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A Contribution to the Thermal Ecology of Lacerta agilis bosnica Schreiber, 1912 (Reptilia: Lacertidae)

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Abstract. Thermal ecology is critical for the study of the biology of every living species. Accordingly, body temperature affects the performance and fitness of animals. In the particular case of ectotherms, this effect is further reinforced since they rely on environmental conditions for heat adjustment. Current air and substrate temperatures are among the crucial sources of regulation for their body heat. In the case of lizards, various behavioral techniques are employed to insure their thermoregulation and therefore survival. The aim of the present study is to identify the basic thermal preferences of *Lacerta agilis bosnica*, including possible age and sex related variations. This lacertid subspecies is endemic to the high mountains across the Balkan Peninsula and limited between 1100 and 2200 m above sea level. Our results revealed highest body temperature of 35.9°C and lowest – of 19.5°C both measured in adult females. We calculated the average thermoadaptation index (Ti) of 1.27 for all individuals. Furthermore, the positive correlations of lizard body temperature (Tb) compared to air (Ta) and to substrate (Ts) temperatures were highest in immature males, while lowest - in adult males. We found statistically significant differences between the age/sex groups within Tb and Ta. Regarding Ts and Ti, the tests did not show significant differences between the groups. We concluded that *L. agilis bosnica* is a taxon adapted to low ambient temperatures. With this discovery we contributed to the exploration of the understudied subspecies *Lacerta agilis bosnica*.

Key words: Sauria, lizard, Balkan Peninsula, Bulgaria, thermoadaptation.

Introduction

Lizards, as ectotherms, rely on environmental sources for heat gain, while all metabolic and physiological processes are temperature dependent. Body temperature affects the entire animal performance and fitness, therefore thermal ecology has a key role in explaining their biology and behavior. Body temperature depends on several environmental variables. Some of them, such as air and substrate temperature, have a great impact. Others like wind speed, atmospheric pressure, and radiation are probably equally important (Ortega et al., 2016).

Body temperatures in lacertids can vary widely in association with seasonal and daily acclimation (Huey et al., 1977; Castilla & Bauwens, 1991; Díaz et al., 2006; Ortega & Pérez-Mellado, 2016), habitat complexity (Monasterio et al., 2009, Neel & McBrayer, 2018), size, age, and sex (Van Damme et al., 1987; Paulissen, 1988). As a whole, the lizards manage their body temperature behaviorally, such as by changing active time, by choosing appropriate microhabitats, or by selecting heating or cooling sites (Huey, 1974; Castilla & Bauwens, 1991).

The Sand lizard (*Lacerta agilis* Linnaeus, 1758) is a widespread species which inhabits an area from southern Sweden to northern Greece in the South, and from Great Britain and north-eastern Spain to the Baikal Lake in the East (Ananjeva et al., 2004; Sillero et al., 2014). According to Andres et al. (2014), at least nine subspecies are known, but the intraspecific taxonomy is still not elucidated in full. *Lacerta agilis bosnica* Schreiber, 1912, the subject of this study, is an endemic taxon, whose distribution is limited to the high mountains across the Balkan Peninsula (Bischoff, 1984; Džukić & Kalezić, 2004; Valakos et al., 2008; Stojanov et al., 2011; Mizsei et al., 2017).

The sand lizard, like almost all other species in the Lacertidae family, is a diurnal and heliothermic species (Arnold, 1987; Castilla et al., 1999). Its activity is usually bimodal, but in some populations, it is unimodal (Korsos & Gyovai, 1988). The sand lizard can prolong the period and duration of its activity by taking advantage of different temperature conditions in diverse microhabitats (House et al., 1980). Some aspects of *L. agilis* ecology have been subject of research, including thermoecology in certain parts of its range (House et al., 1980; Bischoff, 1984; Castilla et al., 1999; Amat et al., 2003; Litvinov & Ganshchuk 2010; Litvinov et al., 2010), as well as the effect of temperature on sprint speed (Bauwens et al. 1995), and incubation time (Kjaergaard, 1981; Li et al., 2013). However, it should be emphasized that nearly all studies have been conducted in other parts of the vast range of the species, while the Balkan populations are still very poorly studied in ecological terms, and the only data on the thermal ecology (Grozdanov et al., 2011) refers to *L. agilis chersonensis* Andrzejowski, 1832.

