

On the thermal ecology of two coexisting lizard species (Reptilia, Lacertidae) in a hot and dry area of Bulgaria

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Abstract

Thermal ecology plays a key role in shaping the behavior and distribution of reptiles, particularly under climate stress. Understanding how sympatric lizard species manage their thermal preferences is essential for predicting their responses to changing climate. We studied thermo-ecological parameters in two lizard species, *Lacerta viridis* and *L. trilineata* that occur under syntopy in SW Bulgaria. We recorded *in situ* body, air and substrate temperatures, humidity and UV radiation for 190 individuals. Both species maintained body temperatures higher than air and substrate temperature. Comparison of the two species showed statistically significant differences between them in terms of body and substrate temperature, with a trend towards higher values in *L. viridis*. For *L. trilineata*, statistically significant differences were found only between immatures and females in terms of substrate temperature (higher values in females). For *L. viridis*, there were significant differences between immatures and males in each of the temperature parameters (with a trend for higher values in males) as well as between immatures and females in air temperature (higher values in females). No significant differences were found in humidity or UV exposure among groups. Our findings reveal species- and sex-specific thermal strategies in two co-existing lacertids, likely reflecting different ecological or physiological demands. The results provide valuable baseline data for predicting species responses to increasing temperatures in hot and dry habitats.

Key Words

Balkan Peninsula, body temperature, humidity, *Lacerta*, Sauria, syntopy, UV radiation

Introduction

As ectotherms, lizards exhibit a strong dependence on environmental temperature with regard to all life processes, like foraging (Harwood 1979; Du et al. 2000), locomotion (Herrel et al. 2007), reproduction (Licht 1972), sex determination (Yamaguchi and Iwasa 2018), etc. They can maintain their body temperature higher than the air temperature, and keep it constant mainly by behavioral thermoregulation (Huey 1974; Castilla and Bauwens 1991; Kirchhof et al. 2017). The air and substrate temperature, along with humidity, are the main environmental factors that affect lizard activity, as ectotherms rely on external sources to regulate their body temperature and maintain

water balance (Rozen-Rechels et al. 2019; Bodensteiner et al. 2020). Solar radiation, wind speed, availability of shade and microhabitat structure are also important and interact with ambient temperature and humidity to influence lizard thermoregulation and overall physiological performance (Monasterio et al. 2009; Ortega et al. 2016a; Ortega and Pérez-Mellado 2016; Rozen-Rechels et al. 2019; 2020). UV light exposure plays an important role in the thermoeology of lizards (Conley and Lattanzio 2022) and is linked to physiological processes such as vitamin D₃ synthesis (Holick et al. 1995), which is essential for calcium metabolism and reproductive success. However, excessive UV, particularly in open and arid habitats, may lead to overheating or skin damage,

and even can be lethal (Ferguson et al. 1996), prompting lizards to balance sun exposure with shade-seeking behaviors. Thus, UV radiation indirectly shapes thermal strategies by influencing both behavioral and physiological responses (Karsten et al. 2009; Conley and Lattanzio 2022). Despite the important role of UV radiation in lizard ecology, relatively few studies have quantified UV exposure under natural conditions (Ferguson et al. 2010, 2014), with the majority of available research focusing on captive or laboratory-maintained individuals (Baines et al. 2016 and references therein).

The relatively low mobility and small size do not allow reptiles and lizards in particular, to move long distances, which makes them highly vulnerable to climate change (Huey et al. 2009; Sinervo et al. 2010; Biber et al. 2023). In addition to habitat loss, degradation, and fragmentation, the increased temperature and changes in precipitation patterns are causing the decline of many reptiles worldwide (Choudhary and Chishty 2021). Moreover, species inhabiting arid environments, such as the Mediterranean regions, are particularly threatened by the higher risk of drought (Foufopoulos et al. 2010; Belasen et al. 2017) and lizards dealing with seasonal changes in temperatures require more precise thermoregulation (Díaz et al. 2006; Huey et al. 2009). As environmental temperatures increase reptiles need to spend more time to thermoregulate (Kearney et al. 2009; Díaz et al. 2022), and are constrained to select thermally favorable microhabitats (Žagar et al. 2015; Sagonas et al. 2017) and spend more time in shade or in shelters, thus reducing their time for activity, feeding, and reproduction (Kirchhof et al. 2017; Sannolo and Carretero 2019).

Therefore, collecting data on the temperature requirements of reptiles is particularly important in the context of global climate warming. For Bulgaria, the only published data on the thermal ecology of lizards refer to *Lacerta agilis* Linnaeus, 1758 (Grozdanov et al. 2011; Popova et al. 2023) and *Ophisops elegans* Ménétries, 1832 (Krastev et al. 2023). Thus, there is a fairly large knowledge gap, given that 16 lizard species are found in the country (Stojanov et al. 2011; Kotsakiozi et al. 2018; Kornilios et al. 2019). In many areas of Bulgaria, two or more lacertid species inhabit the same territory (Tzankov 2005; Vacheva et al. 2020), but their thermal preferences have not been the subject of comparative analysis so far. Lizards that occupy the same habitat must differentiate their thermal preferences or select different microhabitats (Pianka 1986; Heltai et al. 2015; Sagonas et al. 2017), but closely related species often display similar thermal tolerances (Schall 1977; Grigg and Buckley 2013). It should be noted that there are two main approaches in this type of research: *in-situ* and *ex-situ*, but they reflect different aspects of thermal ecology, i.e. the field measurements mostly reflect the ecological optimum of reptile body temperature, while the laboratory measurements (under a constant thermal gradient) mostly reflect the physiological optimum of body temperature (see Meek 1995 and references therein).

The Balkan Green Lizard (*Lacerta trilineata* Bedriaga, 1886) and European Green Lizard (*Lacerta viridis*

(Laurenti, 1768)) (Fig. A1 in Appendix 1) are the largest species of the family Lacertidae in Bulgaria. The maximum known snout-vent lengths, in Bulgaria, are 153 mm for *L. trilineata* and 150 mm for *L. viridis* (Stojanov et al. 2011; Vacheva et al. 2022). The range of *L. trilineata* includes only the southwesternmost part of Bulgaria up to about 550–700 m a.s.l. (Stojanov et al. 2011; authors' unpublished data from 2023–2024), while *L. viridis* is widespread from the sea level up to ca. 1200, and in some places up to 1600–1800 m a.s.l. (Stojanov et al. 2011). *Lacerta trilineata* prefers habitats with xerophytic grass, shrubs or trees, and rocky terrain. *Lacerta viridis* is a more generalist species, inhabiting open landscapes or meadows with sparse bush vegetation and forest edges, but can also be found in areas that are densely covered with bushes or sparse forests (Stojanov et al. 2011; Vacheva et al. 2020).

Previous studies on the thermal ecology for these species are scant: *Lacerta trilineata* has been studied only in Greece by Sagonas et al. (2013), while for *L. viridis* the only available data is provided by Arnold (1987) from Bosnia and Herzegovina. To fill this gap, we conduct an *in-situ* comparison of *L. trilineata* and *L. viridis* (including potential sex- and age-related variations), in relation to body and ambient temperatures, as well as to UV radiation and humidity, under syntopy. Our working hypothesis was as follows: because the two species are closely related and very similar in size we hypothesized that they would exhibit no substantial differences in field body temperatures and will have similar preferences for some basic abiotic factors. Understanding how these species regulate their body temperature and respond to environmental variation provides valuable insight into their ecological adaptability and helps predict their vulnerability under future climate scenarios.

