

Population and Thermal Ecology of *Testudo hermanni hermanni* in the Tolfa Mountains of Central Italy

ERNESTO FILIPPI¹, LORENZO RUGIERO¹, MASSIMO CAPULA², RUSSELL L. BURKE³, AND LUCA LUISELLI¹

¹FIZV (Ecology), Centro di Studi Ambientali Demetra srl, via Olona 7, 00198 Roma, Italy
[ernesto.filippi@fastwebnet.it; Lrugiero@hotmail.com; lucamlu@tin.it];

²Museo Civico di Zoologia, Herpetological Section, viale Ulisse Aldrovandi 18, 00197 Roma, Italy [m.capula@comune.roma.it];

³Department of Biology, Hofstra University, Hempstead, New York 11549 USA [biorlb@hofstra.edu]

ABSTRACT. – Herman's tortoise, *Testudo h. hermanni*, is an endangered subspecies in Italy, France, and Spain. We studied a Herman's tortoise population in the Riserva Naturale Regionale Monterano in the Tolfa Mountains of central Italy. We found that, unlike most other studies of this and related species, sex ratios were 1:1, and half the population was made up of juveniles. Sexual maturity was reached at 12 years in males and 12–13 years in females, and females were significantly larger. Tortoises greatly preferred open maquis habitat over other habitat types and thermoregulated so that body temperatures stayed consistently above ambient temperatures, especially under low ambient temperature conditions. Presence of ticks was significantly associated with an index of thermoregulatory behavior. We suggest that maintenance of open maquis habitat in this reserve is essential to high hatchling production and effective thermoregulation.

KEY WORDS. – Reptilia; Testudines; Testudinidae; *Testudo hermanni*; *Eurotestudo*; tortoise; ecology; thermoregulation; ticks; demography; habitat; Italy

Herman's tortoise, *Testudo hermanni*, is endemic to southern Europe, with *T. hermanni boettgeri* in the eastern part of the range and *T. h. hermanni* in the west, including scattered populations in Italy (Mazzotti 2006). In Italy, *T. h. hermanni* occupies a wide range of dry habitats, mostly in coastal areas of western central Italy and less commonly in the mountains of central Italy (Mazzotti 2006). Population levels have been greatly reduced by humans; for thousands of years, tortoises were harvested for food, as they were considered to be "fish" and therefore acceptable food on Fridays under Catholic doctrine (e.g., Bruno and Maugeri 1977). Consumption for food may be increasing again because homeless immigrants may use *Testudo* habitat as home sites (Filippi et al., unpubl. data). More recently, they were heavily collected for the local and international pet trade (Carpaneto 2000; Hailey 2000; Willemsen and Hailey 2001; Mazzotti 2004); this has ceased mostly because of recent legislation at both Italian and European Union levels. However, the coastal habitats of this species continue to be under heavy pressure from human development for urbanization and agriculture. The subspecies *T. h. hermanni* is endangered in western Europe, particularly in France (Corbett 1989), Spain (Pleguezuelos et al. 2002), and Italy (Mazzotti 2004), and is listed on the Turtle Conservation Fund's "Extinction Row" (Turtle Conservation Fund 2002). In addition, the species *Testudo hermanni* is listed as near threatened at the global range scale on the IUCN Red List (IUCN 2009).

Most of the ecological work on *T. hermanni* focuses on *T. h. boettgeri* (e.g., Stubbs et al. 1985; Hailey 2000;

additional references in Loy et al. 2007); somewhat less is known about *T. h. hermanni* (e.g., Cheylan 1981, 1984; Stubbs and Swingland 1985; Bertolero et al. 1995, 2005; Henry et al. 1998; Corti and Zuffi 2003; Mazzotti 2004; Mazzotti et al. 2002; Bertolero et al. 2007; Loy et al. 2007). Here we report basic ecological information on a population of *T. h. hermanni* from the Riserva Naturale Regionale Monterano in the Tolfa Mountains of central Italy to provide baseline data for future conservation work. The populations of tortoises from this hilly territory are considered among the most important for their conservation in central Italy (e.g., Carpaneto 2000).

