



Ecophysiological conservativeness and size-mediated plasticity in the High Mountain Lizard *Atlantolacerta andreanskyi* confirm its vulnerability to climate change

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Abstract. Recent studies have reported declines in lizard populations associated with local warming trends and alteration of thermal niches. These studies suggest that there are some key physiological and behavioural parameters that determine the sensitivity of each species to the local context of global warming. The Atlas Dwarf Lizard, *Atlantolacerta andreanskyi*, is a small lacertid lizard endemic to the High Atlas Mountains (2200–3500 m a.s.l.) of Morocco. Its populations display a disjointed distribution across 440 km, consisting of phylogenetic lineages separated since the Miocene, suggesting a morphologically cryptic species complex. To establish whether this deeply rooted evolutionary divergence has promoted shifts in fundamental niche occupancy, but also whether there is any ecophysiological plasticity, preferred body temperatures (T_{pref}) and evaporative water loss (EWL) were examined in populations of the central and western High Atlas. Our results revealed no differences in T_{pref} across populations, but lower thermal preferences in (gravid) females. In contrast, EWL varied between populations, but not between sexes. In both cases, sexual patterns remained similar between populations, and differences were not evident between body sizes. Importantly, T_{pref} and EWL traded off, even if with a common trend across populations. These results suggest that ecophysiology remains mostly conservative within this species complex and that hydric ecology, which is more closely associated with body size, is more flexible than thermal ecology. The increase of lizard body size with altitude, in agreement with life history trends with seasonality observed in other Mountain Lizards, cannot be interpreted in terms of ecophysiological adaptation since the low-altitude populations of small lizards are the ones most exposed to dehydration. Rather, local environment and possibly aspects of evolutionary history could be involved. Overall, the ecophysiological conservativeness of this endemic species confirms its vulnerability to climate change, and indicates that hydric ecology needs to be incorporated into general models regarding how behavioural plasticity may mediate resilience to such changes. In this context, the low-altitude populations are the most vulnerable ones, and local management measures should focus on the conservation of vegetation and water bodies.

Key words. Squamata, Lacertidae, Eremiadini, thermal and hydric ecophysiology, climate change.

Introduction

Global climate change and associated alterations to thermal niches are predicted to have a catastrophic impact on lizard diversity, with models suggesting local extinctions reaching 39% being validated by observed local extinction rates (SINERVO et al. 2010). It is therefore essential to establish how plasticity of thermal ecology within lizard species may mediate their resilience to climate change. Temperature and humidity are among the most important environmental factors that define a reptile's niche, influencing

metabolic reactions, heat and water balance within a given environment, and ultimately the performance and fitness of individuals (PORTER & TRACY 1983, ANGILLETTA 2009, SANNOLO & CARRETERO 2019). Unlike endotherms, which produce heat to maintain their body temperature, ectotherms, including lizards, typically thermoregulate through behavioural adjustments (STEVENSON 1985, COSSINS & BOWLER 1987). For lizards, it was hypothesized that the preferred body temperature (T_{pref}) would be either an evolutionary labile or static trait (HERTZ et al. 1983). The labile hypothesis supposes fast local adaptation to the ther-

mal environment, while the static hypothesis emphasises the evolutionary rigidity of the T_{pref} , with adjustments to local conditions being mediated by phenotypic plasticity, and in particular by microhabitat selection or activity patterns (GVOŽDÍK 2002). For a long time, most studies on lizards were focused exclusively on the thermal aspect of their ecology, but recent studies have since illustrated its interdependency with water balance; for example demonstrating that dehydrated lizards tend to prefer lower body temperatures (CROWLEY 1987, SANNOLO & CARRETERO 2019).

In lacertid lizards, T_{pref} varies in response to season, reproductive condition, and feeding status (CASTILLA et al. 1999, CARRETERO et al. 2005). However, within comparative classes, lacertid T_{pref} is generally considered evolutionarily rigid (VAN DAMME et al. 1990, but see SAGONAS et al. 2013), responding very slowly to long-term changes in the thermal environment (GARCIA-PORTA et al. 2019). As a consequence, T_{pref} tends to remain similar within species even under different climate regimes (VAN DAMME et al. 1989, 1990, TOSINI et al. 1993, GVOŽDÍK & CASTILLA 2001, CARRETERO et al. 2005). This is because behavioural thermoregulation is expected to buffer the effects of thermal heterogeneity in the environmental context, restraining adaptation in thermal physiology (LOGAN et al. 2019). Nevertheless, recent studies found individual variation in thermal preferences within species, suggesting that natural selection may still act on these traits (ARTACHO et al. 2013, 2015, MUÑOZ & BODENSTEINER 2019). Consequently, both interspecific and intraspecific variation in T_{pref} must be analysed taking into account the phylogeny and biogeographic history of the groups under study. Different thermal responses depend on variable thermal requirements due to ontogenetic state, sex or reproductive cycle (STEVENSON 1985, ATKINSON 1994, CULLUM 1998, CLUSELLA-TRULLAS et al. 2009). In particular, reproductive females often differ in their thermoregulatory behaviour, since embryonic development requires a particular thermal regime that will often differ from that of adults (GEORGES et al. 2005).

