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1 Impacts of temperature on O₂
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9 **Abstract**

10 Global warming impacts biodiversity worldwide, leading to species' adaptation, migration, or
11 extinction. The population's persistence depends on the maintenance of essential activities, which is
12 notably driven by phenotypic adaptation to local environments. Metabolic rate – that increases with
13 temperature in ectotherms - is a key physiological proxy for the energy available to fuel individuals'
14 activities. Cold-adapted ectotherms can exhibit a higher resting metabolism than warm-adapted ones
15 to maintain functionality at higher elevations or latitudes, known as the metabolic cold-adaptation
16 hypothesis. How climate change will affect metabolism in species inhabiting contrasting climates
17 (cold or warm) is still a debate. Therefore, it is of high interest to assess the pace of metabolic
18 responses to global warming among populations adapted to highly different baseline climatic
19 conditions. Here, we conducted a physiological experiment in the endemic Pyrenean brook newt
20 (*Calotriton asper*). We measured a proxy of standard metabolic rate (SMR) along a temperature
21 gradient in individuals sampled among 6 populations located from 550 to 2189 m a.s.l. We
22 demonstrated that SMR increased with temperature, but significantly diverged depending on

23 populations' origins. The baseline and the slope of the relationship between SMR and temperature
24 were both higher for high-elevation populations than for low-elevation populations. We discussed the
25 stronger metabolic response observed in high-elevation populations suggesting a drop of performance
26 in essential life activities for these individuals under current climate change. With the increase of
27 metabolism as the climate warms, the metabolic-cold adaptation strategy selected in the past could
28 compromise the sustainability of cold-adapted populations if short-term evolutionary responses do not
29 allow to offset this evolutionary legacy.

30

31

32 **Keywords**

33 Metabolic rate, temperature, elevation, ectotherms

34

35 **Introduction**

36 All organisms process energy from their environment through their metabolism to maintain
37 functionality, develop, grow, and reproduce (Brown et al. 2004). The factors which explain variation
38 in this metabolic rate are thus central to understanding ecological interactions across levels of
39 biological organization. For example, both long-term evolutionary forces and immediate environment
40 can shape energy exchange rates, with important consequences for individuals and populations (Iles
41 2014; Schulte 2015). The “Standard Metabolic Rate” (SMR) is a particularly important physiological
42 trait to consider because it indicates the minimum energy cost necessary to sustain life in an organism
43 (Careau, Killen, and Metcalfe 2014). This measure of energy requirement for physiological
44 maintenance is subject to trade-offs and influences the energy available for locomotion (i.e., escape,
45 foraging behavior), growth, or reproduction (Clarke 1993). Also, SMR can vary according to several
46 biotic characteristics such as size, age, sex, or ecological niches (Brown et al. 2004). SMR is
47 quantified as the O₂ consumption of resting and unfed individuals, at a completed growth stage and
48 during a non-reproductive period (Hulbert and Else 2004). Under conditions of limited energy
49 availability, high SMR can reduce the energy available for non-maintenance functions. In other
50 contexts, such as when energy input is not limited, high SMR can suggest high energy turnover for all
51 vital activities (Kooijman 2000). Moreover, ectothermic animals adapted to cold environments (such
52 as at high-elevation or latitude) may be characterized by higher SMR at a given temperature as a
53 physiological adaptation to cold climates. This phenomenon, known as the metabolic cold adaptation
54 (MCA) hypothesis, suggests that having a higher metabolic rate in cold environments may be
55 advantageous for ectotherms by allowing them to be active at lower temperatures and thus compensate
56 for shorter daily or seasonal warm periods (Clergue Gazeau 1983; Gaston et al. 2009). Support for this
57 hypothesis has been demonstrated in arthropods (Terblanche et al. 2009), amphibians (Kreiman et al.
58 2019), and reptiles (Dupoué, Brischoux, and Lourdaïs 2017), though these patterns are not universal
59 (Lardies, Catalán, and Bozinovic 2004; Lardies, Bacigalupe, and Bozinovic 2004).

