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Abstract. Despite the abundant data on habitat use of *Vipera ammodytes*, most studies are purely descriptive, merely listing the habitats in which the species is most often found. More complete studies evaluating the habitat preference of the species are lacking. The intraspecific variation (i.e., interpopulation or seasonal) in habitat and microhabitat utilization of the species also remains a poorly studied topic. In the current study, we assessed the general patterns of habitat and microhabitat use of *V. ammodytes* and their interpopulation and seasonal variations, based on habitat/microhabitat availability. To achieve that, we studied five different populations along a latitudinal gradient in western Bulgaria. In all of the studied areas, *V. ammodytes* showed a clear preference for various stony and rocky habitats and microhabitats, overgrown with herbaceous and shrub vegetation, while it avoided bare habitats, dark deciduous forests as well as cultivated agricultural lands. There were clear interpopulation and seasonal variations in habitat and microhabitat preference and spatial niche utilization. Our results suggest that habitat and microhabitat use of *V. ammodytes* depends on a

combination of many factors such as season, locally specific characteristics like habitat structure and availability, population dynamics, food availability, physical and microclimatic conditions, and possibly on the extent of the interspecific competition.

Key words. Reptilia, spatial niche, Viperidae, snakes, Nose-horned viper.

INTRODUCTION

A species' habitat is defined as the biotic and abiotic conditions that allow the survival and reproduction of this species (Hall et al., 1997; Morrison, 2009). A microhabitat is a smaller-scale subset of a habitat, which represents a specific place or a physical requirement of the species in a given habitat (Connell, 1961; Lugo et al., 1999; Petren, 2001; Bailey, 2009; Keith et al., 2020). A habitat can include several microhabitats, which may differ in their structure or conditions (i.e. vegetation, light exposure, humidity, temperature, air circulation) (Connell, 1961; Lugo et al., 1999; Petren, 2001; Bailey, 2009; Keith et al., 2020). Therefore, when researching the spatial niche of a particular species, it is important to assess both its habitat and microhabitat requirements to better understand its utilisation of the environment. Such assessments are crucial for properly and effectively delivering conservation actions on a target species.

Many snakes are generally sedentary animals with low dispersal abilities so their distribution usually depends on both the climatic and habitat characteristics of the environment. The microclimatic and microhabitat conditions play a major role in snakes' habitat selection (Vitt and Caldwell, 2014). For instance, the presence of stony microhabitats often plays a major role in the hierarchical selection of habitats as they provide snakes with favorable thermal conditions for thermoregulation and easy access to shelter from extreme environmental conditions or predators (Reinert, 1993; Kurek et al., 2018). Habitat use may vary across seasons, age groups, and populations of the same species, or depending on the reproductive status of individuals (Reinert, 1984, 1993; Sweet, 1985; Shine, 1986; Seigel, 1986; Burger and Zappalorti, 1989; Luiselli et al., 1994; Charland

and Gregory, 1995; Webb and Shine, 1998). Habitat use variability can be due to different factors, such as habitat and microhabitat availability, presence and location of suitable areas for hibernation and/or thermoregulation, or differences in food abundance between habitats (Reinert and Kodrich, 1982; Huey et al., 1989; Madsen and Shine, 1996). Moreover, variability is also common in microhabitat use (Neumeyer, 1987; Brito, 2003; Martínez-Freiría et al., 2010; Strugariu et al., 2011).

European vipers usually adhere to a certain small to medium-sized home range territory throughout most of their lives (Neumeyer, 1987; Naulleau et al., 1996; Saint Girons, 1997; Brito, 2003; Weinmann et al., 2004; Graitson, 2008; Plasinger et al., 2014; Dyugmedzhiev et al., 2020). When hibernating sites, sites for thermoregulation, shelters from unfavorable climatic conditions or predators, and a sufficient food base are all available within a given small territory, vipers can inhabit it throughout the entire activity period (Saint Girons, 1952, 1980; Neumeyer, 1987; Naulleau et al., 1998; Thomas, 2004; Wollesen and Schwartze, 2004). However, places suitable for hibernation, those with high food availability or with suitable summer' microclimatic conditions, often do not coincide. In such places, vipers conduct seasonal migrations from the hibernating areas to the summer habitats, and in autumn, they return to the hibernating areas (Duguy, 1963; Viitanen, 1967; Prestt, 1971; Saint Girons, 1980; Naulleau et al., 1998; Anderson, 2003; Wollesen and Schwartze, 2004; Graitson, 2008). The scales of these migrations depend on individual locality, with the biggest documented migrations being for *Vipera berus* (Linnaeus, 1758), from England and Finland, where some individuals may travel up to 1.5-2 km from the hibernating areas to the summer habitats (Viitanen, 1967; Prestt, 1971).

