The turtle is in the details: microhabitat choice by *Testudo hermanni* is based on microscale plant distribution

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Abstract

Although research on habitat use and habitat selection is essential for understanding population ecology and behavior, most such zoological studies have used only general habitat categories describing main habitat features instead of using modern plant ecological approaches. Here, we analyze *Testudo hermanni* microhabitat use at a coastal Mediterranean site in central Italy by modeling tortoise presence/absence at three spatial scales, using a logistic regression design and quantitative vegetation and plant community analysis to reveal correlates of tortoise habitat use on a fine scale. Our analyses showed that only a few plant species among the many present, and these on a very small spatial scale, are important determinants of tortoise presence and site selection. We also find that tortoises chose a paradoxical combination of high levels of bare soil and high total vegetation cover. This suggests that these tortoises are selecting small patches of habitat in a matrix of less desirable habitat. Our findings also have important implications for habitat management, in that increasing the number of habitat patches containing the few significant plants is likely to be desirable, whereas increasing the size of such patches is probably less relevant.


Keywords

Tortoises; habitat selection; phytocoenosis; ecology; spatial scale; central Italy

Introduction

It is widely accepted that microhabitat niche is one of the most important characteristics of each species’ ecology, and that a good knowledge of this niche is necessary to understand organism-environment adaptation in free-ranging reptiles.

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(Reinert, 1993; Shine and Fitzgerald, 1996). Indeed, numerous studies have documented patterns of habitat utilization in reptiles, either in terms of auto-ecology or synecology (e.g., Fitzgerald et al., 2002; Ives et al., 2008; Freeman and Freeman, 2009). Three important problems are typically encountered by herpetologists investigating microhabitat niche dimensions: characterization of the microhabitat types available in a given environment, quantification of the relative abundance of the different microhabitats, and determination of the habitat spatial scale relevant to the focal organism. These problems occur because herpetologists typically ignore in-depth vegetation structure, unlike plant ecologists (e.g., van Der Maarel, 1979; Stortelde et al., 1986; Scoppola and Caporali, 1998). As a result practically no studies have tested whether a particular reptile species’ use of available microhabitats depends on the abundance of specific plant species, or on plant associations, or on structural characteristics of the microhabitats themselves. In addition, plant species diversity itself is often not documented in herpetological studies (e.g., see Fitzgerald et al., 2002; Ives et al., 2008; Freeman and Freeman, 2009), and this limits our understanding of whether reptiles prefer diverse microhabitats. In this paper, we combined typical plant ecology and herpetological approaches and attempted to understand the microhabitat characteristics of a tortoise species (*Testudo hermanni hermanni*) in Mediterranean central Italy on three spatial scales, whereas previous studies analysed only descriptive aspects of microhabitat (e.g., Rugiero and Luiselli, 2006; Filippi et al., 2010). We analyzed whether there were particular structural and plant diversity characteristics of the microhabitat correlated with resting sites chosen by free-ranging tortoises, whether plant species richness was important, and if so, on what spatial scale. In order to analyse these issues, we used a combination of multivariate and logistic regression analyses.

### Materials and methods

#### Study species

Herman’s tortoise, *Testudo h. hermanni*, is a small (up to 800-1000 g on average) terrestrial turtle (fig. 1) that eats mainly plants (up to 132 species belonging to 46 families identified, mainly Asteraceae, Fabaceae, and to a lesser extent Poaceae and Ranunculaceae (Cheylan et al., 2010). They are long lived (up to about 90 years; Cheylan et al., 2010), have high adult survivorship (over 95% annually; Cheylan et al., 2010), have home ranges that vary from 4.6 to 7.4 ha in males and 3.6 to 4.9 ha in females (Chelazzi and Calzolai, 1986), and tend to remain in the same area for many years (Cheylan et al., 2010). They use vegetation and other landmarks to navigate their range and thus are likely to know detailed aspects of their home range (Cheylan et al., 2010). They spend over 60% of their time resting and thermoregulating (Huot Daubremont et al., 1996; Cheylan et al., 2010). Resting and thermoregulation sites can be extremely important, because maintaining high body temperatures can be important to digestion, immune function, mating activity, etc. (Chelazzi and Calzolai, 1986; Meek, 1988; Huot Daubremont et al., 1996).
Figure 1. *Testudo hermanni*, basking around midday at the study area. (Photo: Massimo Capula.) This figure is published in colour in the online version.

