

Variation in field body temperature and total evaporative water loss along an environmental gradient in a diurnal ectotherm

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Abstract

The maintenance of optimal body temperatures has profound consequences on all aspects of ectotherms life history, like fitness and performance, and has been the subject of research for decades. In contrast, for reptiles, comparatively less is known on the potential effects of water balance on physiology, ecology and behaviour. In recent years, several recent studies are pointing out the importance of studying thermoregulation and water balance within the same framework. Here, we used a Mediterranean lizard, *Psammotromus algirus*, to investigate how field body temperature and water loss rates may vary among populations, between sexes and along an altitudinal gradient. We found little variation in field body temperatures among populations, while within each population, field body temperature may change daily, seasonally and differ between sexes. On the contrary, water loss rates decreased with elevation and showed no seasonal trend or difference between sexes. Microclimatic data indicated that highland lizards experienced more fluctuating conditions, a factor that may explain their lower water loss rates. Other factors, like inter-population differences in ectoparasite intensity, might also contribute in explaining the observed patterns. We present here the first data for a lacertid lizard on the inter-population variability and sexual difference in water loss rates and point out that water balance may play a fundamental role in regulating lizard activity during the hottest and driest period of the year.

Introduction

Temperature has deep effects on every facet of ectotherms biology, including fitness and performance (Angilletta, 2009). Ectotherms can regulate their body temperature using behavioural adjustments (Stevenson, 1985), physiological mechanisms, and by changing the daily and seasonal patterns of activity (Adolph & Porter, 1993). Ectotherms can adjust their body temperature by exposing to or retreating from direct or indirect solar radiation (Cossins & Bowler, 1987), and body temperature is often kept within often narrow species-specific ranges (Cowles & Bogert, 1944; Cossins & Bowler, 1987). Thermoregulation is thus used to optimize performance (Angilletta, Niewiarowski & Navas, 2002) and maximize fitness (Andrews & Schwarzkopf, 2012), by achieving and maintaining optimal temperatures for digestion (Van Damme, Bauwens & Verheyen, 1991), locomotion (Angilletta, Hill & Robson, 2002), reproductive output (Van Damme *et al.*, 1992), avoid predators (Christian & Tracy, 1981) and fighting infections (Rakus, Ronsmans & Vanderplasschen, 2017).

While the ecological and adaptive significance of thermoregulation has been the subject of studies for decades (Cowles & Bogert, 1944; Licht *et al.*, 1966; Huey, 1982; Angilletta, 2009), comparatively less is known on the role of water in reptile ecophysiology, at least when compared to other ectotherms like amphibians. Dehydration status or access to water is often used as modulatory factors to explain patterns in reptile life history, ecology and behaviour. For example, restriction in water availability may constrain growth rate (Lorenzon *et al.*, 1999), alter habitat selection (Neilson, 2002), affect immune function (Moeller, Butler & DeNardo, 2013) and trigger mother-offspring conflicts (Dupoué *et al.*, 2015a). In a few cases, morphological features are used to reduce dehydration (Davis & DeNardo, 2007) or to exploit water from non-conventional sources, like in the case of rain-harvesting species (Bentley & Blumer, 1962). Still, several research areas, particularly regarding the potential interaction between thermal and water ecology, remain mostly unexplored.

Several recent studies are pointing out the importance of studying thermoregulation and water balance within the same framework. For example, it has been shown that lizards'

thermoregulatory activity may be affected by short-term rainfall regime (Ryan *et al.*, 2016) and dehydration status (Sannolo *et al.* in review). Also, while their thermal preferences may be similar, lizard populations that have access to limited free-standing water may be more resistant to water loss than those populations with unrestricted access to water (Dupoué *et al.*, 2017). However, the research on the ecological, physiological and adaptive role of water in reptiles, and especially in lizards, still lags behind if compared with the information available on thermal biology. For example, it is mostly unknown to what extent water loss rates may vary across environmental gradients, or whether it is subject to daily and seasonal plasticity, like thermal preferences often are.