METHODS

The field study was carried out at the Riserva Naturale Regionale Monterano (RNRM), about 50 km north of Rome (Tolfa Mountains, Latium, central Italy). This area has been officially protected since the end of the 1980s, and since the 1990s, it has also been considered a European Community Interest Site under the European Union's Natura2000 program (site code: IT6030001, Fiume Mignone—medio corso). This hilly area (488.3 ha, from 150- to 250-m elevation above sea level) is currently characterized by a patchy mosaic of habitat types, primarily plantation, maquis, and forest (Spada 1977). Plantation habitat includes olive (*Olea europaea*) and various fruit trees, and each field is surrounded by stony walls with spiny bushes, mostly *Rubus* spp. Maquis vegetation includes *Cytisus scoparius* bushlands, *Rubus* spp., *Rosa canina*, *Crataegus monogyna*, and *Thero-*

Brachypodetalia and *Brometalia* in the grassy pastures. Forest habitat is characterized by many plant species, primarily *Ulmus*, *Populus*, *Salix*, *Alnus*, and *Fraxinus angustifolia* in the riparian areas and *Quercus cerris*, *Q. pubescens*, *Ostrya*, *Carpinus orientalis*, *Acer monspessulanum*, *Cercis*, *Paliurus*, and *Castanea sativa* as mixed woodlands (Spada 1977). At one elevated spot, the ruins of an ancient town (Monterano) dominate the area. These ruins were completely surrounded by dense bushes of *Rubus* spp., *Rosa canina*, *Crataegus monogyna*, and *Cytisus scoparius* up to 2002, but this vegetation was subsequently almost entirely cleared under an archaeological-historical program supported by the European Union.

The study site itself was approximately 80 ha in the center of the park surrounding the ruins of Monterano, which was the least anthropogenically disturbed area of the reserve. The climate of the study area is Mediterranean-temperate, with cold winters (usually without snow), rainy springs and autumns, and dry and hot summers (hypomesaxeric subregion [type B] according to Tomasselli et al. 1973).

The field study was conducted from August 2002 to September 2003. Fieldwork was conducted under all climatic conditions, and a total of 359 person-hours were spent in the field. The time spent surveying in plantation, maquis, and forest habitat types was recorded. The tortoises were found by random walks throughout the study area. Once captured, we marked them individually by carapace scute notching (e.g., see Bury and Luckenbach 1977; Luiselli 2003; Rugiero and Luiselli 2006), measured straight-line carapace length (CL) (Stubbs and Swingland 1985), noted whether ticks were present, measured cloacal temperatures, palpated adult females for eggs (Bertolero and Marín 2005), collected other morphometric data, and released them unharmed at the capture site. Cloacal temperatures (T_b) were measured using a rapid-recording Schultheis thermometer (Miller & Weber, Inc.) within 1 min of tortoise capture and before any further handling or marking. Air temperatures (T_a) were recorded 100 cm above the capture spot. To avoid pseudoreplication, we analyzed temperature data only for the first capture of any individual. Habitat type (plantation, maquis, and forest) in the area of each tortoise capture was also recorded.

Age of each captured tortoise was estimated by counting growth annuli on the carapace, assuming that 1 growth annulus was added each year (Saint Girons 1965; Castanet and Cheylan 1979; Castanet 1988; Germano and Bury 1998). Although this method is sometimes applied without prior validation (Wilson et al. 2003), growth annuli counts are reliable methods for assessing tortoise ages in *T. hermanni* (Bertolero et al. 2005) as well as in the closely related *T. graeca graeca* from Spain (Diaz-Paniagua et al. 2001). This method is especially sound with juvenile and subadult aging (Castanet and Cheylan 1979).

We estimated sexual maturity by assuming that maturity coincided with the end of the fast growth phase (Congdon et al. 1993), which is associated with a transition from broad to narrower growth annuli (Castanet and Cheylan 1979; Castanet 1988). Our assumption that individuals reaching sexual maturity experience a deceleration of absolute growth that can be detected through a clearly visible narrowing of the growth annuli has been verified in other *Testudo* populations (Castanet and Cheylan 1979; Diaz-Paniagua et al. 2001; Rouag et al. 2007). Growth rates (estimated as mm/d) were determined by successive measurements of same individuals that were recaptured multiple times during our study.