Material and methods

Study area

Fieldwork was conducted in an area situated between the villages of Vlaha and Stara Kresna in the western foothills of the Pirin Mountains (SW Bulgaria). The area is characterized by well-developed microrelief and heterogeneous land cover (a mosaic of low-stem forests, open stony terrains with grass and shrub vegetation, bare rocks, etc.) (Fig. A2 in Appendix 1). Human activity in this area is low (free grazing of small flocks of sheep and goats, low-traffic roads, minimal hiker presence), and hence probably little impact on local lizard populations. For the purpose of the study, two test sites were selected, located in the southeastern (41.74°N, 23.22°E; 500–700 m a.s.l.) and northwestern (41.76°N, 23.17°E; 450–600 m a.s.l.) parts of the area (Fig. 1). The two sites are physiographically similar but sufficiently distant from each other (ca. 4 km), which, in our opinion, excludes the possibility of the same individual being caught in both sites. The

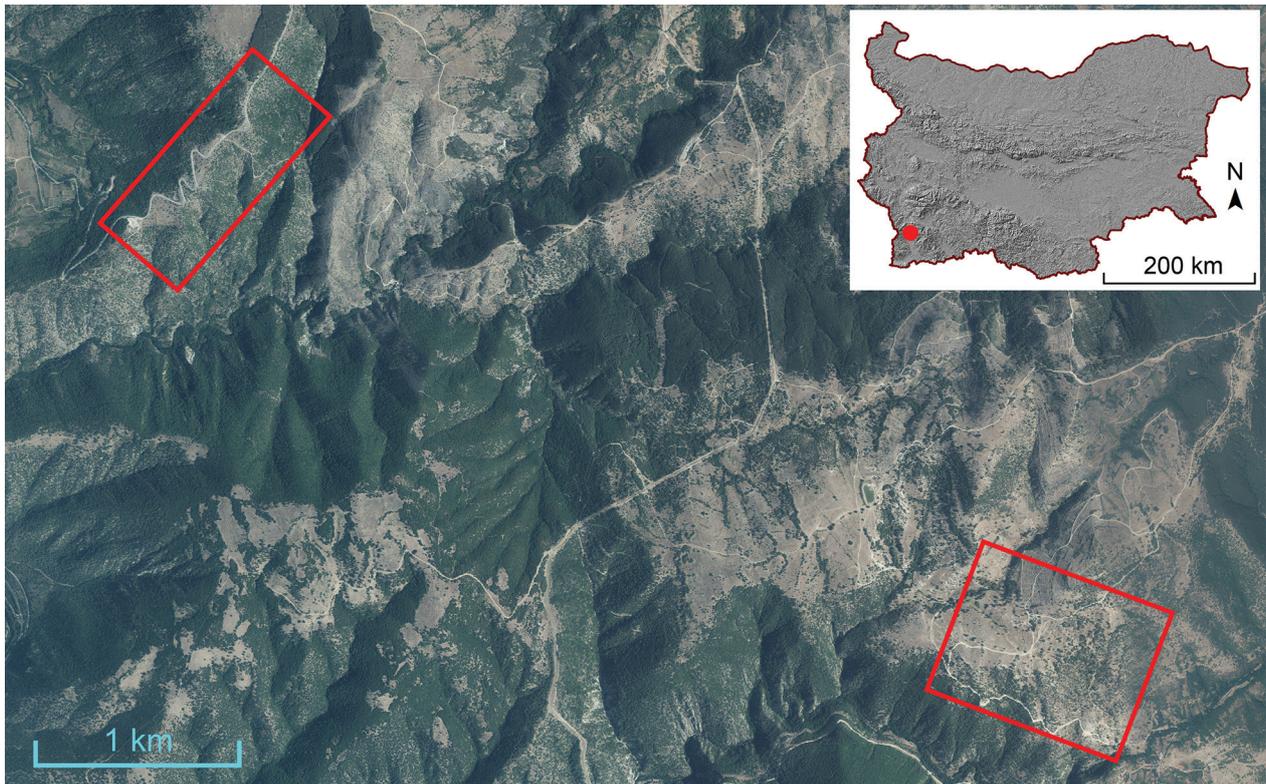


Figure 1. Landscape of the study area (source: <https://kais.cadastre.bg/>) with rectangular outlines of the visited sites, and position of the study area on a relief map of Bulgaria.

climate of the study area (https://www.meteoblue.com/en/weather/historyclimate/climatemodelled/vlahi_bulgaria_72582), is characterized by mean daily maximum air temperature ranging from 6 °C in January to 29 °C in July/August, mean daily minimums from -2 °C in January to 17 °C in July/August, and mean monthly precipitation between 32 mm in August and 79 mm in December.

Sampling

The study area was visited monthly in the period March–October in 2023 and 2024. The individual visits lasted two or three consecutive days, always visiting both sites and in some cases – one of them twice. The specific dates, duration, and time intervals of individual site visits varied widely, ranging from 2 to 9 hours (average: 5 hours) between 08:30 and 21:00 local time (UTC + 3), considering the activity time of both species. Visits were primarily conducted in favorable weather conditions, thus avoiding periods of low or no lizard activity due to prolonged rain, sudden cold, or strong wind. On each visit, attempts were made to capture (by hand or using a noose) every individual of *Lacerta trilineata/viridis* that was spotted. Handling of animals was carried out in accordance with the Ministry of Environment and Water of Bulgaria’s Permit No. 861/13.01.2021.

The body temperature (T_b) of the lizards captured was measured in the cloaca immediately after capture using a Digital probe thermometer (TFA Dostmann Ltd. – Germa-

ny with a precision of 0.5 °C). Thereafter, individuals were measured (using a plastic ruler with an accuracy of 1 mm), sexed (adults only), tagged with a permanent marker (red dot on the back) to avoid repeated capture/measurement within the same visit, and released. At the exact place of the initial registration of each lizard captured, the following environmental parameters were recorded: substrate surface temperature (T_s) and air temperature at 20 cm above the ground (T_a) (via the above-mentioned thermometer); UV radiation intensity (UV) in the range 260–390 nm (via UV meter LUTRON YK-35UV with a precision of 0.01 mW/cm²); relative humidity (RH) of the air at the substrate level and at 20 cm above the ground (via digital thermo-hygrometer TFA Dostmann Ltd. – Germany with a precision of 5%). Such or similar methods for assessing environmental parameters are widely used in ecological studies on reptiles (Amat et al. 2003; Huey and Pianka 2007; Arribas 2013).

Data processing and statistics

Data from the individual visits were pooled and analyzed as a whole, i.e. without taking into account possible seasonal/time/site specific variation in lizard activity. This approach was chosen because, in our opinion, the pattern of the collected data does not allow for such a degree of detail. The individuals registered were divided into two groups according to the values of snout-vent length (SVL). In *L. trilineata*, individuals with SVL > 81 mm

were conditionally considered adults [according to Sagonas et al. (2019) the minimum SVL values for sexually mature individuals recorded in mainland Greece are 81.3 mm for females and 80.6 mm for males], and in *L. viridis*, individuals with SVL > 79 mm were conditionally considered adults [according to Sagonas et al. (2018) the minimum SVL values for sexually mature individuals recorded in Greece are 76.1 mm for females and 82.8 mm for males]. In our experience, sex determination by external features in individuals with SVL less than 80 mm is uncertain (in both species), therefore the sex of these individuals was not taken into account and they were conditionally considered immatures.