Adding an extra layer of complexity, many lizard species range across steep altitudinal gradients. Generally, environmental temperature decreases as altitude and latitude increase, which makes the thermal environment at high latitudes and altitudes (e.g., high-mountain systems) particularly hostile to ectotherms (GVOŽDÍK 2002, ANGILETTA 2009, AGUADO & BRAÑA 2014). In such cold environments, ectotherms may not be able to thermoregulate effectively for long periods and, hence, they must either be efficient thermoregulators or become inactive (HUEY & SLATKIN 1976, VAN DAMME et al. 1989, HERTZ et al. 1993, ANGILETTA et al. 2002, ANGILETTA 2009). Since thermal physiology may respond slowly to such pressures and thermal requirements remain conservative in populations and species (HERTZ et al. 1983, CROWLEY 1985, ANDREWS 1998, SEEBACHER & SHINE 2004, DIAZ DE LA VEGA-PÉREZ et al. 2013), species inhabiting contrasting environments (e.g., high vs. low altitudes) often exhibit similar thermal requirements but incur different thermoregulatory costs (BOGERT 1949, HERTZ et al. 1983, CROWLEY 1985, S'KHIFA

et al. 2020a). In such cases, the organisms must compensate for poor thermal quality by highly efficient thermoregulatory behaviour or other phenotypic shifts (DIAZ 1997, ASHTON & FELDMAN 2003, OSOJNIK et al. 2013).

Thermoregulating species can respond to thermal heterogeneity by selecting specific microhabitats and body postures to optimise heat gain and loss (HUEY & BENNETT 1987). While doing so, they will also experience water loss through evaporation, and since evaporation increases with rising mean temperatures, a compromise is made between thermoregulation and water balance (MAUTZ 1982a, b). Thus, dehydration could pose another constraint to activity when the availability of water is limited. Indeed, recent research on lizards suggests that humidity, ambient moisture, and the availability of water might be even more limiting than the thermal environment in warm areas (CARRETERO et al. 2016, FERREIRA et al. 2016, S'KHIFA et al. 2020a). Also, while their thermal preferences may be similar, lizard populations with limited access to water may be more resistant to water loss than those populations with unrestricted access to water (DUPOUÉ et al. 2017). Moreover, gravity may also affect water loss because embryo needs may shift the balance between thermoregulation and hydroregulation in the mothers. While ecophysiological shifts in gravid females were traditionally interpreted as being due to the higher oxygen needs of large embryos at higher temperatures (MADERSON & BELLAIRS 1962), there is now evidence that dilated bellies may increase water loss rates, inducing mothers to counteract the dehydration of their offspring (BONNET et al. 2017), with both probably acting in combination. However, generalisation of these findings is problematic due to a lack of comparative data, since most of the current literature on lizard ecophysiology remains focused on thermal traits (HERRANDO-PÉREZ et al. 2020). For example, it is almost unknown to which extent water loss rates may vary along environmental gradients, or whether they are subject to diel and seasonal plasticity, as thermal preferences often are (SANNOLO et al. 2020). Incorporation of such information into modelling approaches will be essential to establish whether behavioural responses of lizards may mediate the worst impacts of global climate change.

Here, we investigated the thermal and hydric ecophysiology of the Atlas Dwarf Lizard *Atlantolacerta andreanskyi* in contrasting mountain systems of the High Atlas range. We compared the T_{pref} and evaporative water loss (EWL) rates between three populations representing widely divergent lineages within this species complex (BARATA et al. 2012). In accordance with recent findings in the family Lacertidae (GARCIA-PORTA et al. 2019, SANNOLO et al. 2020), we would expect more flexibility across lineages in hydric physiology than in thermal physiology. Moreover, we predict that gravity will induce phenotypic plasticity in females, affecting the balance between thermoregulation and hydroregulation. Furthermore, as the combined study of thermal and hydric ecology is the basis of the delimitation of fundamental niche occupancy in lizards (BOWKER 1993), our aim was to establish whether temperature and

water requirements trade off, and if this is the case, if the same balance is maintained across populations/lineages. This information may be critical for validating correlative ecological models based on realized niche occupancy, which already indicate that precipitation plays a prominent role in the distribution of Moroccan endemic reptiles and forecast considerable range retractions due to climate change (MARTÍNEZ-FEIRÍA et al., 2013).

Material and methods

Species and areas studied

The Atlas Dwarf Lizard, *Atlantolacerta andreanskyi* (WERNER 1929) is a small lacertid lizard endemic to Morocco, where it is found from 2200 to 3500 m a.s.l. in the mountains of the High Atlas and the Siroua Massif (MARTÍNEZ DEL MÁRMOL et al. 2019). Its distribution (Fig. 1) is clearly discontinuous, and extends from Jbel Ayachi in the east to Jbel Aoulime in the west for about 440 km (straight line). Associated with this spatial disjunction, almost no current gene flow between the different populations has been identified (BARATA et al. 2012). A mountain specialist, this ground-dwelling lizard is often found near watercourses and around the bases of cushion-like thorny plants (BONS & GENIEZ 1996, SLIMANI et al. 1996) that offer a buffered microclimate with higher humidity, more food, and protection against predators and wind (SCHLEICH et al. 1996). Genetic studies conducted by BARATA et al. (2012) and GIOVANNOTTI et al. (2020) suggest that *A. andreanskyi* may be a species complex with at least seven distinct lineages grouped in two major clades, i.e., a western (Oukai-

meden + Jbel Siroua) and an eastern clade (Jbel Ayachi + Outabati + Jbel Azourki + Tizi-N-Tichka). Although the cladistic relationships of the Jbel Aoulime population are poorly supported, it may be the sister taxon to the rest of the eastern lineage (BARATA et al. 2012). These distinct lineages separated during the Miocene (7.6 Mya), while the separation between the seven lineages (likely species) inhabiting separated mountain massifs is estimated to be Plio-Pleistocenic. Contrasting with this deep phylogenetic divergence, morphological variation showed considerable overlap across lineages, limited diagnostic traits, and only some size differences, suggesting niche conservatism within this species complex (BARATA et al. 2015).

