot{V}\text{O}_2$  and TEWL at the same lifetime stage. BLUPs and associated standard errors were extracted from univariate mixed models.

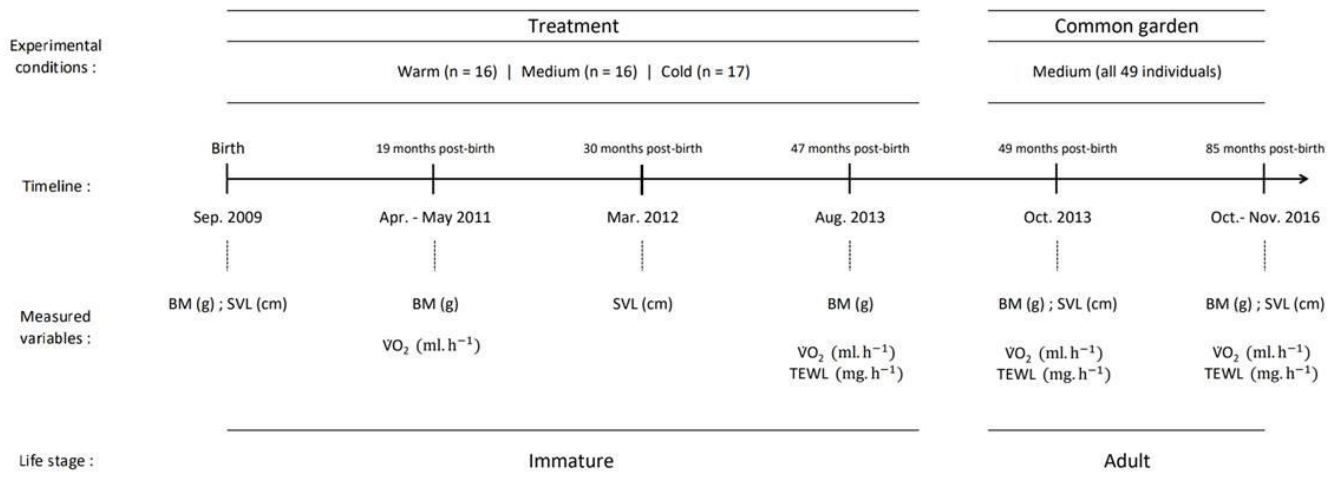
**Figure 4.** Daily expenditure during inactivity (DEEi), A) and daily total evaporative water loss (DEWL), B) calculated at different time points through lifetime (see Figure 1A) for each treatment group. White points represent mean values ( $\pm$  SE) of calculated DEEi and DEWL. Black points represent single values of DEEi and DEWL predicted by a null model. Arrows symbolize the degree of metabolic or water loss compensation. Because the acclimation response in SMR did not persist at adulthood in the common garden (49 and 85 months post-birth, see Figure 2A), we calculated and predicted DEEi only at 19 and 47 months post-birth.

