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

Mathias Dezetter, Andréaz Dupoué, Jean François Le Galliard, Olivier
Lourdais

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1 Running title: determinant of lifetime metabolic and water loss rates of a dry-skinned ectotherm

2

3 **Additive effects of developmental acclimation and physiological**
4 **syndromes on lifetime metabolic and water loss rates of a dry-skinned**
5 **ectotherm**

6

7 Mathias Dezetter^{1,2,*}, Andréaz Dupoué¹, Jean François Le Galliard^{1,3}, Olivier Lourdais^{2,4}

8

9 ¹ CNRS Sorbonne Université, UMR 7618, iEES Paris, Université Pierre et Marie Curie, Tours 44-
10 45, 4 Place Jussieu, 75005 Paris, France

11 ² Centre d'étude biologique de Chizé CNRS, UMR 7372, 79360, Villiers en Bois, France

12 ³ Ecole normale supérieure, PSL Research University, Département de biologie, CNRS, UMS 3194,
13 Centre de recherche en écologie expérimentale et prédictive (CEREPEP-Ecotron IleDeFrance), 78
14 rue du château, 77140 Saint-Pierre-lès-Nemours, France

15 ⁴ School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501, USA

16

17 * Correspondence:

18 Mathias Dezetter, CEBC UMR 7372 CNRS-ULR

19 79360 Villiers en Bois, FRANCE

20 Phone: + 33 (0) 5 49 09 35 52

21 Fax: + 33 (0) 5 49 09 65 26

22 E-mail: mathias.dezetter@gmail.com

23 ORCID ID: 0000-0002-5083-624X

24

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37

38 **Authors' contributions**

39 O.L. and A.D designed the study and collected the data. M.D. led the data analyses together with
40 O.L., J.-F.L.G. and A.D. M.D. led the writing of the manuscript and all authors contributed
41 critically in result interpretation and manuscript writing. All authors gave final approval for
42 publication.

43

44 **Conflict of interest**

45 The authors declare no competing or financial interests.

46

47 **Data accessibility**

48 **Data will be deposited in Zenodo after acceptance.**

49

50 **Abstract**

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

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

57 3. We used climatic chambers to simulate three contrasted daily thermal cycles (warm, medium and
58 cold) differing in mean temperatures (28, 24, and 20°C respectively) and amplitudes (5, 10 and
59 13°C respectively) during immature life (0 to 4 years of age). Individuals were then maintained
60 under common garden conditions (medium cycle) for an additional 3-years period (4 to 7 years of
61 age). SMR and TEWL were repeatedly measured in the same individuals throughout life during and
62 after the climate manipulation.

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

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

69 6. Our study demonstrates the capacity of long-lived organisms to flexibly shift their SMR to reduce
70 daily maintenance costs in warmer and less variable thermal environments, which might be
71 beneficial for low energy specialist organisms such as vipers. It further suggests that thermal
72 conditions provide cues for developmental changes in TEWL. Beside plasticity, contrasted
73 individual physiological syndromes could be selected for and contribute to the response to climate
74 change.

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

76 **Introduction**

77 Physiological plasticity refers to the ability of an organism to change its physiological state in
78 response to external cues and is an important process for coping with thermal variations (Bonamour
79 et al., 2019; Fuller et al., 2010). In particular, physiological plasticity in response to changes in
80 thermal conditions might determine ‘winner or losers’ in the context of global warming (Seebacher
81 et al., 2015; Somero, 2010). Thermal plasticity involves a range of distinct but interconnected
82 mechanisms including flexible acclimation responses (shift in physiological routines throughout
83 life) and developmental plasticity (persistent effects induced during development; da Silva et al.,
84 2019; Healy et al., 2019; Sultan, 2017). Recent attention has focused on physiological acclimation
85 capacity to a persistent increase in environmental temperatures (Angilletta, 2009; Huey et al.,
86 2012). Thermal acclimation response for ectotherms involves shifts in physiological responses,
87 which thereby changes the optimal temperature for biological process, and the temperature
88 tolerance limits (Angilletta Jr., 2009; Seebacher, 2005). Such responses are critical to cope with
89 global warming for long-lived ectotherms because of their presumed limited potential for rapid
90 genetic evolution (Chevin et al., 2010; Seebacher et al., 2015; Urban et al., 2014). Yet, uncertainty
91 surrounds the speed and breadth of thermal acclimation responses and their efficiency as a means to
92 buffer ectotherms from global warming (Gunderson & Stillman, 2015; Havird et al., 2020; Morley
93 et al., 2019; Rohr et al., 2018; Seebacher et al., 2015).

94 There are several reasons why we might underestimate the true acclimation capacity of long-
95 lived terrestrial ectotherms such as some species of amphibians and reptiles. First, most experiments
96 on these organisms do not last long enough to ensure that individuals are fully acclimated (Rohr et
97 al., 2018; Seebacher et al., 2015). Time to acclimate is typically longer for large-bodied species,
98 based on energetic models and empirical evidence (Kingsolver & Huey, 2008; Pörtner et al., 2017)
99 and recent comparative analyses across a diversity of ectothermic organisms (Rohr et al., 2018).
100 Long-term (> 1 year) acclimation studies are crucial to investigate the plastic responses of long-
101 lived ectotherm but are very rare. Second, the flexibility of phenotypic responses to acclimation

102 temperatures is rarely tested for, and how persistent are the effects of developmental plasticity
103 remains unknown for most organisms (Beaman et al., 2016; Ligon et al., 2012). Irreversible
104 acclimation responses to developmental conditions might lead to maladaptive mismatches between
105 the phenotype and ecological conditions in highly variable and unpredictable environments
106 (Gluckman et al., 2007; Piersma and Gils, 2011). Therefore, the ability of organisms to flexibly
107 adjust their phenotype to environmental variations throughout their lifetime may determine their
108 capacity to cope with climate change (Morley et al., 2019; Pallarés et al., 2020). Similarly, whether
109 plastic responses supersede fixed inter-individual differences in physiology has been poorly
110 investigated so far, despite evidence of consistent physiological syndromes in some ectotherms
111 (Goulet et al., 2017a; Mell et al., 2016).

112 In ectotherms, SMR represents the minimal energy requirements to fuel maintenance energy
113 demands, and variation in SMR within and between species may faithfully reflect life history
114 strategies (Clarke, 2006; Seebacher et al., 2015). Considerable variation in MR exists within and
115 between species (Careau et al., 2019), potentially reflecting thermal adaptations (Addo-Bediako et
116 al., 2002) and additive constraints from habitat aridity (Dupoué et al., 2017). Within species,
117 individuals can respond to thermal fluctuations through MR modulation (Noble et al., 2018; While
118 et al., 2018). For instance, after exposure to warm temperatures, MR may decrease (“negative
119 compensation”) to limit the energy costs of self-maintenance and minimize oxidative stress (see
120 Costantini, 2008, Angilletta, 2009; Norin et al., 2014). Temperature is also a proximate cue for
121 water constraints inducing adjustment of evaporative water loss (TEWL see Riddell et al., 2019). In
122 terrestrial species, a universal cost of metabolic gas exchange is the loss of water vapor such that
123 there could be a functional trade-off between SMR and TEWL (Woods & Smith, 2010). Because
124 climate change will affect both energy and water balance, considering jointly SMR, TEWL and
125 associated tradeoffs between both is required (Riddell & Sears, 2020; Riddell et al., 2018b).
126 Individual variation in metabolism can co-vary with variation in other physiological or in
127 personality traits through consistent pace-of-life syndromes, where “fast” phenotypes are

128 characterized by a higher SMR (Biro & Stamps, 2010; Careau et al., 2019, 2014). Thus, phenotypic
129 correlations may also exist between SMR and TEWL and could influence individual sensitivity to
130 temperature and water constraints. Despite its relevance, the long-term developmental effect on
131 variation and co-variation in SMR and TEWL has not been addressed to date, and the pace-of-life
132 syndrome framework has not been extended yet to these functionally related traits.