In an ecophysiological study conducted in Oukaimeden, S'KHIFA et al. (2020a) reported that *Atlantolacerta* had similar thermal requirements to sympatric species of the genera *Podarcis* and *Scelarcis*, but was more sensitive to dehydration. Given the long evolutionary time frame, it seems that aridification of climate, together with niche conservatism due to the interaction with other lowland lacertids, may have resulted in its range retraction and fragmentation while preventing niche shifts. However, this hypothesis is based solely on evidence from Oukaimeden. Therefore, we here investigate if the fundamental niche occupancy of *A. andreanskyi* has remained conserved in the long term by comparing representatives of three lineages. Some aspects of the biology of *A. andreanskyi* are already well known, at least at Oukaimeden (BUSACK 1987, CARRETERO et al. 2006), but it remains unclear how the different climatic events that occurred during the Miocene and Pleistocene may have shaped the ecophysiological variation in this species complex.

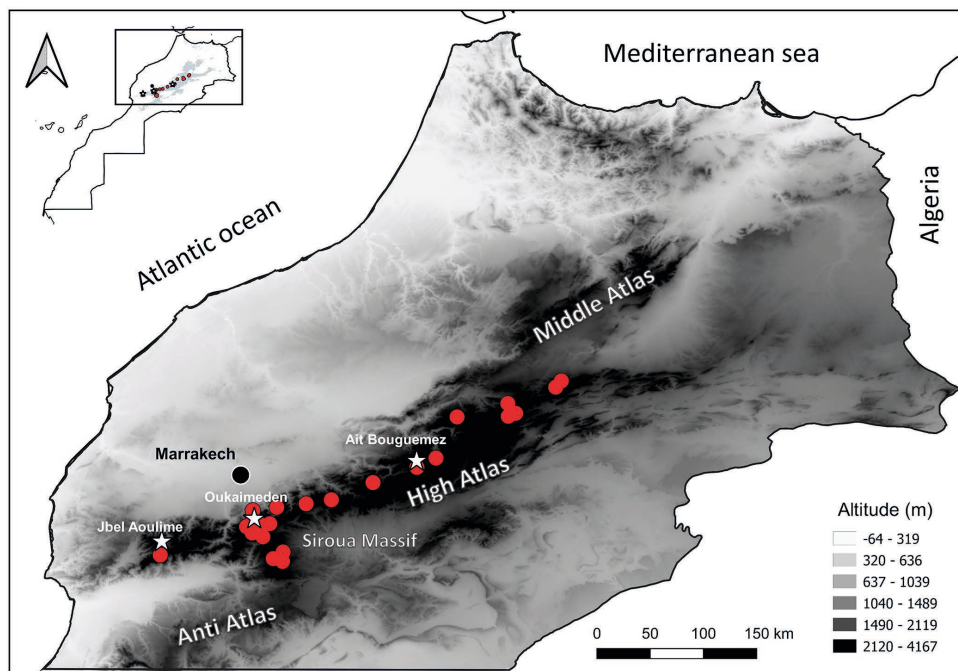


Figure 1. Distribution map of *Atlantolacerta andreanskyi* (red dots) and localities studied (stars) in the High Atlas Mountains.

Populations representing these lineages (eastern, western and the ambiguously placed Jbel Aoulime) were investigated in three high-altitude localities (Fig. 1) in the High Atlas in Morocco. All are characterized by a mountain climate with a cold winter and sport vegetation consisting of xerophilous thorny bushes (*Alyssum spinosum*) that offer excellent protection against predators: 1) Ait Bouguemez (51 km southeast of Azilal in the Central High Atlas; 31°45'45.8" N, 6°16'50.6" W, altitude 2,723 m a.s.l. – this corresponds to the population called “Jbel Azourki” in the previously published genetic analyses), 2) Oukaïmeden (75 km south of Marrakech on the southern limits of the Central High Atlas; 31°12'09.5" N, 7°51'24.4" W, altitude 2,600 m a.s.l.), and 3) Jbel Aoulime (64 km south of Imintanoute in the Occidental High Atlas; 30°58'50.7" N, 8°45'11.1" W, altitude 2,512 m a.s.l.). Climatic data (Supplementary Table S1) of the three study areas, gathered from www.power.larc.nasa.gov for the last 30 years (1990–2020), show that Jbel Aoulime was less humid and warmer than Oukaïmeden and Ait Bouguemez during this period. The ombrothermic diagrams (Supplementary Fig. S1) produced for the 30 years of recording for the three localities indicate a dry season (precipitation < 2*temperature) extending over 5, 6, and 7 months, respectively, at Ait Bouguemez, Oukaïmeden, and Jbel Aoulime.

Sampling

Sixty adult individuals (11 males and 9 females from Ait Bouguemez, 10 males and 10 females from Oukaïmeden, and 8 males and 12 females from Jbel Aoulime) were captured by hand along random one-way transects during their activity periods from 9:00 to 18:00 h GMT in the first week of June 2019. We recorded the snout–vent length (SVL) for each individual, using a digital calliper (precision 0.01 mm), and the body mass (BM) with a digital scale (precision 0.01 g). Due to the sampling period, all females captured and used in our different experiments were gravid (apparent through palpation). After capture, the lizards were kept captive in individual plastic terraria (30 × 19 × 17 cm) for no more than five days with water and food (*Tenebrio molitor* larvae) available to them *ad libitum* daily during our ecophysiological experiments. The experiments were carried out in the Water, Biodiversity and Climate Change Laboratory, Biodiversity and Ecology of Vertebrates Unit, of the Faculty of Sciences Semlalia Marrakech, Morocco.