The large psammodromus, *Psammodromus algirus*, is a medium-sized lacertid lizard (snout-to-vent length, SVL up to 93 mm; Fig. 1), widely distributed in Mediterranean environments of the Iberian Peninsula, SW France and NW Africa (Sindaco & Jeremčenko, 2008). This species serves as an excellent model for the present study because while patterns of variation in body temperature have been studied extensively in lizards, relatively little information is available on the potential variability of water loss rates. It can be found in a variety of environments (e.g. from arid to more humid ones). The large psammodromus prefers high body temperatures if compared with most other lacertid species: its preferred temperature, measured in standard thermal gradients, is around 35.6°C (Díaz, 1997). In the field, body temperatures ranged 30–33°C and are subjected to daily and seasonal variation (Carrascal *et al.*, 1989; Díaz, Iraeta & Monasterio, 2006). This species thermoregulate carefully by selecting the appropriate microhabitats and heat sources (Belliere & Carrascal, 2002). Little variability in selected body temperatures was detected along altitudinal gradients (Díaz *et al.*, 2006; Zamora-Camacho, Reguera & Moreno-Rueda, 2016). This species is polygynic and older males are strongly territorial. If territories vary in thermal quality, inter-sexual differences in body temperature may result as a consequence of reproductive behaviour (Díaz, 1993; Iraeta, Salvador & Díaz, 2012). On the contrary, scarce information is available on water loss rates and its potential variability among populations or across environmental gradients. Only a previous study reported the amount of water loss at low body temperature (~24°C) throughout a day from a single locality (Ferreira *et al.*, 2016). The populations studied here (see below) all belong to the same clade and show both little genetic differentiation and high gene flow (Verdú-Ricoy *et al.*, 2010; Díaz *et al.*, 2017). Consequently, we would expect to attribute variability among populations to acclimation to local conditions (i.e. phenotypic plasticity) rather than to genetic adaptation.

In this study, we examine the variation in body temperatures and water loss of *P. algirus* lizards along an elevation gradient. The hypotheses of the present work were multiples. (1) Field body temperatures and water loss rates should vary depending on the elevation, even though previous evidence suggested that the thermal ecology of *P. algirus* may be somewhat conservative and insensible to changes along elevations (Díaz *et al.*, 2006; Zamora-Camacho *et al.*, 2016). Water loss rates in lizards may differ among species due to adaptation (Cox &



Figure 1 An adult male (top) and an adult female (bottom) of *Psammodromus algirus* used in this study. The male belonged to the highland population, while the female came from the lowland population. While the female showed no ectoparasite, note the high number of ticks parasitizing the male. The prevalence and intensity of ticks is higher in the highland population than in the lowland population. [Colour figure can be viewed at [zslpublications.onlinelibrary.wiley.com](https://onlinelibrary.wiley.com)].

Cox, 2015), as well as among populations as a result of acclimation to local conditions (Dml'el *et al.*, 1997). However, no information is available on the potential variability of water loss rates across along an elevation gradient. If along the altitudinal gradient sampled in this study air temperature decreases with elevation while humidity increases with elevation (but see Körner, 2007), we would expect to find a pattern of lower water loss rates in lowland populations, provided that lizards are acclimatized or adapted to local meteorological conditions. On the contrary, high-elevation populations should be less resistant to water loss. (2) We expect that both field body temperatures and water rates loss should vary according to daily and seasonal patterns. Previous research reported both daily and seasonal plasticity in *P. algirus*' field body temperature (Díaz *et al.*, 2006; Zamora-Camacho *et al.*, 2013), while no data are available on the potential variability of water loss

rates. (3) Finally, we would expect to find inter-individual differences in field body temperature and water loss rates, especially when the comparison is made on body size (small vs. large individuals) and between sexes (Diaz, 1993; Diaz *et al.*, 2012).

Materials and methods

Study sites

We sampled three populations, distributed along an elevation gradient (650–1300 m.a.s.l.) approximately 40 km long. The lowest elevation location, Monte de El Pardo (onward ‘Lowland’; 40°30’N, 3°45’W; mean 650 m above sea level, m. a.s.l.), is situated close to the city of Madrid. It is characterized by sandy soil and open *Quercus ilex* woodland. Lizards are mainly found within the undergrowth composed by oak sprouts and bushes of *Cistus ladanifer*. The mid-elevation location, Puente Medieval de Colmenar Viejo, (onward ‘Midland’; 40°40’N, 3°49’W; mean 850 m. a.s.l.) is situated at midway between the other two locations (20 km from El Pardo and 20 km from the Guadarrama range). It is characterized by a sandy clay loam substrate, with abundant rock outcrops. Tree cover is mostly composed by sparse *Juniperus oxycedrus* and few *Quercus ilex*. Lizards are mainly found close to oak sprouts or in the bush matrix of *Thymus* spp., *Lavandula stoechas* and *Festuca* sp. Finally, the high-elevation location is at the base of the Sierra de Guadarrama mountain range (onward ‘Highland’; 40°43’N, 4°01’W; mean 1250 m. a.s.l.). The tree coverage is composed almost entirely by the deciduous oak *Quercus pyrenaica*, while the undergrowth is dominated by *Cistus laurifolius*. Lizards are found mostly at the ground level within the bush matrix. The lizards from these populations belong all to the same haplogroup and showed significant gene flow among them (Verdú-Ricoy *et al.*, 2010).