133 We used a 7-years long experiment to characterize the long-lasting consequences of thermal
134 conditions on the asp viper (*Vipera aspis*), a temperate ectotherm with a late sexual maturation (age
135 at maturation between 3 to 4 years depending on growth; Bonnet et al., 1999) and a relatively long
136 generation time (maximum lifespan >10 years) compared to other squamate reptiles. We
137 hypothesized that thermal conditions should shape both SMR and TEWL, and that chronic exposure
138 would result in persistent effects. We applied three contrasted daily thermal cycles (warm, medium,
139 and cold) with contrasted temperature and thermal amplitude from birth to sexual maturity (4
140 years). Individuals were then placed in a common garden treatment (medium thermal cycle) during
141 their adult life (from 4-7 years old). Building up from previous findings in ectotherms, we tested the
142 following three predictions. First, individuals raised in the warmest thermal cycle should exhibit the
143 lowest SMR due to a negative compensation (Bruton et al., 2012; Clarke, 2006; Zari, 1996).
144 Second, chronic exposure to the thermal treatments during growth should lead to persistent
145 acclimation responses in both SMR and TEWL (Nettle & Bateson, 2015; Refsnider et al., 2019).
146 Third, SMR should correlate with TEWL among and within individuals because of a functional
147 relationship between the two traits (e.g., water loss caused by ventilation) and/or to some persistent
148 early environment or genetic effects on an underlying trait influencing both SMR and TEWL
149 (Riddell et al., 2018a; Rozen-Rechels et al., 2019).

150

151 **Material and Methods**

152 Thermal treatment and experimental design

153 The use of realistic thermal treatment mimicking natural condition is of critical importance to
154 address the impact of climatic changes on physiological responses (Morash et al., 2018; Potter et
155 al., 2013). The asp viper is a diurnal species that behaviorally thermoregulates to reach preferred
156 temperature close to 30°C (Lorioux et al., 2013). At night this species will usually hide in a shelter
157 and body temperature will fluctuate with the temperature of the resting habitat. This implies that
158 warmer climate conditions within the thermal critical limits will lead to an extended time spent at
159 the basking temperature, and exposure to warmer nighttime temperatures in vipers (Davy et al.,
160 2017), as predicted by mechanistic models and as seen in empirical studies of heliothermic
161 squamate reptiles (Blouin-Demers & Weatherhead, 2001). This implies higher mean body
162 temperature and to lower thermal variability in a warmer climate.

163 Therefore, we simulated three realistic daily thermal cycles (Fig. 1A, here after designated
164 as “warm”, “medium” and “cold” cycles) that differed in mean temperature (28, 24 & 20°C
165 respectively) but also in the time at preferred temperature and daily amplitude. Preferred
166 temperature (30°C) was simulated in all of our treatments but with different durations (14, 9 and 5
167 hours in warm, medium and cold cycles). We also simulated different night-time temperatures (25,
168 20°C and 17°C respectively in in warm, medium and cold cycles; see Fig. 1A). Thus, daily thermal
169 amplitude differed between groups (5,10, and 20°C respectively). For all treatments, relative
170 humidity was set at a constant value of 50% and water vapor pressures deficit were respectively,
171 1.91 kPa (warm cycle), 1.55 kPa (medium cycle) and 1.24 kPa (cold cycle).

172 We used 49 captive-born snakes from a colony of asp viper maintained in the Centre
173 d'Etudes Biologiques de Chizé, France. Mothers were collected in west-central France from
174 neighboring populations and were reproduced in captivity where they were maintained in the same
175 standard conditions during pregnancy (see Lorioux et al., 2013 for details on maintenance
176 conditions and thermal cycles). From birth (September-October 2009) to maturity (October 2013),
177 individuals were randomly assigned within families to one of three treatment (n=16, 16 and 17
178 respectively for warm, medium and cold cycle; see Fig. 1B). In November 2013, we transferred all

179 adult individuals to a common garden at the medium cycle for an additional 3-year period (Fig. 1A).
180 Individuals exposed to the medium cycle during development were thus kept in the same conditions
181 across the experimental timeframe. Individuals from hot and cold cycles were exposed to new
182 thermal conditions when transferred to the common garden, allowing us to test for the flexibility of
183 acclimation responses in these treatment groups.

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

193

194 Laboratory measurements

195 To investigate how experimental treatments affected growth rate, we weighed ($\pm 0.01\text{g}$) and
196 measured (snout-vent-length, SVL $\pm 0.5\text{cm}$) all individuals throughout their life. Body mass (BM)
197 was measured at birth and then at the same time points as the respirometry exchanges
198 measurements (see below), while we measured SVL at birth, and when snakes were 30, 49 and 85
199 month-old. To assess both short-term and long-term phenotypic responses, we quantified SMR and
200 TEWL during four successive measurement sessions (Fig. 1B). We measured respiratory exchanges
201 when individuals were 19 and 47 month-old in order to examine to pace of the acclimation response
202 during the development period exposed to contrasted thermal treatments. We conducted
203 measurements 3 months after the beginning of the common garden situation (at 49 month-old) in
204 order to detect any quick changes in SMR or TEWL that may occur following the change in thermal

205 conditions. Later, we realized a last measurement session 3 years later (at 85 month-old) in order to
206 test for long-term persistent effects of developmental plasticity.

207

208 Respiratory exchanges

209 We measured the rate of oxygen consumption (VO_2 proxy for SMR; $ml.h^{-1}$) and the total
210 evaporative water loss (TEWL; $mg.h^{-1}$) on resting individuals at two test temperatures (20°C and
211 30°C), to calculate Q10 and acclimation response (Einum et al., 2019; Havird et al., 2020). We
212 randomized temperature trials for each individual. To measure VO_2 , we used a closed-system
213 respirometry at 19 months post-birth measurements because of the small size of the snakes, and an
214 open-flow respirometry system at 47, 49 and 85 months post-birth measurements (see detailed
215 methods regarding respirometry systems on Appendix S1 in Supporting Information). We measured
216 TEWL together with VO_2 using the open flow-system (Fig. 1A). We measured gas exchanges at
217 night on post-absorptive snakes after a minimum of 7 days of fasting. To stabilize the test
218 temperatures and avoid exploratory behaviors, we placed each individual in the temperature-
219 controlled chamber to acclimate for 3 h prior to each measurement.

220

221 Statistical analyses

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

224

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

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

229

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

231 We fitted linear mixed models using a Bayesian inference approach with Markov Chain Monte
232 Carlo (MCMC) techniques from the *MCMCglmm* library (Hadfield, 2009). Results from Bayesian
233 models are further presented as median estimates \pm 95% highest posterior density intervals (HPDI).
234 To investigate independently the effects of experimental conditions on body mass and physiological
235 trait, we first ran univariate mixed effects models with individual identity and mother identity
236 included as random intercepts. In these univariate models, the variance attributed to individual
237 identity represents the intra-individual variance V_I , whereas the variance attributed to maternal
238 identity represents the intra-litters variance V_m . For each univariate model, treatment group (warm,
239 medium, or cold cycle), effects of session (see Figure 1), and their interaction were included as
240 fixed factors. The SMR, TEWL, BM trait values were treated as the dependent variable in each
241 univariate model. In univariate models for BM, we included sex as a factor to account for sexual
242 dimorphism. In univariate models for SMR and TEWL, we tested the effect of the ambient
243 temperature during the measurement by including trial temperature (20 or 30°C), along with its
244 interactions with treatment and session, as factors. Because BM can strongly influence SMR and
245 TEWL, individual mass at the time of measurement was also included as a covariate in these
246 univariate models but we did not include sex based on preliminary analyses indicating no sexual
247 differences in metabolism and water loss rates.