Preferred body temperature and water loss experiments

Evaporative water loss (EWL) and preferred body temperature (T_{pref}) were evaluated for a target sample size of 20 adult lizards per population. Each lizard was subjected to the two experiments (once each). The experiments of water loss were always conducted after the experiments of pre-

ferred body temperature, with an interval of two days in between. For both experiments, the lizards were left to fast for a day before handling. All lizards were released at the exact locations of their capture after the end of the experiments.

The experiments of T_{pref} were executed in a closed room in the laboratory to prevent wind and sun from affecting the temperature in the terrarium. Each lizard was placed in a thermal gradient corridor (min–max 13–50°C; 100 × 30 × 30 cm) generated by a thermoSpotlight 100 W (HOBBY®), fixed 20 cm above the substrate at one extremity of the gradient. The bulb was switched on one hour before a lizard was placed into the corridor, and the first measurement was taken one hour later. The experiments were conducted between 9:00 and 19:00 h GMT. Due to logistic constraints (no thermal camera available) and to prevent the disturbance of gravid females due to the permanent or repeated introduction of thermal probes to the cloaca (KOZIEL et al. 2021), body temperature was measured every hour on the dorsal skin of the lizards using an infrared thermometer (FLUKE 572 CE, precision 0.01°C), perpendicularly orientated to the lizard and at very close distances (approximately 10–20 cm) to the animal to ensure accurate measurements of body temperatures (HARE et al. 2007) without handling the lizards.

For the experiment of EWL, each lizard was housed individually in a plastic box (15 × 10 × 3 cm, ~ 0.45 l) with ventilation holes on the top, bottom and sides. Each box was placed into a second box including 5 g of silica gel. All individual plastic boxes were placed in a large container (40 × 30 × 25 cm, ~ 30 l), with 100 g of silica gel in a gauze bag. The quantity of silica gel used facilitated a low level of relative humidity (20 ~ 30%) inside each box, where we installed a thermo-hygrometer (Beurer HM 16, precision 0.1%; 0.1°C) to monitor humidity and temperature (OSOJNIK et al. 2013, CARNEIRO et al. 2015, S'KHIFA et al. 2020a). The container with the boxes in it was then placed in a climatic chamber (POL-EKO-APARATUR SP. J type ST3+/ST3+) set at 24°C. This temperature was selected to allow lizard activity while preventing acute desiccation stress, which may occur if lizards remain at T_{pref} for long periods without an opportunity for thermoregulation (SANNOLO et al. 2018, SANNOLO & CARRETERO 2019). This experiment ran from 8:00 to 19:00 h GMT. Lizards were individually removed from the container at hourly intervals, weighed together with the box using a digital scale (RADWAG, AS 110/C/2, precision ± 0.0001 g), and immediately placed back into their respective containers in the climatic chamber. To minimize stress and hyperventilation, which may increase EWL, lizards' weights were obtained by subtracting the weights of the respective individual boxes. The whole measuring procedure took no longer than 20 seconds for each lizard.

From our data, we calculated three measures of relative water loss. The accumulated water loss (EWLa) after each hour of the experiment (from 8:00 to 19:00 h GMT), was calculated according to the formula:

$$EWLa = [(W_0 - W_n) / W_0] \times 100,$$

Table 1. Sample size, mean, and standard deviation (SD) of the body mass (BM), snout-vent length (SVL), preferred body temperature (T_{pref}), total evaporative water loss (EWL_t), and instantaneous evaporative water loss (EWL_i) calculated for each sex and population of *A. andreanskyi*. Jbel Aoulime (JAO), Oukaimeden (OUK), and Ait Bouguemez (BOU). * Groups whose elements are homogeneous after performing post hoc tests at $\alpha = 0.05$.

Sex	Localities	Number	BM (g) mean \pm SD	SVL (mm) mean \pm SD	T _{pref} (°C) mean \pm SD	EWL _t (%) mean \pm SD	EWL _i (%) mean \pm SD
Gravid females	Jbel Aoulime	12	1.83 \pm 0.69	46.50 \pm 2.61	33.2 \pm 1.1	3.41 \pm 1.02	0.31 \pm 0.09
	Oukaimeden	10	1.86 \pm 0.27	47.90 \pm 2.23	34.5 \pm 0.9	4.23 \pm 1.77	0.38 \pm 0.16
	Ait Bouguemez	9	3.36 \pm 0.59	50.67 \pm 4.24	34.3 \pm 1.5	3.00 \pm 0.89	0.27 \pm 0.08
Males	Jbel Aoulime	8	1.66 \pm 0.49	43.38 \pm 4.43	34.5 \pm 1.7	4.16 \pm 1.96	0.38 \pm 0.18
	Oukaimeden	10	1.89 \pm 0.24	46.30 \pm 1.77	34.9 \pm 1.0	3.28 \pm 1.62	0.30 \pm 0.15
	Ait Bouguemez	11	2.65 \pm 0.27	49.18 \pm 2.48	35.0 \pm 1.0	2.29 \pm 0.65	0.21 \pm 0.06
*Statistical group: between populations			P<0.001 {JAO-OUK} {BOU}	P<0.001 {JAO-OUK} {BOU}	P=0.055 {JAO-OUK-BOU}	P=0.016 {JAO-OUK} {BOU}	P=0.016 {JAO-OUK} {BOU}

where W is weight. The instantaneous water loss (EWL_i) was calculated using the formula

$$EWL_i = [(W_n - W_{n+1}) / W_0] \times 100.$$

And the total water loss (EWL_t) was calculated by subtracting the mass of the lizard at the end of the experiment, and dividing it by the initial mass at 8:00 h (W₀) and multiplying it by 100 to obtain a percentage value (%). While EWL_a provides information on the overall volume of water evaporated since the start of the experiment, EWL_i values record the pattern of water loss over time (constant or variable), and EWL_t is the total amount of water lost by the animal at the end of the experiment (OSOJNIK et al. 2013, S'KHIFA et al. 2020a).