The nose-horned viper, *Vipera ammodytes* (Linnaeus, 1758), is distributed from the western foothills of the Alps across the entire Balkan Peninsula and many Aegean islands to north-western and northern Asia Minor and the Lesser Caucasus (Speybroeck et al., 2016). Throughout its range, it inhabits a wide variety of habitats. However, the species is most frequently found in different types of open and sunny stony or rocky habitats with shrubs and grasses, also in different types of open deciduous forests (Tuleshkov, 1959; Bruno, 1967; Beshkov, 1993; Ioannidis and Bousbouras, 1997; Stumpel and Hahn, 2001; Heckes et al., 2005; Crnobrnja-Isailović et al., 2007; Plasinger et al., 2014;

Mebert et al., 2015; Ghira, 2016). Within this wide variety of habitats, however, nose-horned vipers usually adhere to stony and rocky microhabitats (Beshkov, 1993, Mebert et al., 2015; Ghira, 2016). The microhabitat type is considered one of the main determinants for population density of the species because optimal microhabitats provide more access to shelter and a richer food base for the vipers (Ghira, 2016).

Despite the abundant data on the habitat use of *Vipera ammodytes,* most studies only describe the variety of habitats in which the species is found, More complete studies, taking into consideration habitat availability, in order to evaluate the habitat preference of the species, are lacking. To date, the intraspecific variation (i.e., interpopulation or seasonal) in habitat and microhabitat use of the nose-horned viper also remains a poorly studied topic, with data mainly on the seasonal variations in habitat and microhabitat use. In Serbia, Montenegro, and Northern Macedonia, males are usually detected in spring, exploiting open deciduous forests with southwest exposure; females are most often detected in summer, in rocky habitats with east and south exposure (Crnobrnja-Isailović et al., 2007). In Bulgaria, in early spring and late autumn, nose-horned vipers mainly inhabit rocky and stony sunny terrains with scarce vegetation (Beshkov, 1993). From the late spring until the beginning of autumn, vipers conduct short migrations to adjacent habitats, such as herbaceous vegetation, shrublands, and forests, often close to water sources (Beshkov, 1993). To date, there are no studies comparing habitat and microhabitat use among different populations of the nose-horned viper.

In Bulgaria, *V. ammodytes* is widespread throughout the country, except in the high mountains and urbanized or intensively cultivated agricultural lands (Stojanov et al., 2011). The current study aims to assess the general patterns of habitat and microhabitat use of *V. ammodytes*, based on habitat/microhabitat availability. In light of the available literature on vipers' habitat and microhabitat use, and under the assumption that nose-horned viper habitat and microhabitat use can vary among populations, the following hypotheses were tested: 1) *V. ammodytes* prefers various stony and rocky habitats and microhabitats, overgrown with shrubs and herbaceous vegetation; 2) habitat and microhabitat preference are highly dependent on their respective availability; 3) habitat and microhabitat preferences vary among different populations of *V. ammodytes*; 4) habitat and microhabitat use vary between the different seasons of the activity period.

MATERIALS AND METHODS

Study sites

Five sites along the latitudinal gradient in western Bulgaria were studied: 1) near Karlukovo Village, north-western Bulgaria (43°10'N, 24°3'E; 111-250 m a.s.l.); 2) near Gara Lakatnik Village, north-western Bulgaria (43°5'N; 23°23'E; 352-733 m a.s.l.); 3) near Balsha Village, the central parts of western Bulgaria (42°51'N; 23°15'E; 652-853 m a.s.l.); 4) near Bosnek Village, the central parts of western Bulgaria (42°29'N, 23°11'E; 942-1332 m a.s.l.); 5) the "Gabrovitsa" area in the Kresna Gorge, south-western Bulgaria (41°46'N, 23°9'E; 165-488 m a.s.l.; presented as "Kresna" in the tables and figures). Both sites 1 and 2 are karst valleys along the Iskar River, with steep rock cliffs, and patches of deciduous forests. Site 3 is an abandoned quarry, surrounded by fields, bare hills, and deciduous forests. Site 4 is a middle-mountain karst valley along the upper reaches of the Struma River, with rocky slopes, vegetated with shrubs and thin deciduous forests. Site 5 is a plain area along the middle reaches of the Struma River, vegetated with grass, scattered shrubs, and abandoned vineyards and surrounded by steep stony slopes overgrown with forest vegetation. Map and photographs of the sites are presented in Dyugmedzhiev et al. (2020). All sites fall in the temperate-continental climate zone except site 5, which lies in the continental-Mediterranean zone (Kopralev, 2002).

