



Ecophysiology of a lacertid community in the high Moroccan mountains suggests conservation guidelines

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ABSTRACT

Lizard species may differ in their ecophysiology due to adaptation, plasticity and/or phylogeny. In restrictive environments, ecophysiological differences of species living in sympatry are expected to reveal long-term evolutionary responses to the abiotic environment while competitive interactions should be limited. These influences can be disentangled by combining field monitoring with experimental tests. Here, three lacertid lizard species, *Atlantolacerta andreanskyi*, *Scelarcis perspicillata* and *Podarcis vaucheri* sharing high mountain habitats in Oukaimeden (High Atlas, Morocco), were studied. In the field, spatiotemporal variation of the thermal and hydric environment used by the lizards was monitored using data-loggers. In the lab, thermal and hydric ecophysiology was estimated through assessments of preferred temperatures (Tp) and water loss (WL) rates. Species differed in microhabitat use and, hence, in their exposure to variations in temperature and humidity. However, they only differed in their WL ($A. andreanskyi > S. perspicillata > P. vaucheri$) while their Tp were similar. Such partial differences of species in the fundamental niche, likely derived from their long-term independent phylogenetic trajectories, can be used to predict their responses to climate and habitat shifts in this and other parts of their respective ranges. Results also confirm previous suggestions that, together with thermal physiology, hydric physiology plays a prominent role in the organisation of lizard communities in the temperate region.

1. Introduction

Species distribution is moulded by a combination of ecological suitability, geographic opportunity (e.g. dispersal pathways, lack of barriers), interaction with other organisms (prey, predators, competitors, parasites) and evolutionary history (i.e., selective pressures acting in the past) (Lomolino et al., 2010). As such, the assemblage of local communities depends on 1) the fundamental niche of each species (sensu Sillero, 2011), 2) their historical biogeographies allowing or preventing them to contact their ranges, and 3) their current biological interactions (Webb et al., 2002; Pianka et al., 2017). Both 2) and 3) may cause a deviation of the realized niche from the fundamental niche (Sillero, 2011), as reconstructed from organismal physiology (Kearney and Porter, 2009).

Lizards provide excellent models for ecophysiological studies due to their life history traits (Carretero et al., 2016). On one hand, as they are ectotherms with restricted vagility and short lifespan, their populations become exposed to local environmental fluctuations with limited homeostatic responses. On the other hand, they are abundant, conspicuous and easily kept in captivity, which allows efficient field sampling and experimental manipulation (Pianka and Vitt, 2006). Of the multiple environmental features shaping a lizard's niche, temperature and water are among the most important (García-Porta et al., 2019). Temperature shapes metabolic reactions, heat balance with the environment and many other biological functions determining individual fitness (Angilletta, 2009). In a continuum between thermoregulators and thermoconformers, the first tend to cope with environmental fluctuations by adopting behavioural postures and selecting favourable thermal

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microhabitats while the second rely more on wide thermal performance of their biological functions (Angilletta et al., 2010). In both cases, inactivity may be the only response when thermal conditions are unsuitable either during the day or throughout the year (Adolph and Porter, 1993) but this also carries substantial fitness costs (Sears and Angilletta, 2015). Because species may differ in thermal physiology, it is frequent that they display divergent patterns of activity and microhabitat use even in syntopy (e.g. Carretero and Llorente, 1995).

The preferred body temperature (T_p ; the body temperature selected in the absence of thermoregulatory constraints, Huey and Bennett, 1987) is a biological indicator widely used to characterise the thermoregulation in ectotherms, including heliothermic lizards. This is because it varies within a narrow range (Huey, 1982), correlates with many physiological optima (Huey and Bennett, 1987; Bauwens et al., 1995), is phylogenetically conservative (see below) and remains stable across conspecific populations under contrasting climatic conditions (e.g. Gvozdík and Castilla, 2001). While individual T_p may suffer some variation with time, season, body condition, reproductive status and ontogeny, these biases can be removed by restricting the lizards tested and the experimental conditions (Carretero et al., 2005; Camacho and Rush, 2017). It is also worth noting that, in mountain ectotherms, preferred temperatures are more informative to characterise thermal niche than critical thermal maxima (CT_{max}) and minima (CT_{min}). This is because body temperatures at high elevations do not approach CT_{max}, which is phylogenetically very conservative (Araújo et al., 2013), while both CT_{max} and CT_{min} show little signals of short-term adaptation (Labra et al., 2009).

Conversely, although water would be expected to be as important for life as temperature, hydric ecology has remained largely neglected in lizards. This is probably the consequence of most studies on lizard communities being conducted in deserts, where species tend to be resistant for water loss, or in rainforests where water restrictions are rare. Nevertheless, the evaporative water loss (EWL), either instantaneous or accumulative, is a simple robust physiological measure to assess the hydric trends in the fundamental niche of reptiles (Mautz, 1982; Eynan and Dmi'el, 1993). Remarkably, it was early suggested that thermoregulation and water balance may trade-off in reptiles (Mautz, 1982). Indeed, recent research on temperate species confirms that water loss may constrain thermoregulation, activity, habitat use and distribution (Rato and Carretero, 2015; Ryan et al., 2016; Carneiro et al., 2017; Sannolo et al., 2018; Rozen-Rechels et al., 2020). Not surprisingly, hydric ecophysiology has already been reported to play a prominent role in structuring some lizard communities when access to water is restricted (García-Muñoz and Carretero, 2013; Osojnik et al., 2013; Carneiro et al., 2015; Carretero et al., 2016).

Some of this ecophysiological evidence comes from lacertids. This is a family of generalised lizards, which primarily evolved in the temperate climates of what is now Europe, although later diversified to other environments across Africa and Asia (García-Porta et al., 2019). Lacertids remain the dominant squamate group in the Mediterranean region, not only in terms of evolutionary diversity but also regarding the variety of habitats occupied, from hot desertic regions to high mountains (Arnold, 1987). Remarkably, these studies found a strong evolutionary signal in distribution range, habitat use, activity patterns and ecophysiology. A recent phylogenomic reconstruction and biogeographic analysis (García-Porta et al., 2019) suggests that, leaving the stem group of Gallotiinae apart, a major event in the early Eocene split this family in two branches, Lacertini and Eremiadini, which both developed cool adapted clades that diverged from the rest after the Eocene-Oligocene boundary under relatively stable conditions. This deep phylogenetic signal is, however, less marked in hydric physiology than in thermal physiology, suggesting that adaptation and niche shift are more frequent in the first while niche conservativeness and plasticity (e.g. thermoregulation) prevail in the second (García-Porta et al., 2019).