248 We ran the full model including all variables and interactions following standard guidelines
249 of the *MCMCglmm* package (Hadfield, 2009). The analysis was performed on raw variables,
250 assuming a Gaussian distribution of the residuals, and poorly informative priors for fixed effects
251 (Gaussian distributed with zero mean and large variance), and uncorrelated poorly informative
252 priors for variance components (inverse Wishart distribution with $nu = 0.004$ and V set at 1). The
253 MCMC algorithm was set to 120,000 iterations after a minimum burn-in phase of 10,000 iterations
254 to allow convergence and using a thinning interval of 50 to avoid autocorrelation issues. We
255 examined the posterior distribution of model estimates, calculated autocorrelation terms to evaluate
256 independence of the samples in the posterior distributions, and performed half-width tests of

257 convergence, which showed satisfactory properties of the sampling distribution. Using the full
258 model, we then tested for the significance of random effects using model comparison with the
259 deviance information criterion (DIC).

260 We next performed a model selection procedure of the fixed effects based on DIC and the
261 posterior probability associated to the null hypothesis or pMCMC. To ease interpretation of the
262 results, we further conducted Tukey post-hoc comparisons using the posterior chains of parameter
263 estimates converted into posterior samples of contrasts with the *emmeans* package (Lenth et al.,
264 2020). Note that the results of the *MCMCglmm* model selection for univariate models were similar
265 to those of classical, maximum likelihood based approaches fitted with the *lme* procedure (Pinheiro
266 & Bates, 2006).

267
268 *Bivariate mixed model to estimate phenotypic correlation between SMR and TEWL*

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

282

283 Quantification of the compensation

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

$$288 \quad R(T) = R(20^\circ\text{C}) \times Q_{10}^{\frac{(T-20)}{10}} \quad (1)$$

289 where $R(T)$ is the predicted process rate at temperature T , $R(20^\circ\text{C})$ is our reference process rate at
290 20°C and Q_{10} is the ratio of the process rate at 30°C and 20°C . We calculated Q_{10} for each viper at
291 each measurement session. We then used equation (1) to calculate the predicted SMR and TEWL
292 over each 30 minutes' time bin from the daily nycthemeral cycle of ambient temperatures for each
293 treatment group of the viper. Daily energy expenditure during inactivity (DEEi; $\text{cal}\cdot\text{day}^{-1}$) and daily
294 total evaporative water loss (DEWL; $\text{mg}\cdot\text{day}^{-1}$) were calculated by summing the predicted SMR and
295 TEWL over each 30-minute time bin through a day. This calculation therefore accounts for both
296 acute effects of temperature (through individual Q_{10} and the nycthemeral cycle) and acclimation
297 effects of temperature (through changes in $R(20^\circ\text{C})$ and Q_{10} among treatments. To quantify the
298 degree of metabolic and water loss compensation induced by the observed acclimation response, we
299 compared the DEEi and DEWL of each treatment group at each session with those predicted by a
300 null model. Individuals had lower SMR in response to warmer and less variable temperatures (see
301 results below). Thus, in order to quantify how much individuals saved energy through acclimation
302 to warmer and less variable temperatures, the null model for DEEi assumed no warm acclimation
303 response. We therefore used the mean $R(20^\circ\text{C})$ and Q_{10} of vipers from the cold cycle to predict the
304 DEEi from the warm and medium cycle treatment in the absence of metabolic acclimation. We
305 estimated the degree of metabolic compensation only at 19 and 47 months post-birth because the
306 acclimation response in SMR did not persist at adulthood in the common garden (49 and 85 months
307 post-birth, see Fig. 2A). In addition, individuals had lower TEWL in both cold and warm cycle
308 treatment than in medium treatment (see results below). In order to quantify the compensation in

309 DEWL induced by lower TEWL, the null model for DEWL therefore used the mean $R(20^{\circ}C)$ and
310 Q_{10} from vipers from the medium cycle treatment as a baseline reference for the two other groups.

311

312 **Results**

313 Morphological differences between treatments

314 There was strong inter-individual variation in body mass but a negligible variation among
315 littermates in the full model (DIC, full model: -929.76, model without V_i : -644.09, model without
316 V_m : -929.57). The change in body mass through time was weakly influenced by a sex-specific effect
317 of treatment groups (DIC, full model: -929.76; full model without three-way interaction: -927.7). In
318 females, body mass did not change between treatments but increased through time faster than in
319 males, especially between session 2 and session 3 (analysis on subset of females data; DIC, model
320 with Treatment \times Time: -453.2, model without Treatment \times Time: -457.7). In males, body mass
321 was weakly influenced by treatments (analysis on subset of males data; DIC, model with Treatment
322 \times Time: -475.02, model without Treatment \times Time: -473.98) because males from the cold cycle
323 were slightly bigger at 19 months post-birth than males from the two other treatments (Tukey post-
324 hoc contrast between cold and warm cycle: 0.104 [-0.034, 0.244] 95% HPDI, warm and medium
325 cycle: 0.099 [-0.034, 0.237] 95% HPDI see Table 1). After accounting for these fixed effects, the
326 inter-individual variation in body mass was highly significant ($R_I = 0.660$ [0.523, 0.743] 95%
327 HPDI).

328

329 Growth rate measured in each life stage differed between sexes consistently (faster growth in
330 females, all $P < 0.001$) and also between treatments from birth to 30 months post-birth (
331 $F_{2,44} = 6.69$, $P = 0.003$) and from 49 months post-birth to 85 months post-birth ($F_{2,44} = 3.75$, $P = 0.03$
332). During the acclimation phase, vipers grew longer in the warm cycle than in the medium and cold
333 cycle. During the common garden phase at adulthood, there was a compensatory growth response in

334 vipers from the medium and cold cycle, which reached slightly higher SVL than vipers from the
335 warm cycle group at 85 months post-birth (see Table 1).

336

337 Univariate models for SMR and TEWL

338 According to our model selection procedure, there was strong inter-individual variation in SMR and
339 comparatively little variation among littermates in the full model (DIC, full model: -186.69, model
340 without V_i : -171.7, model without V_m : -186.03). Regarding the fixed effects (see Table S2), SMR
341 increased with body mass (pMCMC > 0.001) and was higher at 30°C than at 20°C (pMCMC >
342 0.001), but neither the treatment group nor the trial temperature influenced the allometric
343 relationship with body mass (pMCMC > 0.10). Independently from body mass, treatment groups
344 influenced SMR differently through time and trial temperature levels (DIC, model without V_m and
345 without Treatment × Time × Temperature: -182.3). At 20°C, vipers raised in the warm cycle
346 treatment had slightly lower SMR than vipers raised in the medium and cold cycles during the
347 acclimation phase (19 and 47 months post-birth) and this difference was significant three months
348 after the end of the manipulation (49 months post-birth; post-hoc contrasts of the median posterior
349 distribution, see Fig. 2A). Two years later, at 85 months post-birth, SMR values were not different
350 between treatments irrespective of body temperatures. At 30°C, vipers raised in the warm cycle
351 treatment had significantly lower SMR than vipers raised in the medium and cold cycles during the
352 acclimation phase (19 and 47 months post-birth), but this difference vanished right after the end of
353 the manipulation (49 and 85 months post-birth, see Fig. 2A). After accounting for these fixed
354 effects, the inter-individual variation in SMR was significant but relatively small ($R_I = 0.129$ [0.049,
355 0.231] 95% HPDI).