**Figure 1**

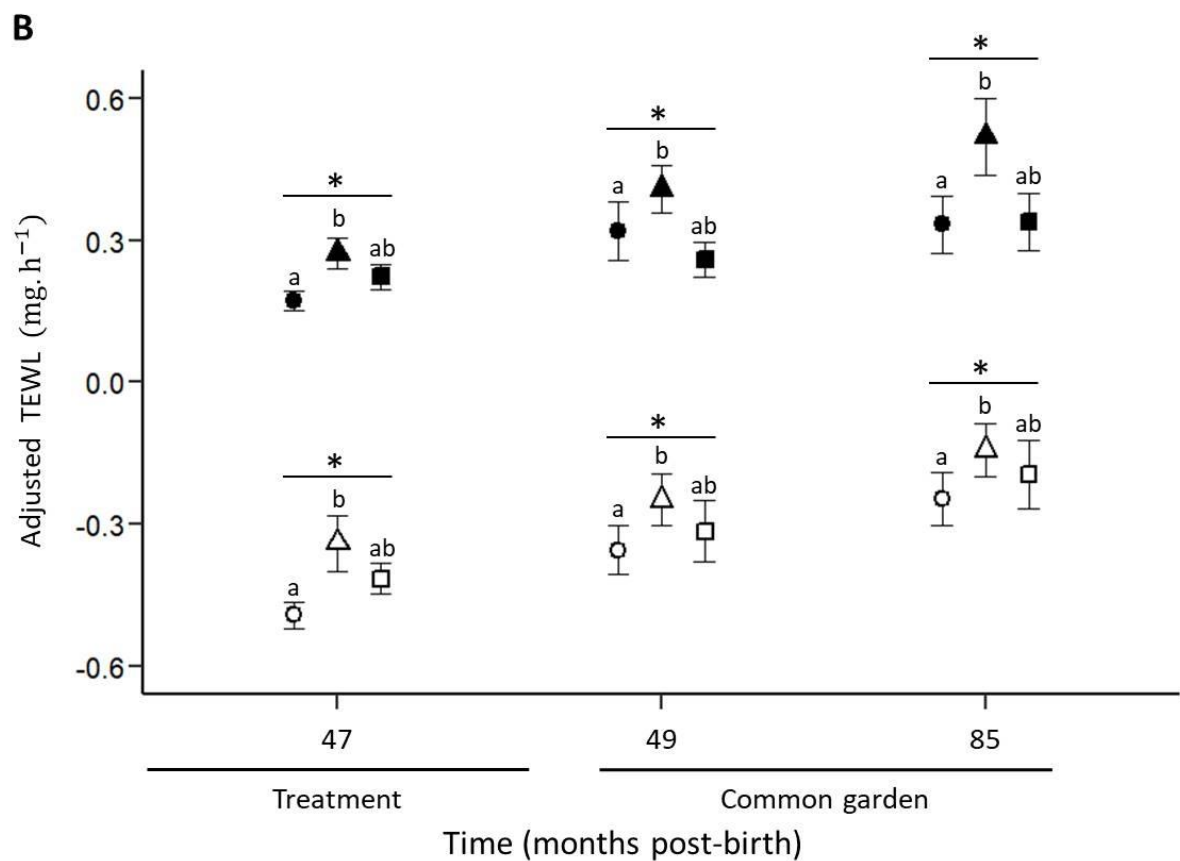
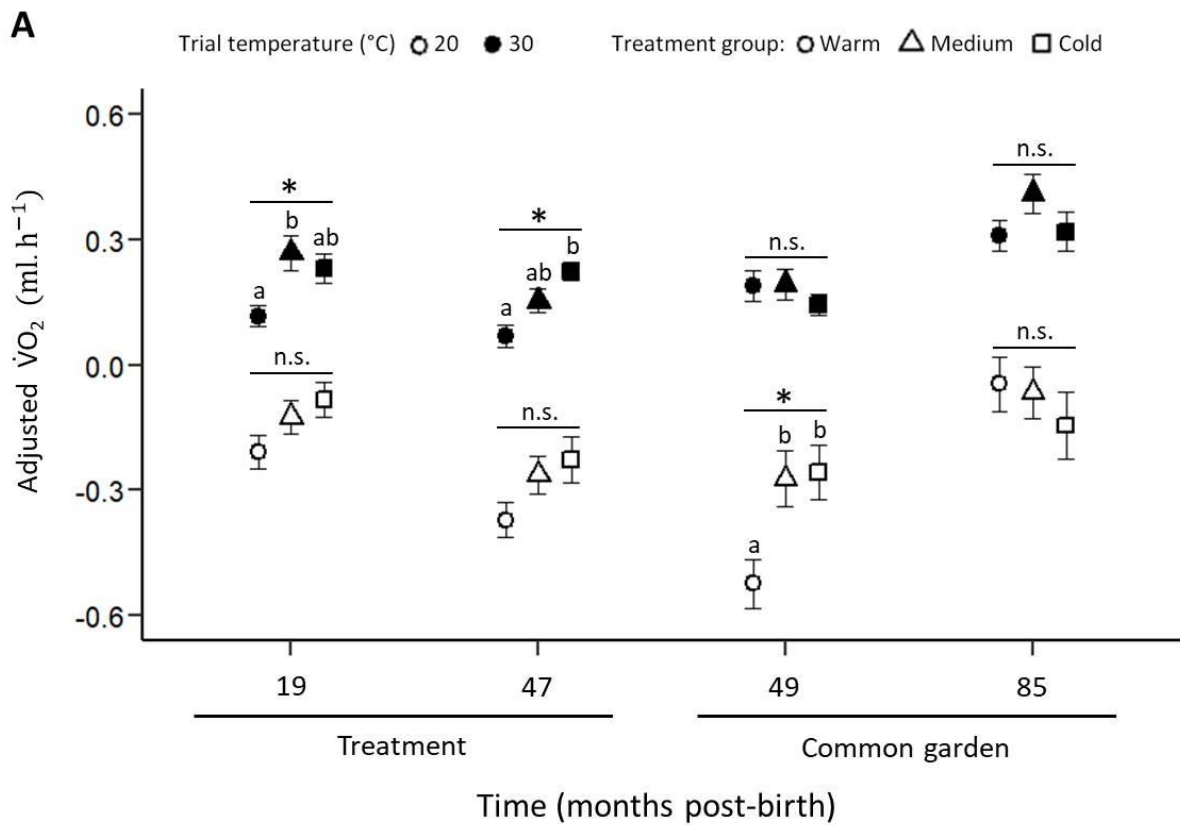
**A**



