

## Notes on the thermal ecology of *Testudo hermanni hermanni* in Menorca (Balearic Islands, Spain)

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**Abstract.** We studied the thermal ecology of *Testudo hermanni hermanni* in Menorca during late spring. We measured body temperatures of adult individuals, together with air and substrate temperatures at points of capture. Sunlight exposure (full sun, filtered sun, or shade) and type of substrate were also recorded. Body temperatures were similar between sexes (mean = 29.95°C) as were air temperatures between gender capture sites (mean = 28.33°C). Conversely, females were found in areas with a higher substrate temperature (31.60°C) than males were (29.15°C). Moreover, the correlation between body and air temperatures was stronger than it was between body and substrate temperatures, as found in other populations of this species. The tortoises were usually found in full sun, and the proportion of animals found in each sunlight category was similar between sexes. Our results contribute to the knowledge of the thermal ecology of the western Hermann's tortoise.

**Keywords:** microhabitat, temperature, Testudinidae, thermoregulation, tortoises.

Temperature fundamentally affects the biology of ectotherms (Bogert, 1949; Pough, 1980; Adolph and Porter, 1993; Angilletta, Niewiarowski and Navas, 2002; Angilletta, 2009). Thermoregulation in ectotherms is the ability of an animal to maintain its body temperature close to its thermal preference, independently of the environmental temperature (Hertz, Huey and Stevenson, 1993). Mechanisms used by reptiles to regulate their body temperature include adjusting their activity periods, shuttling between different microhabitats, and changing their body postures (Adolph and Porter, 1993; Bauwens, Hertz and Castilla, 1996; Angilletta, 2009). The mechanism or combination of mechanisms used are dependent on the costs and benefits in each case (Huey and Slatkin, 1976; Sears and Angilletta, 2015). Of these, tortoises are more dependent on adjusting their activity period and shuttling between microhabitats to thermoregulate, as their ability to make postural adjustments is limited by the carapace (Hailley and Coulson, 1996; McMaster and Downs, 2013).

The Hermann's tortoise, *Testudo hermanni* (Gmelin, 1789), is a medium size testudinid that is distributed throughout most of the European Mediterranean and sub-Mediterranean regions (Cheylan, 2001). There are two sub-species: the eastern Hermann's tortoise, *T. hermanni boettgeri*, and the western Hermann's tortoise, *T. hermanni hermanni* (Fritz et al., 2006). The geographic range of *Testudo h. hermanni* is from northwest Spain to the Po River Valley in Italy, where its distribution is restricted and fragmented. Most populations of *T. hermanni* are declining due to habitat loss, illegal animal trade (Bertolero et al., 2011), and climate change (Fernández-Chacón et al., 2011). In the Balearic Islands, *T. h. hermanni* inhabits Menorca and Mallorca, where it was probably introduced by humans around 3000 years ago (Fritz et al., 2006). In Menorca, this sub-species is distributed throughout the main island, with strong populations (Bertolero and Petrus, 2012).

The Hermann's tortoise shows sexual size dimorphism: female mean straight carapace length (SCL) is approximately 12% greater than that of males (Cheylan, 2001). Body size of adults is variable among populations, with western populations (from Spain, France and Italy) being smaller than eastern populations;

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the average SCL of western populations is 150–179 mm in females and 130–149 mm in males (Cheylan, 2001). Hermann's tortoises are heliothermic, and thermoregulate behaviourally, mainly by basking in the sun (Willemsen, 1991). Their body temperatures are more related to air than to substrate temperatures (Panagiota and Valakos, 1992; Carretero, Bertolero and Llorente, 1995). Their thermal preferences are close to 30°C (Huot-Daubremont, Grenot and Bradshaw, 1996), and they have a thermal tolerance of between –2 and 44°C (Cherchi, 1956).