In this evolutionary scenario, lacertid communities in the Western Palearctic are complex and their assemblage results from ancient

overlap and subsequent segregation between the evolutionary lineages (e.g. genera) of their components (Arnold, 1987). This ancient niche partition makes current competition between lineages less likely than within lineages (Seligmann, 2003; Escoriza, 2018). Of the three classic dimensions of the ecological niche, food, time and space (Pianka, 1986), the latter was once suggested to take precedence in structuring lacertid communities (Arnold, 1987). However, since relations between niche dimensions are intricate, disentangling them may reveal problematic if not artificial. For instance, prey consumption is constrained by microhabitat use, activity time and metabolic requirements (Carretero et al., 2005). Overall, physiology provides a compendium of biological properties of an organism that can be used to reconstruct its fundamental niche projected against the abiotic environment (Kearney and Porter, 2009; Kearney et al., 2013). In absence of barriers, deviations of realized niche from fundamental niche are attributable to biotic interactions, including competition (Sillero, 2011). As commented above, the contribution of species interactions to the community structure will depend on their phylogenetic relationships. However, the environmental context is also relevant. In particular, the same set of species inhabiting suboptimal environments (e.g. in terms of temperature and humidity) is expected to be less interactive and more limited by the abiotic conditions, then revealing their respective historical constraints. This is what has been described for mountain communities when compared to their counterparts in the plain (MacArthur, 1972; Cunningham et al., 2015).

Here, we investigate the ecophysiological patterns of a lacertid community composed of three mountain species belonging to different genera living in sympatry in a high mountain environment from Morocco. By combining systematic field observations and lab experiments on well-recognised ecophysiological indicators, T_p and EWL, we infer the thermal and hydric aspects of their fundamental niches as well as their eventual deviations from realized niche in the field. We explicitly test the hypothesis that intrinsic (historical) differences in thermal and/or hydric ecophysiology shape the activity and habitat use of these species in that community, and whether they mirror their biogeographic trends. Based on the deep evolutionary differentiation between the species in this community and on the restrictive environment, we predict substantial ecophysiological differences and little influence of current interspecific interactions.

Furthermore, the outcomes of this work are also expected to shed light on the vulnerability of lizard species to environmental disturbance, namely, climate change and habitat degradation. Specifically, examining their functional responses to environmental fluctuations instead of correlating them will provide better predictions under novel conditions (Kearney and Porter, 2009) and more objective species prioritization (Ferreira et al., 2016).

2. Material and methods

2.1. Study site

The plateau of Oukaimeden is located in the central block of the High Atlas at 75 km south of Marrakesh (31°12' N, 7°52' W, altitude 2650 m). It is characterized by mountainous vegetation rich in endemic species (Alaoui Haroni et al., 2009). The bioclimate is of the subhumid Mediterranean type, with cold winter variant. Rainfall ranges between 400 and 500 mm per year and snowfall is important between December and March, while summer draught is very short. Freezing days are between 82 and 139 days per year. The temperatures are rather low, the minima in the coldest month average -2.69 °C and the maxima of the hottest month average 23.5 °C (Alaoui Haroni et al., 2009).

2.2. Study community

A lizard assemblage composed of three small-sized lacertids (a fourth large species, *Timon tangitanus* is also present but in very low numbers)

inhabits the Oukaimeden plateau. Remarkably, all three species display a mountain distribution in Morocco although their altitudinal trends differ (Martínez del Marmol et al., 2019). *Podarcis vaucheri*, belongs to a genus with multiple representatives across the Mediterranean Basin with one species complex entering the Maghreb region (Lima et al., 2009; Kalliontzopoulou et al., 2011). In Morocco, it is found in some coastal locations but mostly in mountain ranges with the exception of Beni-Snassen and Anti-Atlas (Caeiro-Dias et al., 2018). *Scelarcis perspicillata* is endemic to the western Maghreb (Morocco and north-western Algeria), mostly in mountains, where it constitutes another species complex (Perera et al., 2007). Finally, *Atlantolacerta andreanskyi* is the only representative of the genus and endemic to Morocco, with populations restricted to the highest peaks of the High Atlas, between 2000 and 3800 m, and its taxonomy also pends revision (Barata et al., 2012).

While found in strict syntopy in Oukaimeden, the three species have been reported to differ in habitat use with *A. andreanskyi* occupying debris and low vegetation at ground level, *S. perspicillata* preferably using vertical rocky surfaces and *P. vaucheri* being more eclectic and also using human buildings (Busack, 1987; Schleich et al., 1996). Patterns of trophic ecology are constrained by lizard body size but essentially parallel these spatial trends, with *P. vaucheri* having a wider trophic niche than the other two species (Carretero et al., 2006a). Phylogenetic relationships identify *A. andreanskyi* as a sister to all other Eremiadini (last common ancestor ~60 Mys), while the other two species are Lacertini although only distantly related (last common ancestor ~35 Mys) (García-Porta et al., 2019).

2.3. Sampling and field body temperature of lizards

Adult lizards of the three studied species were captured in random, one-way transects by using nooses for *S. perspicillata* and *P. vaucheri* (García-Muñoz and Sillero, 2010) and by hand for *A. andreanskyi* during their activity time from 9h00–18h00 GMT during their reproductive season between April and May 2017 (Busack, 1987; Schleich et al., 1996). Within less than 15s upon capture, the cloacal temperature was measured using a K-thermocouple probe associated with a digital thermometer (GHM-Greisinger: GTH 1170, precision 0.1 °C) for measuring the field body temperature (T_b). We recorded the snout–vent lengths (SVL) for each individual using a digital calliper (precision 0.01 mm), and the body mass (BM) with a digital balance (precision 0.01 g).

A random subset of lizards representing all three species were kept in individual plastic terraria (20 × 40 × 25 cm) for no more than five days in captivity with water and food (*Tenebrio molitor* larvae) given ad libitum daily for performing ecophysiological experiments (see below). Only adult males were tested to avoid the influences of reproduction, body condition and ontogeny (Carretero et al., 2005). The experiments of EWL were always performed after experiments of preferred body temperature and with an interval of two days in between, when the lizards were kept in the terraria. The lizards were fed on the first day and fasted the next day before the experiment. At the end of all experiments, the animals were released at the exact locations of their capture.