The aim of the present study was to identify the basic thermal preferences of *Lacerta agilis bosnica*, including possible age and sex related variations.

Materials and Methods

The field research was conducted in three of the mountains of western Bulgaria: Plana, Vitosha and Osogovo Mts. (Fig. 1). In each of the mountains, we selected specific sites with relatively numerous local populations of *Lacerta agilis bosnica* (according to preliminary data). The studied sites represented vast meadows (or a complex of smaller ones), located between 1100 and 1700 m above sea level (more detailed descriptions of the areas are provided by Popova et al., 2021). The particular sites were visited irregularly during the months from April to September in the period 2014 – 2019.

The lizards were detected visually, followed by an attempt to capture each individual manually. Subsequently, we measured body temperature (by placing the thermometer probe in the cloaca), air temperature, substrate temperature, and body length (SVL: from the tip of the snout to the cloaca), and determined sex. Upon completion of the measurements, each individual was released at the place of capture. Temperature measurements were done by a digital thermometer with a probe (Thermo TA - 288, with an accuracy of 0.1°C), and those of body length – with a transparent ruler with an accuracy of 1 mm. Data collected this way includes a total of 369 specimens of *Lacerta agilis bosnica* (respectively 129 from Plana Mtn., 162 from Vitosha Mtn., and 78 from Osogovo Mtn.). Two age classes were defined according to the body length (see Popova et al. (2019) and references therein): adults (SVL > 55 mm) and immatures (SVL ≤ 55 mm). Sex affiliations were determined based on the coloration pattern, number of ventral scales, and size of the femoral pores (see e.g. Darevsky et al. (1976), Majláth et al. (1997), and Popova et al. (2021)). Thereby, the captured lizards were divided in four age/sex groups: adult males (AdM), adult females (AdF), immature males (ImM), and immature females (ImF).

Since the intensity of fieldwork was not equal across months/seasons, all of the collected data was combined and analyzed without considering possible seasonal variations. Based on the temperature data, the thermoadaptation index (It) was also calculated for each individual, according to the formula:

$$It = Tb/(0.5(Ta+Ts)),$$

where Tb, Ta, and Ts are respectively body, air, and substrate temperatures (see Litvinov & Ganshchuk, 2010).

Statistical descriptions and analyses were done using non-parametric statistics, as the data did not have a normal distribution and the attempt to normalize it (by logarithmisation) was only partially successful. Spearman's correlation coefficient (rho) was used to test for correlation between body temperature and the temperatures of air and substrate. The possible differences between age/sex groups were tested through "Kruskal-Wallis ANOVA" and "Multiple comparisons of mean ranks" as a post-hoc test (after confirmation of homogeneity of variance via Levene's test ($p > 0.05$ in all cases)). Statistical procedures were done using STATISTICA v.10 (StatSoft, Inc. 2011).

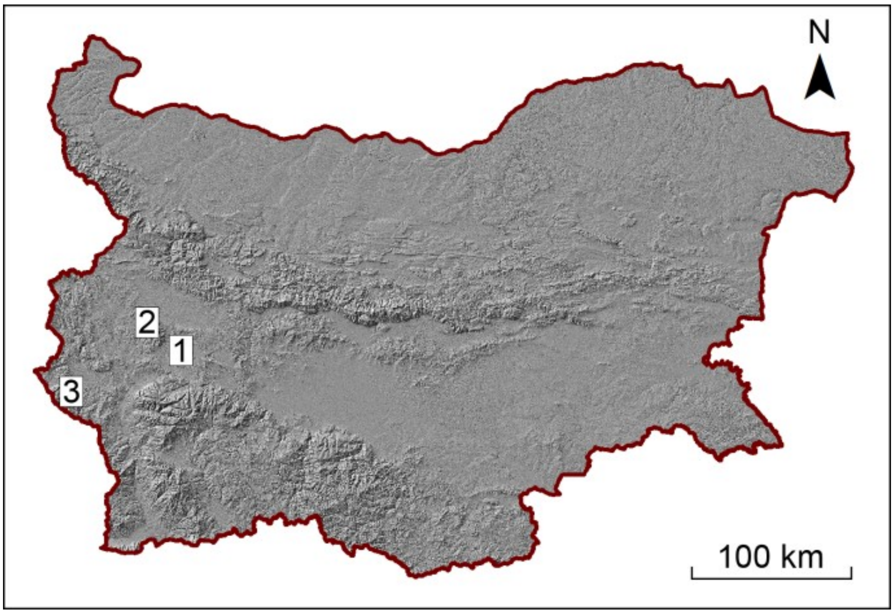

Fig. 1. Location of the study sites on a map of Bulgaria: 1) Plana Mtn.; 2) Vitosha Mtn.; 3) Osogovo Mtn.