Currently, no data is available about the thermal biology of the Menorca population. Here we aim to assess the basic thermal ecology of *T. hermanni hermanni* of Menorca. First, we studied basic morphometrics and evaluated if there was sexual size dimorphism in the population of Menorca. Then, we described body temperatures ( $T_b$ ) of the tortoises and environmental temperatures (air temperatures,  $T_a$ , and substrate temperatures,  $T_s$ ) of their capture places. In order to study the thermoregulation ability of this population, we assessed the linear relationships between  $T_b$  and  $T_a$  and  $T_b$  and  $T_s$ : the weaker the dependence of  $T_b$  of environmental temperatures, the greater the thermoregulation ability. Lacking the necessary data to calculate the indexes of thermoregulation proposed by Hertz, Huey and Stevenson (1993), this approach at least provide some valuable information about the thermoregulation of the population. Our results will deepen the knowledge of the thermal biology of tortoises, and will also provide important information for conservation management of this population under climate change.

We collected data between the 1st and the 16th of June of 2013 in Menorca (Balearic Islands, Spain). We captured adult tortoises (21 males and 23 females) by hand during their daily activity period, from 07:00 to 17:00. Immediately after capture, we measured the cloacal body temperature ( $T_b$ ) in degrees °C with a Testo® 925 digital thermometer, shadowing the probe, together with air temperature 10 cm above the capture place ( $T_a$ ), and substrate temperature ( $T_s$ ) at the capture place. We recorded the type of substrate (dry grass, leaf litter, *Posidonia oceanica*, rock, sand and soil)

and the sunlight exposure (full sun, filtered sun – that is, when a tortoise was not in full sun or full shade –, or full shade). In addition, we measured the SCL, the straight carapace width and height (both at the 6th marginal scutes), and the plastron width and length (at the midline distance between the gular and anal scutes) of each tortoise. All measurements were taken using a steel ruler ( $\pm 0.01$  mm), and carapace measurements were made in a straight line, without considering carapace curvature.

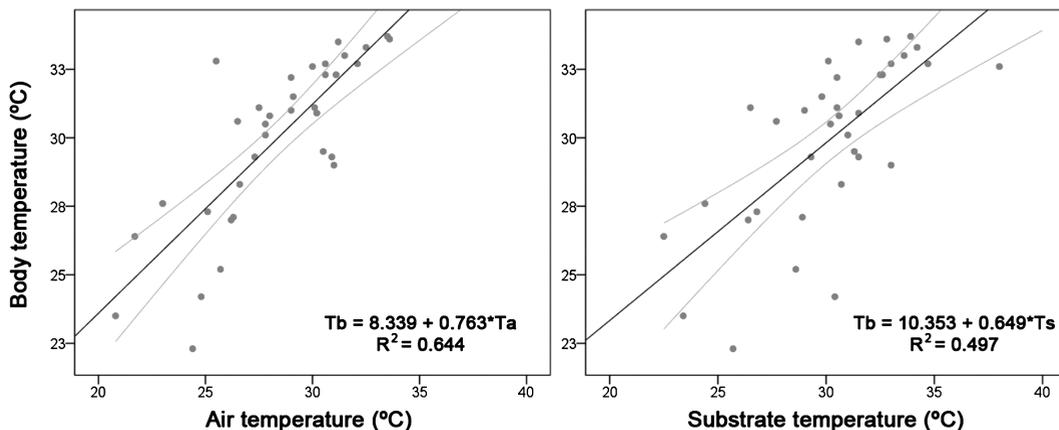
Data followed the assumptions of normality and homoscedasticity, so we used parametric tests for all analyses (Crawley, 2012). We used one-way ANOVA in order to test for possible effects of sex on the morphometric measurements and the temperature ( $T_b$ ,  $T_a$ ,  $T_s$ , SCL, straight carapace width, plastron width, plastron length and carapace height as dependent variables on each test and sex as factor). We used the ANCOVA slope test in order to test for the effect of sex in the linear relationships between  $T_b$  and  $T_a$  and  $T_b$  and  $T_s$  (since it was not significant, we pooled the data for males and females for regressions). We calculated the Pearson correlation coefficient ( $r$ ) to evaluate the direction and the intensity of the linear relationships between  $T_b$  and  $T_a$  and between  $T_b$  and  $T_s$ , where a weaker relationship indicated a greater extent of thermoregulation. Since body size is positively related to thermal inertia, we also used  $r$  for testing the possible effect of plastron length (a proxy of body size) on  $T_b$ . We calculated simple linear regression models between  $T_b$  (dependent variable) and  $T_a$  (independent variable) and between  $T_b$  (dependent variable) and  $T_s$  (independent variable) in order to assess the linear relationships between body and environmental temperatures. Finally, we used the G-test of goodness of fit in order to test if the proportion of tortoises on the sunlight categories differed between sexes. We conducted the analyses using R, version 3.1.3 (R Core Team, 2015). We reported mean values ( $\pm$ SD). Significance level was set at  $\alpha = 0.05$ .