Statistical analyses

We first tested for morphological variation in body mass (BM) and snout-vent length (SVL) across populations and between sexes with a two-way ANOVA, as well as variation in robustness with the same factors by comparing body masses with SVL as a covariate in an ANCOVA. Morphological variables were log-transformed for linearizing the relations. The residuals of the ecophysiological data were normally distributed (Shapiro-Wilk test, $p > 0.05$), homoscedastic (multivariate Box M and univariate Levene's tests, $p > 0.05$), and non-spherical (Mauchly's tests, $p > 0.05$ in most cases). Two-way AN(C)OVA tests (populations and sex as between-subject factors) of T_{pref} and water loss total rate were performed based on the values of both ecophysiological parameters of males and females of each population. Then, to establish the variation in body temperature and water loss rates (EWL_a and EWL_i) according to population, sex and time interval, we used a (co)variance analysis with repeated measurement (AN(C)OVA-rm). Subsequently, in both types of analysis (two-way and one-way AN(C)OVA and AN(C)OVA-rm), Log BM and Log SVL were incorporated as covariates to account for the effects of size, shape and body condition. Tukey HSD post hoc tests

were performed between population pairs and sexes to explore any significant differences. In the end, a partial correlation was performed between T_{pref} and EWL_t at individual level, taking SVL, BM, population and sex into account.

All our analyses were computed in IBM SPSS Statistics version 22.0 (IBM Corporation 2011). Significance was evaluated at an α -value of 0.05.

Results

Our statistical analyses of snout-vent length (Table 1) revealed variation between populations (the Ait Bouguemez population being larger) and sexual dimorphism (females being larger), but no sexual dimorphism across populations. The results for body mass were similar, except that there was no sexual dimorphism (two-way ANOVA test: for SVL, Populations $F_{2,54} = 13.415$, $p < 0.001$, Sex $F_{1,54} = 6.941$, $p = 0.011$, Populations \times Sex $F_{2,54} = 0.447$, $p = 0.642$; for BM, Populations $F_{2,54} = 15.140$, $p < 0.001$, Sex $F_{1,54} = 0.164$, $p = 0.687$, Populations \times Sex $F_{2,54} = 1.177$, $p = 0.316$). Similarly, our analysis of the body mass when SVL was used as a covariate showed a significant difference in robustness between populations and sexes, but no interaction between these factors (two-way ANCOVA test for BM, Populations $F_{2,53} = 4.166$, $p = 0.021$, Sex $F_{1,53} = 9.523$, $p = 0.003$, Populations \times Sex $F_{2,53} = 1.035$, $p = 0.362$).

The analyses of two-way (co)variance of preferred body temperature and total water loss produced different results for both parameters. The analyses of T_{pref} (Table 1) indicated no differences between the three populations, but did so between sexes (females having a lower T_{pref}) and no interaction Populations \times Sex (two-way ANOVA; Populations $F_{2,54} = 3.069$, $p = 0.055$, Sex $F_{1,54} = 6.916$, $p = 0.011$, Populations \times Sex $F_{2,54} = 0.755$, $p = 0.475$). However, after accounting for lizards' size, the opposite pattern was found, with differences between populations, but neither between sexes nor the interaction Populations \times Sex (two-way ANCOVA with log BM and log SVL as covari-

ates, $\log\text{BM } F_{1,52} = 0.001, p = 0.973, \log\text{SVL } F_{1,52} = 2.709, p = 0.106, \text{Populations } F_{2,52} = 5.975, p = 0.005, \text{Sex } F_{1,52} = 2.343, p = 0.132, \text{Populations} \times \text{Sex } F_{2,52} = 0.415, p = 0.662$). Finally, ANOVA-rm (Fig. 2) indicated temporal variations in T_{pref} with different patterns for populations and sexes, which mostly disappeared after accounting for body size/shape (Supplementary Table S2).

For EWLt (Table 1), the analyses of raw data revealed a difference between populations, but not between sexes or interaction Populations \times Sex (two-way ANOVA; Populations $F_{2,54} = 4.446, p = 0.016, \text{Sex } F_{1,54} = 0.738, p = 0.394, \text{Populations} \times \text{Sex } F_{2,54} = 2.199, p = 0.121$). After accounting for size, no differences either between populations, sexes, or the interactions between them were found (two-way ANCOVA with log BM and log SVL as covariates, $\log\text{BM } F_{1,52} = 4.255, p = 0.044, \log\text{SVL } F_{1,52} = 0.022, p = 0.882, \text{Populations } F_{2,52} = 0.400, p = 0.672, \text{Sex } F_{1,52} = 0.307, p = 0.582, \text{Populations} \times \text{Sex } F_{2,52} = 1.543, p = 0.223$).

Through time, we found significant differences in all analyses with two-way ANOVA-rm (Time- T_{pref} , Time-EWL_a and Time-EWL_i, $p < 0.001$). The analysis of T_{pref} produced significant differences in time between populations as well as between sexes (ANOVA-rm; time \times Populations $F_{20,540} = 1.971, p = 0.007; \text{time} \times \text{sex } F_{10,540} = 3.137, p = 0.001$). In EWL_a rates, we found differences in time between populations (ANOVA-rm; time \times Populations $F_{22,594} = 3.576, p = 0.019$), but no differences between sexes (ANOVA-rm; time \times sex $F_{11,594} = 0.807, p = 0.413$). In EWL_i rates, no differences were detected in time either between populations or between sexes (ANOVA-rm; time \times population $F_{20,540} = 0.783, p = 0.606; \text{time} \times \text{sex } F_{10,540} = 0.617, p = 0.634$). Likewise, no significant difference was found in the interaction time \times populations \times sex ($p > 0.05$) in T_{pref} , EWL_i or EWL_a (Figs 2–4, respectively).