356

357 Similarly, there was inter-individual variation in TEWL and comparatively little variation among
358 littermates in the full model (DIC, full model: -97.7, model without V_i : -66.2, model without V_m : -
359 97.9). The TEWL increased with body mass similarly between treatment and temperature groups

360 (pMCMC > 0.05) and was higher at 30°C than at 20°C (pMCMC > 0.001). Contrary to SMR,
361 treatment groups significantly influenced TEWL independently from time and trial temperature (see
362 Table S2). On average, vipers raised in the medium cycle treatment displayed consistently higher
363 TEWL rates through lifetime relative to the warm treatment (Tukey post-hoc contrast: 0.1203
364 [0.0281, 0.2141] 95% HPDI) and also slightly higher TEWL than in the cold cycle treatment
365 (Tukey post-hoc contrast: 0.092 [-0.0035, 0.1827] 95% HPDI) irrespective of trial temperature (see
366 Fig. 2B). After accounting for these fixed effects, the inter-individual variation in TEWL was
367 significant and twice than the one for SMR ($R_I = 0.237$ [0.115, 0.373] 95% HPDI).

368

369 Bivariate models for SMR and TEWL

370 The bivariate mixed models indicated that both the intra-individual and inter-individual covariance
371 between SMR and TEWL were positive and significantly different from zero as shown by the DIC
372 (full model: DIC = 1173.91, COV_e set to zero: DIC = 1268.6; COV_I set to zero: DIC = 1198.21; see
373 Table 2). The inter-individual correlation was slightly larger ($r_I = 0.69$ [0.46, 0.88] 95%CI) than the
374 intra-individual correlation ($r_e = 0.60$ [0.51, 0.68] 95%CI) and the total phenotypic correlation ($r_P =$
375 0.44 [0.15, 0.75] 95%CI, see Fig. 3A and 3B).

376

377 Metabolic and water loss compensation

378 Individuals from warm and medium cycle treatment groups had lower predicted DEEi than
379 expected in the null model without an acclimation response. Individuals from the warm cycle
380 treatment compensated for approximately 40% of their DEEi at both 19 and 45 months post-birth.
381 In the medium cycle treatment group, individuals compensated for 28.5 % of their DEEi at 45
382 months post-birth (Table 3, Fig. 4A). Similarly, DEWL was lower than expected in the null model
383 without an acclimation response for individuals from the warm and cold cycle treatment groups. At
384 47 months post-birth, DEWL was reduced by 36.4 % for individuals acclimated to the warm cycle
385 treatment, and by 24.2 % for individuals acclimated to the cold cycle treatment. At 49 months post-

386 birth individuals acclimated to the cold cycle treatment compensated for 32.7 % of their DEWL,
387 and individuals acclimated to the warm cycle treatment compensated for 49.5 % of their DEWL at
388 85 months post-birth (Table 3, Fig. 4B).

389

390 **Discussion**

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

397

398 *Negative compensation and flexible acclimation in SMR*

399 During the time period of exposure to contrasted treatments (until the age of 4 years old), snakes
400 exposed to the warmest and less variable thermal conditions adjusted their SMR with a negative
401 compensatory response (Clarke, 2006; Dupoué et al., 2017) when compared to snakes maintained at
402 lower and more variable temperatures (medium and cold cycle). This physiological response to
403 thermal conditions did not persist in a common garden, providing unambiguous evidence that
404 acclimation in SMR was flexible. The compensatory response was quantified and allowed vipers to
405 save as much as 40% of their DEEi relative to a null model hypothetical estimate simulating no
406 acclimation response. The asp viper is a typical capital breeder with a low feeding frequency and a
407 slow pace of life strategy. Therefore, lower SMR is likely to be advantageous according to the
408 “allocation model” of energy metabolism where individuals with lower SMR benefit from more
409 energy to spend in maintenance (e.g., survival), activity and/or reproduction (Careau et al., 2008;
410 Mathot & Dingemanse, 2015). In support of this scenario, we found that warm-acclimated vipers
411 had both the lowest SMR and the highest growth rate. Thus, warm-acclimated snakes were able to

412 advantageously invest more energy into growth, given their lower energetic demands. Even though
413 the relationship between SMR and fitness can be quite variable across organisms (Arnold et al.,
414 2021), these results confirm earlier findings where MR reduction in warm-acclimated ectotherms
415 minimized the daily energetic costs of self-maintenance and limited oxidative stress by decreasing
416 respiratory activity (Norin et al., 2014; Seebacher et al., 2010). In addition, the covariation between
417 SMR and TEWL suggests that lower SMR is likely to positively impact water balance through
418 reduced respiratory water loss (Dupoué et al., 2015). In colder conditions, MR may remain high to
419 support performance, activity, and physiological functions despite lower temperatures (Berg et al.,
420 2017). We further found that treatment-induced changes in SMR differed at body temperatures of
421 20 and 30°C, suggesting that thermal acclimation changed also the acute effects of temperature on
422 metabolism (Xie et al., 2017).

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

436 Some SMR differences persisted among groups for a month after the beginning of the
437 common garden (49 months post-birth, at 20°C), but no group difference in SMR was found 3 years

438 later (85 months post-birth). This indicates that SMR responds dynamically to the thermal
439 environment to optimize performance and satisfy daily energetic costs (Berg et al., 2017). Such
440 reversibility is adaptive in seasonal, variable, or unpredictable environments (Piersma & Gils,
441 2011), and thus was likely selected for in temperate ectotherms such as the asp viper (Little &
442 Seebacher, 2016). In turn, the inherent capacity of these organisms to reversibly shift their SMR in
443 response to fluctuating temperatures significantly buffers their energetic sensitivity to climate
444 change and to extreme weather events by allowing them to compensate for the energy costs of life
445 at warmer temperatures (Pallarés et al., 2020; Seebacher et al., 2015). Flexible metabolic shifts may
446 be achieved by a range of short-term proximal mechanisms, such as changes in the structure
447 (membrane fluidity, protein activity) and density of mitochondria (Chung et al., 2017; Norin &
448 Metcalfe, 2019; Seebacher et al., 2010). Exploring the mechanisms underlying thermal acclimation
449 of SMR in vipers would require further functional studies of their energy metabolism, especially
450 detailed analyses of the thermal plasticity of mitochondrial activity. Additional experimental design
451 could also help to fully disentangle between developmental plasticity and thermal acclimation *sensu*
452 *stricto* by investigating how flexible is SMR plasticity to temperature at different stages through life
453 (Beaman et al., 2016).