*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 portions of their range (for instance, in South-Western Sardinia), however, tortoises populations are more continuous (Cheylan et al., 2010). 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). Previous studies revealed that these animals are especially linked to maquis habitats (Rugiero and Luiselli, 2006; Filippi et al., 2010).

It is important to understand the habitat requirements of these tortoises because they are declining in many parts of their range. 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 (Van Dijk et al., 2004).

Study area and protocol

The field study was conducted between April-October 2009 and April-June 2010 in the coastal pine-forest of Castelfusano (Roma, central Italy).

We searched for tortoises using standardized routes across the study site, crossing through the main habitat types available. When encountered, tortoises were
sexed (sexing methods as in Cheylan et al., 2010), carapace and plastron length measured, and individually marked by scute notching. Tortoise recaptures were excluded from analysis to avoid pseudoreplication. We chose random points by first randomly generating a list of time intervals, and then walking at slow but steady pace (ca. 1.6 km/hr) along the standardized routes while concurrently measuring the time we were walking. We stopped when we either encountered a tortoise or the previously generated time interval had elapsed. When we encountered tortoises, we stopped the time measurement and recorded data as described below. When we were done recording data associated with an encountered tortoise, we resumed the time measurement and resumed walking along the route until either we encountered another tortoise or until the generated time interval elapsed. When the time interval elapsed we stopped, we recorded data as described below for random points, and then began the next time interval on the list.

Plant rélevés (lists of plant species and their abundances in sampling plots) were compiled to characterize the plant species communities at the study area immediately surrounding locations where the tortoises were encountered (presence points) and at nearby but randomly chosen locations where tortoises were never found (random points). Plant rélevés were performed only during spring (April, May, and June) due to the difficulty of identifying many plant species out of season. Rélevés were conducted around encountered tortoises and random points on three spatial scales, all centered at the tortoise or random point: i) circular plots with 5 m radii; ii) circular plots with 15 m radii; iii) circular plots with 25 m radii. These circular plots were the three spatial scales. All plant species in each plot were identified to species and their coverage value was estimated using the method of Braun-Blanquet (1928). According to this method, every plant species identified in each plot had an associated cover-abundance value, determined from estimates of vertical plant shoot-area projection as a percentage of quadratic area. The scale range was from 1 to 5 (5 for coverage > 75%, 4 for coverage between 50-75%, 3 for coverage between 25-50%, 2 for coverage between 5-25% and 1 for coverage < 5%). Cases where only very few individuals or a single individual were present were recorded as “+” (Wikum and Shanholtzer, 1978).

At each tortoise location and each random plot we also recorded eight measures of vegetation structure: i) total plant coverage; ii) average height (visually estimated) of the tree stratum; iii) coverage of the tree stratum; iv) average height (visually estimated) of the bush stratum; v) coverage of the bush stratum; vi) average height (visually estimated) of the grass stratum; vii) coverage of the grass stratum; viii) percent bare soil. Coverages could sum to greater than 100% where layers overlapped.

Statistical analyses

For all rélevés, we used the plant species distribution data to calculate a biological spectrum of ‘plants life form’. These plant data were subjected to a multivariate analysis (DCA, Pc-ord 5.1). From the resulting ordination plot we determined the
main vegetation types present at the study site, which we used to characterize tortoise microhabitats (fig. 1). We tested the independence of these variables via Spearman’s rank correlation coefficient (in all cases, $r < 0.553, P > 0.197$) and analyzed the matrix of relevés × variables by Canonical Correspondence Analysis (CCA, P-ord 5.1) (fig. 2). ‘Plant life form’ is a morphological type independent from a species’ phylogenetic classification, and it is characterized by plant adaptation to specific ecological conditions (Mera et al., 1999; Pignatti et al., 2001). Plant life form was expressed according to the acronyms reported by Pignatti (1982) and based on the classification by Raunkiaer (1934); this classification depends on the position and degree of protection of the renewing buds, which are responsible for the renewal of the plant’s aerial body during the growing season. The biological spectrum is the percent representation of the number of species belonging to each life form in a given plant community, and it expresses both the growth form distribution and the phytoclimatic under which the prevailing growth forms evolved.