Finally, partial correlation analyses revealed a significant negative relationship between EWLt and $\log\text{BM} \cdot \log\text{SVL}$

($n = 60; \text{df} = 58, r_{\text{partial}} \log\text{BM} \cdot \text{EWLt} = -0.518, p < 0.001; \text{df} = 58, r_{\text{partial}} \log\text{SVL} \cdot \text{EWLt} = -0.369, p = 0.004$), with all populations following the same trend. In contrast, no significant association of T_{pref} with either $\log\text{BM}$ or $\log\text{SVL}$ was observed ($n = 60; \text{df} = 58, r_{\text{partial}} \log\text{BM} \cdot T_{\text{pref}} = -0.017, p = 0.895; \text{df} = 58, r_{\text{partial}} \log\text{SVL} \cdot T_{\text{pref}} = -0.182, p = 0.165$). No significant association was observed even after accounting for $\log\text{BM}$, $\log\text{SVL}$, populations and sex ($n = 60; \text{df} = 54, r_{\text{partial}} \text{EWLt} \cdot T_{\text{pref}} = 0.146, p = 0.282$).

Discussion

Our ecophysiological comparisons across three widely divergent lineages within the *Atlantolacerta andreanskyi* species complex, encompassing more than 7 Mya of independent evolution, is in line with previous findings suggesting that thermal physiology is more rigid than hydric physiology is along the phylogenetic axis in Lacertidae (GARCIA-PORTA et al. 2019). However, they also provide evidence of phenotypic plasticity, since thermoregulation in gravid females was biased towards lower temperatures (CARRETERO et al. 2005) and mainly because variations in body size also had repercussions on the lizards' hydric physiology (FERREIRA et al. 2016).

As in other lacertids from cold climates, sexual dimorphism in SVL of *A. andreanskyi* indicates that females are larger, likely due to stronger fecundity selection rather than sexual selection under these conditions (BRAÑA 1996, ROITBERG et al. 2015). Moreover, in populations with shorter annual activity periods, growth takes precedence over reproduction and lizards tend to delay sexual maturity in favour of attaining larger sizes first (HORVÁTHOVÁ et al. 2013, ROITBERG et al. 2020). Whereas robustness of male lacertids compared to females is considered a result of sexual selection (KALIONTZOPOULOU et al. 2008), robust-

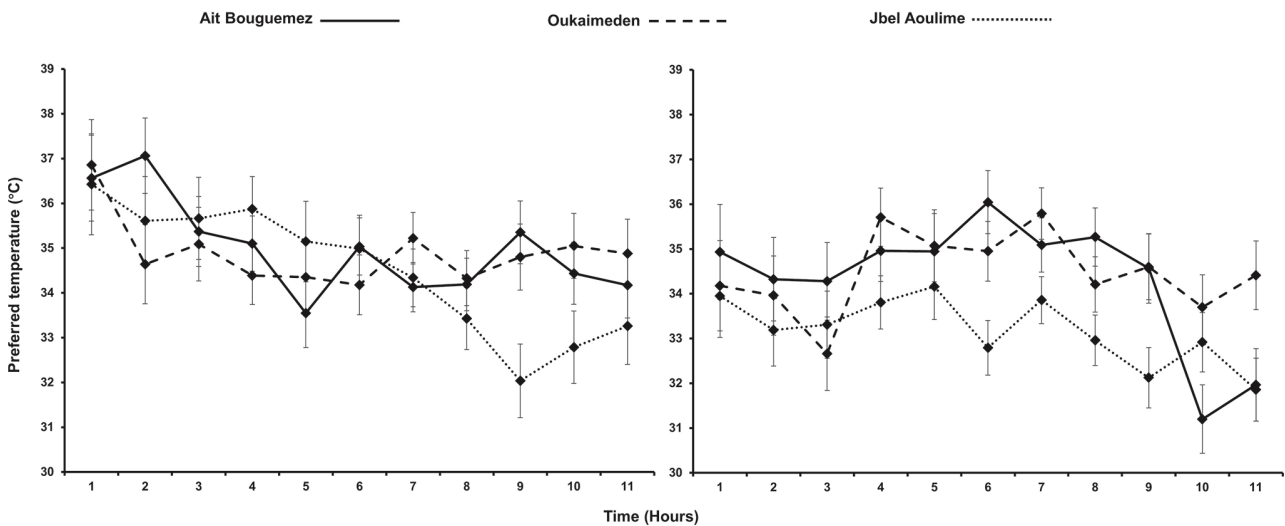


Figure 2. Daily variations in the preferred body temperatures (T_{pref}) of *Atlantolacerta andreanskyi* (males left, females right) between the three studied populations and sexes. Displayed are mean values and standard deviations.

ness of both sexes rising with altitude is probably the result of allometric growth (KALIONTZOPOULOU et al. 2010a, b).

Despite their considerable genetic separation, preferred body temperatures were similar across the studied populations of *A. andreanskyi*. This conservativeness is not surprising and has been observed along other wider phylogenetic distances in this lizard family (GARCIA-PORTA et al. 2019). However, the preference of lower temperatures by (gravid) females indicates there is phenotypic plasticity also for this trait. In Mountain Lizards with short activity seasons, female phenology tends to be accentuated and synchronized (CARRETERO et al. 2005, 2006), suggesting the gestation status to have been similar across examined

populations and individuals, and mirrored by similar thermal shifts across populations. While an effect of thermal stress on advanced embryos cannot be ruled out (MADERSON & BELLAIRS 1962), the fact that the statistical removal of size/shape effects made the shift disappear suggests other causative factors, namely, hydric physiology.