60 Temperature is a major driver of variation for most biological processes, from biochemical
61 reactions at the cell level to the fitness of organisms (Kingsolver 2009). Warmer temperatures can

62 result in (i) earlier phenology of organisms that can lead to an imbalance in interspecific interactions
63 (Kharouba et al. 2018), (ii) a modification of population's dynamic with a biased sex ratio for some
64 reptile species (Thompson et al. 2018), (iii) a change in the spatial distribution of organisms with
65 poleward and/or upward shifts mainly (Lenoir et al. 2020) leading community reshuffling with
66 uncertain sustainability due to novel species interactions (Williams and Jackson 2007; Gibson-
67 Reinemer, Sheldon, and Rahel 2015), and (iv) ultimately local extirpations when organisms are unable
68 to cope with rapid climate change (Whitfield et al. 2007; Bodensteiner et al. 2021). This is especially
69 true in ectotherms in which most physiological functions are directly influenced by temperature
70 (Angilletta 2009). As described by a thermal reaction norm, metabolism increases (ramping phase)
71 with temperature until it reaches a pre-mortal plateau and then drops (mortal fall) as critical limits are
72 approached (Lighton and Turner 2004; Taylor et al. 2020). The slope and shape of these thermal
73 reaction norms depend upon species or populations (Evans 1939; Brown and Fitzpatrick 1981;
74 Homyack, Haas, and Hopkins 2010; Londoño et al. 2015; Catenazzi 2016; Gangloff et al. 2016). Thus,
75 global warming may lead to temperatures exceeding the thermal tolerance of ectotherms (Deutsch et
76 al. 2008), or impose important sublethal effects such as altering body size, growth rates, immunity,
77 and generating physical deformities (Weerathunga and Rajapaksa 2020). This could have negative
78 consequences on individual fitness and populations in the long term, and many ectotherm populations
79 are therefore - or will shortly be - at risk of extirpation (Novarro et al. 2018).

80 To understand the potential response of a species to changing climates, it is necessary to
81 characterize the thermal-dependence curves of metabolic rate across a relevant latitudinal or
82 elevational gradient (Sears 2005; Muir, Biek, and Mable 2014; Bulgarella et al. 2015; Hu et al. 2019).
83 Here, we investigated the physiological sensitivity of the endemic Pyrenean brook newt *Calotriton*
84 *asper* through the measure of SMR across a range of ecologically relevant temperatures (5°C to 25°C)
85 and for individuals from populations living at elevations ranging from 250 to 2500 m a.s.l. By
86 characterizing the temperature-dependence of SMR in *C. asper*, we test whether this relationship
87 varies along an elevational gradient. We predict a linear increase in SMR as assay temperatures rise

88 and in accord with the MCA hypothesis - a higher metabolic rate in high-elevation populations than in
89 low-elevation populations at a given temperature (Terblanche et al. 2009; Kreiman et al. 2019).

90 **Material and Methods**

91 **Study species**

92 The Pyrenean brook newt (*Calotriton asper* Dugès, 1852) is an amphibian species from the
93 Pyrenees mountains (Andorra, Spain, France) commonly found in brooks, lakes, and springs ranging
94 from 250 to 2500 m a.s.l. (Nicol 1990; Martinez Rica and Clergue Gazeau 1977; Clergue Gazeau and
95 Martinez Rica 1978; Montori, Llorente, and García-París 2008). This species is listed in appendix IV
96 of the Habitats Directive (92/43/CEE, 21 May 1992), appendix II of the Bern Convention, and as near-
97 threatened species in the national Red List of amphibians of metropolitan France (UICN France).
98 Major threats to *C. asper* include climate change, habitat change, and predation by introduced
99 predatory fish (Dalibard 2021). *Calotriton asper* is characterized by a low dispersal rate (and gene
100 flow) among populations (Mila et al. 2009; Lucati et al. 2020), which favors high phenotypic variation
101 among populations (Serra-Cobo, Uiblein, and Martinez-Rica 2000). For instance, previous work
102 highlighted among-population variation according to site elevation in terms of different biological and
103 ecological factors. First, in terms of life-cycle with reproduction that takes place in spring and autumn
104 in low-elevation individuals and summer in high-elevation ones due to water temperature (Clergue
105 Gazeau 1983). Then, in terms of morphology with an increase of body size with elevational gradient
106 according to the Bergmann's rule (Trochet et al. 2019). Finally, in terms of thermal preferences with a
107 negative relationship between thermal preferences and elevation in low-elevation individuals and a
108 positive relationship in high-elevation ones (Trochet et al. 2018).