2.4. Preferred body temperature

The preferred temperature (T_p) was determined for a total of 56 adult male lizards (24 *A. andreanskyi*, 21 *P. vaucheri* and 11 *S. perspicillata*). The experiments were performed in a closed room in the field at Oukaimeden to prevent wind and sun from affecting the temperature in the terrarium. Each lizard was placed in thermal gradient corridor (Min-Max 13–50 °C; 100*30*30 cm) generated by a thermo-Spotlight100W (HOBBY®), fixed 20 cm above the substrate at one extremity of the gradient. The minimum and maximum temperatures of the thermal gradient were recorded using an infrared thermometer pointed to the substrate (FLUKE 572 CF). The bulb was switched on one hour before we placed a lizard into the gradient, and two hours before the first measurement. The experiments were performed from

09h00–19h00 GMT, and the T_p was measured each hour by inserting in the cloaca (~5 mm) a K-thermocouple probe associated with a digital thermometer (GHM-Greisinger: GTH 1170). We limited the between catching the lizard and the measurement of the T_p, to no more than 15 s, to minimize thermal shifts due to stress or contact with the researcher's hand.

2.5. Evaporative water loss rate

For this experiment, our logistical means limited the determination of the rates of EWL to 30 adult male lizards (10 *A. andreanskyi*, 10 *P. vaucheri* and 10 *S. perspicillata*) out of the 56 used to determine the preferred temperature. For each individual, we used two small plastic boxes (15 × 10 × 3 cm ~ 0.45 L). The first one with ventilation holes (in the bottom and at the top) where we placed the lizard, then we placed it in the second box (without ventilations holes or lid) where we installed in the bottom 5 g of silica gel. After that, in groups of five boxes, lizards were placed into an opaque and larger box (40 × 30 × 25 cm ~ 30 L) where we fixed in the bottom of the lid 100 g of orange silica gel in a bag made of gauze. The amount of silica gel used guaranteed a low relative humidity (20–30%) inside each box (Osojnik et al., 2013; Carneiro et al., 2015). Finally, the ensemble was placed in a climatic chamber (POL-EKO-APARATUR SP. J type ST3+/ST3+) fixed at 24 °C. This temperature was selected to allow lizard activity while preventing desiccation stress, which may be the case if lizards remain at T_p for long periods without chance for thermoregulation (Sannolo et al., 2018; Sannolo and Carretero, 2019). The experiment ran from 08h00–20h00 GMT. Conditions inside the chamber were monitored with a hygrometer (Beurer HM 16, precision 0.1%). To standardize both factors, the experiments were conducted under room temperature (~24 °C) and low humidity conditions (20–30%). Every hour, the lizards were individually removed from the climatic chamber, weighted together with the box using a digital balance (precision ± 0.0001 g; RADWAG, AS 110/C/2), and immediately placed back into their respective boxes in the climatic chamber. Lizards weights were obtained by subtracting the weights of the respective individual boxes, to minimize stress and hyperventilation of them, which may increase EWL. The whole measurement procedure took no longer than 20 s to let the scale stabilize.

2.6. Thermal and hydric characterization of habitats

For thermal and hydric characterization of habitats and microhabitats use (Tracy, 1982; Bakken, 1992; Walsberg and Wolf, 1996; Bouazza et al., 2016), we installed 12 iButton dataloggers (DS1923-F5# Hygrochron Temperature and Humidity Data Logger. Maxim Integrated, San José, CA, USA, precision 0.001 °C, 0.001%) randomly placed in two microhabitat types; under rocks, in full shade and on open ground in full sun, which were expected to provide an indication of the spectrum of environmental conditions available to the lizards during the sampling period. To define the thermal conditions of the study area in Oukaimeden plateau, these dataloggers were programmed to record the site temperature and humidity every hour during the entire study period (April–May 2017). These data are detailed in Supplementary materials.

2.7. Statistical analyses

We first used (co)variance analyses with repeated measurements (AN(C)OVA-rm) to determine the variation in T_p according to species and time interval (within-subject factor). In the second step, SVL and body mass of the lizards were incorporated as covariates to account for the effect of size and shape (Ferreira et al., 2016). For water loss experiments, we also used AN(C)OVA-rm to determine the differences in instantaneous water loss (EWLi = [(W_n - W_{n+1})/W₀] where W is the weight) between species and time intervals, adding as variables the SVL and body masses of the lizards. We also calculated the accumulated water loss for the 11 intervals (EWLa = [(W₀ - W_n)/W₀] where W is the

weight) and compared it between species using AN(C)OVA, with also SVL and body mass as covariates. Where significant, Duncan's post hoc tests were performed between species pairs to detect any significant differences.

All the analyses were performed in IBM SPSS Statistics version 22.0 (IBM, 2013).

3. Results

3.1. Sampling and field body temperature

During the period of study, we sampled 356 lizards for the field data (152 *A. andreanskyi*, 119 *P. vaucheri* and 85 *S. perspicillata*). The three species of Oukaimeden plateau did not differ significantly in their Tb (ANOVA, $F_{2,353} = 0.63$; $p = 0.53$), but they were statistically different in their SVL (ANOVA, $F_{2,353} = 777.06$; $p < 0.001$) and robustness (weight accounting for SVL; $F_{2,352} = 52.81$; $p = 10^{-6}$). *Scelarcis perspicillata* was the most robust followed by *P. vaucheri* and finally the most gracile *A. andreanskyi* (Duncan tests, $p < 0.001$) (Table 1).

3.2. Preferred body temperature

The results of the preferred temperature (Table 1, Fig. 1), showed no difference between the three Lacertidae species of Oukaimeden (ANOVA-rm, species $F_{2,53} = 0.12$; $p = 0.89$; time*species $F_{20,350} = 0.60$; $p = 0.80$), but a significant variation among time interval with trend of decrease of Tp (time $F_{10,350} = 3.58$; $p = 0.005$). Neither SVL or weight had an influence on these results (species $F_{2,51} = 0.26$; $p = 0.77$; time $F_{10,510} = 0.80$; $p = 0.54$; time*species $F_{20,510} = 0.60$; $p = 0.80$).

3.3. Evaporative water loss

Our analysis of EWL_a (Fig. 2) rate as function of species and among time interval showed a significant difference (time $F_{12,324} = 160.35$; $p < 0.001$; species $F_{2,27} = 3.38$; $p = 0.049$; time*species $F_{24,324} = 3.26$; $p = 0.022$), with *A. andreanskyi* having the higher trend of losing water followed by *S. perspicillata* and then *P. vaucheri*. Rate of EWL_i (Fig. 3) differed between species and among time interval (time $F_{11,297} = 3.29$; $p = 0.015$; species $F_{2,27} = 3.77$; $p = 0.036$) but not for the time profile by species (time*species $F_{22,297} = 0.87$; $p = 0.54$), with no clear trends. Analysis of EWL_t rate by ANOVA test showed a significant difference ($F_{2,27} = 3.94$ $p = 0.031$) and that *A. andreanskyi* have the highest rate (mean \pm SD %) of $6.34 \pm 1.24\%$ followed by *S. perspicillata* with a rate of $5.42 \pm 2.95\%$, and by *P. vaucheri* with the lowest rate of $3.88 \pm 1.26\%$.