Sampling, measuring and husbandry

Adult lizards (SVL > 60 mm) were captured by noosing in the three areas during their activity time (~9:00–18:00 h) in the reproductive season in April and May 2018. Within less than 15 s since capture, the cloacal temperature was measured using a thermometer fitted with a K-thermocouple probe (Hibok 18, precision: 0.1°C, accuracy: ±0.2%). Time of capture and GPS position were also recorded. To ensure that each lizard was used only once throughout the study and hence avoid potential pseudoreplication (Hurlbert, 1984), in each site, we sampled over a large area, and along different transects. Furthermore, when each lizard was released, it was stained on the neck with white nail varnish that in some individuals lasted up to 14 days (pers. obs.). Finally, we photographed dorsally, laterally and ventrally each lizard. Photo-identification has already been applied several times in lizards (Sacchi *et al.*, 2016) and *P. algirus* has conspicuous features, like lateral blue ocelli variable in number and size and, in males, orange head coloration, which enable the researcher to distinguish among individuals. The cross-check of location, sex, body size and picture

comparison enabled us to reduce to the minimum the risk of using an individual more than once.

Lizards were taken to the ‘El Ventorrillo MNCN-CSIC Field Station’ where small groups (max five lizards of the same sex) were housed in large plastic terraria (~70 × 40 × 40 cm) separately for each locality. Each terrarium had two sources of water replenished *ab libitum*, two shelters and access to natural light. Measurements of total evaporative water loss (TEWL) were carried on after one day of resting in their cages, during which we assumed that lizards could re-hydrate if necessary. After TEWL measurements, we sexed lizards according to clear sexual secondary characters (Mellado & Martínez, 1974) and measured the SVL (with a calliper to the nearest 0.01 mm) of each lizard and then released them back at the site of capture. Lizards did not spend more than five days in captivity. Food (*Tenebrio molitor* larvae) was provided every day only after the TEWL measurements.

Total evaporative water loss measurements

Total evaporative water loss (TEWL) was measured as the individual difference in body mass following the protocol of Sannolo *et al.* (2018). Briefly, we first weighted each lizard to the nearest 0.001 g with a precision balance (Sartorius M-Pact AX224; Sartorius AG, Goettingen, Germany). We then set a thermal chamber (FRIOCELL FC-B2V-M/FC-404, MMM group Germany; accuracy at 37°C = ±0.3°C) at 35°C to mimic the preferred temperature of this species (Díaz, 1997). Each lizard was then placed inside a cylindrical plastic box (11 × 10 cm) provided with holes on all sides. Several boxes with different lizards were placed in the incubator in random order and position with respect to sex and location. The incubator was sealed, and after nine hours (9:00–18:00 h), we measured each lizard again to determine TEWL. The solid portion of waste products was accounted for during the weighing procedure. This procedure has been already used in other studies on lacertid lizards (Sannolo, Barroso & Carretero, 2018) and, in general, the loss of water is small (1–5% of the initial body mass), so that the health of individuals is not compromised. We checked the lizards inside the chamber every two hours to ensure that they were in good condition and did not show stress signs. No lizard suffered any damage during the experiment, and all of them were released with good health at their capture sites.

Microclimate measurements

With the goal of sampling the range of microclimatic variability available to lizards in each sampling area, we placed 12 iButton Thermochron® (Model DS1923; Maxim Integrated Products, Sunnyvale, CA, USA), randomly spaced along a track of approximately 1 km long. All dataloggers recorded temperature every hour, and six of these dataloggers in each sampling area also recorded relative humidity. We placed the dataloggers in two different microhabitat types: under rocks, in full shade and on open ground, in full sun. We considered that

such microhabitats encompass the whole spectrum of temperatures and humidity that lizards may experience in the field. Clearly, lizards may select intermediate values, and we did not assume that such data represent lizard operative temperature. Because the gathered data are inevitably temporally autocorrelated, we summarized the data by pooling together each day measurements.

Data analysis

We first tested for potential differences in body size and body condition among populations for each sex separately. Differences in SVL were calculated using two-way ANOVA with the population and sex as fixed factors. We checked that the residuals of the model followed a normal curve (Shapiro test = 0.99, $P = 0.64$). We calculated body condition as the residuals of the model II regression of the log SVL on the log body mass (Green, 2001). The residuals followed a normal curve (Shapiro test = 0.99, $P = 0.16$).

Before modelling field body temperature and water loss rates, we checked the deviation from normality of these dependent variables. Field body temperature was slightly left-skewed (-0.82), as it is usually the case in lizards (Huey & Pianka, 2018). Raw TEWL data was right skewed (0.62). Because water loss was correlated with initial body mass (t -value_{1,169} = 3.408, $P < 0.0001$) but not with SVL (t -value_{1,169} = 1.652, $P = 0.10$), all analyses involving water loss included initial body mass (BM) as covariate. We then used the R package *fitdistrplus* to plot a Cullen and Frey graph to find the appropriate distribution curve for these variables. AIC comparisons indicated that both field body temperatures and raw TEWL data best approximated normal curves. Hence, we used a linear model to test for the effect of elevation, daily fluctuations (time), sex and seasonal trend (day) on field body temperature (formula: Body temperature ~ SVL + locality × day + locality × sex + locality × [time + time²]). The polynomial term in the model was included to account for potential quadratic patterns in body temperature along the day. We fitted a second LM with the water loss raw data to test for the effect of elevation, the day of the season, and for the potential interaction between sex and locality (formula: TEWL ~ BM + locality × day + locality × sex).