454

455 *Developmental plasticity in TEWL*

456 Thermal conditions during development induced long-lasting effects on TEWL across the
457 experimental timeframe. Thus, our study provides a clear demonstration that, at a given relative
458 humidity, mean temperature and thermal variability can lead to developmental plasticity in a key
459 functional trait related to water balance. Individuals raised in both the warmest and the coldest
460 conditions (warm and cold cycles) had lower TEWL rates on average than those raised in the
461 intermediate conditions (medium cycle treatment). Metabolic compensation associated with the
462 warm cycle treatment may have contributed to the decrease in TEWL that we observed in this group
463 as lower metabolic gas exchanges are associated with reduced loss of water vapor (Dupoué et al.,

464 2015; Riddell et al., 2018a). However, individuals raised in the cold treatment also had low
465 evaporative water loss, despite their high oxygen consumption rates. We calculated the daily
466 evaporative water loss (DEWL) taking into account solely the thermal dependence of TEWL. This
467 showed that individuals from warm and cold cycles were able to reduce their DEWL by
468 approximately 30 %, and even up to 50 % for individuals from the warm cycle treatment group at
469 85 months post-birth. The decrease of TEWL for snakes from the warm cycle treatment may thus
470 be adaptive in anticipation of hotter and drier conditions by reducing DEWL and thus reducing the
471 risks of dehydration. The fact that TEWL also decreased under the cold cycle treatment remains
472 intriguing because the water vapor deficit was minimal and vipers had low predicted DEWL in this
473 treatment group. This reduction in cold conditions could reflect a plastic response of TEWL to the
474 lower absolute air humidity in this treatment or to some behavioral constraints (less frequent
475 drinking behavior) imposed by activity restriction in cold environments. Our results suggest that
476 mean and variance of temperature can provide a relevant cue to regulate water balance (Riddell et
477 al., 2019) probably because temperature, water vapor pressure deficit and water constraints are
478 correlated in natural habitats and organisms cue on temperature to assess dehydration risks (Dai,
479 2013). Manipulating both temperature and humidity levels will be required to elucidate how
480 temperature and humidity levels jointly influence TEWL plasticity (Rozen-Rechels et al., 2019).
481 Heretofore, the pace of TEWL plasticity and the period of sensitivity during development also
482 remain unknown, as well as the proximal mechanisms involved.

483

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

485 Phenotypic (Baškiera & Gvoždík, 2020; Careau et al., 2019; Nilsson et al., 2009) and quantitative
486 genetic (Pettersen et al., 2018) studies of energy metabolism have uncovered that MR and its
487 thermal sensitivity are often consistent over time and variable among individuals within the same
488 species (Nespolo et al., 2003; Nespolo & Franco, 2007; Réveillon et al., 2019). In line with these
489 results, our study shows consistent inter-individual variation in SMR throughout 7 years of life and,

490 for the first time, also in TEWL. In addition, SMR and TEWL were strongly positively correlated
491 both within and among individuals and so independently of developmental treatments. The quite
492 remarkable age span over which we examined these individual differences suggests very strong and
493 persistent effects of pre-natal factors on these two functional traits regardless of post-natal
494 conditions and growth trajectories. Our dataset does not allow to elucidate the origin of these
495 physiological syndromes that possibly include long-lasting effects of early environmental
496 conditions and/or genetic factors (White et al., 2019).

497 The pace-of-life syndrome describes covariation between MR and individual personality,
498 behavior, and life history traits. It is an useful framework to understand how variation in SMR may
499 correlate with maximum MR, behavioral activity and risk-prone behaviors (Careau et al., 2019,
500 2014; Mell et al., 2016). This theoretical framework has recently been extended to thermal
501 physiology where inter-individual in MR and behavior coincide with variation along the cold-hot
502 axis of thermal physiology (Goulet et al., 2017a; Goulet et al., 2017b). Our results suggest that such
503 a physiological syndrome can also integrate functional traits related to water and energy balance.
504 Although this is the first quantitative analysis of this physiological syndrome, the shared proximal
505 mechanisms, joint plasticity and potential co-evolution between functional traits related to energy
506 balance, respiration, thermal physiology and water biology have already been emphasized (Riddell
507 et al., 2019, 2018a; Rozen-Rechels et al., 2019).

508

509 *Conclusion*

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

516 combining plastic, adaptive responses to droughts and heatwaves (Gunderson et al., 2017;
517 Seebacher et al., 2015) and responses to selection on physiological syndromes (Žagar et al., 2018).
518

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759

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

764

		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

765

766

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

772

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]

773

774

775 **Table 3.** Degree of metabolic and water loss compensation induced by acclimation response (%
776 differences between values predicted by a null model and calculated DEEi and DEWL values) of
777 each treatment group at each session. P values are from t-test comparing DEEi and DEWL
778 predicted by a null model with calculated DEEi and DEWL. Because the acclimation response in
779 SMR did not persist at adulthood in the common garden (49 and 85 months post-birth, see Fig. 2A),
780 we estimated the degree of metabolic compensation only at 19 and 47 months post-birth.
781

Time of measurement	Treatment group	Degree of metabolic compensation	
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
Time of measurement	Treatment group	Degree of water loss compensation	
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

782

783 **Figure captions**

784 **Figure 1.** A) Experimental design and variables collected through time. 19 and 47 months post-
785 birth are during the time in which vipers were exposed to thermal treatments, while sessions 49 and
786 85 months post-birth are during the time in which vipers were in a common garden. VO_2 is the
787 oxygen consumption (standard metabolic rate); TEWL is Total Evaporative Water Loss; SVL is
788 Snout-Vent Length; BM is Body Mass. B) Daily temperature ($^{\circ}C$) cycle applied for each thermal
789 treatment (warm, medium, cold).

790 **Figure 2.** Effect of the thermal treatments on the residual standard metabolic rate (log transformed
791 VO_2 provided in mL per hour, A) and residual total evaporative water loss (log transformed TEWL
792 provided in mg per hour, B) measured at different time points through lifetime (see Figure 1) at
793 $20^{\circ}C$ and $30^{\circ}C$ body temperatures. Residual SMR and TEWL scores were calculated from a log-log
794 regression of individual raw values with body mass and thus accounts for ontogenetic and inter-
795 individual variation in body mass. Error bars represent SE. The * sign and different letters indicate
796 significant differences among treatment groups based on Tukey post-hoc tests of contrasts
797 performed A) in each treatment and temperature group, B) on average for all treatment and
798 temperature group. ns = not significant