3.4. Thermal and hydric characterization of habitats

Data of temperature and relative humidity were filtered to retain only daytime measurements (08h00 - 18h00; GMT). We found a significant difference between typical habitat used (site) by the three

species and also between microhabitats (position of dataloggers) characterized by the data recorded for both parameters (Temperature, month $F_{1,3860} = 128,09$ $p < 0,001$; site of species $F_{2,3860} = 92.76$ $p < 0.001$; position $F_{2,3860} = 929.56$ $p < 0.001$; month*site of species $F_{2,3860} = 92.92$ $p < 0.001$; month*site of species*position $F_{3,3860} = 103.61$ $p < 0.001$; Humidity, month $F_{1,363} = 269.05$ $p < 0.001$; site of species $F_{1,363} = 774.17$ $p < 0.001$; position $F_{1,363} = 429.66$ $p < 0.001$; month*site of species $F_{1,363} = 9.65$ $p = 0.002$). For both parameters, we found two groups one with the locations of *A. andreanskyi* and the other grouping those of *P. vaucheri* and *S. perspicillata*.

4. Discussion

The hypothesis that the sympatric lacertid species would differ in ecophysiology according to their phylogeny and biogeography was here confirmed, but only for hydric ecophysiology. Thermal ecophysiology was essentially similar across species and, when monitored throughout time, it appeared subsidiary to hydric ecophysiology (see below). Also as predicted, the use of space and time by the three species suggests ancient niche partitioning rather than current competition as main driver of the community structure.

Despite the prolonged independent evolution (~60-35 Mys) between all three species, they seem to have developed similar thermal preferences. Since each species (but especially *A. andreanskyi*) has closer thermophilic relatives in the phylogeny, our results agree with a scenario of convergent adaptation to cold conditions in lacertid lineages as proposed by García-Porta et al. (2019). Thus, the high mountain environments have likely operated by filtering the occurrence of cold-adapted species to assemble the community rather than by promoting thermal adaptation in situ. Remarkably, the thermal preferences of the three species in the lab were similarly realized as body temperatures in the field despite the considerable thermal restrictions of the environment and the interspecific differences in microhabitat use (Schleich et al., 1996). This contrasts with lowland lacertid communities often resulting from the admixture of cold- and hot-adapted elements inhabiting thermally heterogenous landscapes (Bellière et al., 1996; Ferreira et al., 2016; Li et al., 2017; Pafilis et al., 2017; Sagonas et al., 2017).

In contrast, the three species clearly differed in their resistance to water loss under standard conditions. *Atlantolacerta andreanskyi* lost water the most, *P. vaucheri* the least and *S. perspicillata* attained intermediate rates but different from both. Even if the small size of *A. andreanskyi* might have suggested that this was consequence of body size and surface/volume ratio (Schmidt-Nielsen, 1997), the intermediate rate of *S. perspicillata*, the largest species, contradicted this initial conclusion and advocates for interpreting the differences in hydric physiology as species-specific. Working at a wide phylogenetic scale, García-Porta et al. (2019) concluded that hydric physiology evolved faster than thermal physiology in lacertids. Differences in hydric ecology between *Podarcis* species also seem to support this view (Ferreira et al.,

Table 1

Sample size; mean, maximum, minimum, and confidence interval (CI) of the mean of body mass (BM), snout-vent length (SVL), field body temperature (Tb), preferred temperature (Tp), total accumulated evaporative water loss (EWL_t) and instantaneous evaporative water loss EWL_i calculated for each species, *Atlantolacerta andreanskyi* (Aa), *Podarcis vaucheri* (Pv) and to *Scelarcis perspicillata* (Sp).

Species	BM		SVL		Tb		Tp (°C)		EWL _a (%)		EWL _i (%)	
	N	Mean \pm CI	Mean \pm CI	Mean \pm CI	N	Mean \pm CI	N	Mean \pm CI	Mean \pm CI	Mean \pm CI		
		Min-Max	Min-Max	Min-Max	Min-Max	Min-Max	Min-Max	Min-Max	Min-Max	Min-Max		
<i>A. andreanskyi</i>	152	1,96 \pm 0,04 1,60–2,60	44,02 \pm 0,41 39,00–50,00	28,82 \pm 0,69 19,2–36,40	24	31,75 \pm 1,34 13,90–37,00	10	6,34 \pm 0,77 5,27–8,92	0,47 \pm 0,11 0,17–0,97			
<i>S. perspicillata</i>	85	4,58 \pm 0,12 3,40–5,50	59,86 \pm 0,46 55,00–65,00	28,54 \pm 1,09 21,10–36,00	11	31,64 \pm 2,35 13,50–38,8	10	5,42 \pm 1,83 1,75–10,39	0,37 \pm 0,21 0,05–1,82			
<i>P. vaucheri</i>	119	2,97 \pm 0,12 1,70–4,30	51,65 \pm 0,70 43,00–59,00	27,90 \pm 0,50 23,70–35,20	21	31,46 \pm 1,78 13,60–37,20	10	3,88 \pm 0,78 2,16–5,39	0,31 \pm 0,08 0,12–0,79			
Statistical group	{Aa} {Pv} {Sp}		{Aa} {Pv} {Sp}	{Aa, Pv, Sp}	{Aa, Pv, Sp}		{Aa, Sp} {Sp, Pv}	{Aa, Sp} {Sp, Pv}	{Aa, Sp} {Sp, Pv}			

