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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3   Additive effects of developmental acclimation and physiological

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7   Mathias Dezetter[1,2,][*], Andréaz Dupoué [1], 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 (CEREEP-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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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

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

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 aspic vipers 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 (± 0.01g) and

196 measured (snout-vent-length, SVL ±0.5cm) 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

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 (VO2 proxy for SMR; ml.h^{-1}) and the total

evaporative water loss (TEWL; mg.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 VO$_2$, 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 VO$_2$ 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

19
20

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 medium estimates ± 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 $$R(T) = R(20°C) \times Q_{10}^{\frac{(T-20)}{10}} \quad (1)$$

289 where $R(T)$ is the predicted process rate at temperature T, $R(20°C)$ 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; cal.day^{-1}) and daily

294 total evaporative water loss (DEWL; mg.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°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 $R(20°C)$ 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 $R(20°C)$ and

310 Q_{10} from vipers from the medium cycle treatment as a baseline reference for the two other groups.

311

312 **Results**

313 <u>Morphological differences between treatments</u>

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_l: -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 × Time: -453.2, model without Treatment × Time: -457.7). In males, body mass

321 was weakly influenced by treatments (analysis on subset of males data; DIC, model with Treatment

322 × Time: -475.02, model without Treatment × 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

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 × Time × 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

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 <u>Bivariate models for SMR and TEWL</u>

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 <u>Metabolic and water loss compensation</u>

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 aspic 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 approximatively 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-prove 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

Table 1. Descriptive statistics (mean and SE) for body mass and body size (SVL) of aspic 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 (Ve and COVe) 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 ± 95% CI
SMR	Ve	0.400 [0.33, 0.482]
	V_I	0.252 [0.107, 0.415]
TEWL	Ve	0.569 [0.463, 0.671]
	V_I	0.217 [0.08, 0.367]
SMR-TEWL	COVe	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

Figure captions

Figure 1. A) 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. VO_2 is the

oxygen consumption (standard metabolic rate); TEWL is Total Evaporative Water Loss; SVL is

Snout-Vent Length; BM is Body Mass. B) Daily temperature (°C) cycle applied for each thermal

treatment (warm, medium, cold).

Figure 2. Effect of the thermal treatments on the residual standard metabolic rate (log transformed

VO_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°C and 30°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
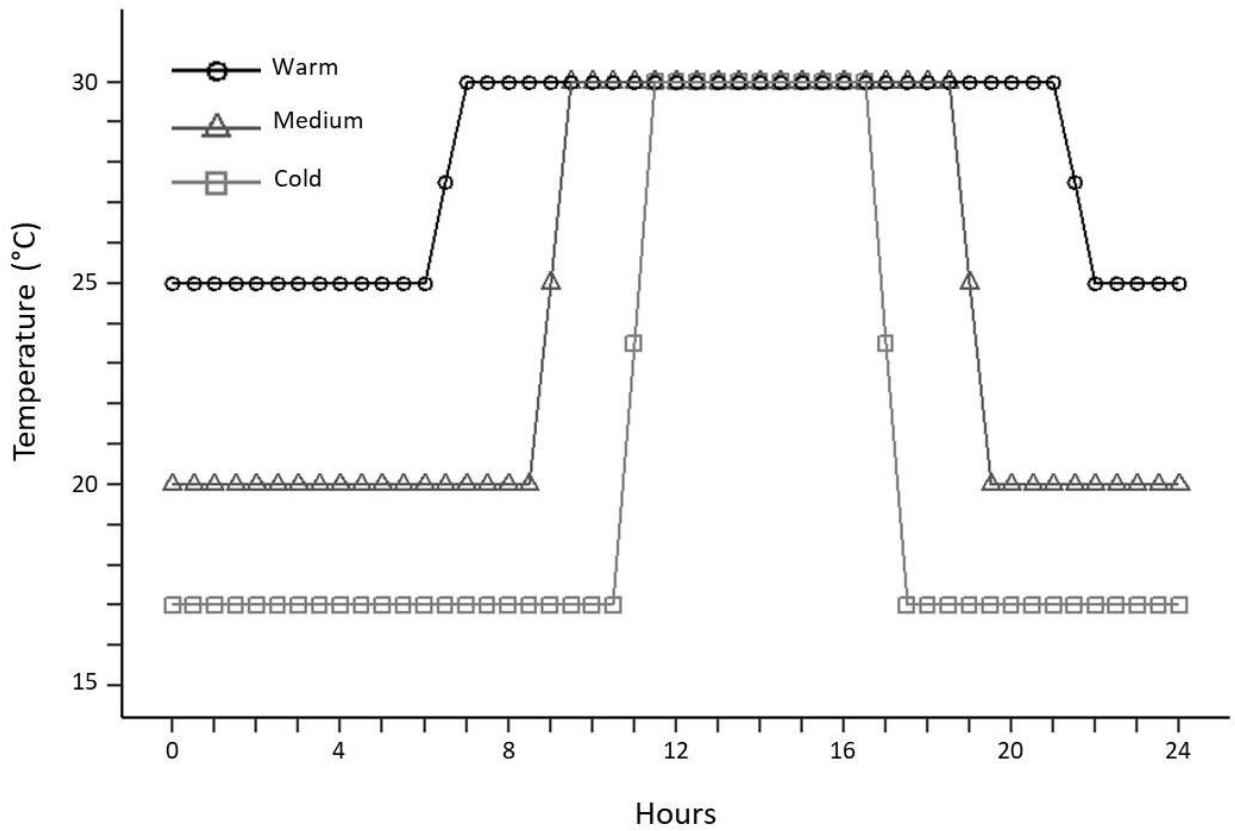
temperature group. ns = not significant