Fieldwork

Fieldwork was conducted mainly between April and September from 2014 to 2017, and each site was visited regularly once per month in 2014 and twice per month from 2015 onwards. Visits were also made between January–March and October–December, however, they were not evenly distributed among sites. Each visit lasted one day. All visits were made on days with daily temperatures above 15°C, on which vipers' activity could be expected (Dyugmedzhiev et al., 2021). Searches started when morning temperatures reached at least 15-16°C: usually around 12:00 in winter, at 11:00 in March, October, and November, at 9:00 in April, May, and September and at 8:00 during the summer. From October to March, searches continued until ambient temperatures dropped below 13-14°C, which was usually in the afternoon. From April to September, searches continued until dusk (i.e., around 30 minutes before dark), however, during some days vipers were also searched throughout parts of the night, usually until 23:00-24:00. Search efforts covered the entire vicinity of the study sites, with the exception of some physically inaccessible areas (e.g., too thick patches of shrubs, very steep rock cliffs). The same route scheme was followed in each visit, which covered parts of each of the different habitat types in a site. However, due to the different size areas of the different habitat types, the search effort was not equal across habitats. Vipers were located by sight as well as by inspection of potential shelters such as under stones and logs or inside rock crevices. Geographic position (Garmin eTrex 20; precision: 5 m), habitat and microhabitat characteristics of the location were recorded for each viper or viper's molt found. Habitat types were categorized visually, based on a list of habitat categories generated from the mobile application SmartBirds Pro (Popgeorgiev et al., 2015). A total of 24 habitat type categories were derived (see Table 1). Microhabitat characteristics of the location were categorized according to the percentage of trees/shrubs, grasses, stones/rocks, water surfaces, and roads within a radius of 2.5 m from the snake's location (Martínez-Freiría et al., 2010; Mebert et al., 2015; Dyugmedzhiev et al., 2019). Based on the period of observation, seasons were categorized as spring (beginning of March-end of May), summer (beginning of June-end of August), and autumn (beginning of September-end of November). Captured vipers were measured (snout to vent [SVL] and tail length [TL]; precision 0.5 cm), weighted (precision: 0.01 g), color marked, and photographed for individual identification (Dyugmedzhiev et al., 2018) and then released on the site of capture, usually within 15-30 min following the capture.

Statistical analyses

Individuals found more than once throughout the day were included in the analyses only with the data from the first observation since the capture and measuring procedures can cause changes in vipers' natural activity patterns. Pre-shedding vipers (2-3 days before the shedding) usually avoided conducting long movements until they shed their skin and mostly basked or hid in shelters within a very small area, until shedding their skin. Therefore, found molts were considered a reliable source of habitat and microhabitat selection of pre-shedding vipers. To avoid collecting data for the same molt in two different field visits (pseudoreplication), each found molt was torn apart and removed from the site. Dead animals were excluded from the analyses, as it was impossible to objectively assess whether they died while passing through the habitat on their way to a neighboring, more suitable one, or whether they actually stayed in this particular habitat prior to their death.

Habitat preference was analyzed with Ivlev's index. The index is calculated by the formula:

$$\frac{U - A}{U + A}$$

where *U* is the number of observed individuals in habitat *i* / number of observed individuals in all habitats, and *A* is the size area of habitat *i* / total size area of all habitats (Kenward, 1992). Positive values of this index indicate that the habitat is used more often than expected, based on its availability, and negative values indicate that it is less used. Values of -1 of this index indicate that the habitat is not used at all. The area of each habitat type was drawn out via satellite pictures, obtained from Google Earth Pro, and its size was calculated with ArcGIS v. 10.4.1. (ESRI, Redlands, CA, USA). Ivlev's index was calculated for each available habitat type, both for the combined data from all populations and for each separate population. In order to assess the local variation in habitat preference, two types of habitat preference were derived, general and local. The estimated values of each habitat type's Ivlev's index based on the combined data from all populations were used as reference values to assess the general species' habitat preference. The estimated index values for each separate population were then used to assess the local preference of habitat types, which were then compared to the general preference for evaluating the interpopulation variations in habitat preference. For this purpose, habitat types were divided into four categories, based on the values of the Ivlev's index: preferred, PR – habitat types with values between 0.5 and 1; often used, OU – habitat types with values between 0.5 and 0; rarely used, RU – habitat types with values between 0 and -0.5; avoided, AV – habitat types with values between -0.5 and -1. Therefore, if a certain habitat type is placed within different categories based on the values for general and local preference, this was considered as an indication of local variation in preference of this habitat type.