Microclimatic data of temperature and relative humidity were filtered to retain only daytime measurements (08:00–20:00 h) and were averaged throughout each day. We then fitted two GLS models (one for the temperature and one for humidity data) to test the potential effect of seasonal trending (day), position of the datalogger (sun or shade) and differences among localities (formula: temperature (or humidity) ~ day × locality × datalogger position). We included in the model an autocorrelation structure to account for the temporal autocorrelation of the data. Finally, we summarized the daily maximum and minimum of both temperature and humidity for each location and computed the range of fluctuation of both types of data. All statistical analysis was run in the R environment (R Development Core Team, 2018). Figures were produced with the *ggplot2* package (Wickham, 2009).

Results

Field body temperatures of active lizards

Over the course of 40 days, we sampled 171 lizards (Lowland $n = 58$; Midland = 47; Highland = 66). SVL showed no significant variation neither among populations ($F_{2,168} = 1.006$, $P = 0.368$) nor between sexes ($F_{1,165} = 2.368$, $P = 0.123$). Body condition showed no differences among populations ($F_{2,168} = 0.954$, $P = 0.387$), but differed between sexes, with males being significantly heavier than females for a given SVL ($F_{1,165} = 59.53$, $P < 0.0001$). Hence, the following results were not due to potential differences in body size or body condition among populations.

Overall, field body temperature showed no consistent pattern with elevation. Indeed, while the lowland population differed from the midland population (Lowland = $31.6 \pm 2.7^\circ\text{C}$, Midland = $32.5 \pm 1.9^\circ\text{C}$; t -value = -2.870 , $P = 0.0047$; Table S1), there was no difference in field body temperature between the low- and highland populations (Lowland = $31.6 \pm 2.7^\circ\text{C}$, Highland = $31.6 \pm 2.1^\circ\text{C}$; t -value = -0.196 , $P = 0.84$; Fig. 2, Table S1). However, field body temperatures showed significant variability depending on the time of day, day of study and sex.

Field body temperatures significantly decreased along the day in the lowland population, while showed no trend in the other two populations (Lowland t -value = -2.179 , $P = 0.031$; Fig. S1, Table S1). Body temperatures also increased

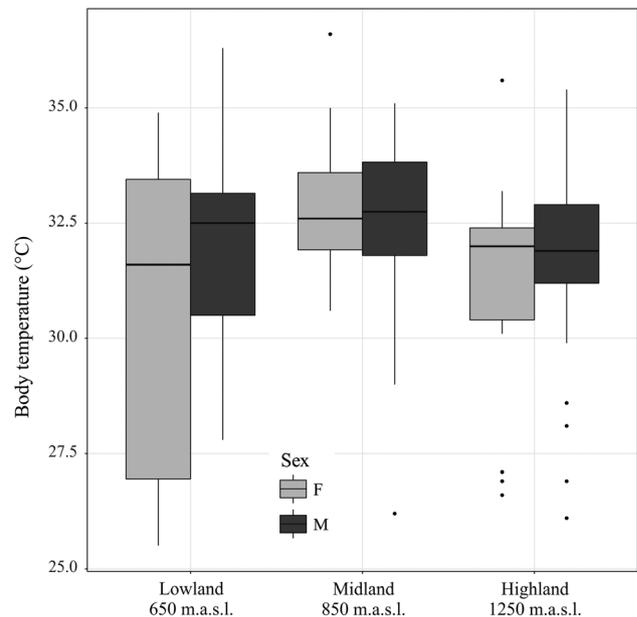


Figure 2 Boxplot of the field body temperatures of lizards along elevation and between sexes. In light grey are represented the females, while in dark grey are the males. Note the absence of an elevation trend in body temperature, nor a consistent difference between the sexes. Males have significantly higher body temperature at lowland and highland, but not at midland.

significantly throughout the season in the lowland and highland populations (Lowland t -value = 5.321, $P < 0.0001$; Highland t -value = 4.355, $P < 0.0001$; Fig. S2, Table S1).

Sexual differences in field body temperatures were significant in both the lowland and highland populations (Lowland t -value = 2.753, $P = 0.0064$; Highland t -value = 2.506, $P = 0.0133$; Fig. 2, Table S1). While in the lowland population males had higher body temperatures than females (t -value = 2.753, $P = 0.0066$; Table S1), the reverse was true for the highland population (t -value = 2.506, $P = 0.013$). Sexes showed no difference in body temperature at the midland population (t -value = -0.894 , $P = 0.37$).