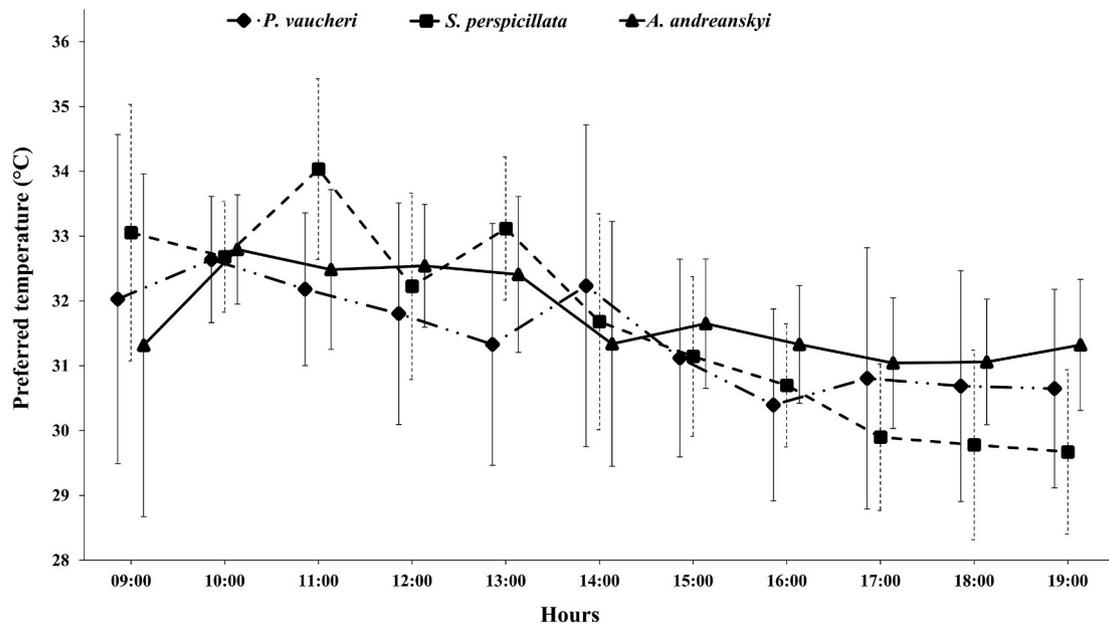


Fig. 1. Daily variation of the preferred body temperatures (T_p) of three Lacertidae species. Displayed are mean values and 0.95 confidence intervals.

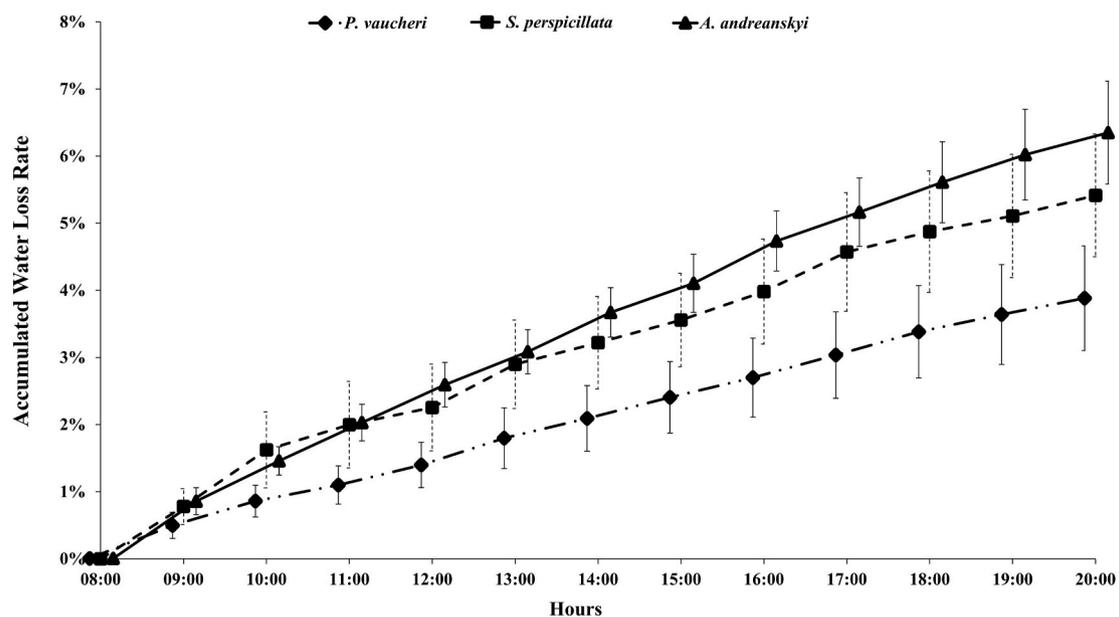


Fig. 2. Daily variation of the accumulated water loss (EWLa) of three Lacertidae species. Displayed are mean values and 0.95 confidence intervals.

2016; Sannolo et al., 2018), which may be also confirmed by further ecophysiological studies within the *A. andreanskyi* and the *S. perspicillata* complexes. Studies on other lacertid genera also indicate flexibility of water loss rates between congeneric species (Carneiro et al., 2017) and even between conspecific populations (Sannolo et al., 2020).

In the field, *A. andreanskyi* apparently compensated its vulnerability to water loss by using more humid habitats, and by restricting its activity window (see also Busack, 1987). Differences in the hydric environment used by *S. perspicillata* and *P. vaucheri* were unclear, although they strongly differ in habitat use, foraging strategy and antipredator behaviour (Schleich et al., 1996; Carretero et al., 2006a, b; Damas-Moreira et al., 2014). Significantly, preferred temperatures of all three species declined throughout time in the experiments and such decline was steeper in *A. andreanskyi* and *S. perspicillata*. In the field, lizards shifted microhabitat selection in the afternoon even when

thermal conditions were similar to those in the morning. Both sources of evidence suggest that, even if water availability is less restrictive in mountain environments than in lowlands on a seasonal perspective, hydric ecology still constraints daily rhythms of thermoregulation in lizards as has already been reported for Mediterranean and arid environments (Belasen et al., 2016; Carretero et al., 2016; Sannolo and Carretero, 2019).

Thus, contrary to the initial expectations, hydric ecophysiology seems to take precedence over thermal ecophysiology in the organisation of this mountain community inhabiting a restrictive environment, namely, by constraining the activity patterns, habitat use (Schleich et al., 1996) and trophic ecology (Carretero et al., 2006a). No apparent effects of competition can be inferred from these results although this is to be confirmed further experimental work (see Damas-Moreira et al., 2020).