109 **Sampling sites and newt maintenance in laboratory**

110 We sampled six riverine populations from six independent streams within the French Pyrenees
111 that span along an elevational gradient ranging from 550 to 2189 m a.s.l. during summer 2019.
112 Localizations and sampling dates for the six streams are provided in Table 1. Slope (which greatly

113 determines streamflow in rivers, and thus the microhabitat experienced by the brook newt) varied
114 among the sampling streams from 9.8% to 75.1%. Every sampled stream had continuous streamflow
115 over the year. Two populations were sampled in a dense forest environment (Fougax, Belesta), three
116 populations in an open field (Orlu, Gavarnie, and Néouvielle), and one population in a low-density
117 forest stand (Aulus). Mean annual air temperatures varied among the sampling streams from 10.4°C to
118 11.4°C for low elevation sites and from 0.5°C to 6.7°C for high elevation sites, on average over the
119 1981-2010 period (Figure S1; Karger et al. 2017). Annual snow duration varied among the sampling
120 sites from 26 to 41 days for low elevation streams and from 106 to 178 days for high elevation
121 streams, on average over the 2000-2010 period (Gascoin et al. 2015). We sampled twenty adults per
122 site by hand found by a visual survey. A total of 120 individuals were sampled with a near-balanced
123 sex ratio (54 females and 66 males; Table 1). Non-gravid females were selected to avoid any influence
124 of the reproductive state on respirometry measurements (Finkler 2006). Individuals were then housed
125 at the laboratory (436 m a.s.l., Moulis, Ariège, France). All individuals were PIT-Tagged for further
126 identification (BIOLOG-ID, France; accreditation of animal experimentation n°A09-1), following the
127 protocol described in Le Chevalier et al. (2017). Newts from each population were all housed in a
128 single aquarium of 80 × 35 × 40 cm, at a water temperature of 15 °C ± 2°C (Sylvestre 2011) and
129 exposed to a natural photoperiod. For each population, the test period began at least 3 days after
130 capture (mean ± SD: 7 ± 2 days) and lasted 4 weeks on average (± 1 week SD; Table S1). Newts were
131 fed *ad libitum* with *Tubifex* sp. live worms throughout the experiment. However, because digestion
132 may influence physiology in ectotherms (Huey 1982; Gvoždík 2003), feeding was halted 48 h before
133 testing bouts. Each animal was weighed with a digital balance (± 0.1 g) before experiments (Table 1).
134 All newts were released at their respective capture sites between 2 and 6 days after their last test (i.e.
135 total mean time of captivity ± SD: 7 ± 1 weeks).

136 Table 1. Characteristics of the 6 studied sites where Pyrenean brook newts were captured. Mean Annual Air Temperatures
137 (MAAT) were extracted over the 1981-2010 period from the CHELSEA database (Karger et al. 2017).

Sampling site	Date of capture	Number of females	Mean mass females (g) ± sd	Number of males	Mean mass males (g) ± sd	Coordinates (latitude/longitude)	Elevation (a.s.l.)	MAAT (°C)
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Fougax	27 May 2019	5	9.0 ± 0.8	15	9.6 ± 2.3	$\frac{42^{\circ}52'9,05'' \text{ N}}{1^{\circ}53'25,30'' \text{ E}}$	550	11.4
Belesta	04 June 2019	10	7.1 ± 2.1	10	9.5 ± 1.7	$\frac{42^{\circ}53'0,17'' \text{ N}}{1^{\circ}56'13,16'' \text{ E}}$	800	10.1
Aulus	13 June 2019	9	7.6 ± 1.2	11	9.4 ± 1.7	$\frac{42^{\circ}47'9,42'' \text{ N}}{1^{\circ}19'20,50'' \text{ E}}$	1100	10.4
Orlu	25 June 2019	10	9.3 ± 1.5	10	9.8 ± 1.4	$\frac{42^{\circ}39'48,21'' \text{ N}}{1^{\circ}58'2,10'' \text{ E}}$	1313	6.7
Gavarnie	22 July 2019	10	4.4 ± 0.9	10	6.2 ± 1.2	$\frac{42^{\circ}44'19,86'' \text{ N}}{0^{\circ}1'51,85'' \text{ O}}$	1724	0.5
Néouvielle	31 July 2019	10	8.5 ± 0.7	10	10.0 ± 1.0	$\frac{42^{\circ}50'20,80'' \text{ N}}{0^{\circ}8'56,30'' \text{ E}}$	2189	2.4