Patterns of total evaporative water loss

Water loss rates, unexpectedly, decreased significantly with elevation, with lizards from the highland population losing less water than lizards from the lowland population (t -value = -5.409 , $P < 0.0001$; Lowland mean 0.279 ± 0.084 g; Midland = 0.237 ± 0.081 g; Highland = 0.202 ± 0.063 g; Fig. 3). However, the water loss rates appeared to be less variable than field body temperature, at least when measured over the course of the season. In fact, TEWL did not significantly change throughout the study for any population (at least $P > 0.25$ for each comparison, Table S2, Fig. S3), and we did

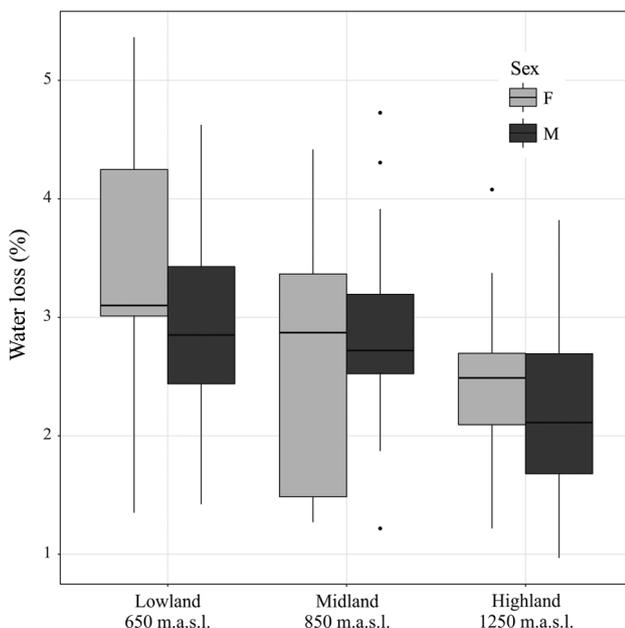


Figure 3 Boxplot of the total evaporative water loss of lizards along elevation and between sexes. In light grey are represented the females, while in dark grey are the males. There is an elevation trend, with highland lizards losing less water than lowland ones. Sex, on the other hand, shows no statistically significant differences, even though females lost more water than males in all populations. Note that, to account for body size in the figure, TEWL is expressed as percentages of body mass lost, while the numerical calculations in the text are done on the raw data.

not detect significant differences between sexes within any populations (at least $P > 0.30$, Table S2, Fig. 3).

Microclimates

The dataloggers showed that temperatures measured in full sun were on average significantly higher than those in full shade for all localities (Lowland t -value = -2.548 , $P = 0.0115$; Midland t -value = -2.947 , $P = 0.0035$; Highland t -value = 2.548, $P = 0.0115$; Table S3). Conversely, humidity in shade was on average significantly higher than in full sun (Midland t -value = -5.278 , $P < 0.0001$; Highland t -value = -6.676 , $P < 0.0001$; Table S4). We did not detect significant trends in temperature variation during the study, either in shade or sun ($P > 0.05$ for all localities; Figs 4 and 5). Relative humidity measured in shade did not show significant trends during the study ($P > 0.05$ for all localities; Figs 4 and 5), while humidity measured in full sun increased significantly in the lowland and highland populations (Lowland t -value = 2.228, $P = 0.0268$; Highland t -value = 4.318, $P < 0.0001$; Table S4). Comparisons among localities showed that temperature did not significantly change with elevation neither when measured in the shade nor in full sun ($P > 0.05$ for all comparisons; Figs 4 and 5). Humidity, when measured in shade, was significantly lower in lowland if compared to the other two locations (Lowland vs. Midland t -value = 2.638, $P = 0.0089$; Lowland vs. Highland t -value = 3.122, $P = 0.0020$; Fig. 4; Table S4). Relative humidity measured in full sun showed no significant variation across localities (for all comparisons; Fig. 5).

When we focused on extremes instead of average values of microclimates, we found that the highland locality experienced both the lower minimum and higher maximum in both temperature and humidity in full sun. Similarly, in full shade, the highland locality experienced broader fluctuations in temperature (Table 1). The temperature range, both in shade and full sun, increased with elevation while the ranges of relative humidity decreased with elevation in shade but showed no pattern in full sun. For all localities and for both temperature and humidity, the amplitudes of extremes were higher for measurements made in full sun respect to those made in full shade (Table 1). As an example, lowland lizards in full sun experienced a day whose mean temperature was over 30°C but then dropped to 12°C the next day (Fig. 5). Lizards in highland were instead exposed to a 24°C drop in just two days, passing from 32°C to only 8°C (Fig. 5).

