Activity patterns and habitat preference of eastern Hermann's tortoise (*Testudo hermanni boettgeri)* in Serbia

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Abstract: We analyzed variation in distribution of eastern Hermann's tortoises (*Testudo hermanni boettgeri*) in a complex landscape consisting of both natural and human-altered habitats in a temperate climate regionin Serbia. Our results indicated non-random distribution of tortoises within different habitat types with large and medium effect size for year and activity pattern, respectively. Additionally, the tortoises expressed relatively weak preference for habitats modified by human activity (e.g. vineyards, orchards, or gardens). The results suggested that these tortoises preferred some particular habitats more than others when performing specific activities. They also did not exhibit the same distribution pattern among habitats over consequent years. In other words, they were not randomly distributed among habitat types with regard to specific activity or year. The information on preferences in complex habitat systems is important for the conservation management of eastern Hermann's tortoise and should be considered when planning activities related to sustainable development within the region of study.

Key words: activity patterns, habitat type, *Testudo hermanni boettgeri*, complex environment

1. Introduction

Tortoises inhabit a wide variety of habitats, show diverse activity patterns, dispersal abilities and home ranges, with variability occurring from species to populations, age classes, sex and seasons (Pough et al., 2004). Among three tortoise species occurring in Europe (*Testudo hermanni*, *T. graeca* and *T. marginata*), Hermann's tortoise has the largest regional distribution area (Gasc et al., 1997; Sillero et al., 2014): The western subspecies -*T. hermanni hermanni*- inhabits parts of the Italian Peninsula, Sardinia, Sicily, Corsica, Provence, the Balearic islands and Massif of Alberes while the eastern subspecies -*T. hermanni boettgeri*- occupies parts of Balkan Peninsula, including number of islands in the eastern Mediterranean region (Fritz et al., 2006) and European Thrace in Turkey (Türkozan et al., 2005). Hermann's tortoise has a regular activity break in winter which is shorter in the semi-arid Mediterranean part of the distribution area (October/November to February/March) (Willemsen, 1991; Huot-Daubremont and Grenot, 1997) than in more temperate climate areas (October to March/April) (Haxhiu, 1995; Mazzotti et al., 2002). In summer, Hermann's tortoises in the Mediterranean area have bimodal daily activity while in the temperate region they tend to be active throughout the day (Cruce and Răducan, 1976; Hailey at al., 1984; Meek, 1988) except in extremely high daily temperatures (Willemsen, 1990; also Stojadinović and Crnobrnja-Isailović's unpublished data).

It is obvious that activity patterns of Hermann's tortoises are constrained by local habitat composition in some parts of distribution area. The preferred habitat for *T. hermanni* is described as "open patchy evergreen Mediterranean oak forest" (van Dijk et al., 2004), but number of studies suggested spatial variation in habitat preferences within the distribution range of the species (Wright et al., 1987; Longepierre et al., 2001; Corti and Zuffi, 2003; Rugiero and Luiselli, 2006; Rozylowicz and Popescu, 2013; Corti et al., 2013; Berardo et al., 2015): In the western part of the distribution area, Hermann's tortoises were frequently recorded in macquis with *Pinus sp.* and *Quercus sp*., in coastal heathland, coastal forests, and coastal dunes, as well as in inland temperate grasslands, shrubs, and forests dominated by *Quercus sp*., *Carpinus sp.*, rarely *Fagus sp.* Artificial habitats such as pastureland and rural gardens are considered marginal (van Dijk et al., 2004). Few studies are done so far in the eastern part of the species range (Cruce and Răducan, 1976; Rozylowicz and Dobre, 2010; Rozylowicz and Popescu, 2013) but they suggested that complex habitat matrices harbor relatively dense tortoise populations due to (still) low human impact e.g. modest alteration of primary habitats (traditional farming).

Hermann's tortoise is considered Near Threatened due to its fragile population status in many places e.g. declining population size (Rozylowicz and Dobre, 2010). Habitat fragmentation and degradation is on the top of the list of threatening factors that jeopardize the species (Swingland and Klemens, 1989; Fernández–Chacón et al., 2011) although overexploitation cannot be neglected (Türkozan and Kiremit, 2007; Ljubisavljević et al., 2011; Celse et al., 2014). In eastern part of distribution range, the most of the countries are currently facing transition where economic development in the area could come into the conflict with conservation of indigenous wildlife because of conversion of pristine habitats into intensively exploited agricultural land (Rozylowicz and Dobre, 2010; Celse et al., 2014). It is possible to reach a compromise if ecological preferences of wild species have been already identified.