We compared plant species and plant structure variables from tortoise location sites and the random points using logistic regression models, with the dependent variable being the presence (score = 1 at the capture sites) or the absence (score = 0 at the random sites) of tortoises (see Luiselli, 2006, for more details of the statistical modeling procedure used). Structural variables were the eight variables described above and plant species variables were presence/absence and relative abundance of each plant species, and plant species richness (= number of species). Structural
variables were assessed only at 5 m radius spatial scale because they did not change over the three scales, whereas plant species data were identified at all three spatial scales.

DCA and CCA analyses were done on Pco-ord; logistic regressions and chi-squares were done using SPSS (version 11.0.1), with all tests being two tailed and alpha set at 5%. In cases of multiple simultaneous comparisons alpha was adjusted by Bonferroni rule, i.e. dividing the alpha value of 0.05 by the number of simultaneous tests. All variables were checked for normality and homoscedasticity before applying parametric tests. Non-parametric tests were applied when data normality was not achieved.

**Results**

Data were collected on 61 random sites and 55 tortoise sites, and 125 plant species were identified. We did not collect all plant data at all tortoise sites because for surveys done early in the spring, plant species could not always be identified reliably. All of the tortoises were found resting/thermoregulating; these two activities could not be distinguished. The plant species observed were those typical for coastal pine-forests of this region (Bianco et al., 2002), tree species were dominated by – *Pinus pinea, Pinus pinaster and Quercus ilex* shrubs by *Phillyrea angustifolia, Cistus creticus, Cistus salviifolius, Pistacia lentiscus,* and *Coronilla emerus,* and grasses by *Carex hallerana, Oryzopsis miliacea, Torylis arvensis,* and *Lagurus ovatus.* Most of the plant species were native (122/125; 97.6%); non-native species included *Conyza canadensis,* *Pinus pinaster* and *Pinus pinea;* although pine wood have an artificial origin (planted in 19th century), they are naturalized, and as such they are included in the Habitat Directive 92/43/EEC (2270 – Wooded dunes with *Pinus pinea* and/or *Pinus pinaster*).

**Structural and plant species descriptions of the tortoise habitat**

Biological spectrum analysis revealed that therophytes dominated the study area (33%), followed by phanerophytes (28%), nanophanerophytes (21%), hemicyryptophytes (10%), chamaephytes (7%) and geophytes (1%); the high presence of therophytes indicate the aridity of the area, typical of Mediterranean systems. DCA and CCA analyses (figs. 2 and 3) indicated three main plant species-structure groups: 1) maquis vegetation of the coastal dune outside of the pine-forest; 2) maquis vegetation of the clearings inside the pine-forest; 3) forest and bush vegetation of the pine-forest. Percent of bare soil decreased from group 1) to 3). More specifically the three groups can be defined as follows; complete species lists for each group can be obtained from the corresponding author.

Group 1: High prevalence of evergreen sclerophyllous bushy which are typical of Mediterranean maquis vegetation, including *Phillyrea angustifolia, Pistacia lentiscus, Arbutus unedo, Rosmarinus officinalis, Quercus ilex, Pinus pinea, Osyris alba, Erica multiflora, Cistus creticus, Lonicera implexa, Daphne gnidium, Dorycnium*
Figure 3. Canonical correspondence analysis (CCA) classifying the main habitat types available to tortoises at the study area. Symbols: coperba = percent of grass coverage; halb = average height of tree stratum; Copalb = percent of tree stratum coverage; Suolonu = percent of bare soil.

Grass stratum was poor, and dominated by Medicago minima, Trifolium campestre, Catapodium rigidum, Arenaria sepyllifolia and Centaurium erythraea. This group had intermediate plant species richness (n = 89 species).

Group 2: High prevalence of bushes and trees of mid height, like Cistus salviifolius, Cistus creticus, Coronilla emerus, Quercus ilex, Rubia peregrina. Grass diversity was high, and included species as Avena barbata, Geranium robertianum, Desyryrum villosum, Cynosurus echinatus, Torylis arvensis, Lagurus ovatus, Carduus pycnocephalus, Hypochaeris achyrophorus, Urospermum picroides, Melilotus neapolitana, Medicago lupulina, and Anagallis arvensis. This group had the highest plant species richness (n = 105 species).