Female's carapace and plastron length and width, and their carapace height were significantly greater than those of males (table 1). Body temperature was similar for males and females (table 1), with a mean value of  $29.95 \pm 3.06^\circ\text{C}$  ( $n = 35$ ). Air temperatures at capture places were also similar between the sexes (table 1), with a mean value of  $28.33 \pm 3.21^\circ\text{C}$  ( $n = 35$ ). Substrate temperatures were significantly higher at male capture places than at female capture places (table 1).

Linear relationships among  $T_b$  and  $T_a$  were similar for males and females (ANCOVA, interaction sex \*  $T_a$ ,  $F_{1,31} = 1.170$ ,  $p = 0.288$ ). There was a large and positive significant correlation between  $T_b$  and  $T_a$  ( $r = 0.802$ ,  $P < 0.001$ ,  $n = 35$ ). Figure 1 shows the linear regression model of  $T_a$  on  $T_b$ . Regarding

**Table 1.** Morphometric measurements and body temperatures of adult individuals of *Testudo hermanni hermanni* on Menorca (Balearic Islands, Spain), together with air and substrate temperatures at their capture sites (all means  $\pm$  SD and (*n*)). Measurements were compared between sexes using one-way ANOVA.

	Males	Females	<i>F</i>	<i>p</i>
Body temperature (°C)	30.91 $\pm$ 2.87 (15)	29.23 $\pm$ 3.06 (20)	2.731	0.108
Air temperature (°C)	29.22 $\pm$ 3.45 (15)	27.66 $\pm$ 2.93 (20)	2.082	0.158
Substrate temperature (°C)	31.60 $\pm$ 3.47 (15)	29.15 $\pm$ 2.86 (20)	5.216	<b>0.029</b>
Straight carapace length (mm)	137.9 $\pm$ 12.8 (13)	171.5 $\pm$ 9.3 (17)	68.979	<b>&lt;0.001</b>
Straight carapace width (mm)	111.2 $\pm$ 10.1 (13)	126.4 $\pm$ 5.2 (17)	28.543	<b>&lt;0.001</b>
Plastron width (mm)	101.3 $\pm$ 8.0 (11)	116.7 $\pm$ 11.2 (17)	15.562	<b>0.001</b>
Plastron length (mm)	117.1 $\pm$ 12.4 (13)	151.9 $\pm$ 6.7 (17)	96.675	<b>&lt;0.001</b>
Carapace height (mm)	69.7 $\pm$ 11.7 (14)	83.9 $\pm$ 6.6 (17)	18.090	<b>&lt;0.001</b>



**Figure 1.** Linear regression models of air temperatures ( $T_a$ ) on body temperatures ( $T_b$ ; left), and substrate temperatures ( $T_s$ ) on body temperatures ( $T_b$ ; right) for adult individuals of *Testudo hermanni hermanni* on Menorca (Balearic Islands, Spain). Air and substrate temperatures were taken at individual capture places. The thin lines accompanying the slope of regression depict the 95% confidence intervals.

substrate temperatures, linear relationships between  $T_b$  and  $T_s$  were also similar for both sexes (ANCOVA, interaction sex  $\times$   $T_s$ ,  $F_{1,31} = 1.548$ ,  $P = 0.223$ ). There was also a significant, positive correlation between  $T_b$  and  $T_s$  ( $r = 0.705$ ,  $P < 0.001$ ,  $n = 35$ ). Figure 1 shows the linear regression model of  $T_a$  on  $T_b$  and  $T_s$  on  $T_b$ . The correlation between  $T_b$  and length of the plastron was not significant ( $P = 0.220$ ).