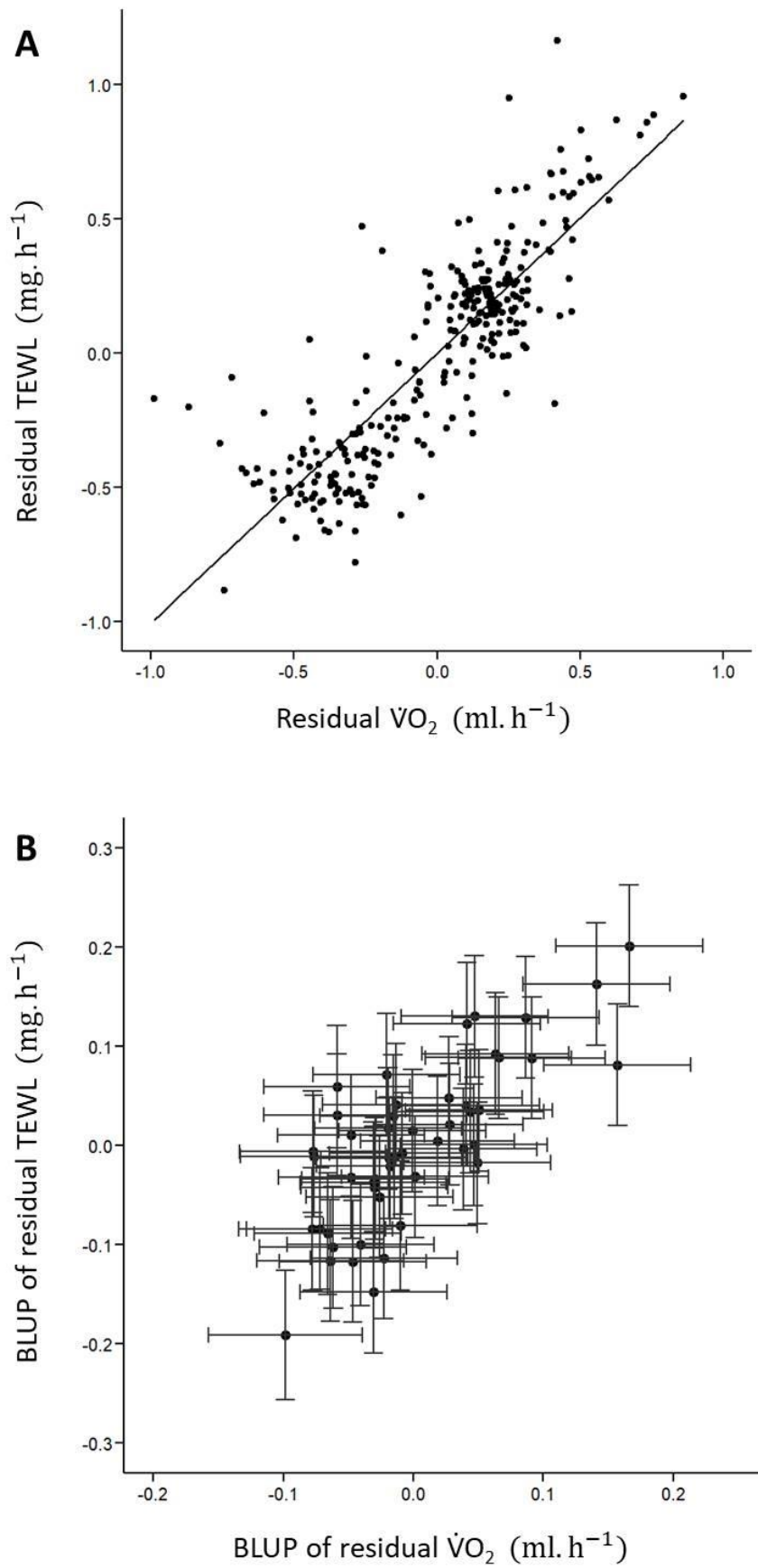
**B**



**Figure 2**



**Figure 3**



**Figure 4**