Sex was determined in most individuals by examining the plastron concavity and the tail length (longer in males than in females). We considered all tortoises that were not clearly sexually dimorphic in this way or that were not sexually mature (see the previous discussion) to be juveniles.

All statistical analyses were done using SPSS (version 11.0.1), with all tests being 2 tailed and alpha set at 5%. All variables were checked for normality and homoscedasticity before applying parametric tests. We report means followed by (\pm SD).

RESULTS

We captured 96 tortoises, excluding recaptures. Of these, 30 were adult females, 18 were adult males, and 48 were juveniles. The sex ratio was 0.6M:1F; the difference from 1:1 was not significant (Yates corrected $\chi^2 = 3$, $df = 1$, $p = 0.083$).

Females were significantly larger than the males, 148.9 (\pm 14.51) and 132.1 (\pm 8.57) mm CL, respectively ($t = 4.46$, $df = 46$, $p = 0.00001$). The average age of females was slightly greater than that of males, 22.5 (\pm 5.39) growth annuli and 20.56 (\pm 4.62) growth annuli, respectively, but this difference was not significant ($t = 1.273$, $df = 46$, $p = 0.210$). As all adult tortoises were of known age, it seems that this population lacks very old adults (without growth rings or very worn carapaces).

There was a strong relationship between number of growth annuli and CL ($r = 0.904$, adjusted $r^2 = 0.817$, $p = 0.0001$, $n = 96$). This relationship did not differ significantly between females and males (analysis of covariance [ANCOVA] $F_{1,46} = 6.306$, $p = 0.341$). For juveniles, the relationship between number of growth annuli and CL was also significant (adjusted $r^2 = 0.823$, $p = 0.0001$). The curved relationship had a $b_0 = 3.952$ and $b = 0.056$ (Fig. 1). The overall annual growth rate (including adults, subadults, and juveniles) averaged 6.5% (6.3% in males, 6.6% in females); adult growth rate (i.e., excluding subadults and juveniles) was 4.7%, and juvenile growth rates averaged 8.6% (Fig. 1). Our sample size was too small to test for a significant difference between males and females. It needs be taken

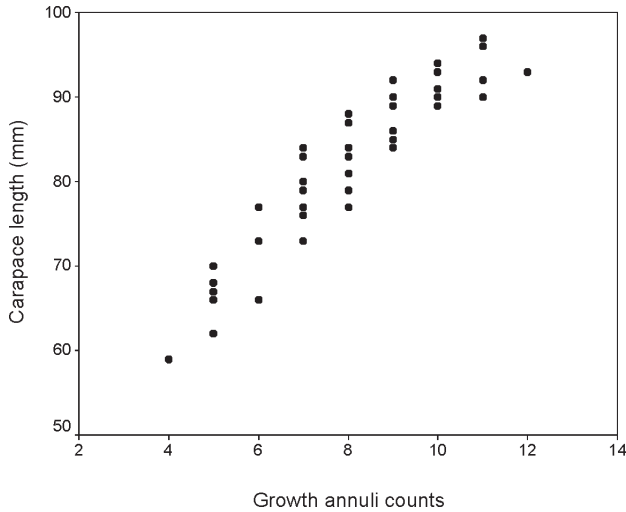


Figure 1. Relationship between age (estimated with growth annuli counts) and size (carapace length) for juvenile tortoises.

into account that many of our adult tortoises were fairly young, so the average population growth rates were probably higher than usual for this species.

Male sexual maturity (based on clearly visible narrowing of growth annuli) occurred at 12 years old without variation ($n = 3$); female sexual maturity occurred at 12 years old ($n = 1$) and 13 years old ($n = 4$).