Data were tested for normal distribution using Kolmogorov-Smirnov test (Lilliefors modification). The test showed that most variables (except Ts) are not normally distributed (Lilliefors $p < 0.05$), therefore only non-parametric statistics were used. Spearman rank order correlation was used to evaluate the relations of lizard Tb with the ambient temperature parameters. Preliminary estimates showed that the correlation between Ts and Ta was moderate (Spearman's Rho = 0.68, $p < 0.001$), while that between substrate and air humidity was very high (Spearman's Rho = 0.91, $p < 0.001$). In view of the latter, the arithmetic mean of measurements at substrate level and 20 cm above the ground was used for relative humidity estimation. Mann-Whitney U test was performed to detect possible differences between Tb and Ts and Ta, as well as between the two species in terms of all parameters studied. Kruskal-Wallis ANOVA (with post-hoc multiple comparisons) was used to compare the sex/size groups with each other at the intrapopulation level. Since the data corresponded to the basic assumptions of these tests (all variables were continuous and the variances in the groups compared were similar (Levene test of homogeneity of variances: $p > 0.05$ in all variables)), the results can be considered sufficiently correct.

The statistical procedures were done using STATISTICA 10.0 (StatSoft Inc. 2011).

Results

A total of 72 measurements of *Lacerta trilineata* and 118 of *L. viridis* were made (Fig. A3 in Appendix 1). The lowest Tb measured in *L. trilineata* was 21.5 °C (an immature individual, caught on April 25 at a Ts of 18.9 °C and a Ta of 17.8 °C), the highest – was 37.5 °C (an adult female, caught on June 21 at Ts of 41.4 °C and Ta of 35.6 °C), and the median was 31.25 °C. In *L. viridis*, these values were 22.0 °C (an immature individual, caught on April 24 at Ts of 17.6 °C and Ta of 17.5 °C), 38.5 °C (an adult female, caught on June 22 at Ts of 32.7 °C and Ta of 33.3 °C), and 33.20 °C, respectively.

No statistically significant correlation was found between the lizard's SVL and Tb (*L. trilineata*: Rho = -0.10, $p = 0.397$; *L. viridis*: Rho = 0.09, $p = 0.322$). The correlation (Table 1) of Tb with Ts was positive and statistically

significant (Table 1). In *L. trilineata*, it can be defined as weak, and in *L. viridis* – as moderate, at least as far as the full samples are concerned. At the intrapopulation level, the following differences are noticeable: in *L. trilineata*, the correlation was significant only in females, while in *L. viridis*, it was significant in both sexes, but higher in females. Correlation between Tb and Ta was statistically significant in both species (full samples) as well as in all of the sex/size groups (Table 1).

The Mann-Whitney test showed statistically significant differences between Tb and Ts and Ta in all combinations, with Tb being higher in both comparisons (Table 2; Appendix 2). In both species, Tb measured was usually higher than both Ts (in 88.73% of measurements in *L. trilineata* and 92.37% in *L. viridis*) and Ta (in 92.96% of measurements in *L. trilineata* and 95.73% in *L. viridis*). Comparison of the pooled samples of the two species showed statistically significant differences between *L. trilineata* and *L. viridis* in terms of Tb and Ts (Table 3; Appendix 2), with a trend towards higher values in *L. viridis* in both cases (Fig. 2). The comparison between species at the level of sex/size group showed significant differences only between males, with each of the temperature parameters trending towards higher values at *L. viridis*, while the opposite trend was evident in regards to air humidity.

Table 1. Spearman rank order correlations (Rho, p) of lizard's body temperature (Tb) with substrate (Ts), and air (Ta) temperatures. Statistically significant correlations are marked with asterisks: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

		<i>L. trilineata</i>		<i>L. viridis</i>	
		Rho	p	Rho	p
Tb w/ Ts	Overall	0.37	0.001**	0.58	0.000***
	Females	0.50	0.010*	0.77	0.000***
	Males	0.40	0.090	0.43	0.002**
	Immatures	0.24	0.241	0.31	0.105
Tb w/ Ta	Overall	0.68	0.000***	0.67	0.000***
	Females	0.72	0.000***	0.73	0.000***
	Males	0.65	0.003**	0.48	0.001**
	Immatures	0.67	0.000***	0.72	0.000***

Table 2. Mann-Whitney U test (U, p) for the difference between lizard's body temperature (Tb) and substrate (Ts) and air (Ta) temperatures based on the entire samples of both species and separately for the sex/size groups. Statistically significant differences are marked with asterisks: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

		<i>L. trilineata</i>		<i>L. viridis</i>	
		U	p	U	p
Tb vs. Ts	Overall	987.50	0.000***	2677.00	0.000***
	Females	184.50	0.003**	337.50	0.000***
	Males	101.00	0.021*	410.00	0.000***
	Immatures	47.50	0.000***	117.00	0.000***
Tb vs. Ta	Overall	823.50	0.000***	2186.50	0.000***
	Females	155.00	0.001**	225.00	0.000***
	Males	70.50	0.001**	442.50	0.000***
	Immatures	46.00	0.000***	53.50	0.000***

Table 3. Mann-Whitney U test for the difference between *L. trilineata* and *L. viridis* in the studied factors (Tb = body temperature; Ts = substrate temperature; Ta = air temperature; UV = UV radiation intensity; RH = relative humidity). Statistically significant differences are marked with asterisks: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; for the values of medians see Appendix 2.

	Overall		Females		Males		Immatures	
	U	p	U	p	U	p	U	p
Tb	3266.00	0.008**	499.50	0.730	193.00	0.000***	352.00	0.680
Ts	3288.50	0.013*	505.50	0.989	310.00	0.027*	307.50	0.245
Ta	3536.00	0.088	496.00	0.888	279.00	0.011*	348.50	0.637
UV	3197.00	0.219	431.50	0.494	344.00	0.299	271.00	0.764
RH	3535.00	0.322	454.00	0.711	276.50	0.020*	313.00	0.680

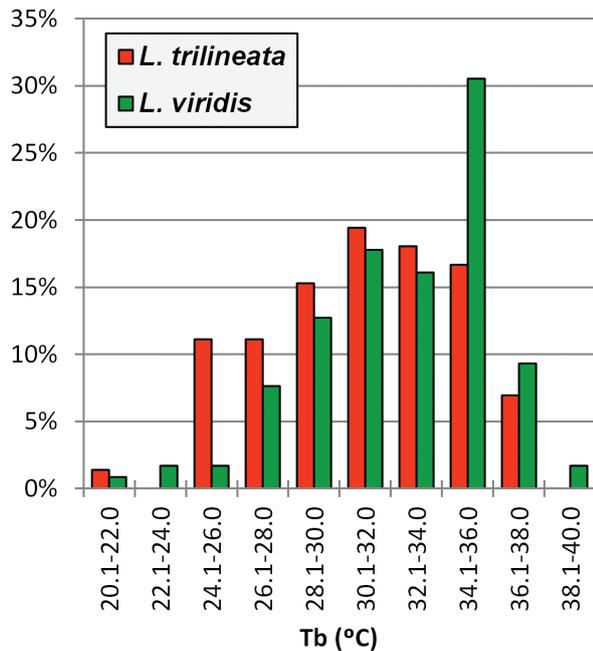


Figure 2. Percentage distribution of the individuals of both species according to their body temperature (Tb) per interval of 2 °C.

For *L. trilineata*, the post-hoc test (Table 4; Fig. 3) showed a statistically significant difference only between immatures and females in terms of Ts (higher values in females). For *L. viridis*, there were significant differences between immatures and males in each of the temperature parameters (with a trend for higher values in males) as well as between immatures and females in Ta (higher values in females). Regarding the other two factors considered (UV and RH) there were no significant differences between the sex/size groups.