138

139 **Physiological response**

140 We measured individual oxygen consumption rate as a proxy for SMR across a range of
141 temperature experienced by *C. asper* in its natural environment during the year (i.e. 5°C ± 1 to 25°C ±
142 1). All individuals from each population were tested at three different temperatures (Table 2): one cool
143 test temperature (from 5°C to 13°C), one warm test temperature (from 17°C to 25°C), and one control
144 temperature (15°C). To complete the experiment within the three-month activity period and to limit
145 stress on the animals, each population of twenty individuals was divided into five groups of four
146 individuals, and each group of four was tested at different cool and warm test temperatures to cover
147 the entire thermal gradient (Table 2). To limit potential confounding effects between temperature *per*
148 *se* and the order (timing) of tests, we alternated order for all populations between cold, control, and hot
149 temperatures presented in Table 2. We let individuals rest for at least one day (mean ± SD: 2.4 ± 1.4
150 days) between tests.

151 Individuals were isolated in a 0.55 L airtight plastic transparent box filled with dechlorinated
152 water (Guillaume et al. 2020). To avoid a thermal shock, we gently acclimatized the newts by
153 increasing or decreasing the captivity water temperature (15°C) to the test water temperature over a 1-
154 hour period. After this 1-hour acclimatization, the water oxygen concentration was measured every
155 minute for 2 hours using FireSting fiber-optical oxygen meters (PyroScience GmbH, Aachen,
156 Germany). An individual measure of oxygen consumption during these 2 hours (hereafter SMR in

157 ppm) corresponding to the individual loss in oxygen concentration was calculated by subtracting water
 158 oxygen concentration at the end of the experiment to water oxygen concentration at the beginning of
 159 the experiment. All tests were performed during the day at fixed and constant hours (9:00 and 14:00).
 160 Moreover, all tests were repeated twice in two sessions separated by two weeks (mean \pm SD: 14.5 \pm 7
 161 days).

162 Table 2. Experimental protocol for the metabolic measurements. The table shows, for one population, the different exposure
 163 of individuals in every test and control water temperature. The same design was applied for each of the 6 populations, in two
 164 sessions separated by two weeks (mean \pm SD: 14.5 \pm 7 days).

Individual ID	Cold test temperatures (°C)		Control temperature (°C)		Hot test temperatures (°C)	
	Target temperature	Effective mean temperature	Target temperature	Effective mean temperature	Target temperature	Effective mean temperature
N1-N4	5	5.7 \pm 1			17	17.4 \pm 0.6
N5-N8	7	7.5 \pm 0.9			19	19.5 \pm 0.5
N9-N12	9	9.7 \pm 0.9	15	15.5 \pm 1	21	21.0 \pm 0.8
N13-N16	11	11.7 \pm 0.8			23	23.0 \pm 0.5
N17-N20	13	13.4 \pm 0.7			25	25.8 \pm 0.7

165

166 **Statistical analyses**

167 To test if SMR (response variable) varied with effective mean temperature (Table 2),
 168 elevation, as well as their first to fourth order interactions (explanatory variables), we built a linear
 169 mixed-effects model (LMM, with a Gaussian error distribution; see Figure S2 for verification of the
 170 model assumption) with sites of capture, individuals, and sessions as random intercept terms (to
 171 control for their potential effect in the estimation of the fixed effects). Because we sampled only 6
 172 populations in a non-continuous elevational gradient, we chose to characterize elevation as a factor.
 173 According to the ecological and thermal behavioral differentiation between sites below and above
 174 1300 m highlighted in Trochet et al. (2018), elevation of origin was categorized into two classes, with
 175 three populations (Fougax, Belesta, and Aulus, 550-1100 m a.s.l) assigned to a “*low elevation class*”

176 and three populations (Orlu, Gavarnie, and Néouvielle, 1300-2189 m a.s.l) assigned to a “*high*
177 *elevation class*”. Body mass and sex were included in the model as covariates. Newts' mass and
178 effective mean temperature variables (Table 1) were standardized to the mean so their respective
179 effects can be compared in the model.