To analyze the breadth of each population's spatial niche, Levins' index (B) was used. This index was calculated by the formula:

$$B = \frac{1}{\sum p_i^2}$$

where p_i is the relative proportion of individuals found in habitat *i* compared to the number of individuals in all habitats (Krebs, 1999). The index was standardized via the formula:

$$B_{st} = \frac{B - 1}{n - 1}$$

where *B* is the Levins' index, and *n* is the number of habitats, thus, the index vary from zero to one, with a value of zero indicating maximum specialization (all individuals are found in only one of the habitats), a value of one – absence of specialization (equal number of individuals in all habitats) (Cooper-Bohannon et al., 2016). A cluster analysis (by the commonly used UPGMA algorithm) based on the Morisita's similarity index was used to compare the different populations in regard to habitat use and to assess potential latitude-based patterns in habitat use. This index was estimated using a frequency matrix representing the number of observations of vipers in each habitat type for each separate population. This similarity index was chosen, as it is the most robust and independent of sample size when the number of individuals is used for its calculation (Wolda, 1981). The combined data for both live individuals and found molts were used for the calculation of each of the three indices, used to assess general and interpopulation patterns of habitat use.

A correspondence analysis was used to evaluate the general seasonal variations in habitat use based on the combined data from all five populations. This analysis was used to clarify which habitat types are associated with each separate season (Rohlf, 1988). A frequency matrix representing the number of observations of live individuals in each habitat type for each of the seasons was used for this analysis. Habitat types in which vipers were never observed were excluded from this frequency matrix. When sample size allowed it, the differences within a separate population between the number of observations of vipers in a particular habitat type during the different seasons were analyzed with a χ^2 test. Information provided by the found molts were excluded from all analyses on seasonal variation of habitat and microhabitat use because often was not possible to assess in which season a particular moult was shed. Due to the smaller and uneven sample sizes for sites 4 and 5, in which most of the vipers were found in spring (Table S1), the seasonal patterns of habitat and microhabitat use of those populations were not analyzed.

Since a normal distribution of the data could not be achieved (Kolmogorov-Smirnov & Liliefors, $p < 0.05$), a Kruskal-Wallis H test was used to analyze the microhabitat use of the species. Due to the low percentage of water surfaces and roads, only the data for trees/shrubs, grasses, and stones/rocks were used as groups in the analyses. The use of each of these three groups was compared between different populations with the combined data for all seasons, as well as between different seasons for each separate population with sufficient sample size (i.e., sites 1, 2, and 3).

Kruskal-Wallis H test, χ^2 test, and correspondence analysis were processed with Statistica 10.0 (StatSoft, Inc. 2011). Morisita's similarity index was calculated using Past 3.25 (Hammer et al., 2001). Statistical significance was accepted at $P < 0.05$.

RESULTS

General habitat preference

A total of 708 records of *Vipera ammodytes* (651 live individuals and 57 molts) from the five study sites were used to analyze the species' habitat preference: 244 from Karlukovo (223 live and 21 molts), 168 from Lakatnik (160 live and 8 molts), 163 from Balsha (149 live and 14 molts), 65 from Bosnek (58 live and 7 molts) and 68 from Kresna Gorge (61 live and 7 molts) (Table S1). Vipers were found in 17 of all 24 available habitat types. The analyses of the combined data for all populations revealed that, based on the values of the Ivlev's index, five habitat types are preferred (PR: H1-H5), four are often used (OU: H6-H9), four are rarely used (RU: H10-H13) and 11 are avoided (AV: H14-H24) (Fig. 1A).