Discussion

The present work aimed to investigate the inter-population variability and potential short-term seasonal changes in field body temperature and water loss rates along an altitudinal gradient in a diurnal lizard. We found only partial support for the first initial hypothesis we formulated, and in the opposite direction. We expected to find an elevation gradient in both field body temperature and water loss rates. Instead, field body temperature showed no significant variation along elevation during the same time period and, contrary to expectations, water loss rates decreased with elevation. We also found partial support

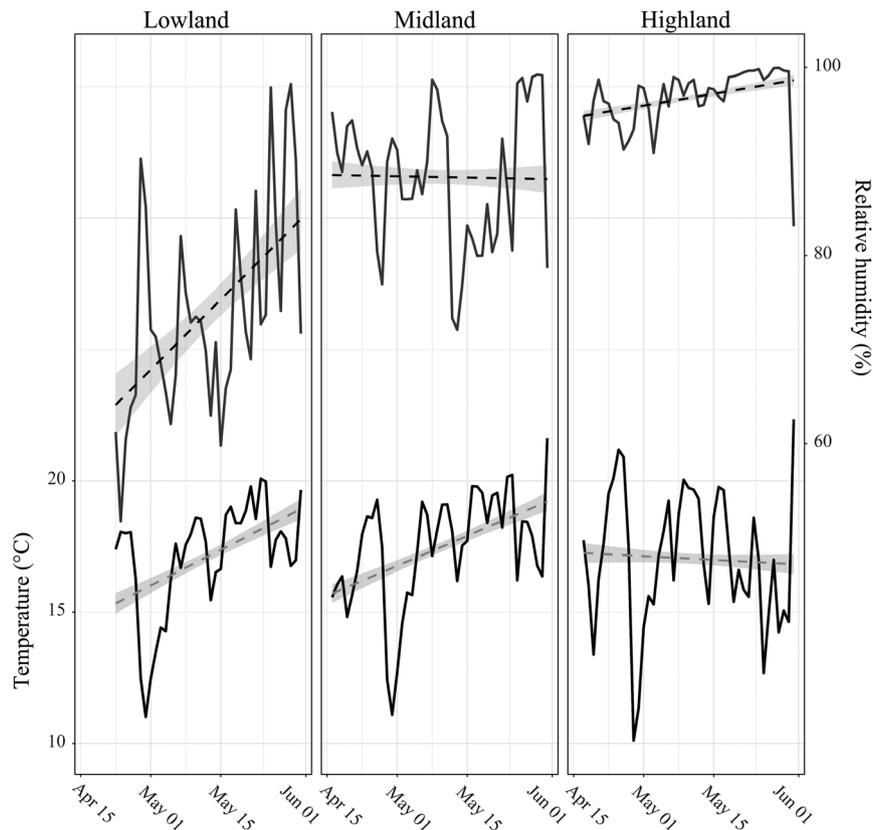


Figure 4 Environmental daytime temperature and relative humidity in each locality measured by the dataloggers in full shade. The top line, in dark grey, shows the daily averaged humidity and the trend over the course of the study. The bottom line, in black, shows the daily averaged temperature and trend. Note that left the scale refers to temperature and right scale refers to humidity.

for our second hypothesis: while field body temperatures showed daily and seasonal variability, water loss rates showed no acclimation changes along the season. Finally, we found significant differences between sexes in field body temperature but not in water loss rates.

The field body temperatures of *P. algirus* lizards appeared to be constant along elevation, a result comparable to that already published for the same species over a broader altitudinal range (Zamora-Camacho *et al.*, 2016; Díaz *et al.*, 2017). Also, depending on the species considered, other lacertid species showed either absent or significant differences in thermal preferences among populations sampled over an elevation range (Van Damme *et al.*, 1989; Gvoždík & Castilla, 2001). The present study focused on a relatively small elevation range (~600 m) and dataloggers indicated that the sampling locations did not differ significantly in temperature during the study. Hence, our results may reflect either conservativeness in thermal preference (*sensu* Hertz *et al.*, 1983) or the lack of variability in environmental temperature during the considered period. In a previous study on the same species, however, thermal preferences of various populations were similar despite decreasing operative temperatures with elevation (Zamora-Camacho *et al.*, 2016). Hence, we inclined towards rigidity in thermal preference for this species. We found that body

temperature was more flexible on a daily and seasonal basis and depending on sex, at least for some populations. In fact, for several lacertid species, daily fluctuations in field body temperature have been reported, as well as season acclimation and sexual differences (Castilla, Van Damme & Bauwens, 1999). When faced with predictable climatic fluctuations, like seasonal changes, individuals may anticipate the change and respond to it accordingly, for example through seasonal shifts in thermal preferences (Angilletta, 2009). In the present case, however, a significant increase in average field body temperature in two populations was not accompanied by an increase in microclimate mean temperature. It should be noted that such a mismatch between lizards' body temperature and microclimatic data may easily arise because lizards can behaviourally adjust their body temperature. As a result, the microclimate data did not necessarily match the body temperature data.