Group 3: High prevalence of tree species, including Quercus ilex, Pinus pinea, Pinus pinaster, followed by bushes as Cistus salviifolius, Phlyrea angustifolia, and Ruscus aculeatus. Abundant populations of Hedera helix, Smilax aspera, Rubia peregrina, Asparagus acutifolius, and Clematis flammula were also observed. Grass stratum was characterized by species like Oryzopsis miliacea, Brachypodium sylvaticum, Conyza canadiensis. This group had the lowest plant species richness (n = 63 plant species).
Distribution of tortoises across the three plant species-structure groups

We collected 71 tortoises (29 males, 33 females, 9 juveniles); adult sex-ratio did not depart from equality ($\chi^2 = 0.258$, df = 1, $P = 0.611$). Based on the respective positions of the various sites within the multivariate space in our DCA, we assigned each site where a tortoise was collected to one of the three main plant species-structure groups defined above (fig. 4). Although tortoises were encountered in all groups, their distribution was uneven at a marginally significant level ($\chi^2 = 5.438$, df = 2, $P = 0.066$), with more individuals in group 2 (fig. 4A). When we subdivided tortoises by sex with juveniles as a third category, their distribution across plant species-structure groups was highly significantly uneven ($\chi^2 = 32.559$, df = 8, $P = 0.000075$), with adult females and juveniles being concentrated in group 2 more significantly than expected by chance (fig. 4B). Adult males also showed a higher concentration of records in group 2, but the difference among groups was not statistically significant (fig. 4B).

Figure 4. Distribution of tortoises along three main plant species-structure groups as defined by DCA and CCA analyses (see text for details). Total sample sizes = 71 individuals (males and females pooled, graphic (A), and divided by sex, graphic (B)).
Structural and plant species variables influencing tortoise presences at three spatial scales

Using structural variables only, the logistic regression model (extraction method = forward conditional; \(-2\)log likelihood = 80.648) allowed correct classification of 64.52% of cases (\(\chi^2 = 10.781, P < 0.03\)), and showed that tortoise presence was significantly and positively influenced by % bare soil (score 5.071, \(P = 0.024\)) and (also positively) marginally by % of total plant coverage (score 3.175, \(P = 0.075\)). The ecological equation was:

\[
g(x) = -0.598 + 5.071 \times \text{bare soil} + 3.175 \times \text{total plant coverage}.
\]

As for plant species distribution variables, at the 5 m radius scale (logistic regression with 91.23% of cases correctly classified; \(-2\)log likelihood = 33.884; \(\chi^2 = 25.015, P = 0.00001\)) we found that presence of several plant species was significantly and positively correlated to the tortoise presence: *Arbutus unedo* (score 1.395, \(P < 0.0001\)), *Arenaria serpyllifolia* (score = 1.374, \(P < 0.0001\)), *Andryala integrifolia* (score = 1.415, \(P < 0.0001\)), *Asparagus acutifolius* (score = 1.04, \(P = 0.001\)), and *Smilax aspera* (score = 1.09, \(P = 0.001\)). In addition, another important variable for predicting the presence of tortoises was the plant species richness (score = 1.04, \(P = 0.001\)).

The resulting ecological equation was:

\[
g(x) = -2.342 + 1.415 \times \text{Andryala integrifolia} + 1.395 \times \text{Arbutus unedo} \\
+ 1.374 \times \text{Arenaria serpyllifolia} + 1.09 \times \text{Smilax aspera} \\
+ 1.04 \times \text{Asparagus acutifolius} + 1.04 \times \text{plant species richness}.
\]

At the other two spatial scales, we did not obtain a significant logistic function model (15 m radii – \(-2\)log likelihood = 17.662; \(\chi^2 = 4.767, P = 0.093\); 25 m radii – \(-2\)log likelihood = 9.172; \(\chi^2 = 3.913, P = 0.116\), thus revealing that the variables we used were important for predicting tortoise presence sites only at a small spatial scale.

Discussion

Our study revealed interesting *Testudo hermanni* microhabitat selection behavioral patterns that were previously undocumented. We found that structural variables were much weaker predictors of *Testudo* habitat use than were the abundances of five plant species and the species richness. This finding is consistent with the fact that we observed tortoises in all three plant species-structure groups (fig. 4A), although with significant frequency differences for at least adult females and juveniles (fig. 4B). Among the three plant species-structure groups identified by our DCA and CCA, it was clear that tortoises were more frequently associated with group 2, as revealed by that i) simple counts of individuals were higher in group 2 (see above); ii) 100% of the plant species we found to be significant to tortoises in
the second ecological equation were also found in group 2, compared to only 80% of those species in groups 1 and 3, and iii) the highest densities of *Smilax aspera* and *Asparagus acutifolius* (two plant species which were found to be statistically significantly influencing tortoise presence in the logit equation) were observed in group 2. However, this was true only at the smallest spatial scale, whilst no variables significantly influenced tortoise presence at the two larger spatial scales. The 15 and 25 m radius spatial scales were very similar, and this may be the reason for which they did not influence tortoise microhabitat selection significantly.