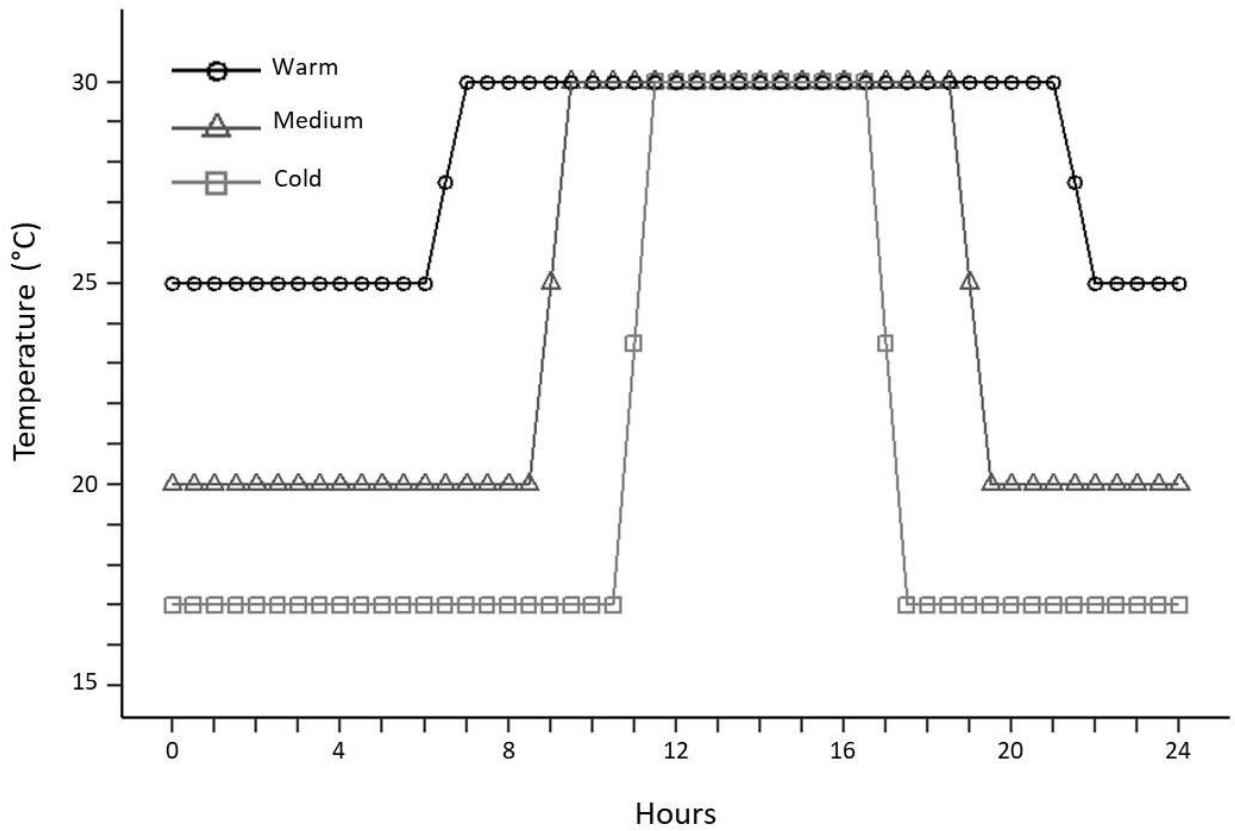
799 **Figure 3.** A) Residual total evaporative water loss (log transformed TEWL provided in mg per
800 hour) against residual standard metabolic rate (log transformed VO_2 provided in mL per hour) for
801 all aspik vipers across all lifetime stages, treatments and temperatures. B) Best linear unbiased
802 predictors (BLUP) of residual VO_2 for the random effect of identity as function of BLUP for TEWL
803 for all aspik vipers with concurrent measurements of VO_2 and TEWL at the same lifetime stage.
804 BLUPs and associated standard errors were extracted from univariate mixed models.

805 **Figure 4.** Daily expenditure during inactivity (DEEi), A) and daily total evaporative water loss
806 (DEWL), B) calculated at different time points through lifetime (see Figure 1A) for each treatment
807 group. White points represent mean values (\pm SE) of calculated DEEi and DEWL. Black points

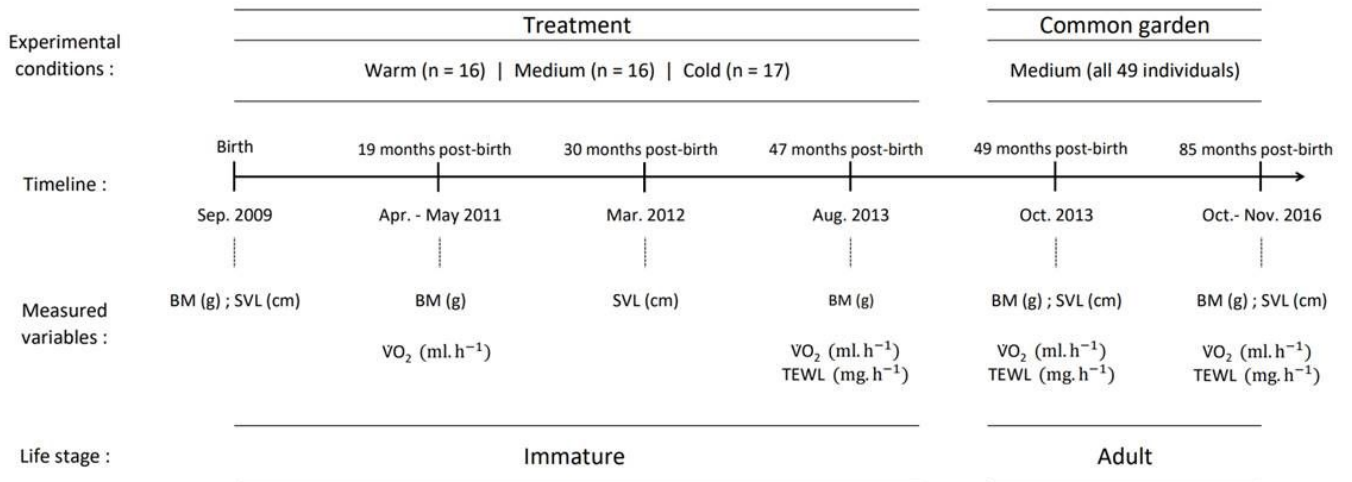
808 represent single values of DEEi and DEWL predicted by a null model. Arrows symbolize the
809 degree of metabolic or water loss compensation. Because the acclimation response in SMR did not
810 persist at adulthood in the common garden (49 and 85 months post-birth, see Figure 2A), we
811 calculated and predicted DEEi only at 19 and 47 months post-birth.