In line with this, we analyzed one population located in the Republic of Serbia where habitat loss, fragmentation, and degradation are considered to be far less intense than in the western part of the species' area (Celse et al., 2014; Ljubisavljević et al., 2014). Our aim was to check following assumptions: First, that eastern Hermann's tortoises in this part of the distribution range prefer specific habitats for specific activities; second, that significant correlations occur between ambient temperatures and variables selected for the study; third, that there are gender or age-specific preferences for a particular habitat type. Additionally, we compared habitat preference of Hermann's tortoises in our study area with literature data to check for regional variations in the eastern part of the species range.

2. Materials and methods

2.1. The study site

Regular monitoring of Hermann's tortoises have been taking place every year in spring and summer since 2010 to 2014 in the area around the village of Kunovica, which administratively belongs to Niška Banja County, a suburban part of the city of Niš. It is a hilly landscape with elevations ranging from 309 to 621 m above sea level, dominated by oak woodlands of *Quercetumfarnetto-cerris* (Ranđelović et al., 1996). The field site of 23 ha is part of Jelašnica gorge nature reserve, and is situated between Jelašnica and Kunovica villages. The district of Kunovica represents a mixture of pristine and rather abandoned human-altered habitats, where forests, pastures & fields, and agricultural land occupy 58%, 16% and 25% of the total space, respectively (Turnšek, 2006). More details on the field locality are presented in Stojadinović et al. (2013). The two important prerequisites for the choice of study population were the adequate population density (Stojadinović et al., 2013) and easy access to the field site.

2.2. Field procedures

The temporal dynamics of monitoring included two visits per year, always in the last week of May (considered to be spring in the analysis) and in the third week of July (considered as summer). On every visit, researchers spent seven consecutive days searching for tortoises using visual encounter survey method. They recorded activity of Hermann's tortoises from 8 a.m. to 7 p.m on a daily basis. Data collection exclusively occurred within the same experimental area (see Figure 1.), with eight people involved in the fieldwork and organized in two teams. Every team was led by one experienced tortoise biologist (DS and JCI) so we considered the sampling effort to be homogeneous throughout the monitoring days. The methodology for general recognition of age/sex (male, female, immature) of the tortoises, as well as a technique of individual marking by shell notching applied in this study, are described in Stojadinović et al. (2013).

Data on diurnal and seasonal activity patterns were collected following the procedure described in Crnobrnja-Isailović et al. (2007), with modifications: only sex, age, season, year, activity pattern and habitat type were recorded and the list of habitat types was different (see next paragraph). Researchers recorded both the time of encounters and the activity performed by each tortoise, as well as the type of habitat where the individual was spotted. Moreover, in order to avoid pseudo replication only first records of individual tortoises during the whole study period were included in the analysis (Rugiero and Luiselli, 2006).

Habitat types were defined following (Rozylowicz and Popescu, 2013) with modifications: Forest was defined as a closed canopy area larger than 100 m^2; open habitat encompassed grassland patches wider than 10 m; forest edge comprised every strip of grassland within the forest narrower than 10 m or an open surface less than 5m distant from the forest line; humanly-modified habitat included all kinds of agricultural land present in the area of study: vineyards, orchards and gardens; a path referred to a local narrow unpaved road which partly represents the borderline of the area of study. We also included into analysis data on substrate temperature (Ts) and air temperature measured at 5 cm (Ta5) and 60 cm (Ta60) above the ground at the capture sites of individual tortoises. Ambient temperature was measured by Dostmann digital Einstich-Thermometer TFA with ± 0.1°C precision.

2.3. Statistical analyses

To analyze the habitat specificity of Hermann's tortoises, data matrices were constructed using the following variables: Sex/age group (1 – adult male, 2 – adult female, 3 - immature); year (1 – 2010, 2 – 2011, 3 – 2012, 4 – 2013, 5 – 2014); season (1 – spring e.g. last week of May, 2 – summer e.g. third week of July); type of activity (1 - basking, 2 - hiding, 3 - moving, 4 - feeding, 5 - reproductive activities including chasing, mating, egg laying, or fighting between males), and type of habitat (1 - forest, 2 - open habitat or grassland, 3 - forest edge, 4 – humanly modified habitat, 5 - local unpaved road). The described habitat types within experimental area occurred in proportions equal to those reported for the overall district of Kunovica (see subsection The Study Site within this section). Departure of frequencies from random distribution was tested using the Shapiro-Wilk's test.