180 We started from a full model including all fixed and random effects with interactions between
181 elevation and other variables (i.e. effective mean temperature, mass, and sex). To avoid having a
182 model where one random variable has no effect on our results because all variability of the intercept is
183 explained by other random variables (i.e. singular model), we first selected the random effect
184 structure. The best random effect structure was identified by testing all the combinations of random
185 factors and selecting the candidate model with the lowest Akaike Information Criterion with a
186 correction for a small sample size (AICc). To compare AICc values among candidate models, we
187 fitted all candidate LMMs using the restricted maximum likelihood parameter estimate (Bates et al.
188 2018). To ensure robust estimations, all the singular fits (i.e. variances closed to zero) were removed
189 from the list of candidate models before model selection. Second, we selected the fixed effect structure
190 based on AICc, keeping the previously identified random structure constant. Third, we fitted a final
191 LMM based on the fixed and random structure selected using the restricted maximum likelihood
192 parameter estimate to ensure a robust estimate of coefficients (Bates et al. 2018). In order to compute a
193 robust estimate and test the significance of the direct effects in the selected model (e.g. Luke 2017),
194 we finally calculated the mean effect of each fixed variable and their respective 95% confidence
195 intervals through a bootstrap approach (N = 5000 iterations). The significance of each variable was
196 determined from the bootstrap distributions for the two alternative hypotheses of a mean coefficient
197 estimate being higher or lower than zero.

198 To assess the goodness-of-fit of the models, we also computed the mean marginal (i.e.
199 variance explained by the fixed effects) and conditional (i.e. variance explained by both the fixed and
200 random effects) R^2 values, as well as their respective 95% confidence interval from the set of bootstrap
201 iterations. All statistical analyzes were performed in the programming language R (version 3.6.2; R
202 Core Team 2019). To fit LMM and conduct bootstraps, we used the lme4 (Bates et al. 2014b) and

203 optimx R-packages (Nash and Varadhan 2011) respectively. The MuMIn (Bartoń 2019) and jtools R-
204 packages (Long 2019) were used to compute marginal and conditional R^2 values and to adjust into the
205 same scale mass and effective mean temperature variables, respectively.

206

207 **Results**

208 Effective mean temperature, elevation, sex, mass, and their first order interactions were
209 retained in the best model (Table 3, Table S2). Marginal and conditional R^2 were equal to 0.447
210 [0.363; 0.529] and 0.558 [0.483; 0.629] respectively (mean [95% CI]). In other words, individuals,
211 population, and session (the random terms) explained a lower proportion of variance in SMR (0.111)
212 than did the effective mean temperature of the tests, elevation's origin of population, sex, and mass of
213 individuals.

214 The mass- and temperature-dependence of SMR varies predictably along the elevational
215 gradient (Table 3). Specifically, the positive relationships between SMR and both test temperature and
216 mass were steeper for individuals sampled at high elevation populations than for those sampled at low
217 elevation (Figure 1), so newts from higher elevations exhibited greater temperature and mass
218 dependencies of standard metabolic rate ($\beta_{\text{mass}} = 0.466$ and $\beta_{T^{\circ}\text{C}} = 0.373$ for low elevation populations;
219 $\beta_{\text{mass}} = 0.696$ and $\beta_{T^{\circ}\text{C}} = 0.485$ for high elevation populations).

220 Individuals sampled at high elevations had on average a higher SMR than those sampled at
221 low elevation populations (mean [95% CI]: + 0.522 ppm [0.732; 0.308]; Table 3). However, this effect
222 depends on the sex of individuals, as highlighted by the significant interaction term between elevation
223 and sex (Table 3): the increase in oxygen consumption from low to high elevations was steeper in
224 females than in males (Figure 2).