Discussion

Our results revealed species-specific thermal differences under syntopic conditions. *L. viridis* exhibited significantly higher Tb and Ts compared to *L. trilineata*, particularly among males. Relative humidity showed an opposite trend, with higher values associated with *L. trilineata*. Intraspecific comparisons indicated sex- and age-related thermal variation: in *L. trilineata*, only females and immatures differed significantly in Ts, while in *L. viridis*,

immatures differed from both males and females. Across both species, Tb was consistently higher than Ts and Ta in most individuals, confirming active thermoregulation under natural conditions.

Information from the literature for variation of Tb and ambient temperature in *Lacerta trilineata* and *L. viridis* in natural conditions is scarce. For adult males of *L. trilineata* from Greece, Sagonas et al. (2013) reported mean values of Tb between 31.3 °C and 32.3 °C, with minimum and maximum values of 28.5 °C and 35.0 °C, respectively. In our case, Tb of males varied in the range 24.8–35.9 °C with a median of 29.4 °C. For *L. viridis* (n = 6) from Bosnia and Herzegovina, Arnold (1987) gave a mean value of 33.9 °C with range from 30–35 °C (information about sex and age of the individuals was absent). In our case, the mean Tb was quite similar (33.2 °C), but with a wider range of 22–38.5 °C which could be due to the larger sample size and extended period of collecting data.

Our results show that Tb (in both species) does not depend on body length. This is also valid for *Lacerta trilineata* from Greece (Sagonas et al. 2013). Other studies on lacertid lizards have shown weak or no significant correlation between body size and field body temperatures as well (Sagonas et al. 2013; Şahin and Kuyucu 2021). This suggests that behavioral thermoregulation plays a more significant role than just passive thermal effects (Verwajen and Van Damme 2007; Beal et al. 2014).

In both species, our results indicated a positive relationship between Tb and environmental temperature. The relationship was more pronounced for Ta than for Ts (in both species). This suggests that both species were more influenced by air temperature for maintaining their body temperature. Thus, they may rely more on behavior thermoregulation such as basking or seeking shelter depending on air temperature fluctuations, rather than solely relying on the temperature of the substrate. To regulate their Tb precisely, both species may prefer microhabitats with more favorable air temperatures, such as more complex habitats (with sparse forests or single trees) or places with available shelters (such as stones and rock crevices). It is possible that there is an additional reason for the stronger correlation with air temperature, i.e. since the air temperature changes faster than substrate temperature, then the body temperature probably also changes faster than substrate temperature, given the small size and physical structure of the lizards.

Table 4. Kruskal-Wallis ANOVA and post-hoc multiple comparisons (z' -values above the diagonal; p -values below the diagonal) for the difference between the sex/size groups (F = females; M = males; IM = immatures) for the studied factors (Tb = body temperature; Ts = substrate temperature; Ta = air temperature; UV = UV radiation intensity; RH = relative humidity). Statistically significant differences are marked with asterisks: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

	<i>Lacerta trilineata</i>			<i>Lacerta viridis</i>				
Tb	H = 5.12, p = 0.077			H = 13.21, p = 0.001**				
		F	M	IM		F	M	IM
	F		2.26	1.07	F		1.47	2.18
	M	0.071		1.27	M	0.422		3.63
IM	0.853	0.613		IM	0.089	0.001**		
Ts	H = 7.53, p = 0.023*			H = 14.55, p = 0.001**				
		F	M	IM		F	M	IM
	F		0.87	2.71	F		1.57	2.26
	M	1.000		1.62	M	0.348		3.81
IM	0.020*	0.315		IM	0.071	0.000***		
Ta	H = 3.75, p = 0.153			H = 21.77, p = 0.000***				
		F	M	IM		F	M	IM
	F		0.74	1.93	F		2.06	2.65
	M	1.000		1.04	M	0.118		4.66
IM	0.161	0.901		IM	0.024	0.000***		
UV	H = 0.99, p = 0.607			H = 0.43, p = 0.805				
		F	M	IM		F	M	IM
	F		0.98	0.23	F		0.61	0.52
	M	0.986		0.73	M	1.000		0.01
IM	1.000	1.000		IM	1.000	1.000		
RH	H = 1.57, p = 0.457			H = 4.71, p = 0.095				
		F	M	imm		F	M	IM
	F		1.25	0.48	F		1.71	0.31
	M	0.635		0.79	M	0.262		1.90
IM	1.000	1.000		IM	1.000	0.171		

In most cases, Tb was higher than the temperature of the environment, and this was valid for all sex/size categories. Higher Tb can enhance metabolic rates, thus leading to increased activity levels, and higher efficiency in foraging and reproduction (Castilla et al. 1999; Verwajen and Van Damme 2007).

When we compared the two species in terms of Tb values, they were higher in *L. viridis* than those in *L. trilineata*. This was true both for the median and for the minimum and maximum values measured (Appendix 2). The same trend was observed for Ts. In both cases, a comparison of sex/size groups showed that these differences applied to adult males, while for adult females and immatures (i.e. individuals with SVL less than 80 mm) the differences between the two species were not significant (Table 3). *Lacerta viridis* inhabits cooler and more diverse environments. Therefore, similar to other species in cooler regions, it probably adopts a more active thermoregulation strategy (Ortega et al. 2016b; Ortega et al. 2016c). In such conditions, lizards may need to actively search for warm microhabitats and maintain higher body temperatures to optimize their physiology and overall functions. In contrast, *L. trilineata* is found in drier and hotter areas, where overheating is a greater risk. To avoid excessive heat stress, it may regulate its Tb at a lower level and use behavioral adaptations such as seeking shade, being active during cooler times of the day, etc. For example, Sannolo et al. (2018) reported a similar result for

Podarcis bocagei (Lopez-Seoane, 1885) inhabiting areas with cooler and wetter microclimate and *P. lusitanicus* Geniez et al. 2014, which prefers warmer and drier microclimate, with *P. bocagei* selecting higher temperatures.

The slight intraspecific differences that display the tendency for higher temperatures in males (Table 4 and Fig. 3) could be due to their larger body size. This means that they have greater thermal inertia, compared to females and subadults (Herczeg et al. 2007). In addition, males are more active, especially in the reproductive season while searching for mates, or guarding their territories. This increased activity requires a higher metabolic rate, which in turn benefits from a higher Tb (Verwajen and Van Damme 2007). Another explanation could be that males may occupy more sun-exposed areas to thermoregulate efficiently. On the other hand, females and subadults might be forced to select less favorable microhabitats to reduce intraspecific competition or to avoid predators (Childers and Eifler 2015; Delaney and Warner 2017). Adult females may also depend on their reproductive status (Tosini and Avery 1996), and could be forced by the reproductive costs of pregnancy to choose habitats that provide them both safe places and better thermoregulation opportunities.