We tested the first and third assumption (e.g. that Hermann's tortoises prefer particular habitats when performing specific activities: there are gender or age- specific preferences for a particular habitat type) by calculating strength of the association among nominal variables (habitat type, sex/age group, year, season and type of activity) using Chi-square test and then Cramer's V as an indicator of small, medium, or large strength of association (effect size) (Cohen, 1977).

Second assumption was tested by analyzing variables with major interaction effects against ambient temperatures recorded at capture spots of adult tortoises. Normal distribution of ambient temperatures was confirmed by Kolmogorov-Smirnov test for continuous data. We tested three variables (activity type, habitat type and year) with major interaction effects against ambient temperatures recorded at capture spots of adult tortoises. We accordingly performed Factorial ANOVA with ambient temperatures Ts, (Ta5 and Ta60) as dependent variables and variables showing significant interactions in the previous analyses as factors.

All analyses were performed using Statistica 5.0 and SPSS version 15.0 software.

3. Results

During the five years of monitoring 449 individuals were marked. Among them, 389 (86%) were adults, and 63 (14%) were immatures (Shapiro-Wilk's $W = 0.41$, $P < 0.001$). In the adult subgroup, females outnumbered males (male:female = 0.56; Shapiro-Wilk's $W = 0.61$, $P < 0.001$). Calculation of Chi-square test and then Cramer's V values for pairs of nominal variables (habitat type, sex/age group, year, season and type of activity) revealed seven significant associations, but four of them had small effect (Table 1) and therefore were not considered in the discussion. For example, distribution of three main sex/age groups among habitat types or with regard to activity patterns was random ($P > 0.10$ in all cases). In contrast, their distribution among years was non-random ($P < 0.05$, Table 1.) but the significance of those interactions was small (Cramer's $V = 0.140$ for $df = 2$, Table 1.).

Three interactions of paired variables had large or medium effect: distribution of sex/age groups with regard to season ($V = 0.316$; medium effect size), and distribution of tortoises among habitat types with regard to year (Cramer's V = 0.255; large effect size), or activity type (Cramer's V = 0.170, medium effect size) (Table 1).

The frequency of occurrence of three sex/age groups in two seasons indicated the predominant occurrence of adult females in the spring (70% in comparison to 30% in summer). In contrast, the most of adult males were detected in the summer (63% in comparison to 37% in spring). Occurrence of immature individuals was almost equal in spring and summer (43% and 57%, respectively).

In general, records were not randomly distributed among habitat types (forest – 111 individuals, grassland – 116 individuals, forest edge – 92 individuals, humanly altered habitats – 53 individuals and the path – 77 individuals; $X^2 = 29.61$, df = 4, $P < 0.001$). There were significant differences between observed and expected frequencies of records on the basis of relative presence of three main habitat types within experimental area (in forested habitat 25% observed vs. 58% expected, difference test P = 0.0000; on grassland 26% observed vs. 16% expected, difference test P = 0.0001; in humanly altered habitats 19% observed vs. 25% expected, difference test P = 0.015).

The frequencies of recorded distribution of all tortoises in different habitat types with regard to year or activity pattern, which departed significantly from randomness with medium or large effect size, are presented in Figures 2 and 3, respectively. In year 2010 most of the records occurred at the forest edge; in 2011 on the grassland, in 2012 in the forest, in 2013 again at the forest edge, while in 2014 most of the records were noted in the forest & the grassland (Figure 2). Basking individuals were recorded most frequently at the forest edge, while hiding was the most common activity on the grassland. Moving was the most frequently recorded behaviour in the forest and on the path. Feeding was mostly seen in tortoises occurring on the path. Reproduction-related activities were the most frequently recorded events in the forest (see Figure 3).

The sample of temperature data in total included 353 records collected over five consecutive years. Most of these records or 295 (84%) were related to adult tortoises, 18 (5%) were of subadult ones and 40 (11%) of juvenile tortoises. Only data collected from sample of adults were subjected to further analysis due to the small sample of ambient temperatures taken on subadult and juvenile tortoises. Over the years of study and among the habitats, ambient temperatures at spots selected by tortoises were similar except Ta60 which differed over the years (Table 2). On the contrary, ambient temperatures were different for specific combinations of year and habitat type (Table 2). Additionally, adult tortoises performed all activites under similar ambient temperatures (Table 3 – variation of all three ambient temperatures showed $P > 0.10$ for factor Activity Type) but Ta5 and Ta60 were not similar in different habitats. Variation of ambient temperatures was not significantly different among interactions between activity patterns and habitat types (Table 3).