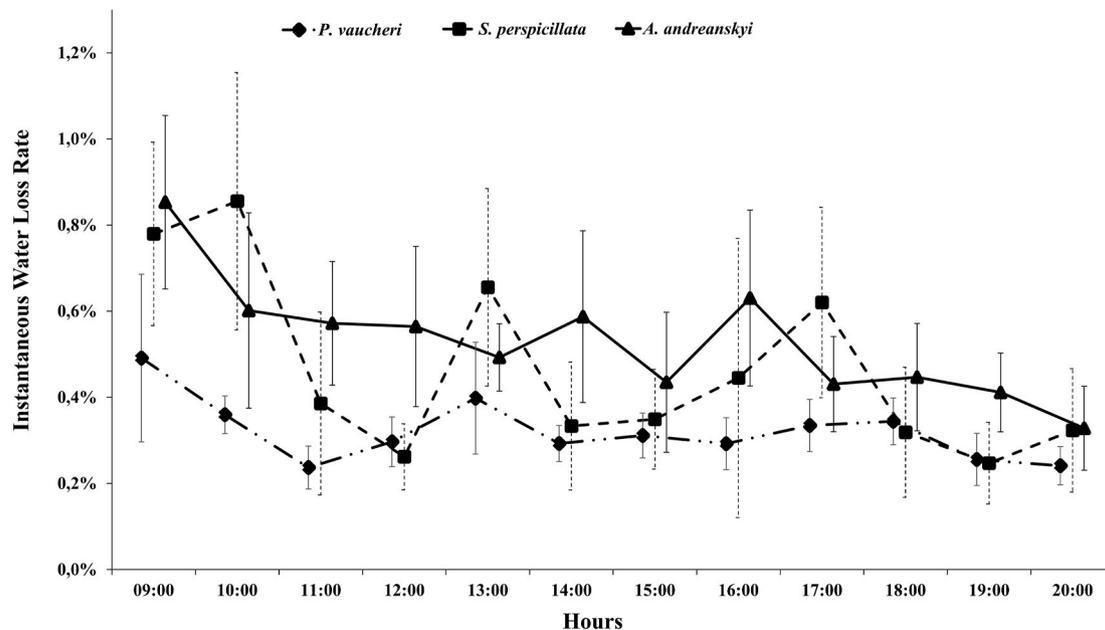


Fig. 3. Daily variation of the instantaneous water loss (EWLi) of three Lacertidae species. Displayed are mean values and 0.95 confidence intervals.

Our ecophysiological results mirror the biogeographic patterns of these species in Morocco (Martínez del Marmol et al., 2019), namely, that the degree of range restriction paralleled the sensitiveness to dehydration across species. In fact, in the correlative ecological models of occurrence of these species, precipitation variables contributed more than temperature variables, while vegetation cover also carried substantial explanatory power (Kaliontzopoulou et al., 2008; Martínez-Freiría et al., 2013). Relevant implications for conservation can be derived from these findings. Moroccan mountain lacertids are certainly cold-adapted when compared to other species living in lowlands (García-Porta et al., 2019), which makes them vulnerable to global warming as lizards worldwide (Sinervo et al., 2010). However, their weak resistance to water loss increases such vulnerability. If warming is accompanied by aridification as forecasted by climate change scenarios for the next decades in this region (Hijmans et al., 2005), *A. andreanskyi* and, secondly, *S. perspicillata* will be suffering stronger range contractions and population declines than *P. vaucheri*, and hydric ecophysiology will play a role in these processes. In this context, conserving natural habitats, and especially plant cover and water bodies, seems the best management strategy at local level, while deforestation, forest fires, water overexploitation and intensification of agricultural practices will only increase such negative trend (Ferreira et al., 2016; Chergui et al., 2020). From this perspective, a functional approach based on physiological evidence, not restricted to thermal parameters, will improve our understanding of the current organisation of lizard biodiversity at different spatial scales and also of their responses to human disturbance, including global change (Huey et al., 2012; Logan et al., 2015; Wang et al., 2016; Nowakowski et al., 2018).

Declaration of competing interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jtherbio.2020.102743>.

Ethics approval

All sampling and experimental work was performed in accordance with the scientific permit Decision N° May 2013 HCEFLCD/DLCDPN/DPRN/CFF provided by the High Commissariat for Water and Forest, Morocco.

References

- Adolph, S.C., Porter, W.P., 1993. Temperature activity and lizard life histories. *Am. Nat.* 142, 273–295.
- Alaoui Haroni, S., Alifriqui, M., Simonneaux, V., 2009. Recent dynamics of the wet pastures at Oukaimeden plateau (high Atlas mountains, Morocco). *Biodivers. Conserv.* 18 (1), 167–189.
- Angilletta, M.J., 2009. *Thermal Adaptation: A Theoretical and Empirical Synthesis*. Oxford University Press. <https://doi.org/10.1093/acprof:oso/9780198570875.001.1>.
- Angilletta, M.J., Cooper, B.S., Schuler, M.S., Boyles, J.G., 2010. The evolution of thermal physiology in endotherms. *Front. Biosci.* E2, 861–881.
- Araújo, M.B., Ferri-Yañez, F., Bozinovic, F., Marquet, P.A., Valladares, F., Chown, S.L., 2013. Heat freezes niche evolution. *Ecol. Lett.* 16, 1206–1219.
- Arnold, E.N., 1987. Resource partition among lacertid lizards in southern Europe. *Journal of Zoology London. (B)* 1, 739–782.
- Bakken, G.S., 1992. Measurement and application of operative and standard operative temperatures in ecology. *Am. Zool.* 32 (2), 194–216.
- Barata, M., Carranza, S., Harris, D.J., 2012. Extreme genetic diversity in the lizard *Atlantolacerta andreanskyi* (Werner, 1929): a montane cryptic species complex. *BMC Evol. Biol.* 12 (1), 167. <https://doi.org/10.1186/1471-2148-12-167>, 2012.
- Bauwens, D., Garland, T., Castilla, A.M., Van Damme, A., 1995. Evolution of sprint speed in lacertid lizards: morphological, physiological, and behavioural covariation. *Evolution* 49, 848–863.
- Belasen, A., Brock, K., Li, B., Chremou, D., Valakos, E.D., Pafilis, P., Sinervo, B., Foutoulas, J., 2016. Fine with heat, problems with water: microclimate alters water loss in a thermally adapted insular lizard. *Oikos* 126, 447–457.
- Belliure, J., Carrascal, L.M., Diaz, J.A., 1996. Covariation of thermal biology and foraging mode in two mediterranean lacertid lizards. *Ecology*. <https://doi.org/10.2307/2265585>.
- Bouazza, A., Slimani, T., El Mouden, E.H., Blouin-Demers, G., Lourdaux, O., 2016. Thermal constraints and the influence of reproduction on thermoregulation in a high-altitude gecko (*Quedenfeldtia trachyblepharus*). *J. Zool.* 300, 36–44.
- Busack, S.D., 1987. Notes on the biology of *Lacerta andreanskyi* (Reptilia: Lacertidae). *Amphibia-Reptilia* 8, 231–236.