Regarding exposure to sunlight, 48.5% of the individuals were in full sun, 39.4% in filtered sun, and 12.1% in shaded microhabitats ( $n = 33$ ), and these proportions were similar between sexes (Goodness of fit test,  $G = 0.614$ ,  $P = 0.736$ ). The substrates used by the studied individuals are variable: dry grass ( $n = 12$ ), leaf

litter ( $n = 3$ ), dry *Posidonia oceanica* ( $n = 6$ ), rock ( $n = 1$ ), sand ( $n = 5$ ), and soil ( $n = 6$ ).

Our results report higher mean body temperatures than those of another population of *T. h. hermanni* in Catalonia (Spain), although this study reported limited data of body temperatures of adult individuals in summer (Carterero, Bertolero and Llorente, 1995). Mean body temperatures *T. h. hermanni* of Menorca were similar to those found for *T. h. boettgeri* at coastal sites in northern Greece (Wright, Steer and Hailey, 1988), and *T. h. hermanni* in France (Pulford, Hailey and Stubbs, 1984) both during summer. Panagiota and Valakos (1992) studied *T. h. boettgeri* in Greece and reported a mean summer body temperature of 30.0°C, a mean air temperature of 28.5°C and a mean

substrate temperature of 28.7°C (they included the whole month of June within summer data). These tortoises also exhibited a similar correlation between body and air temperatures to our study population, and a smaller correlation ( $r = 0.58$ ) between substrate and air temperatures than the ones reported here (Panagiota and Valakos, 1992). Body temperatures of *T. h. hermanni* of Menorca are also similar to those reported by Huot-Daubremont, Grenot and Bradshaw (1996) for a population of *T. h. hermanni* in the southeast of France. In addition, we agree with Huot-Daubremont, Grenot and Bradshaw (1996), that *T. h. hermanni* are not thermoconformers because the value of the slopes of the regressions between environmental and body temperatures were close to 1, but tortoises would rather select microhabitats that approach their thermal preferences. Filippi et al. (2010) reported mean body temperatures for a population of *T. h. hermanni* of central Italy that were similar to our study, although they found that females (mean  $T_b = 31.40^\circ\text{C}$ ) had significantly higher body temperatures than males (mean  $T_b = 30.55^\circ\text{C}$ ). They found no linear relation between air and body temperature, but they measured air temperature at a height of 100 cm, and the effect of wind may have biased their results (Filippi et al., 2010). Finally, the mean body temperatures recorded for *T. h. hermanni* here are also higher than that of a population from the north of Italy (Mazzotti et al., 2002).

Some studies reported that females of *T. hermanni* exhibit lower body temperatures than males when they are in the same thermal environment (Panagiota and Valakos, 1992; Carretero et al. 1995; Huot-Daubremont, Grenot and Bradshaw, 1996; Mazzotti et al., 2002). Without being contradictory, we suggest a slightly different interpretation of this based on our results. We found that mean male and female body temperatures and their respective mean capture place air temperatures were similar. The proportion of males and females in the different sunlight categories were also similar.

However, males appeared to select microhabitats with significantly higher substrate temperatures than females did. Thus, we propose that males in this study may probably select warmer substrates than females because of their lower thermal inertia.

If we consider the thermal preferences of *T. h. hermanni* close to 30°C (Cherchi 1956; Huot-Daubremont, Grenot and Bradshaw, 1996), then 60% of the temperatures observed in this study's population are higher than the thermal preference range of the species, and this population have achieved a very low accuracy of thermoregulation. Although several studies have addressed the thermal ecology of the Hermann's tortoise, there is a need for a more comprehensive study that includes behavioral observations, body temperatures, operative temperatures and thermal preferences, including seasonal variation in the same.

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