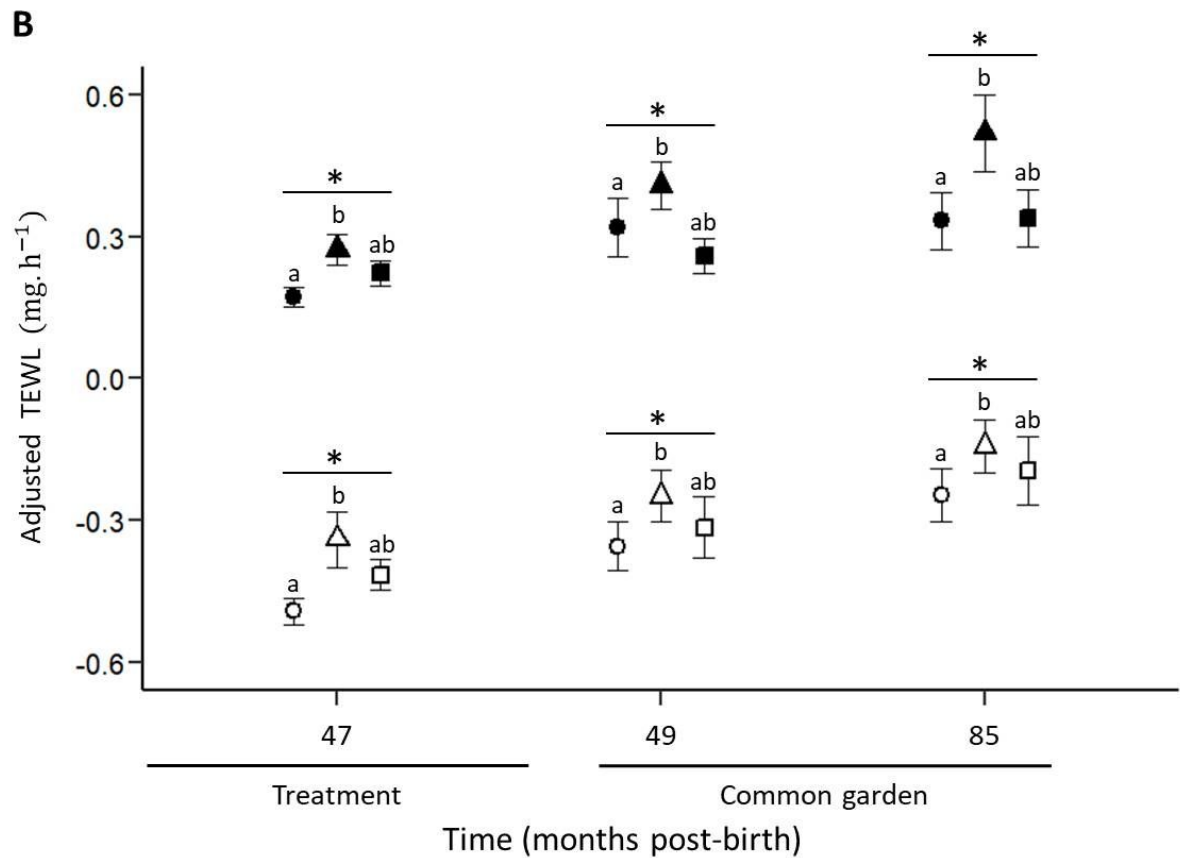
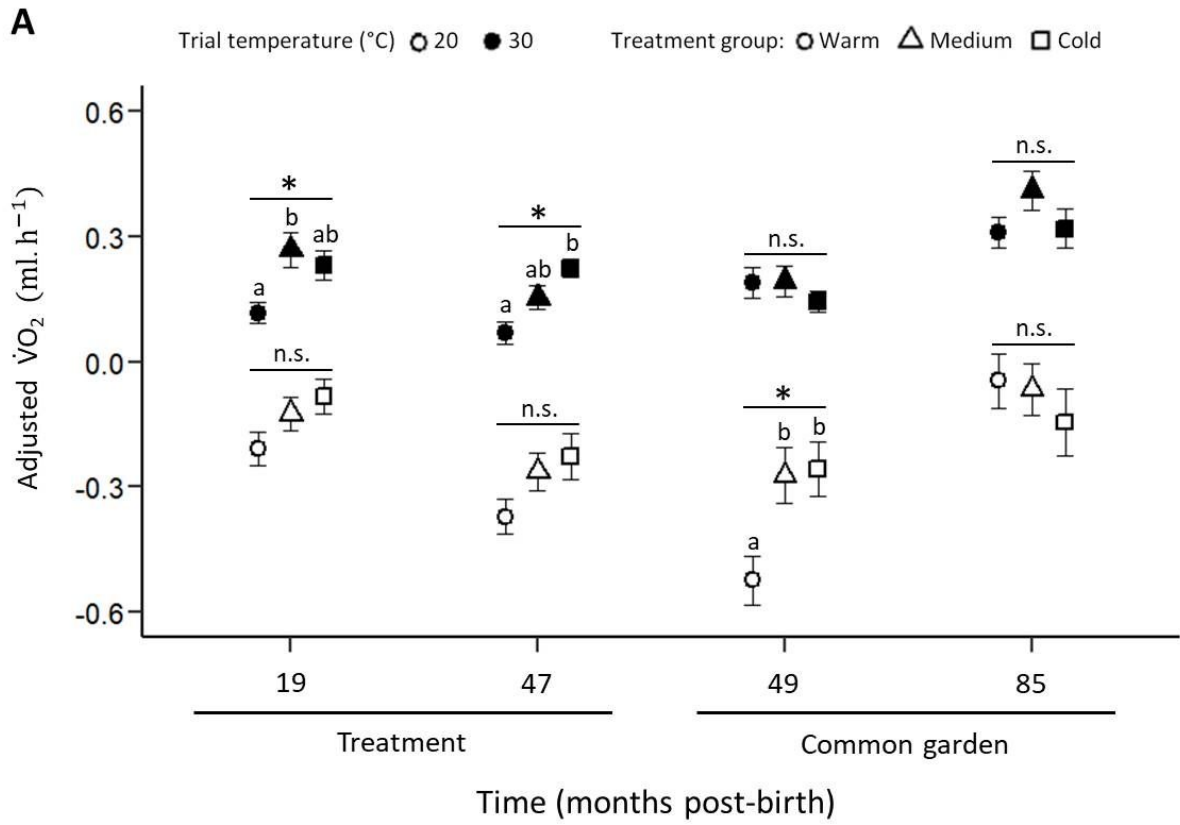
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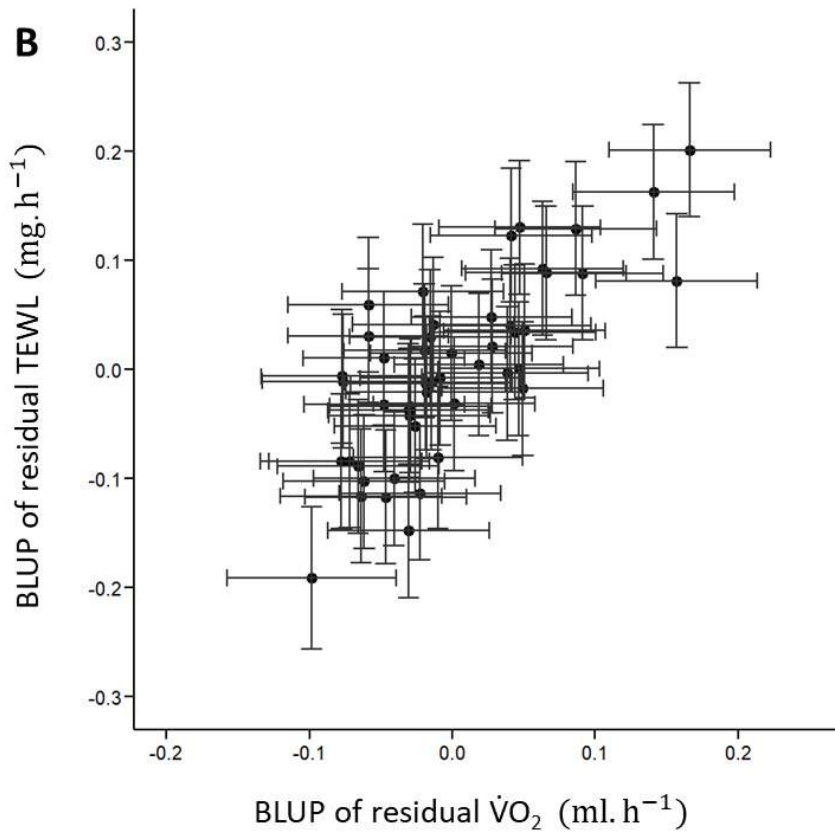
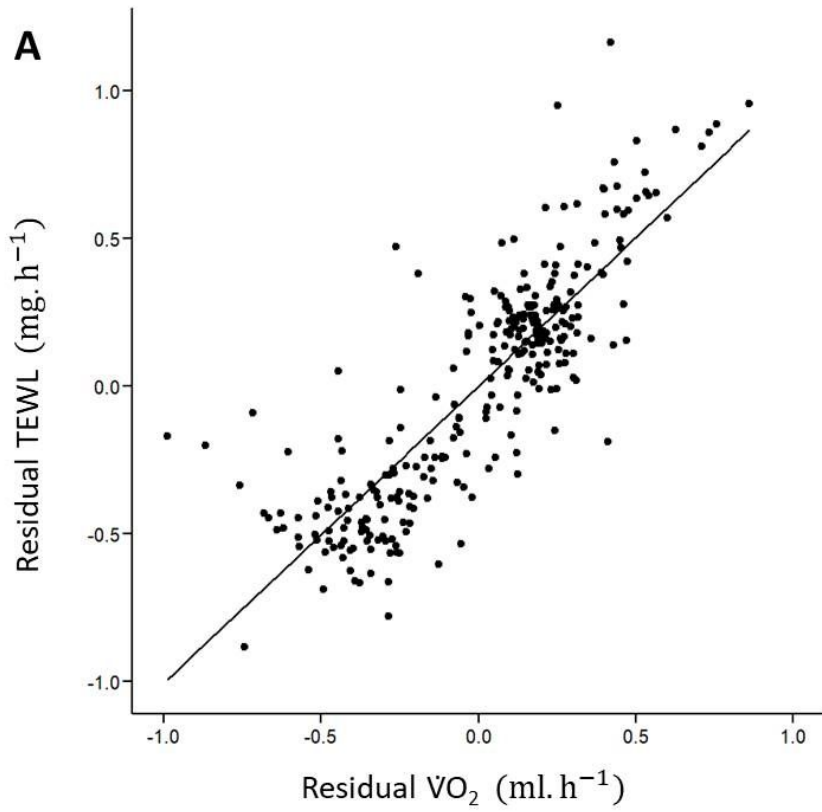
Daily temperature cycles