Figure 3. A) Residual total evaporative water loss (log transformed TEWL provided in mg per

hour) against residual standard metabolic rate (log transformed VO_2 provided in mL per hour) for

all aspic vipers across all lifetime stages, treatments and temperatures. B) Best linear unbiased

predictors (BLUP) of residual VO_2 for the random effect of identity as function of BLUP for TEWL

for all aspic vipers with concurrent measurements of VO_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

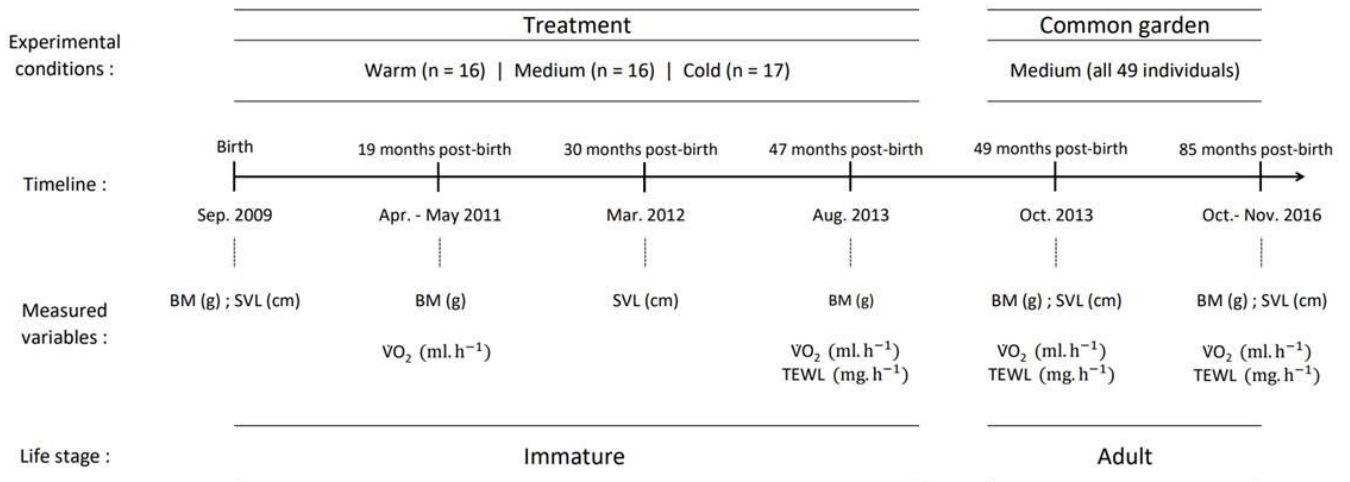group. White points represent mean values (± SE) of calculated DEEi and DEWL. Black points