No differences were found in UV intensity (measured at the sites of capture) between the two species as a whole or between the sex/size groups in particular. The limits of lizard activity against UVI values vary between 0.0 to

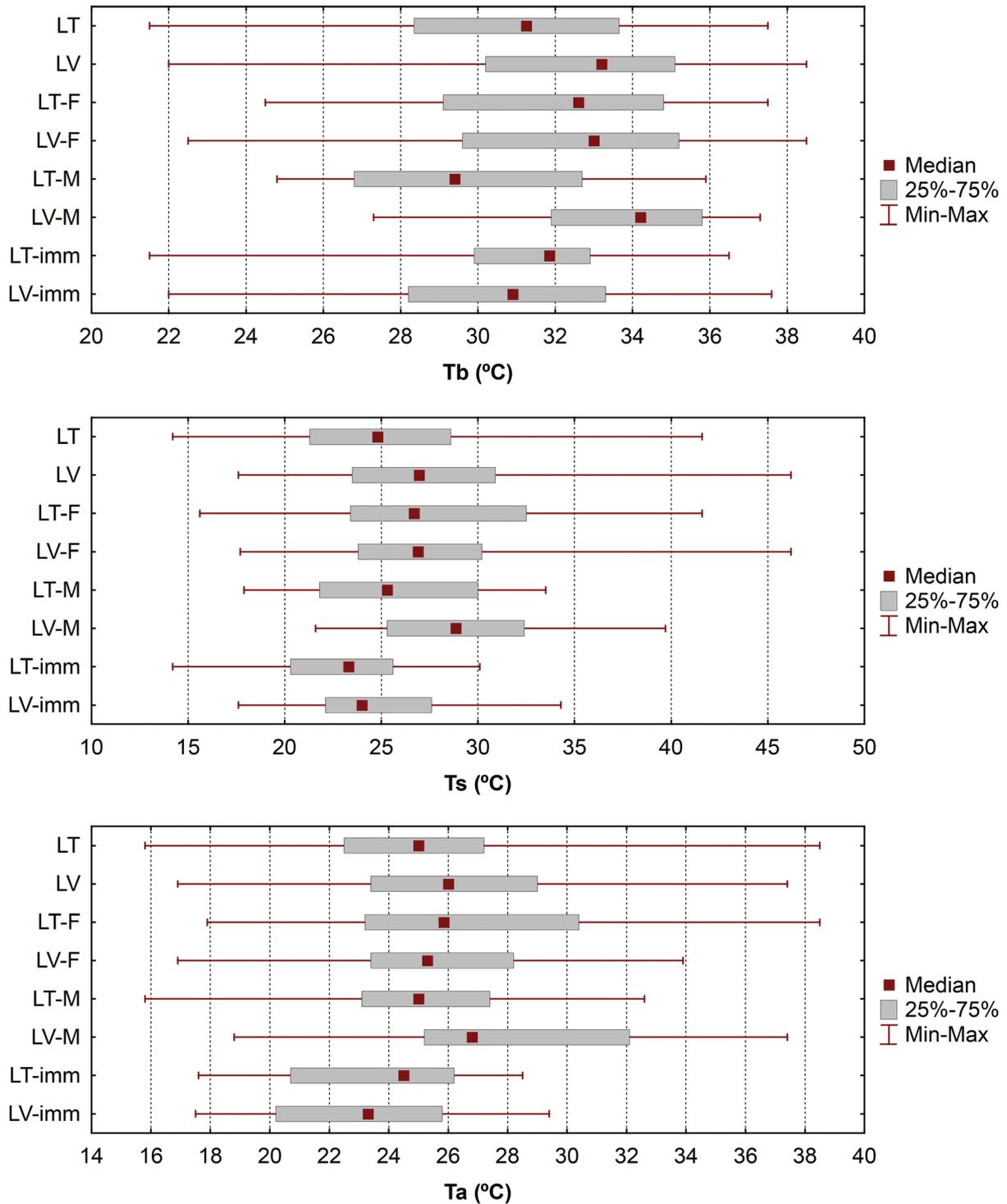


Figure 3. Boxplots of descriptive statistics of body, substrate and air temperature (respectively Tb, Ts and Ta) for the sex/size groups of both species (LT = *L. trilineata*; LV = *L. viridis*; F = females; M = males; imm = immatures).

4.5 mW/cm² in *L. viridis* (with a median of 1.14) and 0.1 to 4.1 mW/cm² (median 1.6) in *L. trilineata*. The UV vision is widespread in lacertid lizards (Pérez i de Lanuza and Font 2014), which indicates they have a complex color vision, including a specific UV-sensitive photoreceptor. Consequently, thermoregulatory behaviors should serve not only to regulate body temperature but also to regulate

the UV exposure (Conley and Lattanzio 2022). Nevertheless, further research is needed to clarify the importance of UV light and lizards' adaptation in view of UV exposure.

No differences were found for air humidity, except for a slightly significant difference between the two species in adult males – a tendency for higher values in *L. trilineata*. It is known that to cope with the drought and risk

of dehydration, lizards in dry environments must balance thermoregulation with water preservation, by changing their activity time or spending less time at higher temperatures. For example, Sannolo and Carretero (2019) showed that dehydration may negatively affect thermoregulation in lacertid lizards, thus forcing them to select body temperatures lower than the preferred ones. However, this was beyond the scope of our study and the effect of evaporation was not taken into account. The importance of humidity and its significance on the studied species, requires further investigation. Overall, long-term studies on the impact of temperature and other climatic factors on lacertid lizards, as well as their response to climate change, are scarce and primarily conducted in high-mountain areas (Chamaillé-Jammes et al. 2006; Ortega et al. 2016b; Díaz et al. 2022). In this context, the present study emphasizes the thermal ecology of lacertid species in a hot and dry area and lays the necessary foundation for future research, as well as monitoring of the impact of rising temperatures on lizard ecology.

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References

- Amat F, Llorente GA, Carretero MA (2003) A preliminary study on thermal ecology, activity times and microhabitat use of *Lacerta agilis* (Squamata: Lacertidae) in the Pyrenees. *Folia Zoologica* 52(4): 413–422.
- Arnold EN (1987) Resource partition among lacertid lizards in southern Europe. *Journal of Zoology* 1: 739–782. <https://doi.org/10.1111/j.1096-3642.1987.tb00753.x>
- Arribas OJ (2013) Thermoregulation, activity and microhabitat selection in the rare and endangered Batuecan Rock Lizard, *Iberolacerta martinezricai* (Arribas, 1996) (Squamata: Sauria: Lacertidae). *Herpetozoa* 26(1/2): 77–90.
- Avery RA, Bedford JD, Newcombe CP (1982) The role of thermoregulation in lizard biology: predatory efficiency in a temperate diurnal basker. *Behavioral Ecology and Sociobiology* 11: 261–267. <https://doi.org/10.1007/BF00299303>
- Baines FM, Chattell J, Dale J, Garrick D, Gill I, Goetz M, Skelton T, Swatman M (2016) How much UVB does my reptile need? The UV-Tool, a guide to the selection of UV lighting for reptiles and amphibians in captivity. *Journal of Zoo and Aquarium Research* 4 (1): 42–63.
- Beal MS, Lattanzio MS, Miles DB (2014) Differences in the thermal physiology of adult Yarrow's spiny lizards (*Sceloporus jarrovi*) in relation to sex and body size. *Ecology and Evolution* 4(22): 4220–4229. <https://doi.org/10.1002/ece3.1297>
- Belasen A, Brock K, Li B, Chremou D, Valakos E, Pafilis P, Sinervo B, Foufopoulos J (2017) Fine with heat, problems with water: microclimate alters water loss in a thermally adapted insular lizard. *Oikos* 126(3): 447–457. <https://doi.org/10.1111/oik.03712>
- Biber MF, Voskamp A, Hof C (2023) Potential effects of future climate change on global reptile distributions and diversity. *Global Ecology and Biogeography* 32: 519–534. <https://doi.org/10.1111/geb.13646>
- Bodensteiner BL, Agudelo-Cantero GA, Arietta AZA, Gunderson AR, Muñoz MM, Refsnider JM, Gangloff EJ (2020) Thermal adaptation revisited: How conserved are thermal traits of reptiles and amphibians? *Journal of Experimental Zoology* 335: 173–194. <https://doi.org/10.1002/jez.2414>
- Castilla AM, Bauwens D (1991) Thermal biology, microhabitat selection, and conservation of the insular lizard *Podarcis hispanica atrata*. *Oecologia* 85: 366–374. <https://doi.org/10.1007/BF00320612>
- Castilla AM, Van Damme R, Bauwens D (1999) Field body temperatures, mechanisms of thermoregulation and evolution of thermal characteristics in lacertid lizards. *Natura Croatica* 8(3): 253–274.
- Chamaillé-Jammes S, Massot M, Aragón P, Clobert J (2006) Global warming and positive fitness response in mountain populations of common lizards *Lacerta vivipara*. *Global Change Biology* 12: 392–402. <https://doi.org/10.1111/j.1365-2486.2005.01088.x>
- Childers JL, Eifler DA (2015) Intraspecific behavioural variation in the lacertid lizard *Meroles cuneirostris* (Strauch, 1867) (Sauria: Lacertidae). *African Journal of Herpetology* 64(1): 54–66. <https://doi.org/10.1080/21564574.2014.998725>
- Choudhary NL, Chishty C (2021) Factors responsible for global decline of reptilian population: A review. *International Journal of Zoological Investigations* 7(2): 549–556. <https://doi.org/10.33745/ijzi.2021.v07i02.033>
- Conley DA, Lattanzio MS (2022) Active regulation of ultraviolet light exposure overrides thermal preference behaviour in eastern fence lizards. *Functional Ecology* 36: 2240–2250. <https://doi.org/10.1111/1365-2435.14114>
- Delaney DM, Warner DA (2017) Adult male Density influences juvenile microhabitat use in a territorial lizard. *Ethology* 123: 157–167. <https://doi.org/10.1111/eth.12586>
- Díaz JA, Iraeta P, Monasterio C (2006) Seasonality provokes a shift of thermal preferences in a temperate lizard, but altitude does not. *Journal of Thermal Biology* 31: 237–242. <https://doi.org/10.1016/j.jtherbio.2005.10.001>
- Díaz JA, Izquierdo-Santiago R, Llanos-Garrido A (2022) Lizard thermoregulation revisited after two decades of global warming. *Functional Ecology* 36(12): 3022–3035. <https://doi.org/10.1111/1365-2435.14192>
- Du W-G, Yan S-J, Ji X (2000) Selected body temperature, thermal tolerance and thermal dependence of food assimilation and locomotor performance in adult blue-tailed skinks, *Eumeces elegans*. *Journal of Thermal Biology* 25: 197–202. [https://doi.org/10.1016/S0306-4565\(99\)00022-4](https://doi.org/10.1016/S0306-4565(99)00022-4)
- Ferguson GW, Brinker AM, Gehrmann WH, Bucklin SE, Baines FM, Mackin SJ (2010) Voluntary exposure of some western-hemisphere snake and lizard species to ultraviolet-B radiation in the field: how much ultraviolet-B should a lizard or snake receive in captivity? *Zoo Biology* 29 (3): 317–334. <https://doi.org/10.1002/zoo.20255>
- Ferguson GW, Gehrmann WH, Brinker AM, Kroh GC (2014) Daily and seasonal patterns of natural ultraviolet light exposure of the western