The evidence above suggest that tortoises seek out small patches of desirable habitat interspersed within a larger matrix of less desirable habitat, and that there are large areas of habitat they do not use except to move through on the way to small patches of desirable habitat. This is consistent with patterns of movements demonstrated in previous radiotracking studies (Cheylan, 1981; Mazzotti et al., 2002; Luiselli et al., 2009). Our study indicates that the reason these small patches are chosen is because of the presence of five plant species (*Arbutus unedo*, *Arenaria serpyllifolia*, *Andryala integrifolia*, *Asparagus acutifolius*, and *Smilax aspera*). Tortoises may actively search for some of these species for food, although our unpublished data on the diet of this tortoise population suggests that these plants are not among the most important food types. For example, fruits of *Arbutus unedo* are known to be an important food for Italian *T. hermanni* (Mazzotti et al., 2007), and more generally the diets of these tortoises usually includes a large variety of plant species (up to 132 species belonging to 46 families in Croatia; see Cheylan et al., 2010), as well as mushrooms and a few invertebrates. Thus, it is also likely that the positive correlation between presence of tortoises and high plant species richness locations depends at least in part on their search for food. It is also possible that, instead of choosing microhabitats for food plants alone, instead tortoises choose microhabitats that optimize refuge and basking sites and food plants simultaneously.

The fact that females and juveniles showed a greater preference for plant species-structure group 2 than did males is intriguing. Male *Testudo* behavior differs from that females in numerous ways – males typically have larger home ranges (Mazzotti et al., 2002; Luiselli et al., 2009; Cheylan et al., 2010), males are active for a larger percentage of the time (Cheylan, 1981), and they are active both earlier and later in the year (Cheylan, 1981). These differences are usually attributed to males searching for mates (Mazzotti et al., 2002; Luiselli et al., 2009) and possibly loosely defending territories (see Lagarde et al., 2003 for *Testudo horsfieldi*). There are no previous reports that males also differ in microhabitat use from females or juveniles, and it seems surprising that they would not tend to be found in the same microhabitats as adult females. For example, there are no reports that the sexes differ in diet. This pattern should be investigated in greater detail. It remains possible that the weaker dependence of males for a specific microhabitat may be an artifact of the fact they simply move more than females, thus increasing the probability of being observed in microhabitat types different from the preferred ones. Indeed, al-
though not at a statistically significant level, it is evident from our study that the higher frequency of males was also seen in group 2 microhabitat (see fig. 4B).

On the other hand, growth form did not have any demonstrated effect on tortoise presence, as two of the five significant species included in the second ecological equation were therophytes (*Andryala integrifolia, Arenaria sepyllifolia*), two were nanophanerophytes (*Smilax aspera, Asparagus acutifolius*), and one was phanerophyte (*Arbutus unedo*). This suggests that the functional shape of the plants did not influence presence/absence of tortoises.

Overall, our study showed that a detailed analysis of *Testudo* microhabitat niche carried out using a plant ecological approach may produce i) a better understanding of the variables influencing tortoise microhabitat selection, and ii) concurrent presence of some plant species, of a high plant species richness, and of bare soil may be positively correlated to the probability of observing a tortoise at a given site. That is, tortoises clearly showed small scale microhabitat preferences for ecotone microhabitats between high vegetation (tree and bushes) and open ground, especially where plant species richness is high. It is possible that this selection pattern depends on a suite of antipredatory, foraging, and thermal strategies (i.e., tortoises need to keep their body temperature constantly higher than ambient temperature, hence they would need thermally efficient resting spots; Filippi et al., 2010).

These results have conservation implications that are potentially valuable to this rare species. For example, they could be employed to identify *Testudo* habitat from which they had been extirpated but which is suitable for reintroduction efforts. In addition, our results could be an important step towards identifying goals for plant management in areas where *Testudo* are being conserved. Increasing the number of habitat patches containing *Arbutus unedo, Arenaria serpyllifolia, Andryala integrifolia, Asparagus acutifolius*, and *Smilax aspera* is likely to be desirable, whereas increasing the size of such patches is probably less relevant.

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