4. Discussion

In this study we have analyzed data on the eastern Hermann's tortoise population in an environment consisting of both pristine and humanly altered habitats. There were no evidence that tortoises exclusively choose specific habitat for performing specific activity, but rather that certain particularities exist in their habitat preferences and activity patterns in the mixed landscape of oak forests, meadows, gardens, orchards and vineyards. Our study site represents a habitat system dominated by deciduous mostly oak forest, but during the five consecutive years the tortoises were mostly not been recorded in the forested habitat type; moreover, the frequency of their occurrence in the same habitat varied significantly among the years of study (Table 1 and Figure 2). Additionally, the frequencies of performing defined activity types were significantly different among the habitats (see Table 1 and Figure 3).This indicates that eastern Hermann's tortoises at least in this part of species area require heterogeneous environment, or in other words, complex habitat system.

Our analysis of ambient temperatures at spots "used" by the adult tortoises suggested that they probably actively maintain suitable range of environmental temperatures in their closest surrounding by moving between different habitat types: Ts and Ta5 at occupied spots were not significantly different over the years of study or among the habitats where they were detected (see Table 2, factors Year and Habitat Type for Ts and Ta5), while Ta60 differed among habitat types. Also, all activities were performed under similar ground temperatures, while air temperatures differed among habitat types (Table 3, factors Activity Type and Habitat Type).

Unequal number of males, females and immatures in the overall sample could not impact on non-random associations between activity patterns and habitat types as three sex/age groups were randomly distributed among habitats. However, absence of sex/age specific habitat requirements in analyzed sample cannot be interpreted as the absence of need for complex habitat system. It is rather confirmation that the entire population should have more than one habitat type at disposal for successful overcome of negative effects of environmental variation.

To the certain extent, our results resembled the general conclusions that arised from the research conducted in South-western Romania (Rozylowicz and Popescu, 2013). Both studies confirmed significantly different frequency of occurrence of tortoises among analyzed habitat types. We detected relatively weak preference of Hermann's tortoises toward habitats modified by humans (e.g. vineyards, orchards, or gardens) which was also indicated elsewhere (see for example in: van Dijk et al., 2004; Rozylowicz and Popescu, 2013; Couturier et al., 2014). Those authors also underlined necessity of having more than one particular habitat type in the tortoise reserve (see results and discussion in their study) which is in accordance with our findings, illustrated by the non-random occurrence of tortoises performing certain activity in certain habitat.

Due to relatively low number of ecologically-oriented studies on eastern Hermann's tortoise in this part of the range, the outcomes of our relatively short-term monitoring also could serve as an argument for promoting habitat protection in local traditional farming systems. Our results strongly suggest that in Eastern Europe landscapes already modified by humans need promotion of carefully planned and monitored traditional land use so the protection of Hermann's tortoise can be realized (see for example Anadón et al., 2007). Due to prolonged existence of extensive cultural practices and to relatively abandoned countryside (see in Rozylowicz and Popescu, 2013) Eastern Europe still has relatively pristine habitats or complex habitat systems that could be comfortable for Hermann's tortoises at least because of the wide array of suitable thermal environments. Moreover, preserving a spatially heterogeneous environment for eastern Hermann's tortoises is a prerequisite for minimizing the harmful effects of climate change, whose deteriorating impact should not necessarily be restricted only to the Mediterranean part of the distribution area (Fernández–Chacón et al., 2011).

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Pairs of Variables	X^2 test				
	Pearson X^2	df	P	Cramer's V	Size effect
Sex/Age type × Year	17.826	8	0.023	0.140	Small
Sex/Age type × Season	**45.268**	**2**	**0.000**	**0.316**	**Medium**
Year x Season	10.709	4	0.030	0.154	Small
Year × Habitat type	**116.923**	**16**	**0.000**	**0.255**	**Large**
Season × Activity pattern	18.300	4	0.001	0.202	Small
Season × Habitat type	27.737	4	0.000	0.249	Small
Activity pattern × Habitat type	**51.754**	**16**	**0.000**	**0.170**	**Medium**

Table 1. Strength of departure from randomness when considering significantly non-random distribution of tortoises in regard to sex/age groups, seasons, years, habitat types and activity types analyzed in this study. df = degrees of freedom; P = significance.