We spent 126 person-hours searching in maquis habitat, 124 person-hours searching in forest habitat, and 109 person-hours searching in plantation habitat. The search time-corrected distribution of captures among habitats was strongly uneven for all groups (Fig. 2), with most individuals captured in maquis habitat. This difference was significant ($\chi^2 = 49.56$, $df = 2$, $p = 0.0001$). We had too few data overall to test for seasonal differences in habitat use. We captured 5 gravid females (detected by palpation), 1 in the last week of May, 1 in the first week of June, and 3 in the second week of June.

Mean and standard deviation value for T_b and T_a are reported in Table 1, and distribution of T_b is shown in

Table 1. Mean and (\pm SD) for body temperatures (T_b), air temperatures 100 cm above capture site (T_a), and the mean differences between T_b and T_a for all tortoises ($^{\circ}$ C).

	T_b	T_a	$T_b - T_a$
Males	30.55 (\pm 2.47)	24.89 (\pm 2.17)	5.67 (\pm 3.32)
Females	31.40 (\pm 1.77)	23.67 (\pm 2.32)	7.73 (\pm 2.89)
Juveniles	29.36 (\pm 2.25)	24.87 (\pm 2.99)	4.48 (\pm 3.30)

Fig. 3, showing that most tortoises kept their T_b between 27° C and 34° C. There was no significant relationship between T_a and T_b overall ($r = 0.051$, $n = 96$, $p = 0.621$). Mean T_b differed significantly among the groups (one-way analysis of variance [ANOVA] $F_{2,92} = 8.417$, $p = 0.0001$), and a Tukey HSD post hoc test revealed that females maintained a higher T_b than did males or juveniles but that males and juveniles did not differ. Concerning the mean T_a , there were no significant differences among the groups ($F_{2,92} = 2.136$, $p = 0.124$). However, as T_a was recorded during the course of 1 year (i.e., at different months and hours and thus different T_a) for T_b comparisons between groups, we also used an ANCOVA analysis with T_a as covariate. These statistical results ($F_{2,89} = 11.334$, $p < 0.03$) also demonstrated that, for any given thermal environment, females had significantly higher T_b .

In every case, tortoises kept their T_b higher than the T_a (Table 1), but there were significant differences in this relationship between T_b and T_a ($F_{2,92} = 9.520$, $p = 0.0001$). A Tukey HSD post hoc test revealed that females kept the difference between T_b and T_a higher than did either males or juveniles ($p = 0.001$) and that males kept the difference between T_b and T_a higher than did juveniles ($p = 0.010$). The higher the T_a , the smaller the difference between T_b and T_a (Fig. 4) ($r = -0.742$, $n = 96$, $p = 0.0001$); therefore, tortoises thermoregulated most dramatically at lower T_a . We used partial correlations to control the general relationship T_b vs. T_a for independent covariates, and we found that neither sex

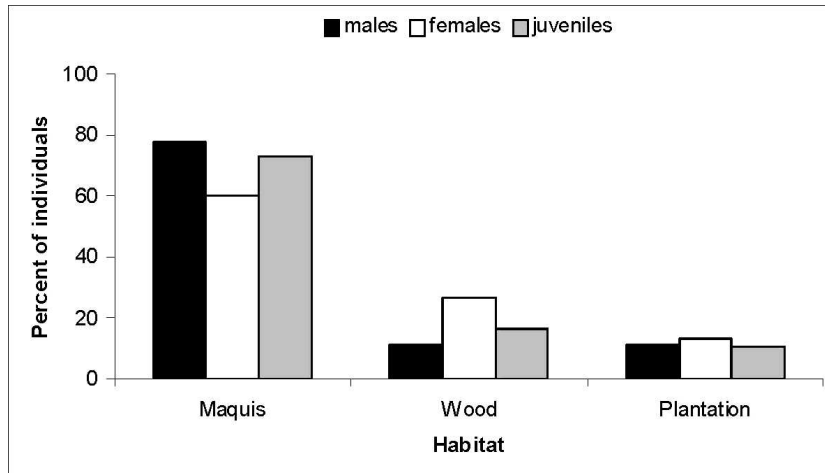


Figure 2. Habitat use by tortoises in relation to demographic category.