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

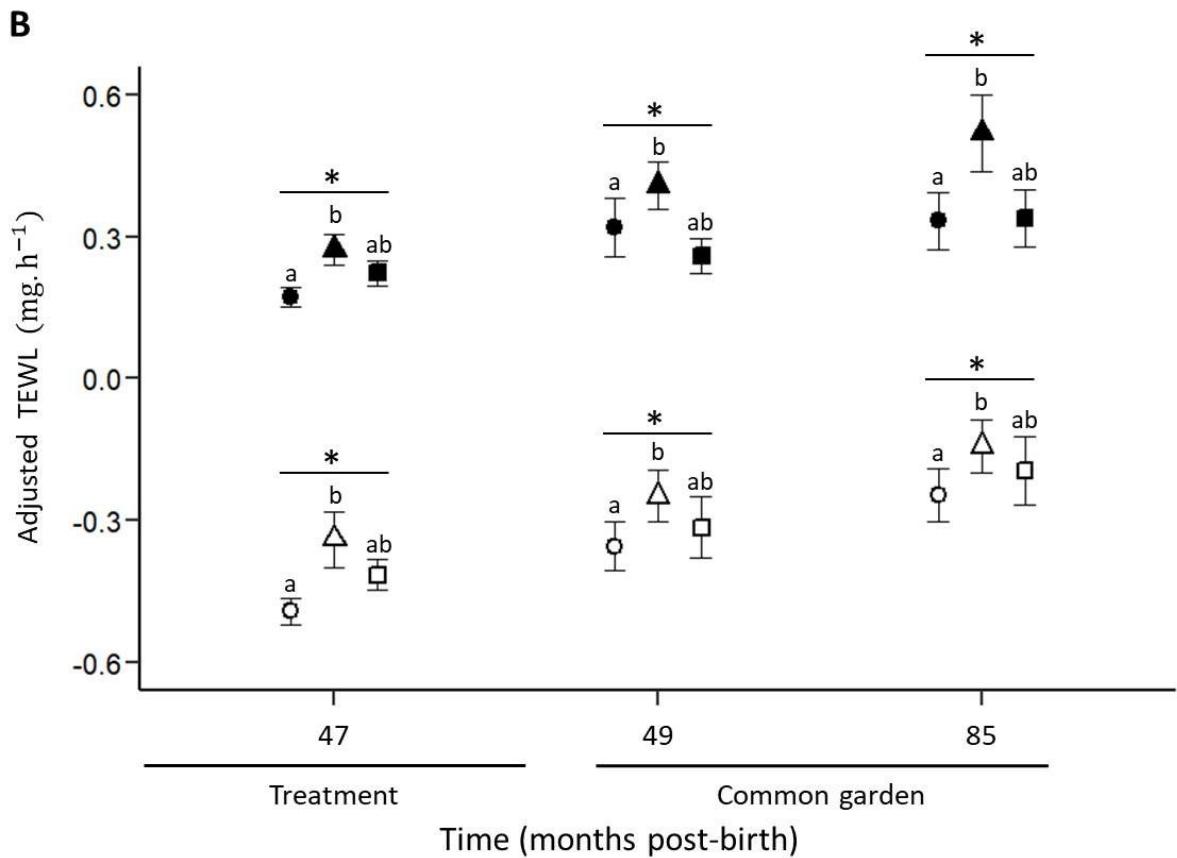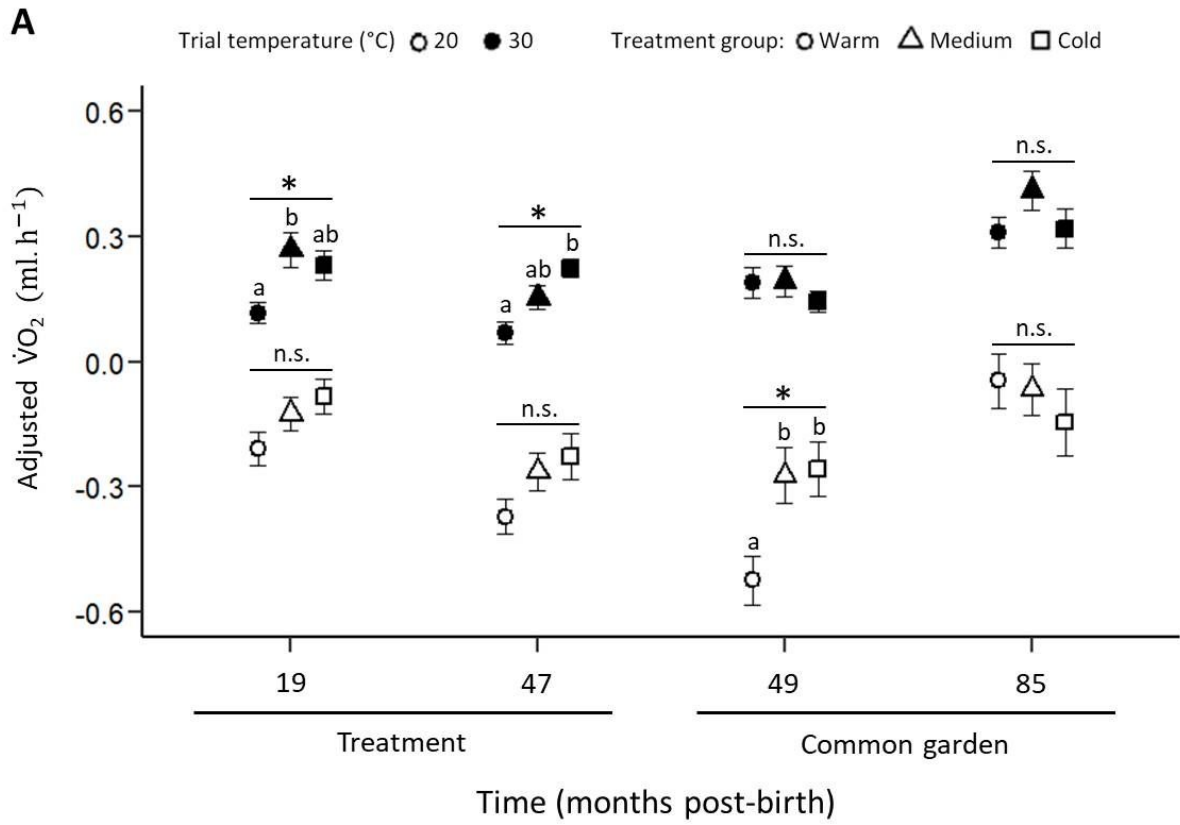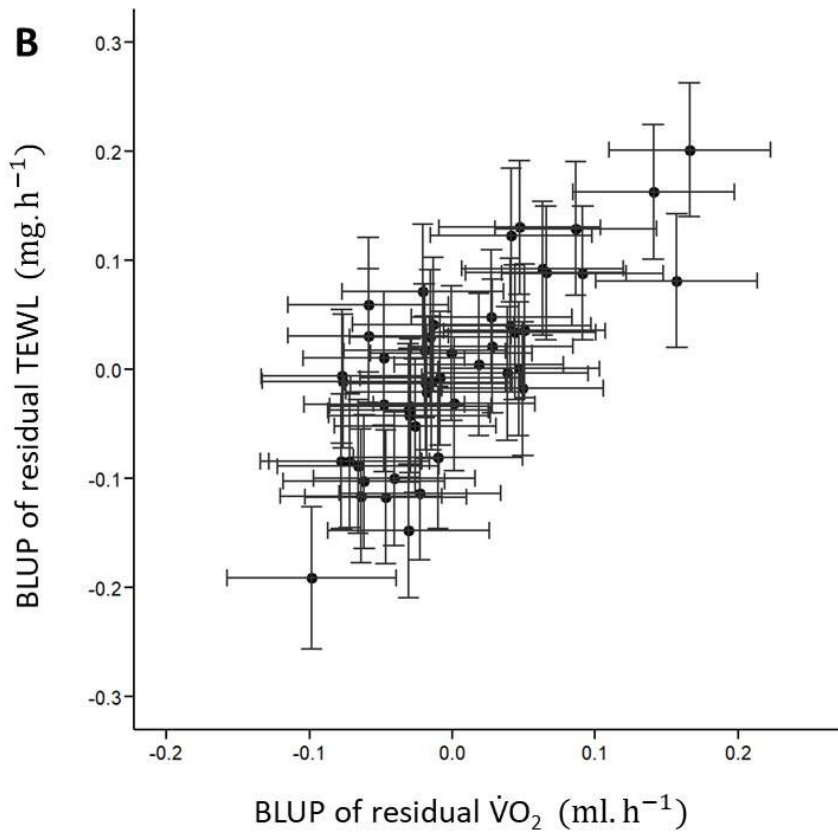811 calculated and predicted DEEi only at 19 and 47 months post-birth.

Figure 1

A

Daily temperature cycles

B

Figure 2

816   **Figure 3**

Figure 4

A

B

819