FACTOR	df	MS	F	P
Factorial ANOVA for Ts				
Year	3	18.48	0.94	0.424
Habitat type	3	44.39	2.24	0.083
Year × Habitat type	**15**	**47.31**	**2.39**	**0.003**
Error	271	19.77		
Factorial ANOVA for Ta5				
Year	3	19.95	1.03	0.380
Habitat type	3	36.42	1.88	0.133
Year × Habitat type	**15**	**44.69**	**2.31**	**0.004**
Error	271	19.38		
Factorial ANOVA for Ta60				
Year	3	37.47	2.06	0.105
Habitat type	**3**	**52.73**	**2.90**	**0.035**
Year × Habitat type	**15**	**42.75**	**2.35**	**0.003**
Error	271	18.16		

Table 2. Factorial ANOVA on ambient temperatures recorded at capture spot of individual adult tortoises with Year and Habitat type as factor variables. Significant effects were bolded. Ts = ground surface temperature, Ta5 = air temperature at 5 cm from the ground surface, Ta60 = air temperature at 60 cm from the ground surface.

FACTOR	df	MS	F	P
Factorial ANOVA for Ts				
Activitytype	3	34.75	1.69	0.169
Habitat type	3	53.52	2.61	0.052
Activity type × Habitat type	15	24.72	1.20	0.268
Error	271	20.53		
Factorial ANOVA for Ta5				
Activity type	3	24.92	1.26	0.288
Habitat type	**3**	**72.18**	**3.65**	**0.013**
Activity type × Habitat type	15	26.94	1.36	0.166
Error	271	19.77		
Factorial ANOVA for Ta60				
Activity type	3	27.87	1.48	0.222
Habitat type	**3**	**71.24**	**3.77**	**0.011**
Activity type × Habitat type	15	28.14	1.49	0.108
Error	271	18.89		

Table 3. Factorial ANOVA on ambient temperatures recorded at capture spot of individual adult tortoises with Activity type and Habitat type as factor variables. Significant effects were bolded. Ts = ground surface temperature, Ta5 = air temperature at 5 cm from the ground surface, Ta60 = air temperature at 60 cm from the ground surface.

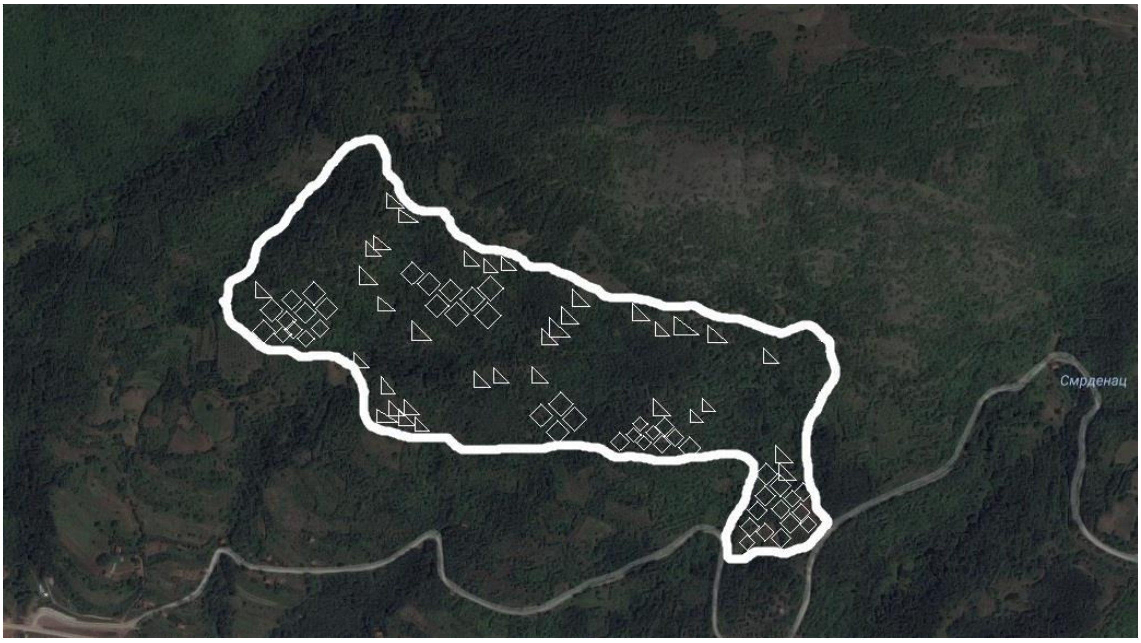

Figure 1. The study area. The map was constructed via Google Earth. White line borders the area where monitoring has been conducted. Triangles assign position of open habitat or grassland. Squares assign position of humanly modified habitat. Surface without symbols presents forest.