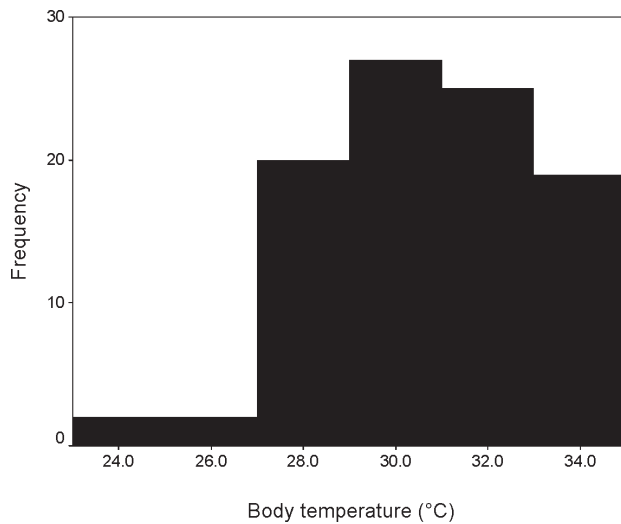


Figure 3. Frequency diagram of tortoise body temperatures (T_b).

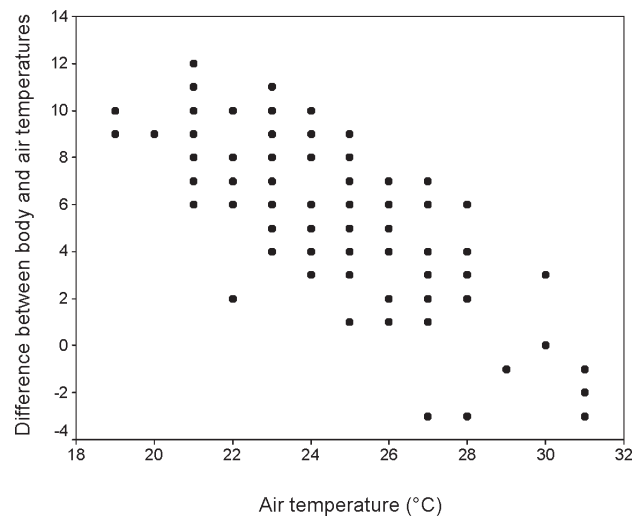


Figure 4. The relationship between air temperature (T_a) and the difference between body temperature (T_b) and T_a ($T_b - T_a$), indicating that at higher T_a , tortoises were less effective thermoregulators.

($r = 0.069$, $df = 92$, $p = 0.507$) nor size ($r = 0.140$, $df = 92$, $p = 0.177$) had any effect on this relationship.

Ticks (species not identified, but probably *Haemaphysalis* sp. and *Hyalomma* sp.; see Vetter 2006) were found on 50% of males, 40% of females, and 25% of juveniles; these differences were significant ($\chi^2 = 7.737$, $df = 2$, $p = 0.01$). A logistic regression (backward stepwise model) produced a significant model ($-2\log$ likelihood model = 114.165, goodness of fit $\chi^2 = 94.233$), allowing us to build the following logit function:

$$g(x) = -0.631 + 2.017(T_b - T_a)$$

This function showed that only the variable “ $T_b - T_a$ ” significantly influenced the presence of ticks on the tortoises, while the other covariates (sex, age, habitat, month, and size) were not important.

DISCUSSION

Half of the tortoises we captured were juveniles (48 of 96); this is in contrast to the results of Corti and Zuffi (2003), who found only 6 juveniles out of 156 *T. hermanni* captured on Sardinia, and Mazzotti et al. (2007), who found 30 juveniles out of 1445 *T. hermanni* captured in northern Italy. These differences in juvenile frequency are common; for example, in areas of Greece the ratio between juveniles and adults is extremely variable (Hailey et al. 1988; Willemsen and Hailey 1999). The ratio of juveniles to adult females, which was proposed as an index of population stability in *T. hermanni* (Hailey et al. 1988), ranges from about 0.1 at the most disturbed sites to about 4 in protected areas in the studies cited previously. At RNNRM, this ratio is 1.6, an intermediate value.