Like all lacertids, all populations of *A. andreanskyi* tended to raise their T_{pref} in the early morning when environmental temperatures in the wild would be low, that is, when the thermal environment is more unfavourable and thermoregulation becomes a priority relative to other physiological requirements (RISMILLER & HELDMAIER 1982, TOSINI & AVERY 1994, CARRETERO et al. 2005). In-

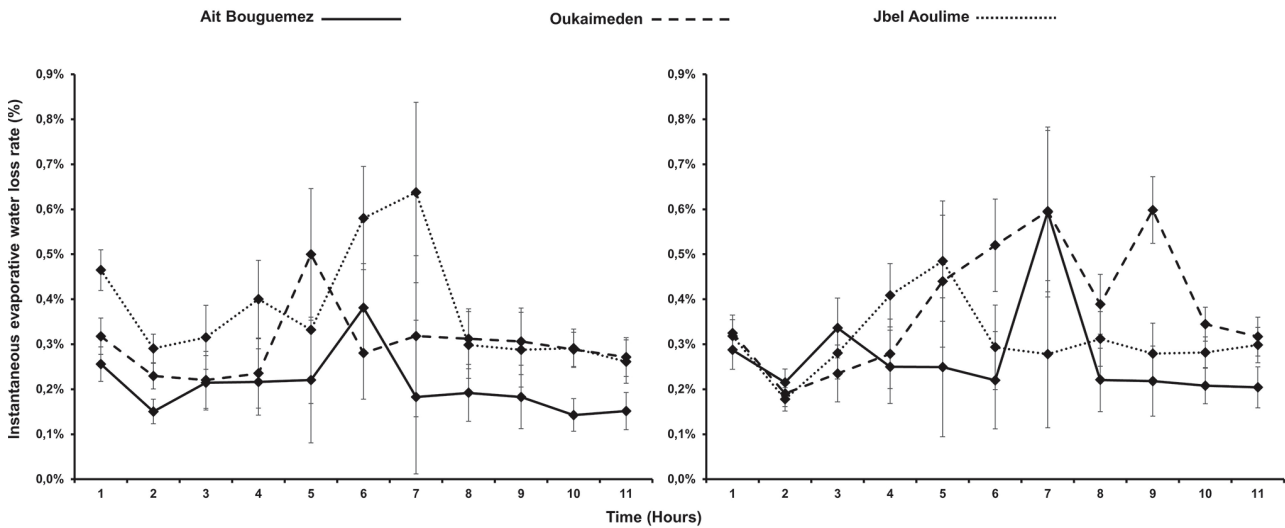


Figure 3. Daily variations in the instantaneous water loss rates (EWLi) of *Atlantolacerta andreanskyi* (males left, females right) between the three studied populations and sexes. Displayed are mean values and standard deviations.

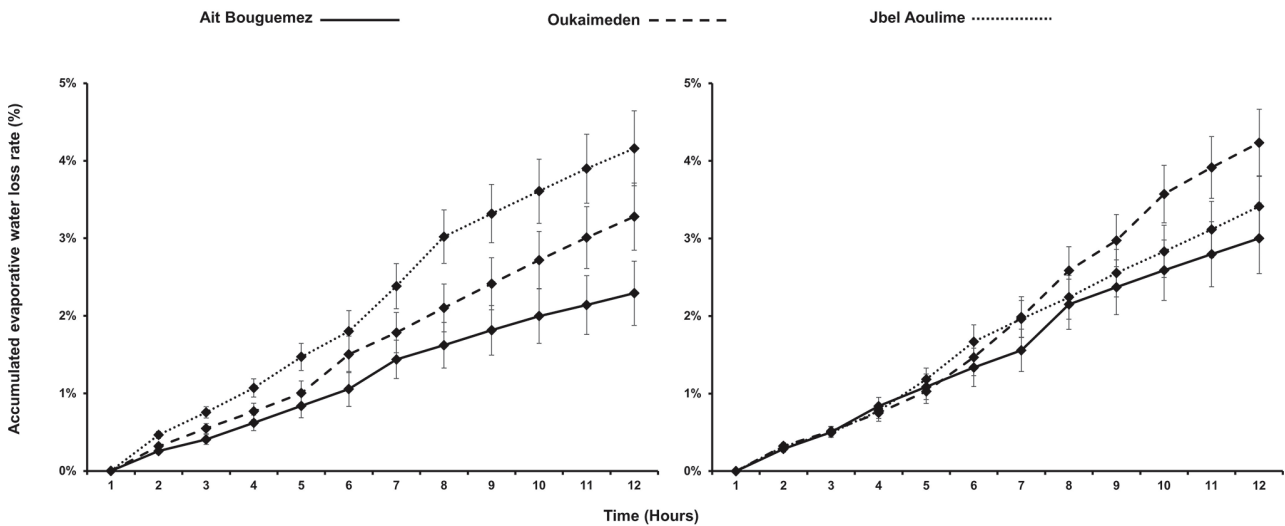


Figure 4. Daily variations in the accumulated water loss rates (EWLa) of *Atlantolacerta andreanskyi* (males left, females right) between the three studied populations and sexes. Displayed are mean values and standard deviations.

terestingly, this was more accentuated in males than in females. However, the fact that T_{pref} values in the late afternoon tended to decline rather than increase suggests that water loss was constraining thermoregulation towards the end of the day (S'KHIFA et al. 2020a). Once more, these shifts were dependent on lizard size and shape and mostly disappeared after accounting for SVL and mass. In a study comparing lacertid species representing different sizes, climatic regions and clades, thermal and water ecophysiology showed signs of a trade-off, but patterns varied between species and were independent of size/form (FERREIRA et al. 2016). Here, the trade-off was also evident (lizards with high T_{pref} had lower EWL and vice versa), but all three populations followed a common trend and the trade-off was clearly size/form-dependent, pointing again to conservatism of fundamental niche occupancy.