- sagebrush lizard (*Sceloporus graciosus gracilis*) and the dunes sagebrush lizard (*Sceloporus arenicolus*). *Herpetologica* 70 (1): 56–68. <https://doi.org/10.1655/HERPETOLOGICA-D-13-00022>
- Foufopoulos J, Kilpatrick AM, Ives AR (2010) Climate change and elevated extinction rates of reptiles from Mediterranean islands. *The American Naturalist* 177: 119–129. <https://doi.org/10.1086/657624>
- Grigg JW, Buckley LB (2013) Conservatism of lizard thermal tolerances and body temperatures across evolutionary history and geography. *Biology Letters* 9: 20121056. <https://doi.org/10.1098/rsbl.2012.1056>
- Grozdanov A, Tzankov N, Peshev D (2011) Thermal ecology of the lowland subspecies of sand lizard *Lacerta agilis chersonensis* Adrejewski, 1832 (Squamata: Lacertidae) in Bulgaria. In: Odjakova M (Ed.) *The youth scientific conference “Kliment’s days”: Conference proceedings. (Second book)*. Sofia University “St. Kliment Ohridski”, Sofia, 81–83.
- Harwood RH (1979) The effect of temperature on the digestive efficiency of three species of lizard, *Cnemidophorus tigris*, *Gerrhonotus multicarinatus* and *Sceloporus occidentalis*. *Comparative Biochemistry and Physiology* 63: 417–433. [https://doi.org/10.1016/0300-9629\(79\)90613-3](https://doi.org/10.1016/0300-9629(79)90613-3)
- Heltai B, Sály P, Kovács D, Kiss I (2015) Niche segregation of sand lizard (*Lacerta agilis*) and green lizard (*Lacerta viridis*) in an urban semi-natural habitat. *Amphibia-Reptilia* 36(4): 389–399. <https://doi.org/10.1163/15685381-00003018>
- Herczeg G, Török J, Korsós Z (2007) Size-dependent heating rates determine the spatial and temporal distribution of small-bodied lizards. *Amphibia-Reptilia* 28(3): 347–356. <https://doi.org/10.1163/156853807781374674>
- Herrel A, James RS, Van Damme R (2007) Fight versus flight: physiological basis for temperature-dependent behavioral shifts in lizards. *Journal of Experimental Biology* 210: 1762–1767. <https://doi.org/10.1242/jeb.003426>
- Holick MF, Tian XQ, Allen M (1995) Evolutionary importance for the membrane enhancement of the production of vitamin D₃ in the skin of poikilothermic animals. *Proceedings of the National Academy of Sciences of the United States of America* 92: 3124–3126. <https://doi.org/10.1073/pnas.92.8.3124>
- Huey RB (1974) Behavioural thermoregulation in lizards: importance of associated costs. *Science* 184(4140): 1001–1003. <https://doi.org/10.1126/science.184.4140.1001>
- Huey RB, Deutsch CA, Tewksbury JJ, Vitt LJ, Hertz PE, Pérez HJA, Garland T (2009) Why tropical forest lizards are vulnerable to climate warming. *Proceedings of the Royal Society B* 276: 1939–1948. <https://doi.org/10.1098/rspb.2008.1957>
- Huey RB, Pianka ER (2007) Lizard Thermal Biology: Do Genders Differ? *The American Naturalist* 170(3): 473–478. <https://doi.org/10.1086/520122>
- Karsten KB, Ferguson GW, Chen TC, Holick MF (2009) Panther chameleons, *Furcifer pardalis*, behaviorally regulate optimal exposure to UV depending on dietary vitamin D₃ status. *Physiological and Biochemical Zoology* 82: 218–225. <https://doi.org/10.1086/597525>
- Kearney M, Shine R, Porter WP (2009) The potential for behavioral thermoregulation to buffer “cold-blooded” animals against climate warming. *Proceedings of the National Academy of Sciences* 106: 3835–3840. <https://doi.org/10.1073/pnas.0808913106>
- Kirchhof S, Hetem RS, Lease HM, Miles DB, Mitchell D, Müller J, Rödel MO, Sinervo B, Wassenaar T, Murray IW (2017) Thermoregulatory behavior and high thermal preference buffer impact of climate change in a Namib Desert lizard. *Ecosphere* 8(12): e02033. <https://doi.org/10.1002/ecs2.2033>
- Kornilios P, Thanou E, Lymberakis P, Ilgaz Ç, Kumlucaş Y, Leaché A (2019) A phylogenomic resolution for the taxonomy of Aegean green lizards. *Zoologica Scripta* 49(1): 14–27. <https://doi.org/10.1111/zsc.12385>
- Kotsakiozi P, Jablonski D, Ilgaz Ç, Kumlucaş Y, Avcı A, Meiri S, Poulakakis N (2018) Multilocus phylogeny and coalescent species delimitation in Kotschy’s gecko, *Mediodactylus kotschyi*: hidden diversity and cryptic species. *Molecular Phylogenetics and Evolution* 125: 177–187. <https://doi.org/10.1016/j.ympev.2018.03.022>
- Krastev G, Vacheva E, Naumov B (2023) Winter activity of the snake-eyed lizard *Ophisops elegans* (Reptilia: Lacertidae) in the north-westernmost part of its range. *Historia Naturalis Bulgarica* 45(4): 83–88. <https://doi.org/10.48027/hnb.45.042>
- Licht P (1972) Environmental physiology of reptilian breeding cycles: Role of temperature. *General and Comparative Endocrinology* 3: 477–488. [https://doi.org/10.1016/0016-6480\(72\)90178-5](https://doi.org/10.1016/0016-6480(72)90178-5)
- Meek R (1995) Reptiles, thermoregulation and the environment. *Testudo* 4(2): 56–78.
- Monasterio C, Salvador A, Iraeta P, Díaz JA (2009) The effects of thermal biology and refuge availability on the restricted distribution of an alpine lizard. *Journal of Biogeography* 36(9): 1673–1684. <https://doi.org/10.1111/j.1365-2699.2009.02113.x>
- Ortega Z, Mencía A, Pérez-Mellado V (2016a) Wind constraints on the thermoregulation of high mountain lizards. *International Journal of Biometeorology* 61: 565–573. <https://doi.org/10.1007/s00484-016-1233-9>
- Ortega Z, Mencía A, Pérez-Mellado V (2016b) Behavioral buffering of global warming in a cold-adapted lizard. *Ecology and Evolution* 6(13): 4582–4590. <https://doi.org/10.1002/ece3.2216>
- Ortega Z, Mencía A, Pérez-Mellado V (2016c) The peak of thermoregulation effectiveness: Thermal biology of the Pyrenean rock lizard, *Iberolacerta bonnali* (Squamata, Lacertidae). *Journal of Thermal Biology* 56: 77–83. <https://doi.org/10.1016/j.jtherbio.2016.01.005>
- Ortega Z, Pérez-Mellado V (2016) Seasonal patterns of body temperature and microhabitat selection in a lacertid lizard. *Acta Oecologica* 77: 201–206. <https://doi.org/10.1016/j.actao.2016.08.006>
- Pérez i de Lanuza G, Font E (2014) Ultraviolet vision in lacertid lizards: Evidence from retinal structure, eye transmittance, SWS1 visual pigment genes and behaviour. *Journal of Experimental Biology* 217: 2899–2909. <https://doi.org/10.1242/jeb.104281>
- Pianka ER (1986) *Ecology and Natural History of Desert Lizards: Analyses of the Ecological Niche and Community Structure*. Princeton University Press, Princeton, New Jersey, 222 pp. <https://doi.org/10.1515/9781400886142>
- Popova S, Vacheva E, Naumov B (2023) A contribution to the thermal ecology of *Lacerta agilis bosnica* Schreiber, 1912 (Reptilia: Lacertidae). *Ecologia Balkanica* 15(1): 173–180.
- Rozen-Rechels D, Dupoué A, Lourdaïs O, Chamaille-Jammes S, Meylan S, Clobert J, Le Galliard J-F (2019) When water interacts with temperature: Ecological and evolutionary implications of thermo-hydroregulation in terrestrial ectotherms. *Ecology and Evolution* 9(17): 10029–10043. <https://doi.org/10.1002/ece3.5440>
- Rozen-Rechels D, Rutschmann A, Dupoué A, Blaimont P, Chauveau V, Miles DB, Guillon M, Richard M, Badiane A, Meylan S, Clobert J, Le Galliard J-F (2020) Interaction of hydric and thermal conditions drive geographic variation in thermoregulation in a widespread lizard. *Ecological monographs* 91(2): e01440. <https://doi.org/10.1002/ecm.1440>