The presence of large numbers of *T. hermanni* juveniles in the RNNRM population may indicate that the

study area has not suffered any recent catastrophes because Stubbs et al. (1985) and Hailey (2000) showed that fires negatively affected juveniles much more than adults. The high numbers of juveniles may also be caused by lack of habitat fragmentation and high reproductive success. Given the history of RNNRM as a nature reserve where only a few fire accidents have occurred (and these were quickly extinguished), all of these possibilities would not be surprising. However, since many juveniles were found but not many very old tortoises, the most likely interpretation is that this could be a growing population. Finally, Loy et al. (2007) suggested that studies indicating low numbers of juveniles reported in many *T. hermanni* studies may be biased because of the lower detectability of juveniles relative to adults. Although we had too few data to conduct a robust statistical test, Fig. 2 indicates that juveniles made up about the same percentage of captured tortoises in the relatively closed forest and plantation habitats as in the more open maquis habitat. We therefore conclude that the high percentage of juveniles in the RNNRM population is not due to higher detectability in this area.

We found that RNNRM *T. hermanni* females were significantly larger than males; this is consistent with all previous studies (e.g., Meek and Iskeep 1981; Corti and Zuffi 2003; Loy et al. 2007). These tortoises have a positive linear relationship between female size and clutch size (reviewed in Bertolero et al. 2007); presumably, this results from positive selective pressure on female size. However, both males and females in our population were significantly smaller than those from Sardinia (Corti and Zuffi 2003) and from northern Italy (Mazzotti et al. 2002) (in all cases, Student *t*-test, $p = 0.0001$). The sizes of the tortoises from RNNRM fit the latitudinal pattern reported by Sacchi et al. (2007) and

thus adds further support to their hypothesis that size variation in *T. hermanni* is predicted accurately by Bergmann's rule.

Our finding that the sex ratio did not differ from 1:1 is consistent with 2 previous studies in northern Italy (Loy et al. 2007; Mazzotti et al. 2007); other studies of *T. hermanni* have consistently found male biases (Stubbs et al. 1985; Hailey and Willemsen 2000; Corti and Zuffi 2003) or female biases (Tomasetti and Bossuto 2000). Unfortunately, other authors have not always reported whether deviations from 1:1 sex ratios were significant. For example, recalculating data from Corti and Zuffi (2003), we found that the sex ratio of their population, while 1.3M:1F, was not significantly different from 1:1 ($\chi^2 = 2.67$, $p = 0.102$).

We found that growth of juveniles was rapid but decreased dramatically after sexual maturity (see Stubbs et al. 1985). The overall annual percent growth rate was consistent with data reported for *T. h. hermanni* in France, where it ranges from 6.2 to 7.8% (Stubbs and Swingland 1985).

Apparently, sexual maturity in *T. hermanni* is reached at very different ages, depending on the location of the population. For instance, tortoises from Spain reach maturity at 8 years old (both sexes) (Bertolero and Marin 2005), whereas tortoises from the Balkans reach maturity at 18–19 years old (both sexes) (Vetter 2006). Our data are intermediate and are most similar to data from southern France, where males reach maturity at 10–12 years old and females at 12–14 years old (Vetter 2006).

Despite the high local availability of forest habitat (Mantero 1998), the tortoises were clearly concentrated on the maquis areas, independent of sex and age. This is consistent with predictions of a spatial modeling approach developed for *T. hermanni* at an urban nature reserve in Rome (Rugiero and Luiselli 2006) that predicted that maquis vegetation would be crucial for detecting tortoise presence. Long-term management plans for this tortoise will require the maintenance of the relatively open maquis patches and avoiding overgrowth of the maquis by forest succession.