A strong population signal was recovered for EWL in *A. andreanskyi*, namely, a decrease with increasing altitude. These results were expectable since SANNOLO et al. (2020) already demonstrated flexibility in hydroregulation by the lacertid *Psammotromus algirus* from different altitude across a small geographic gradient. While the effect of local acclimatization (e.g., to different wind intensities) and the separate evolutionary histories of different lineages cannot be ignored (DML'EL et al. 1997, COX & COX 2015), the simplest explanation in this case is that these differences in EWL result from body size increasing with increasing altitude, which decreased the surface/volume ratio (SCHMIDT-NIELSEN 1984). Accordingly, the largest lizards from Ait Bouguemez exhibited considerably more resistance to water loss than the smallest ones from Jbel Aoulime, while those from Oukaimeden had intermediate values. This is confirmed by the lack of differences in EWL when SVL and body mass were accounted for. Remarkably, sex had no effect per se on the results, although the larger sizes and more voluminous abdomens of females may have minimised their water loss as a function of gravidity (BONNET et al. 2017). This suggests that thermal ecology is taking precedence over hydric ecology regarding the embryo development inside the mother. One possible explanation would be the reduced oxygen pressure at very high altitudes (GANGLOFF et al. 2019). Certainly, after oviposition and then during the juvenile stage it is clear that hydroregulation will increase in importance. Evidence for this is the finding of communal nests and juvenile aggregations, which suggest that suitable nesting sites are limited (MARCO et al. 2004, S'KHIFA et al. 2020b).

Behavioural thermoregulation is understood to be a key mechanism for buffering the physiological effects of climate change, but the classic overreliance on thermal testing without accounting for water availability have limited the development of generalised models (ROZEN-RECHELS et al. 2020). Our data, combined with other recent evaluations of EWL in lizards (e.g., BELASEN et al. 2017), further support the general pattern that hydric ecology is more flexible than thermal ecology, and therefore is the key component to understanding the impact of climate change on ectothermic vertebrates. The development of worldwide

databases for EWL rates in squamates (e.g., LE GALLIARD et al. 2021) may help further address this issue, although undersampling is evident in various geographic regions, including Africa. Rather than focussing primarily on higher temperatures and available thermal niches, a more nuanced approach including water availability and microhabitat will be needed to better model the impact of climate change on lizards in general.

Our findings also have important repercussions on conservation measures for our target species. *Atlantolacerta andreanskyi* is the only representative of its genus (ARNOLD et al. 2007), sister taxon to all other Eremiadini in the lacertid phylogeny (GARCIA-PORTA et al. 2019), and considered as Near Threatened in the Red List of IUCN due to its reduced and fragmented range and high habitat specificity (GENIEZ 2006). The ecophysiological evidence reported here is also consistent with correlative ecological models, underscoring the importance of precipitation for this species' occurrence and predicting a considerable range retraction under the climate change scenarios (MARTÍNEZ-FEIRÍA et al., 2013). However, this considers the complex as a whole – if the genetic lineages were formally described as distinct species, some would likely need to be reclassified as Endangered due to their small known ranges, especially given that the range of the whole complex is already less than 20,000 km². Because it lives in harsh environments at high altitudes in the Atlas Mountains, its vulnerability to global warming is expected to be very high (SINERVO et al. 2010). Niche conservatism in thermal ecophysiology within this species complex reduces the extent of expectations for fast evolutionary responses to fast environmental shifts. Plasticity in hydric ecophysiology between populations and sexes might provide some optimism, but this was mainly mediated by body size, and the smallest lizards of the populations at lower altitudes would in fact be the ones that are most vulnerable to aridification (HIJMANS et al. 2005, S'KHIFA et al. 2020a). Furthermore, thermal constraints imposed on embryos during gestation, as well as the hydric needs of eggs and hatchlings in an environment with limited hydric resources may compromise reproduction and pose an additional threat. While little can be done locally to reverse the global trends in climate change, conserving natural habitats, and in particular the vegetation coverage and incorporated water bodies, seems the best management strategy at a local level to prevent fast range retractions, population declines and, ultimately, extinction (GENIEZ 2006). Part of the range of this Moroccan endemite lies within a protected area, the Toubkal National Park. However, this would only protect one (Oukaimeden) of the several identified genetic lineages. For the remaining populations, where no conservation measures are currently in place, habitat amelioration focusing on vegetation and habitats that offer moisture is strongly recommended. The IUCN Red List does not currently report any specific threats, although it notes that the species occurs in areas accessible to hikers. Other than in the national park, we suggest that very few hikers traverse the places where these populations occur. Rather, given the importance of source of moisture in habitats, efforts could

be focussed on reducing overgrazing in these fragile localities. Our findings are expected to improve our understanding of the potential repercussions of climate change on the conservation status of this Moroccan endemic (GENIEZ 2006; MARTÍNEZ-FEIRÍA et al., 2013), supply scientific evidence for its management, and provide further data to develop generalized models for assessing the impact of climate change on ectothermic species.

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Supplementary data

The following data are available online:

Supplementary Table S1. Precipitation, relative humidity, temperature, wind speed, earth skin temperature and surface pressure at the three study areas for the last 30 years (1990–2020).

Supplementary Table S2. AN(C)OVA-rm of preferred body temperature and evaporative water loss rates between the three populations of *A. andreanskyi* measured for 11 consecutive hours.

Supplementary Figure S1. Ombrothermic diagrams of the three study areas for the last 30 years (1990–2020).