- Caiero-Dias, G., Luis, C., Pinho, C., Crochet, P.A., Sillero, N., Kaliontzopoulou, A., 2018. Lack of congruence of genetic and niche divergence in *Podarcis hispanicus* complex. *J. Zool. Syst. Evol. Res.* <https://doi.org/10.1111/jzs.12219>.
- Camacho, A., Rush, T.W., 2017. Methods and pitfalls of measuring thermal preference and tolerance in lizards. *J. Therm. Biol.* 68, 63–72.
- Carneiro, D., García-Muñoz, E., Kaliontzopoulou, A., Llorente, G.A., Carretero, M.A., 2015. Comparing ecophysiological traits in two *Podarcis* Wall lizards with overlapping ranges. *Salamandra* 51, 335–344.
- Carneiro, D., García-Muñoz, E., Zagar, A., Pafilis, P., Carretero, M.A., 2017. Is ecophysiology congruent with the present-day relictual distribution of a lizard group? Evidence from preferred temperatures and water loss rates. *Herpetol. J.* 27, 47–56.
- Carretero, M.A., Llorente, G.A., 1995. Thermal and temporal patterns of two Mediterranean Lacertidae. In: Llorente, G.A., Montori, A., Santos, X., Carretero, M.A. (Eds.), *Scientia Herpetologica*. Barcelona, Asociación Herpetológica Española, pp. 213–223.
- Carretero, M.A., Lopes, E., Vasconcelos, R., 2016. An ecophysiological background for biogeographic patterns of two island lizards? *Sci. Nat.* 103, 97.
- Carretero, M.A., Perera, A., Harris, D.J., Batista, V., Pinho, C., 2006a. Spring diet and resource partitioning in an alpine lizard community from Morocco. *Afr. Zool.* 41 (1), 113–122.
- Carretero, M.A., Roig, J.M., Llorente, G.M., 2005. Variation in preferred body temperature in an oviparous population of *Lacerta (Zootoca) vivipara*. *J. Herpetol.* 15, 51–55.
- Carretero, M.A., Vasconcelos, R., Fonseca, M., Kaliontzopoulou, A., Brito, J.C., Harris, D. J., Perera, A., 2006b. Escape tactics of two syntopic forms of the *Lacerta perspicillata* complex with different colour patterns. *Can. J. Zool.* 84 (11), 1594–1603.
- Chergui, B., Pleguezuelos, J.M., Fahd, S., Santos, X., 2020. Modelling functional response of reptiles to fire in two Mediterranean forest types. *Sci. Total Environ.* 732, 139–205.
- Cunningham, H.R., Rissler, L.J., Buckley, L.B., Urban, M.C., 2015. Abiotic and biotic constraints across reptile and amphibian ranges. *Ecography* 38, 1–8.
- Damas-Moreira, I., Riley, J.L., Carretero, M.A., Harris, D.J., Whiting, M.J., 2020. Getting ahead: exploitative competition by an invasive lizard. *Behavioral Ecology and Sociobiology*.
- Damas-Moreira, I., Tomé, B., Harris, D.J., Maia, J.P., Salvi, D., 2014. Moroccan herpetofauna: distribution updates. *HERPETOZOA* 27 (1–2), 96–102.
- Escoriza, D., 2018. Patterns of alpha diversity among Tunisian lizards (Lacertidae). *J. Arid Environ.* 151, 23–30.
- Eynan, M., Dmi'el, R., 1993. Skin resistance to water loss in agamid lizards. *Oecologia* 95, 290–294. <https://doi.org/10.1007/BF00323502>.
- Ferreira, C.C., Santos, X., Carretero, M.A., 2016. Does ecophysiology mediate reptile responses to fire regimes? Evidence from Iberian lizards. *Peer J* 4, e2107.
- García-Muñoz, E., Carretero, M.A., 2013. Comparative ecophysiology of two sympatric lizards. Laying the groundwork for mechanistic distribution models. *Acta Herpetol.* 8, 123–128.
- García-Muñoz, E., Sillero, N., 2010. Two new types of noose for capturing herps. *Acta Herpetol.* 5, 259–263.
- García-Porta, J., Irisarri, I., Kirchner, M., et al., 2019. Environmental temperatures shape thermal physiology as well as diversification and genome-wide substitution rates in lizards. *Nat. Commun.* 10, 40–77.
- Gvozdík, L., Castilla, A.M., 2001. A comparative study of preferred body temperatures and critical thermal tolerance limits among populations of *Zootoca vivipara* (Squamata: Lacertidae) along an altitudinal gradient. *J. Herpetol.* 35, 486–492.
- Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., Jarvis, A., 2005. Very high-resolution interpolated climate surfaces for global land areas. *Int. J. Climatol.* 25, 1965–1978.
- Huey, R.B., 1982. Temperature, physiology, and the ecology of reptiles. In: Gans, C., Pough, F.H. (Eds.), *Biology of the Reptilia*. Physiology (C), vol. 12. Academic Press, New York, pp. 25–91.
- Huey, R.B., Bennett, A.F., 1987. Phylogenetic studies of coadaptation: preferred temperatures versus optimal performance temperatures of lizards. *Evolution* 41, 1098–1115.
- Huey, R.B., Kearney, M., Krokenberger, A., Holtum, J.A.M., Jess, M., Williams, S.E., 2012. Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. *Philosophical Transactions of the Royal Society B* 367, 1165–1679.
- IBM, Corp., 2013. IBM SPSS Statistics for Windows, Version 22.0. IBM Corp. Armonk, Armonk, NY.
- Kaliontzopoulou, A., Brito, J., Carretero, M.A., Larbes, S., Harris, D.J., 2008. Modelling the partially unknown distribution of wall lizards *Podarcis* in North Africa: ecological affinities, potential areas of occurrence and methodological constraints. *Can. J. Zool.* 86, 992–1101.
- Kaliontzopoulou, A., Pinho, C., Harris, D.J., Carretero, M.A., 2011. When cryptic diversity blurs the picture: a cautionary tale from Iberian and North African *Podarcis* wall lizards. *Biol. J. Linn. Soc.* 103, 779–800.
- Kearney, M., Porter, W.P., 2009. Mechanistic niche modelling: combining physiological and spatial data to predict species ranges. *Ecol. Lett.* 12, 334–350.
- Kearney, M., Simpson, S.J., Raubenheimer, D., Kooijman, A.L.M., 2013. Balancing heat, water and nutrients under environmental change: a thermodynamic niche framework. *Funct. Ecol.* 27, 950–965.
- Labra, A., Pienaar, J., Hansen, T.F., 2009. Evolution of thermal physiology in *Liolaemus* lizards: adaptation, phylogenetic inertia, and niche tracking. *Am. Nat.* 175, 204–220.