Bertolero et al. (2007) reported that in a semicaptive population of *T. hermanni* maintained in Corsica, first clutches were oviposited mostly in May, whereas second and third clutches were oviposited in June. In northern Italy, oviposition occurred most frequently in July (Mazzotti 2004). We are confident that our technique of palpating to determine gravidity was accurate (see Bertolero and Marin 2005). We found gravid females only in late May through the second week of June, which is consistent with Mazzotti (2004), who reported ovipositions in July. Indeed, given that our study area has a milder climate than northern Italy, we suspect that oviposition typically occurs in the second half of June at our study site. We did not recapture gravid females, so we cannot address the frequency of multiple clutches in our population.

We have strong evidence that T_b is always higher than T_a , which is identical to the findings of Meek and Inskeep (1981), Meek (1984), and Willemsen and Hailey (1999) for the same species in the Balkans (former Yugoslavia) and Greece. Mazzotti et al. (2002) compared substrate temperature (not T_a) to T_b in *T. hermanni* and similarly found that T_b was consistently higher. This fact also matches the pattern highlighted by Luiselli (2005), who found a latitudinal trend in northern tortoise populations in that $T_b > T_a$ while $T_b < T_a$ in tropical environments, where avoiding overheating and exposure to the sun is important.

T_b was unrelated to T_a , which indicates that these tortoises are good thermoregulators. Not only did T_b of our tortoises nearly always exceed T_a , but the maximum T_b found in our study (34°C) was also exactly the same as that reported in Meek (1984). We found that adult females maintained higher T_b than either adult males or juveniles, indicating important thermoregulatory differences for adult females, and that they maintained an even larger difference between T_b and T_a than other tortoises. We found no significant differences either between adult male and adult female body temperatures or between adult males and juveniles, in contrast to Mazzotti et al. (2002), who found that male *T. hermanni* had significantly higher T_b in spring and summer, apparently associated with basking and feeding activities. We did not record tortoise behavior at time of capture and so could not test for differences in T_b associated with behavior.

We found significant differences in the presence of ticks, being most prevalent on males, then females, and then juveniles. These data are especially interesting when compared to those collected from March 1991 to March 1994 in an area less than 10 km west of RNRM (during a larger research project on the population ecology of snakes; see Filippi et al. 1996). In this previous study of 20 female, 16 male, and 11 juvenile *T. hermanni*, ticks were found on 37% of males, 25% of females, and 18% of juveniles, the same patterns and similar frequencies to those we observed. On the other hand, no ticks were found in tortoises ($n = 51$) from a coastal site in central Italy (Castelfusano; Burke et al., unpubl. data). The importance of ticks and any diseases they may vector to the health of tortoises is completely unknown.

We attempted to explain the presence/absence of ticks on tortoises by testing for relationships with tortoise sex, age, size, habitat, month, and a thermoregulation index ($T_b - T_a$), and found that only $T_b - T_a$ was associated with tick presence. It is difficult to know whether this means that tortoises that thermoregulate relatively actively are more likely to accumulate ticks or whether tortoises with ticks maintain relatively high T_b through thermoregulation, perhaps as a response to infection. This suggests some very interesting areas for further research.

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RIASSUNTO

La testuggine di Herman (*Testudo h. hermanni*) è una specie minacciata sia in Italia che in Francia e Spagna. Noi abbiamo studiato la testuggine di Herman in una riserva naturale dei Monti della Tolfa in Italia centrale. Abbiamo osservato che, a differenza di quanto riportato da studi precedenti, la sex-ratio degli adulti è risultata di 1:1, mentre metà delle catture sono state relative ai subadulti. La maturità sessuale viene raggiunta a 12 anni di età nei maschi e a 12–13 anni nelle femmine. Le femmine sono risultate significativamente più grosse dei maschi. Le testuggini hanno dimostrato di preferire largamente gli habitat relativamente aperti di macchia. Le temperature corporee sono risultate sempre superiori alle temperature ambientali, specialmente durante i giorni con basse temperature ambientali. La presenza di zecche è risultata significativamente associata ad un indice di comportamento termoregolatorio. Noi supponiamo che il mantenimento di ambienti aperti all'interno della riserva sia essenziale per ottenere un elevato tasso riproduttivo e una buona capacità termoregolatoria in queste testuggini.

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