- Sagonas K, Kapsalas G, Valakos E, Pafilis P (2017) Living in sympatry: The effect of habitat partitioning on the thermoregulation of three Mediterranean lizards. *Journal of Thermal Biology* 65: 130–137. <https://doi.org/10.1016/j.jtherbio.2017.02.014>
- Sagonas K, Pafilis P, Lymberakis P, Valakos ED (2019) Sexual maturation and reproduction of the Balkan green lizard *Lacerta trilineata* specimens in mainland and island populations from Greece. *North-Western Journal of Zoology* 15(1): 55–61.
- Sagonas K, Valakos ED, Lymberakis P, Pafilis P (2018) Traits of reproduction and feeding of the European Green lizard, *Lacerta viridis* (Laurenti, 1768), at the southern edge of its distribution (Squamata: Sauria: Lacertidae). *Herpetozoa* 30(3/4): 115–129.
- Sagonas K, Valakos ED, Pafilis P (2013) The impact of insularity on the thermoregulation of a Mediterranean lizard. *Journal of Thermal Biology* 38: 480–486. <https://doi.org/10.1016/j.jtherbio.2013.08.004>
- Şahin MK, Kuyucu AC (2021) Thermal biology of two sympatric Lacertids lizards (*Lacerta diplochondrodes* and *Parvilacerta parva*) from Western Anatolia. *Journal of Thermal Biology* 101: 103094. <https://doi.org/10.1016/j.jtherbio.2021.103094>
- Sannolo M, Barroso FM, Carretero MA (2018) Physiological differences in preferred temperatures and evaporative water loss rates in two sympatric lacertid species. *Zoology* 126: 58–64. <https://doi.org/10.1016/j.zool.2017.12.003>
- Sannolo M, Carretero MA (2019) Dehydration constrains thermoregulation and space use in lizards. *Plos One* 14(7): e0220384. <https://doi.org/10.1371/journal.pone.0220384>
- Schall JJ (1977) Thermal ecology of five sympatric species of Cnemidophorus (Sauria: Teiidae). *Herpetologica* 33(3): 261–272.
- Sinervo B, Mendez-de-la-Cruz F, Miles DB, Heulin B, Bastiaans E, Cruz MS, et al. (2010) Erosion of lizard diversity by climate change and altered thermal niches. *Science* 328: 894–899. <https://doi.org/10.1126/science.1184695>
- StatSoft Inc. (2011) STATISTICA (data analysis software system), version 10. www.statsoft.com
- Stojanov A, Tzankov N, Naumov B (2011) Die Amphibien und Reptilien Bulgariens. Chimaira, Frankfurt am Main, 588 pp.
- Tosini G, Avery RA (1996) Pregnancy decreases set point temperatures for behavioral thermoregulation in the wall lizard *Podarcis muralis*. *Herpetological Journal* 6: 94–96.
- Tzankov N (2005) Sympatric distribution of six lacertid lizards. *Annuaire de l'Université de Sofia "St. Kliment Ohrski"* 96(4): 235–242.
- Vacheva E, Sedefchev H, Stanev N, Tsevtkov M, Lazarkevich I (2022) On the maximum size of the European Green Lizard *Lacerta viridis* (Squamata: Lacertidae) from Bulgaria. *Herpetology Notes* 15: 453–456.
- Vacheva ED, Naumov BY, Tzankov ND (2020) Diversity and Habitat Preferences in Lizard Assemblages (Reptilia: Sauria) from Model Territories in Western Bulgaria. *Acta Zoologica Bulgarica* 72(3): 385–396.
- Verwajen D, Van Damme R (2007) Correlated evolution of thermal characteristics and foraging strategy in lacertid lizards. *Journal of Thermal Biology* 32(7–8): 388–395. <https://doi.org/10.1016/j.jtherbio.2007.05.005>
- Yamaguchi S, Iwasa Y (2018) Temperature-dependent sex determination, realized by hormonal dynamics with enzymatic reactions sensitive to ambient temperature. *Journal of Theoretical Biology* 453:146–155. <https://doi.org/10.1016/j.jtbi.2018.05.023>
- Žagar A, Carretero MA, Osojnik N, Sillero N, Vrežec A (2015) A place in the sun: interspecific interference affects thermoregulation in co-existing lizards. *Behavioral Ecology and Sociobiology* 69: 1127–1137. <https://doi.org/10.1007/s00265-015-1927-8>

Appendix 1



Figure A1. Images of the studied species: on the left – *Lacerta viridis*, from top to bottom: a pair of *Lacerta viridis*, a male with a mating coloration and a female; on the right – *L. trilineata*, from top to bottom: a pair, a male and a female *L. trilineata*. Photographs by Emiliya Vacheva.