- Li, S.R., Wang, Y., Ma, L., Zeng, Z.G., Bi, J.H., Du, W.G., 2017. Thermal ecology of three coexistent desert lizards: implications for habitat divergence and thermal vulnerability. *J. Comp. Physiol. B* 187, 1009–1018.
- Lima, A., Larbes, S., Pinho, C., Carretero, M.A., Brito, J.C., Harris, D.J., 2009. Relationships of *Podarcis* wall lizards from Algeria based on mtDNA data. *Amphibia-Reptilia* 30 (4), 483–492.
- Logan, M.L., Fernandez, S.G., Calsbeek, R., 2015. Abiotic constraints on the activity of tropical lizards. *Funct. Ecol.* 29, 694–700.
- Lomolino, M.V., Riddle, B.R., Whittaker, R.J., Brown, J.H., 2010. *Biogeography*, fourth ed. Sinauer Associates, Sunderland.
- MacArthur, R.H., 1972. *Geographical Ecology: Patterns in the Distribution of Species*. Harper & Row, New York.
- Martínez del Mármol, G., Harris, D.J., Geniez, P., de Pous, P., Salvi, D., 2019. *Amphibians and Reptiles of Morocco*. Edition Chimaira, Frankfurt, Germany.
- Martínez-Freiría, F., Argaz, H., Fahd, S., Brito, J.C., 2013. Climate change is predicted to negatively influence Moroccan endemic reptile richness. Implications for conservation in protected areas. *Naturwissenschaften* 100, 877–889.
- Mautz, W.J., 1982. Patterns of evaporative water loss. In: Gans, C., Pough, F.H. (Eds.), *Biology of the Reptilia*, vol. 12. Academic Press, New York, pp. 443–481.
- Nowakowski, A.J., Watling, J.I., Thompson, M.E., Bruschi, G.A.I., Catenazzi, A., Whitfield, S.M., Kurz, D.J., Suárez-Myorga, A., Aponte-Gutiérrez, A., Donnelly, M.A., Todd, B.D., 2018. Thermal biology mediates responses of amphibians and reptiles to habitat modification. *Ecol. Lett.* 21, 345–355.
- Osojnik, N., Žagar, A., Carretero, M.A., García-Muñoz, E., Vrežec, A., 2013. Ecophysiological dissimilarities of two sympatric lizards. *Herpetologica* 69, 445–454.
- Pafilis, P., Maragou, P., Sagonas, K., Valakos, E.D., 2017. Partitioning thermal habitat on a vertical rock, a herculean task. *J. Therm. Biol.* 70, 54–60.
- Perera, A., Vasconcelos, R., Harris, D.J., Brown, R.P., Carretero, M.A., Pérez-Mellado, V., 2007. Complex patterns of morphological and mtDNA variation in *Lacerta perspicillata* (Reptilia; Lacertidae). *Biol. J. Linn. Soc.* 90, 479–490.
- Pianka, E.R., 1986. *Ecology and Natural History of Desert Lizards*. Princeton University Press, New Jersey.
- Pianka, E.R., Vitt, L.J., 2006. *Lizards: Windows to the Evolution of Diversity*. University of California Press, Berkeley.
- Pianka, E.R., Vitt, L.J., Pelegrin, N., Fitzgerald, D.B., Winemiller, K.O., 2017. Toward a periodic table of niches, or exploring the lizard niche hypervolume. *Am. Nat.* 190, 601–606.
- Rato, C., Carretero, M.A., 2015. Ecophysiology tracks phylogeny and meets ecological models in an Iberian gecko. *Physiol. Biochem. Zool.* 88, 564–575.
- Rozen-Rechels, D., Dupoué, A., Meylan, S., Qitout, K., Decencière, B., Agostini, S., Le Galliard, J.F., 2020. Acclimation to water restriction implies different paces for behavioral and physiological responses in a lizard species. *Physiol. Biochem. Zool.* 93, 160–164.
- Ryan, M.J., Latella, I.M., Giermakowski, J.T., Snell, H., Poe, S., Pangle, R.E., Gehres, N., Pockman, W.T., McDowell, N.G., 2016. Too dry for lizards: short-term rainfall influence on lizard microhabitat use in an experimental rainfall manipulation within a piñon-juniper. *Funct. Ecol.* 30, 964–973.
- Sagonas, K., Kapsalas, G., Valakos, E.D., Pafilis, P., 2017. Living in sympatry: the effect of habitat partitioning on the thermoregulation of three Mediterranean lizards. *J. Therm. Biol.* 65, 130–137.
- Sannolo, M., Barroso, F.M., Carretero, M.A., 2018. Physiological differences in preferred temperatures and evaporative water loss rates in two sympatric lacertid species. *Zoology* 126, 58–64.
- Sannolo, M., Carretero, M.A., 2019. Dehydration constraints thermoregulation and space use in lizards. *PLoS One* 14 (7), 220–384.
- Sannolo, M., Civantos, E., Martín, J., Carretero, M.A., 2020. Variation in field body temperature and resistance to dehydration along an environmental gradient in a diurnal ectotherm. *J. Zool.* 310, 221–231.
- Schleich, H.H., Kästle, W., Kabish, K., 1996. *Amphibians and Reptiles of North Africa*. Koeltz Scientific Books, Koenigstein.
- Schmidt-Nielsen, K., 1997. *Animal Physiology: Adaptation and Environment*. Cambridge University Press, ISBN 0521570980, p. 607, 9780521570985.
- Sears, M.W., Angilletta, M.J., 2015. Costs and benefits of thermoregulation Revisited: both the heterogeneity and spatial structure of temperature drive energetic costs. *Am. Nat.* 185, 94–102.
- Seligmann, H., 2003. Resource partition history and evolutionary specialization of subunits in complex systems. *Biosystems* 51, 31–39.
- Sillero, N., 2011. What does ecological modelling model? A proposed classification of ecological niche models based on their underlying methods. *Ecological Modelling* 222, 1343–1346.
- Sinervo, B., Méndez de la Cruz, F., Miles, D.B., et al., 2010. Erosion of lizard diversity by climate change and altered thermal niches. *Science* 328, 894–899.
- Tracy, C.R., 1982. Biophysical modeling in reptilian physiology and ecology. *Biol. Reptil.* 12, 275–315.
- Walsberg, G.E., Wolf, B.O., 1996. A test of the accuracy of operative temperature thermometers for studies of small ectotherms. *Journal of Thermal Biology* 21, 275–281.
- Wang, Y., Zeng, Z.G., Li, S.R., Bi, J.H., Du, W.G., 2016. Low precipitation aggravates the impact of extreme high temperatures on lizard reproduction. *Oecologia* 182, 961–971.
- Webb, C.O., Ackerly, D.D., McPeck, M.A., Donoghue, M.J., 2002. Phylogenies and community ecology. *Annu. Rev. Ecol. Systemat.* 33, 475–505.