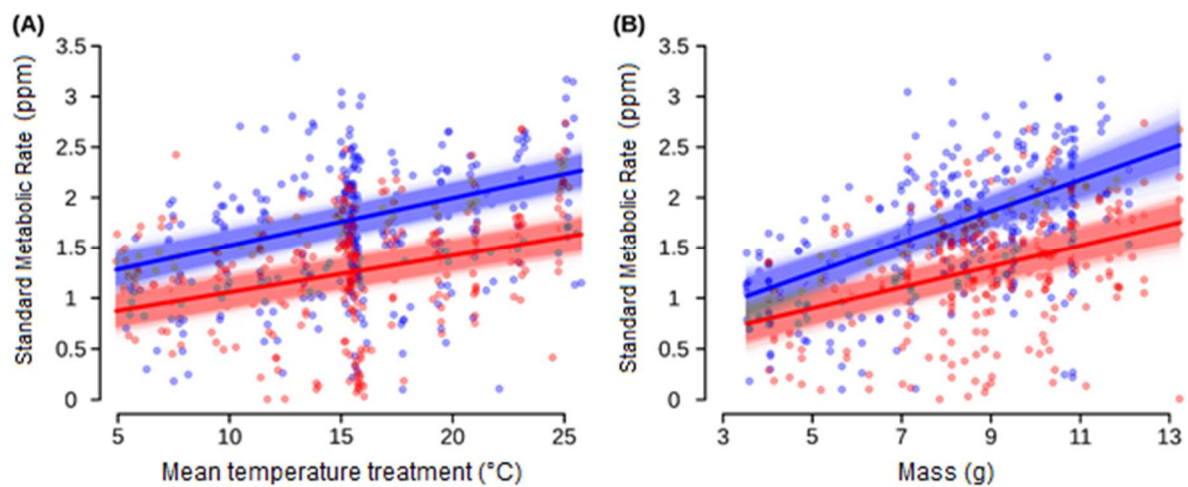
225 Table 3. The effects of effective mean temperature, elevation, mass, and sex on *Calotriton asper*' SMR estimated through
226 bootstrapped mixed-effects linear models (N = 5000). Every fixed effect has been tested through a one-sided test based on the
227 bootstrap distributions of coefficients (null hypothesis: the effect is not higher or lower than 0). Significant effects are
228 displayed in bold (P -value < 0.05).

Variable	Mean estimate	95% CI	P-value
Intercept	1.777	[1.626; 1.927]	< 0.0002
Mass	0.696	[0.500; 0.893]	< 0.0002
Temperature	0.485	[0.397; 0.573]	< 0.0002
Elevation class (Low)	-0.522	[-0.732; -0.308]	< 0.0002
Sex (Male)	-0.135	[-0.270; -0.002]	0.0232
Elevation class (Low) * Temperature	-0.111	[-0.223; 0.01]	0.0368
Elevation class (Low) * Sex (Male)	0.184	[-0.003; 0.375]	0.0274
Elevation class (Low) * Mass	-0.230	[-0.474; 0.015]	0.0318