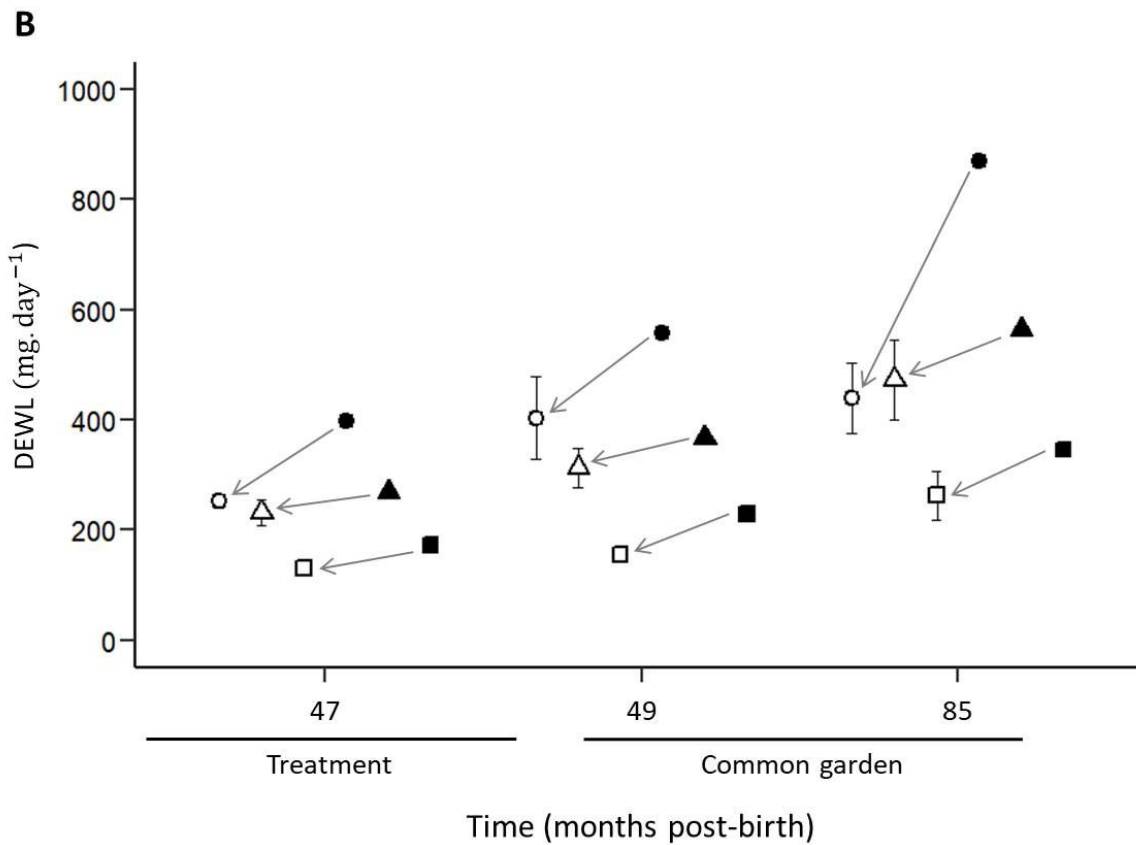
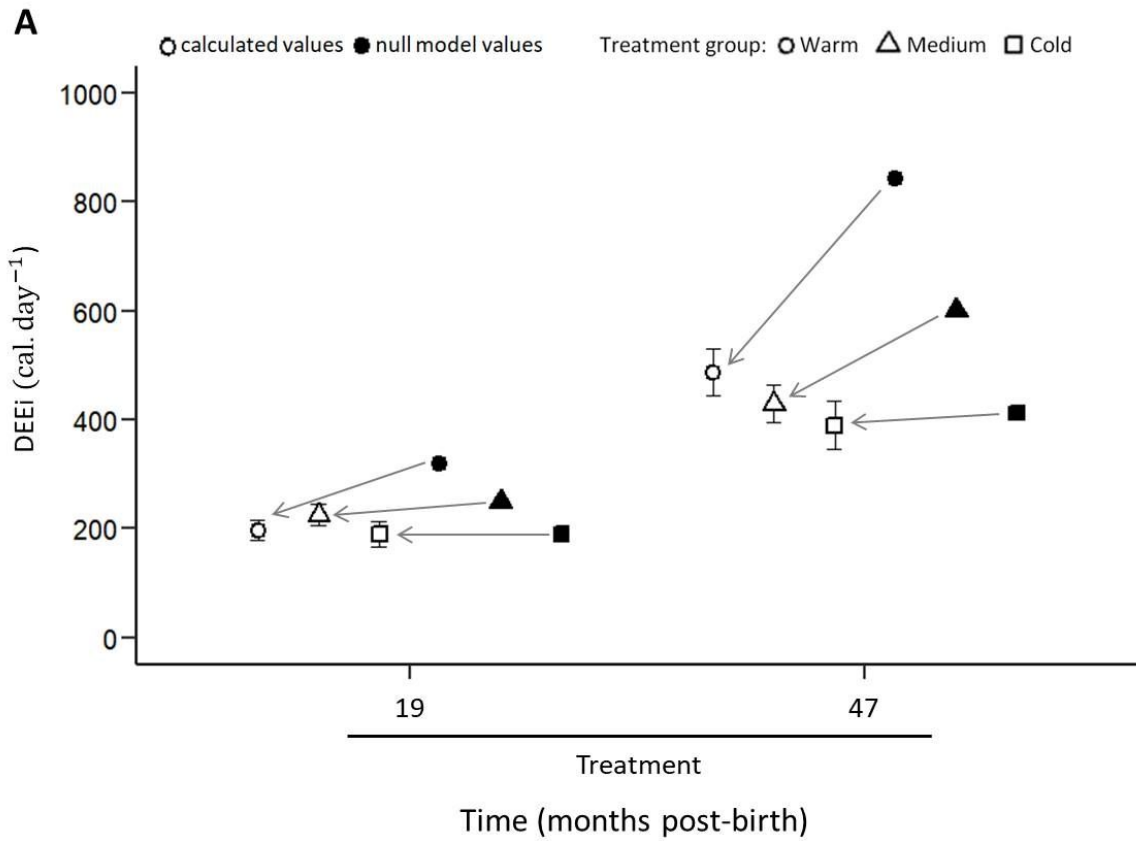
B







818 **Figure 4**



819