Figure A2. Images of the habitats in the two studied parts of the area: above – the northwestern part of the area with the local road; below – the southeastern part of the area and a characteristic habitat with stones and rare shrubs (the small photo). Photographs by Emiliya Vacheva.

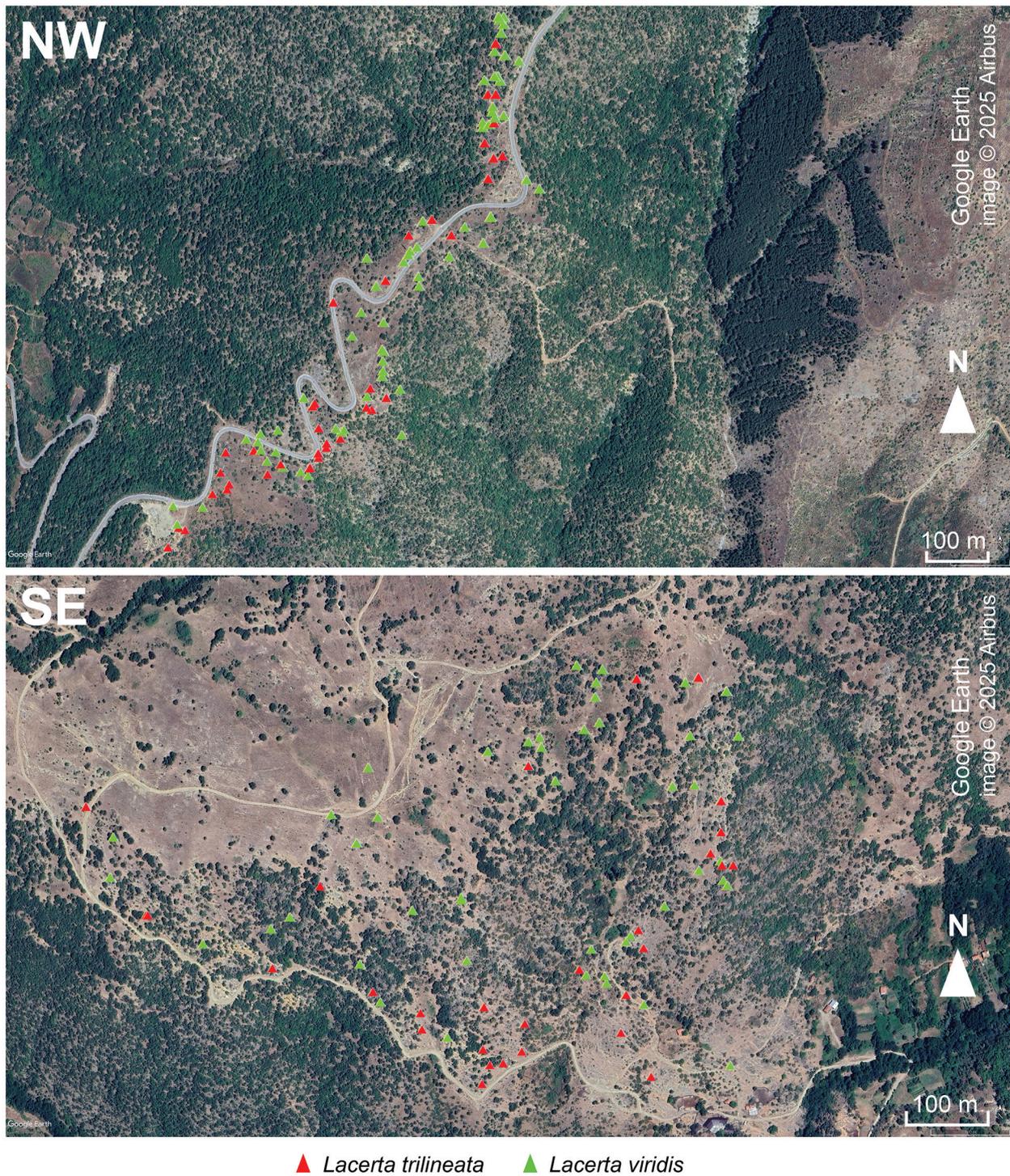


Figure A3. Spatial distribution of *L. trilineata* and *L. viridis* registrations in the study area on satellite images (see also Fig. 1): NW refers to the northwestern part of the area (41 registrations of *L. trilineata* and 69 registrations of *L. viridis*); SE refers to the southeastern part of the area (31 registrations of *L. trilineata* and 49 registrations of *L. viridis*).

Appendix 2

Table A1. Descriptive statistics of the studied factors (SVL = snout-vent length; Tb = body temperature; Ts = substrate temperature; Ta = air temperature; UV = UV radiation intensity; RH = relative humidity) for the full samples (Overall) and separately for the sex/size groups (Females, Males, Immatures) of both species.

		<i>Lacerta trilineata</i>				<i>Lacerta viridis</i>			
		N	Median	Min	Max	N	Median	Min	Max
SVL (mm)	Overall	72	106.50	39	140	118	95.75	36	119
	Females	27	119.00	84	137	39	99.00	84	111
	Males	19	123.00	85	140	50	102.00	80	119
	Immatures	26	59.75	39	81	29	63.00	36	79
Tb (°C)	Overall	72	31.25	21.5	37.5	118	33.20	22.0	38.5
	Females	27	32.60	24.5	37.5	39	33.00	22.5	38.5
	Males	19	29.40	24.8	35.9	50	34.20	27.3	37.3
	Immatures	26	31.85	21.5	36.5	29	30.90	22.0	37.6
Ts (°C)	Overall	71	24.80	14.2	41.6	118	26.95	17.6	46.2
	Females	26	26.70	15.6	41.6	39	26.90	17.7	46.2
	Males	19	25.30	17.9	33.5	50	28.85	21.6	39.7
	Immatures	26	23.30	14.2	30.1	29	24.00	17.6	34.3
Ta (°C)	Overall	71	25.00	15.8	38.5	117	26.00	16.9	37.4
	Females	26	25.85	17.9	38.5	39	25.30	16.9	33.9
	Males	19	25.00	15.8	32.6	49	26.80	18.8	37.4
	Immatures	26	24.50	17.6	28.5	29	23.30	17.5	29.4
UV (mW/cm²)	Overall	66	1.60	0.1	4.1	109	1.41	0.0	4.5
	Females	26	1.35	0.3	3.8	37	0.95	0.1	4.0
	Males	18	2.09	0.1	4.1	46	1.61	0.0	4.0
	Immatures	22	1.60	0.4	3.4	26	1.84	0.0	4.5
RH (%)	Overall	68	38.75	25.5	64.5	114	34.25	23.0	72.6
	Females	26	37.00	25.5	54.5	37	38.00	25.5	63.0
	Males	18	39.75	29.5	64.5	49	33.00	23.0	59.4
	Immatures	24	39.25	26.5	59.5	28	40.00	23.5	72.6