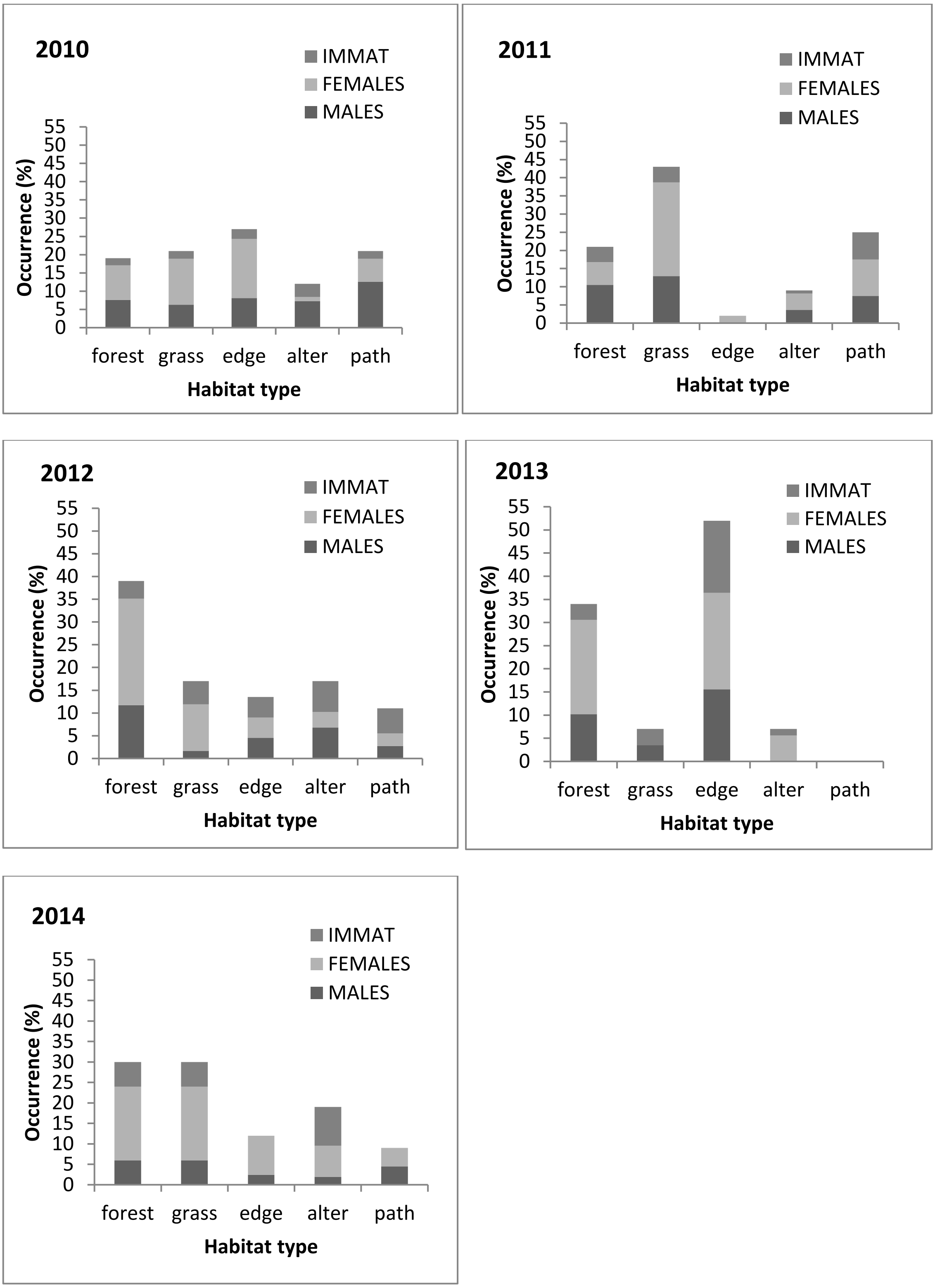

Figure 2. Percent of occurrence of tortoises in specific habitat type in consecutive years. For description of habitat types see section Material and Methods – Field procedures.