Contrary to expectations, water loss rates decreased with elevation, instead of increasing. Our initial hypothesis was based on the assumption that the mean environment temperature would decrease, and humidity increase with elevation. Climatic variables along elevation are not always predictable, especially on a small scale and a reduced period (Körner, 2007) and, in fact, we found significant variation only in humidity, and not in all localities and microhabitats. Also, it should be noted that

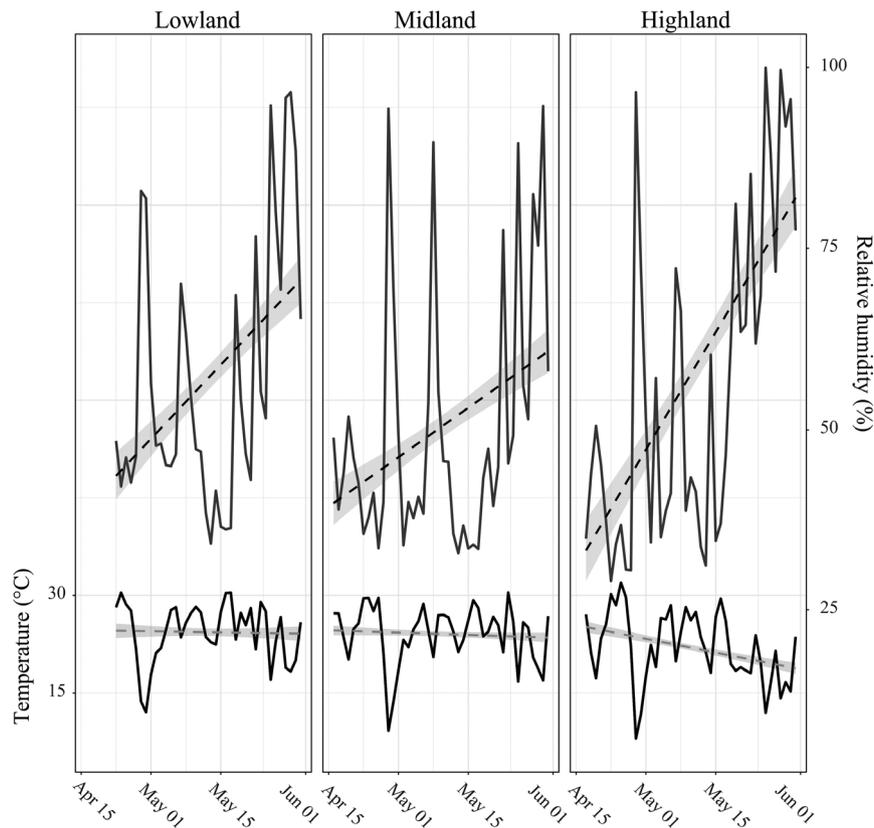


Figure 5 Environmental daytime temperature and relative humidity in each locality measured by the dataloggers in full sun. The top line, in dark grey, shows the daily averaged humidity and the trend over the course of the study. The bottom line, in black, shows the daily averaged temperature and trend. Note that the left scale refers to temperature and right scale refers to humidity.

Table 1 Minimum and maximum daytime (8:00 – 20:00 h) average values of temperature and relative humidity measured by dataloggers placed in full sun or full shade in the three sampling localities

	Full shade		Full sun	
	Temperature	Relative humidity	Temperature	Relative humidity
Lowland (650)	11.0–20.1 (9.1)	51.7–98.3 (46.6)	12.1–30.4 (18.3)	34.2–96.6 (62.4)
Midland (850)	11.1–21.6 (10.5)	72.1–99.3 (27.2)	9.2–30.4 (21.2)	32.8–94.7 (61.9)
Highland (1250)	10.1–22.3 (12.2)	83.1–100 (16.9)	8.0–32.0 (24)	29.0–100 (71)

Within brackets are the ranges. Temperature is expressed in Celsius degrees, relative humidity as % of saturated air. The number near the locality name indicates elevation above the sea level, in meters.

the reduced number of populations and differences in habitat openness may modulate the interaction between elevation and temperature or humidity. Still, highland lizards faced on average cooler and more humid conditions. If lizards from each population were acclimatized or adapted to the local climatic conditions, their TEWL should be higher at lower elevations, where the driving force to lose water was greater (Gates, 1980).

A recent study on water loss rates in lungless salamanders found mixed results, with one species adapted to cope better with dehydration at lower altitudes and a second species better suited for higher altitudes (Riddell & Sears, 2015). The

apparent contradiction between microclimatic and physiological measurements might be explained if we consider variation instead of the average values of microclimatic conditions. A recent work pointed out that extreme operative temperatures are better descriptors than averages of the thermal environment experienced by small animals (Camacho, Trefaut Rodrigues & Arturo Navas, 2015). In the present case, the high-elevation population experienced both the highest and the lowest temperatures and humidity, especially in open areas. Hence, highland lizards might be more resistant to water loss because they are exposed to more extreme and unpredictable conditions than lowland ones. A possible, but not mutually excluding