Results

Descriptive statistics of the measured body, air, and substrate temperatures are presented in Fig. 2 (see also Appendix 1 for more details). The highest measured body temperature is 35.9ºC and applies to an adult female (caught on April 23 at air and substrate temperatures of 25.7ºC and 18.3ºC respectively), while the lowest recorded of 19.5ºC also applies to an adult female (caught on May 27 at air and substrate temperatures of 14.3ºC and 15.9ºC). In immature lizards, the highest and lowest measured temperatures are respectively 35.4ºC (a female, caught on July 25 at air and substrate temperatures of 21.9ºC and 27.0ºC) and 20.6ºC (a male, caught on September 13 at air and substrate temperatures of 17.2ºC and 17.8ºC). Concerning the thermoadaptation index, the total average value is 1.27.

The correlation between lizard body temperature and air and substrate temperatures is positive and statistically significant in each of the age/sex groups, except for adult males with regard to the substrate factor (Table 1). The highest values of correlation are observed in immature males (both with the air and substrate temperatures), while the lowest - in adult males.

Following the Kruskal-Wallis test (Table 2) there are statistically significant differences between age/sex groups according to body and air temperatures. The post-hoc test shows that this is due to significant differences between adult females and immature males, as the central trend is for higher values in females. Regarding the substrate temperature and the thermoadaptation index, the tests do not show significant differences between the groups.