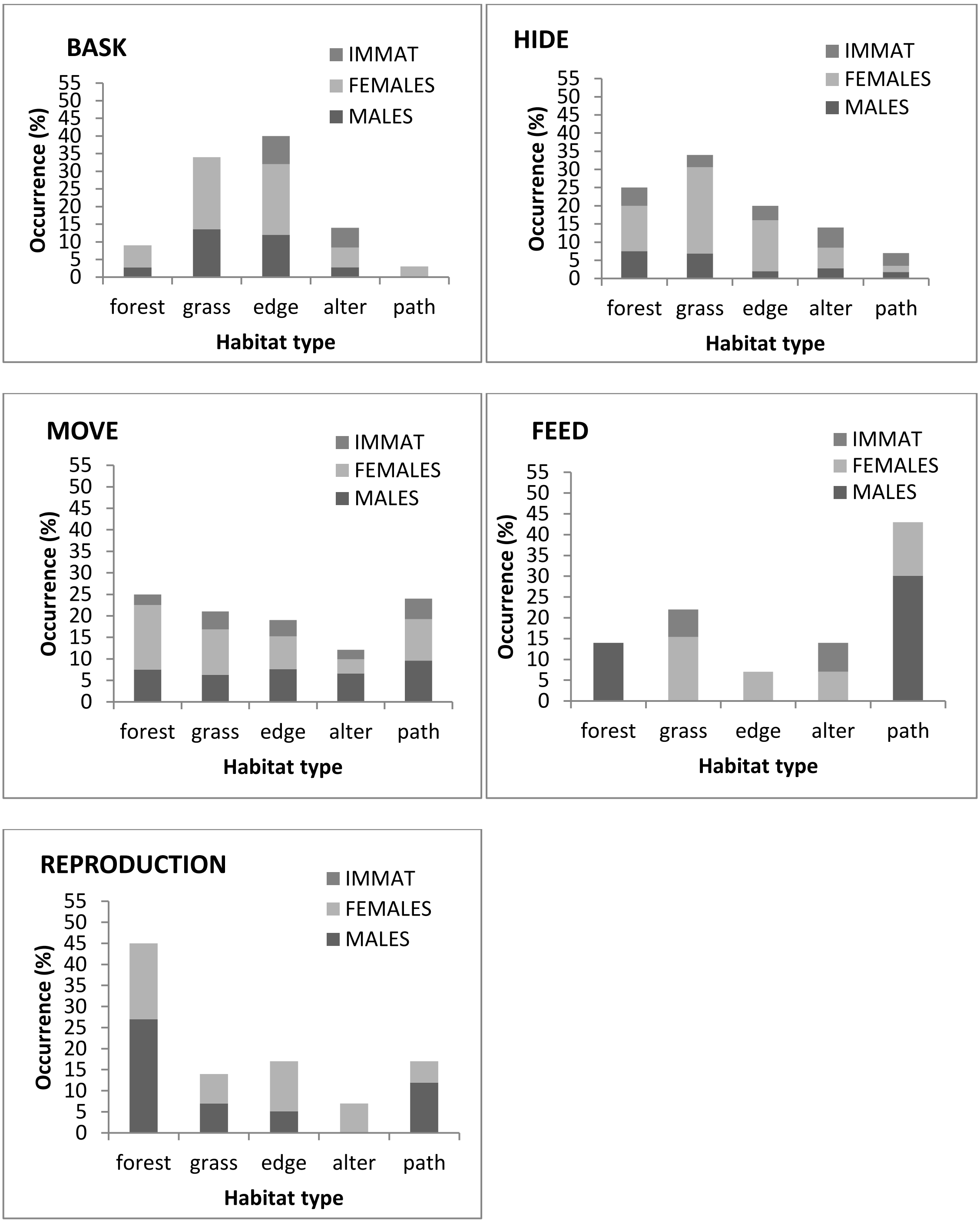

Figure 3. Percent of occurrence of tortoises performing specific activity in specific habitat type. For description of activity types see section Material and Methods – Statistical Analyses.