alternative, is based on a biotic factor. The lizard populations we studied showed different degrees of parasitization (Fig. 1). In particular, the prevalence of ticks was higher in lizards from the high-elevation population (Carbayo, Martín & Civantos, 2019). Ticks are typically found grouped in hot spots just behind the armpit and the neck, where their action often exposed the skin below the scales (Salvador, Veiga & Civantos, 1999). Being more parasitized by ticks, lizards from the highland population may compensate by altering the superficial blood flow, and capillary structure which may reduce water loss rates. Modulation of peripheral blood flow has been recently proposed as a proximate cause to explain daily variability in lizard TEWL (Oufiero & Van Sant, 2018), and anatomical differences between tick-resistant and non-resistant individuals have been demonstrated at least in cattle (Schleger, Lincoln & Bourne, 1981). Clearly, the observed pattern of decreasing water loss rates along the elevation gradient may be due to other factors apart from those we considered. For example, TEWL may change depending on the mountainside considered (north vs. south slope), the openness of each considered area (which in turn influences operative temperatures, humidity and wind speed at ground level) or on long-term climatic fluctuations, rather than seasonal ones. Hence, more effort should be devoted studying proximate and remote causes of population variability in TEWL.

Contrary to field body temperature, water loss rates showed no change along the study, even though other lizard species showed marked seasonal plasticity, especially in response to strong climatic events like droughts (Perry *et al.*, 1999; Perry *et al.*, 2000). We are fully aware that we measured different individuals across the season, and thus lacked repeated measures for the same lizard. However, the same is valid for field body temperatures, which instead showed seasonal variation. Even being cautious, it seems that while field body temperature was variable through time and less flexible along an altitudinal gradient, the opposite conclusion could be drawn for water loss rates. Such a result may bear important ecological consequences. If TEWL is rigid, the need to retain water during the summer months may constrain lizards' activity more than high temperatures. Indeed, a recent study showed that lizard thermoregulation during summer reflected the short-term pattern of rainfall (Ryan *et al.*, 2016). Unfortunately, the potential for a trade-off between thermal preference and dehydration in lizards has received little attention so far (Crowley, 1987) and in lacertid lizards it has been tested only in laboratory conditions (Pollo Mateos & Pérez-Mellado, 1989; Sannolo *et al.* in review).

As far as we know, the present work is the first in testing for sexual differences in TEWL rates in a lacertid lizard. Even though our results indicate a lack of significant difference, females from all population lost more water than males. Such a result may indicate a consistent inter-sexual differences of several kinds, from a higher metabolic stress during the testing to morphological or physiological differences between sexes (in this species, at comparable SVL, female's trunk is longer than male's, while the male's head is bigger; Mellado & Martínez, 1974). For example, pregnant females of a

viviparous snake lost more water than non-pregnant snakes, likely due to the stretching of the skin caused by growing embryos, and shedding snakes lost more water than control snakes (Dupoué *et al.*, 2015b). For lizards, no comparable data are available on the potential effect on TEWL rates of sex, morphology or physiology.

Finally, while comparing field body temperatures across species is relatively straightforward, comparing TEWL rates is challenging due to the scarcity of data and the wide range of temperatures at which lizards are tested. The only published work on lacertid lizards estimating TEWL rates at their preferred temperature is on two *Podarcis* species (Sannolo *et al.*, 2018). At 32°C, *P. bocagei* and *P. guadarramae lusitanicus* lost slightly more water than the large *psammadromus* at its preferred temperature, 35°C, over a comparable amount of time (3.3 % and 2.9 %, respectively). At 37°C, *Podarcis* lizards lost more water than *P. algirus* at its preferred temperature (4.1 % and 3.7 %). This result is not surprising, as *Psammadromus* lizards generally live in drier environments than *Podarcis* (Arnold, 1987). Furthermore, scale morphology in these two groups differs significantly, with *Podarcis* having small semi-beaded non-overlapping scales and *Psammadromus* having large, keeled and overlapping scales. Even though scale morphology in lacertid lizards has been correlated with performance and crypsis (Arnold, 2002), investigations on *Anolis* and *Sceloporus* lizards showed that scale size and number may correlate with climatic factors like temperature, rainfall regime and aridity (Oufiero *et al.*, 2011; Wegener, Gartner & Losos, 2014). Furthermore, even though the composition and thickness of the skin lipid layer is the main factor that determines skin permeability to water (Roberts & Lillywhite, 1980), the potential role of scale morphology in enhancing or reducing water evaporation at the boundary layer remains mostly unknown.

Concluding, we found little variation in field body temperature along an altitudinal gradient, while TEWL rates decreased with elevation. We also provide here the first data on inter-population variability on TEWL for a lacertid lizard. Rigidity in thermal preferences may account for the little variation observed and is congruent with previous findings on other populations of the same species, while both abiotic factors like temperature and humidity fluctuations, as well as biotic factors like parasite intensity, may explain the decrease in TEWL with increasing elevation. The absence of variation in TEWL rates along the study may suggest that during summer months water retention may constrain lizard activity more than temperature.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Summary of the linear, GLM and GLS models used to fit field body temperatures, water loss and microclimate measurements.