229

230

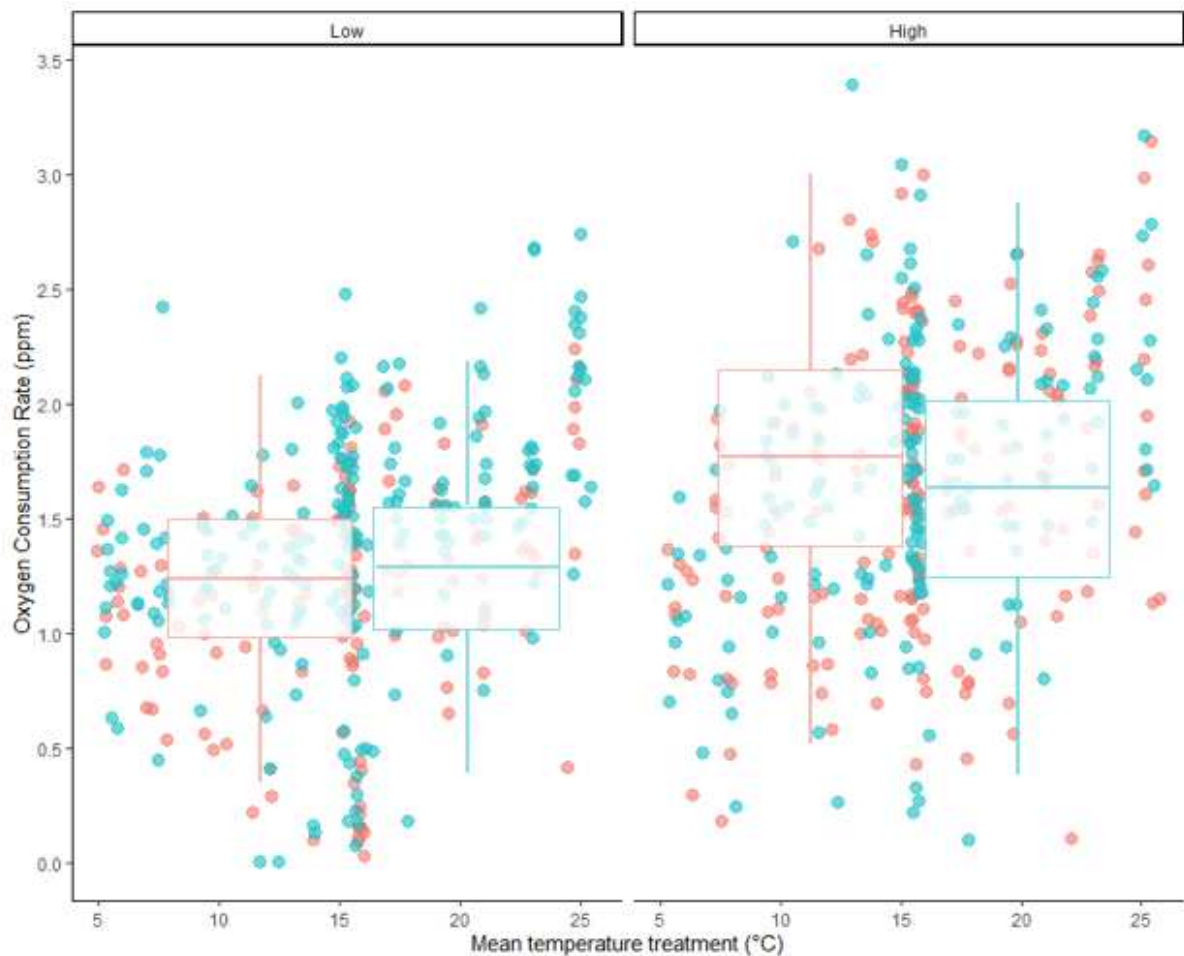
231



232

233 Figure 1. SMR of individual brook newts (*C. asper*) from low (red) and high (blue) elevation populations dependent on (A)
 234 effective mean water temperature treatment and (B) mass. Points are individual observations and lines display the
 235 relationships fitted through the linear mixed-effects model presented in Table 3 (dark color = average model, light color =
 236 individual bootstrap models) for females only with a mean individual mass of 8.35 g (A) and with a total mean temperature
 237 treatment of 15.35 °C (B).

238



239 Figure 2. Temperature dependence of SMR for female (red) and male (blue) brook newts (*C. asper*) sampled in low (left) and
 240 high (right) elevation populations. Points are observations. Boxes display the linear mixed-effects model intercept presented
 241 in Table 3 and calculated from 5000 bootstrap iterations, independent of the mean temperature treatment axis.

242 Discussion

243 Pyrenean brook newts from higher-elevation populations exhibit higher mass-specific
 244 metabolic rates across a range of biologically relevant temperatures compared to conspecifics from
 245 lower elevations. This corroborates the metabolic cold adaptation hypothesis (MCA) which predicts a
 246 higher metabolic rate in individuals from cold environments (i.e high elevation) than individuals from
 247 warmer environments when measured at similar ambient temperatures (Kreiman et al. 2019). In fact,
 248 having a higher metabolic rate in cold environments may be advantageous for ectotherms by allowing
 249 them to be active at lower temperatures and thus compensate for a shorter warm period (Clergue
 250 Gazeau 1983; Gaston et al. 2009). Finally, a higher SMR allows organisms to achieve development,
 251 growth, and reproduction at temperatures that would otherwise be sub-optimal, especially where the

252 life cycle of the species is highly seasonal as in *Calotriton asper* (Clergue Gazeau 1983; Lardies,
253 Bacigalupe, and Bozinovic 2004; Terblanche et al. 2009; Schaefer and Walters 2010).