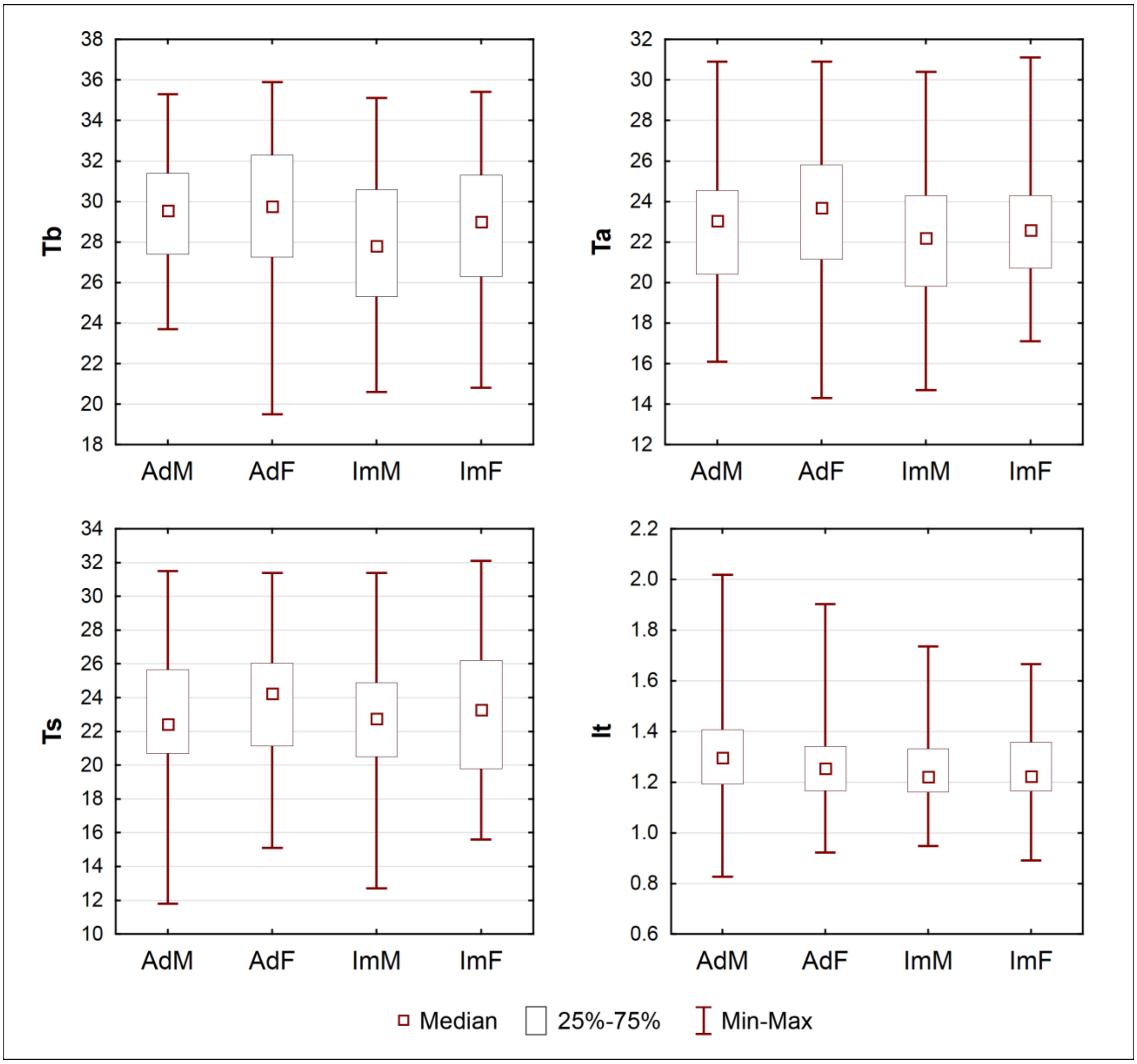

Fig. 2. Median, generalized range of the second and third quartiles (25^{th} – 75^{th} percentile) and range (Min-Max) of body, air, and substrate temperatures (Tb, Ta, and Ts respectively), and of the thermoadaptation index (It) towards the age/sex groups.

Table 1. Correlation (Spearman's rho and p value) between lizard body temperature according to air and substrate temperatures (Ta and Ts respectively) per age/sex group; statistically significant values are indicated by asterisks.

	Ta	**Ts**
AdM	rho = 0.302, p = 0.015*	rho = 0.211, p = 0.095
AdF	rho = 0.534, p << 0.001***	rho = 0.470, p << 0.001***
ImM	rho = 0.741, p << 0.001***	rho = 0.627, p << 0.001***
ImF	rho = 0.668, p << 0.001***	rho = 0.439, p << 0.001***

Table 2. Kruskal-Wallis and post-hoc test results for the differences between age/sex groups with regard to the body, air, and substrate temperatures (Tb, Ta, and Ts), and to the thermoadaptation index (It); statistically significant values are indicated by asterisks.

	Kruskal-Wallis (H, p)	**Post-hoc (p)**				
Tb	H = 14.098, p = 0.003**		AdM	AdF	ImM	ImF
		AdM		1.000	0.051	0.441
		AdF	1.000		0.006**	0.138
		ImM	0.051	0.006**		1.000
		ImF	0.441	0.138	1.000	
Ta	H = 9.597, p = 0.022*		AdM	AdF	ImM	ImF
		AdM		0.598	1.000	1.000
		AdF	0.598		0.030*	0.141
		ImM	1.000	0.030*		1.000
		ImF	1.000	0.141	1.000	
Ts	H = 14.098, p = 0.169		AdM	AdF	ImM	ImF
		AdM		1.000	1.000	1.000
		AdF	1.000		0.221	0.861
		ImM	1.000	0.221		1.000
		ImF	1.000	0.861	1.000	
It	H = 5.650, p = 0.129		AdM	AdF	ImM	ImF
		AdM		0.565	0.165	0.277
		AdF	0.565		1.000	1.000
		ImM	0.165	1.000		1.000
		ImF	0.277	1.000	1.000	