254 Our results showed a steeper increase in SMR in females than in males from low to high
255 elevation populations. This is surprising as males are known to have a higher SMR than females,
256 which has been hypothesized to have a strategy to provide enough energy for spermatogenesis, mate
257 searching, or amplexus and compensate the male-male competition (Ryan and Hopkins 2000; Finkler
258 and Cullum 2002; Finkler 2006). In our case, we speculate that a higher metabolic allocation to
259 baseline functionality in females could be a response to a shorter favorable thermal period in cold
260 environments to ensure reproduction. Reproduction in females is known to drive a large proportion of
261 the energy budget so oogenesis will be more efficient in an extreme environment to maintain total
262 reproductive output (Orrell and Kunz 2016). As a consequence, a greater amount of energy is likely
263 required in females to prepare the egg production and laying in the short time available at high
264 elevation.

265 As expected, our results highlight an increase in SMR as the temperature increases (Whitford
266 1973; Finkler 2006; Gangloff and Telemeco 2018), which confirms that this general rule also holds for
267 the endemic Pyrenean brook newt (*Calotriton asper*). This increase in SMR reflects a rise in metabolic
268 activity that can be advantageous in the short term for ectotherms, leading to a faster growth rate or a
269 longer activity period (Žagar et al. 2015). But, prolonged exposure to high temperatures can lead to a
270 loss of performance in different crucial life activities such as locomotion, foraging, or
271 thermoregulation (Deutsch et al. 2008; Žagar et al. 2015). This loss of performance can be explained
272 by the oxygen- and capacity-limited thermal tolerances (OCLTT) hypothesis which explains that the
273 respiratory system is not able to provide the oxygen needed to meet demand at high temperatures
274 (Pörtner 2002; Pörtner, Bock, and Mark 2017). Chronic heat stress can even induce premature aging in
275 individuals with negative effects on adult fitness and survival, and impact the sustainability of
276 populations (Bestion et al. 2015; Dupoué et al. 2017). In the long term, adaptive responses to high
277 temperatures must be put in place to avoid the inefficiency of subcellular processes, mismatch of
278 oxygen supply and demand, or potentially death.

279 Moreover, our results showed a significant interaction between temperature and elevation that
280 highlights greater temperature-dependence of SMR in individuals from high elevation. This higher
281 sensitivity in cold environments has already been demonstrated in previous studies in salamanders
282 with a differential effect of temperature on the growth rate, heart rate, and activity in individuals from
283 different thermal environments (Catenazzi 2016; Caruso and Rissler 2019). These environmental-
284 dependant characteristics could be the result of an evolutionary legacy, including potentially adaptive
285 responses acquired by natural selection, and could affect the response of individuals to climate change
286 (Uusi-Heikkilä et al. 2015; Bodensteiner et al. 2021).

287 In a global warming context with a rapid increase of temperatures at high elevations, a higher
288 SMR across the experienced temperature range will make high-elevation individuals suffer from
289 increased energetic demands, which could affect energetic allocation to other aspects of life-history as
290 growth, reproductive, or immune functions (Weerathunga and Rajapaksa 2020). While MCA
291 mechanism may have been adaptive in a past context, it could now compromise the sustainability of
292 populations at high elevation. In this situation, the low dispersal ability and gene flow of *C. asper*
293 (Mila et al. 2009; Lucati et al. 2020); the intraspecific variation observed in different traits
294 (morphological, ecological, and behavioral; see Gasser and Clergue Gazeau 1981; Serra-Cobo,
295 Uiblein, and Martinez-Rica 2000; Trochet et al. 2018; 2019) or the possibility for plastic response in
296 metabolic rate that was recently reported in surface populations of *C. asper* (Guillaume et al. 2020)
297 would condition the response of this endemic species to climate change. The diversity observed in our
298 study in metabolic strategies may be of prime importance for this species for coping with future
299 environmental change as it may provide the basis for future adaptation.

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320

321 **Competing interest**

322 We declare no competing financial interest.

323 **Ethics**

324 Our work complies with the international animal care guidelines of the Association for the Study of
325 Animal Behaviour, and all required French (permit no. 2017-s-02) permits relating to authorization of
326 capture, marking, transport, detention, use, and release of protected amphibian species; and animal
327 experimentation (accreditation n°A09-1) were been obtained.

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