Discussion

The mean body temperature, calculated by temperatures recorded from active animals in the field, is commonly used as a measure of the thermal status of a species. Our results show positive and statistically significant correlation between body temperature with air and substrate temperatures in *L. agilis bosnica*. The correlation is least pronounced in adult males, potentially because they are least dependent on ambient temperature. They have developed a better ability for thermosregulation, probably only behavioral, as males inhabit larger territories and can operate with bigger set of microhabitats (Olsson, 1986). Meanwhile, no significant difference between the sexes was found in any of the studied thermal indicators (see Table 2). Differences were found only between adult females and immature males. Generally, the observed relationships between age/sex groups of *L. agilis bosnica* and thermal parameters are similar to those found by Popova et al. (2021) within microhabitat choice: small or no gender differences, opposed to well-expressed age differences. In the other subspecies which occurs in Bulgaria, *L. a. chersonensis*, a similar lack of difference was detected between the sexes in adults, but a distinction was observed between adult females and immature males (Grozdanov et al., 2011). It is known that the body temperature varies among age classes and sexes in many other lacertid species (Castilla & Bauwens, 1991; Carrascal et al., 1992), while in adult females it may also depend on the reproductive status (Van Damme et al., 1987; Tosini & Avery, 1996). It can be assumed that our observations could be due to the various habitat choices within the different age/sex classes. For example, competition with adults may drive immatures to occupy less suitable habitats. Meanwhile, within the adult group, females are motivated by reproductive costs to choose habitats that provide them both with security and better thermoregulation. Another possible explanation could be the role of ontogeny, where juveniles would have higher thermal requirements to maintain high growth rates. However, the potential bias caused by the differences in sample size should also be considered (collected adult females were more abundant than individuals from other age/sex groups).

Regarding the index of thermoadaptation, our study does not show any differences between age/sex groups in *L. agilis bosnica*, while in *L. agilis chersonensis*, adult females differ significantly from immatures according to Grozdanov et al. (2011). According to Litvinov & Ganshchuk (2010), a high value of the index indicates adaptations that allow reptiles to maintain a higher temperature in a relatively cold environment, while a value close to one - the ability to maintain a lower temperature than the environment. Published data on the Sand lizards in western Russia shows that the values of the index are positively related to latitude, with average values ranging from 1.13 in the north to 1.02 in the south (Litvinov & Ganshchuk, 2010; Litvinov et al., 2010). In this study, the calculated average value of the index is 1.27. Therefore, *L. agilis bosnica* should be categorized as a taxon adapted to low ambient temperatures. A potential explanation for this adjustment could be the typically mountainous distribution of the subspecies.

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Appendix 1

Descriptive statistics of the measured temperatures (in degrees Celsius) and of the thermoadaptation index per age/sex groups of *L. agilis bosnica* [AdM, AdF, ImM, and ImF refer respectively to adult males, adult females, immature males, and immature females; Tb, Ta, Ts, and It refer respectively to body, air, and substrate temperature, and thermoadaptation index; N denotes sample size].

		N	Min.	Max.	Mean	St. Dev.	Median	25%	75%
AdM	Tb	64	23.70	35.30	29.53	2.78	29.55	27.40	31.40
	Ta	64	16.10	30.90	22.68	2.94	23.05	20.40	24.55
	Ts	64	11.80	31.50	22.97	3.77	22.45	20.70	25.65
	It	64	0.83	2.02	1.31	0.19	1.30	1.19	1.41
AdF	Tb	132	19.50	35.90	29.53	3.37	29.75	27.25	32.30
	Ta	132	14.30	30.90	23.38	3.32	23.70	21.15	25.80
	Ts	132	15.10	31.40	23.77	3.65	24.25	21.15	26.05
	It	132	0.92	1.90	1.26	0.15	1.26	1.17	1.34
ImM	Tb	90	20.60	35.10	27.97	3.65	27.80	25.30	30.60
	Ta	90	14.70	30.40	22.21	3.15	22.20	19.80	24.30
	Ts	90	12.70	31.40	22.88	3.64	22.75	20.50	24.90
	It	90	0.95	1.74	1.25	0.13	1.22	1.16	1.33
ImF	Tb	83	20.80	35.40	28.39	3.31	29.00	26.30	31.30
	Ta	83	17.10	31.10	22.57	2.90	22.60	20.70	24.30
	Ts	83	15.60	32.10	23.02	3.67	23.30	19.80	26.20
	It	83	0.89	1.67	1.26	0.15	1.22	1.16	1.36
Total	Tb	369	19.50	35.90	28.89	3.39	29.10	26.30	31.60
	Ta	369	14.30	31.10	22.79	3.15	22.70	20.70	24.90
	Ts	369	11.80	32.10	23.25	3.68	23.30	20.70	25.80
	It	369	0.83	2.02	1.27	0.15	1.24	1.17	1.36

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