Interpopulation variations in habitat use

In site 1 (Karlukovo), vipers were found in six of all 12 available habitat types: one habitat type classified as OU, two as RU, and three as AV (Fig. 1B). The habitat types with the largest areas were the ones classified as OU, followed by the AV and the RU categories (Fig. 2). The PR category was the one with the smallest area (Fig. 2; Table S1). The local preference of four habitat types differed from the general habitat preference – H2, H3, H10, and H13 (Figs. 1A and B). In site 2 (Lakatnik), vipers were found in seven of all nine available habitat types: two PR, one OU, and four RU (Fig. 1C). The most available habitat types were the OU and the RU, while the AV and the PR habitat types were with much smaller size (Fig. 2; Table S1). The local preference of three habitat types differed from the general habitat preference – H6, H7, and H9 (Figs. 1A and C). In site 3 (Balsha), vipers were found in eight of all 15 available habitat types: three PR, two OU, and three RU (Fig. 1D). The most available habitat types were the AV, followed by the RU, while the PR and the OU were with much smaller size (Fig. 2; Table S1). The local preference of three habitat types differed from the general habitat preference: H4, H6, and H12 (Figs. 1A and D). In site 4 (Bosnek), vipers were found in six of all 12 available habitat types: four OU, one RU, and one AV (Fig. 1E). The most available habitat types were the OU, followed by the RU and the AV, while the PR covered a negligible size (Fig. 2; Table S1). The local preference of five habitat types differed from the general habitat preference: H1, H6, H10, H11, and H13 (Figs. 1A and E). In site 5 (Kresna Gorge), vipers were found in seven of all 11 available habitat types: three PR, one OU, one RU, and two AV (Fig. 1F). The AV clearly dominated in abundance, while the PR, the OU, and the RU were with much smaller size (Fig. 2; Table S1). The local preference of three habitat types differed from the general habitat preference: H1, H6, and H15 (Figs. 1A and F).

In each of the five sites, *V. ammodyes* had very narrow niche breadth and the species used the available habitat types very unevenly. The values of the Levins' index were close to 0 for each site. They were the lowest in site 1 (B_{st} = 0.03), followed by sites 3 and 4 (B_{st} = 0.06 and 0.07, respectively), and were the highest in sites 5 and 2 (B_{st} = 0.13 and 0.19, respectively). The cluster analysis showed no grouping pattern based on latitude. Site 4 and site 1 were grouped together, followed by site 2. Site 3 was grouped as an outside group from the latter three, and site 5 – as an outside group of the combined cluster of sites 1-4 (Fig. 3).

Seasonal variations in habitat use

The analysis of the combined data from all five populations revealed a clear seasonal variation in habitat use. The first two dimensions of the correspondence analysis explained 100% of the variance (Fig. 4). Separation based on the first dimension was weak. The second dimension, however, clearly separated summer from both spring and autumn. Three habitat types were grouped closer to summer than to spring and autumn – H1, H6, and H7. Four habitat types were grouped between spring and autumn (H2, H8, H11, and H12), and four were closer to spring (H4, H5, H15, and H17). The rest of the habitat types did not group close to any of the seasons.

In site 1, there was a statistically significant difference between the number of observations of vipers in the different seasons for each of the two habitat types with sufficient sample size for the χ^2 test – H7 and H12 (Table 2). H7 was more used in summer than in the other two seasons, while in H12, the opposite trend was present (Table S1). In site 2, four habitat types had a sufficient sample size for the χ^2 test – H1, H7, H8, and H12. A statistically significant difference was present only for H1 and H7 (Table 2), with both being more used in summer than in the other two seasons (Table S1). In site 3, two habitat types had sufficient sample sizes for the χ^2 test, H2, and H5, with statistically significant differences present only for H2 (Table 2). This habitat type was much more used in spring and autumn, while in summer vipers were found rarely in it (Table S1).

Interpopulation variations in microhabitat use

In the studied populations of *V. ammodytes* from the northern and central parts of western Bulgaria (Sites 1, 2, 3, and 4), vipers were found mostly in stony-rocky microhabitats with less presence of grasses and trees/shrubs. Moving south to site 5 there was a gradual decrease in the amount of stones/rocks at the expense of an increase in grasses and trees/shrubs in the microhabitats occupied by the species, with the latter two components having equal presence to that of the stones/rocks (Fig. 5A). Statistically significant differences were found between some of the populations. Regarding the presence of trees/shrubs, statistically significant differences were found between the population from site 5 and those from both sites 1 and 2 (Table 3). Site 5 had the highest values for trees/shrubs presence, compared to all five populations, while site 2 had the lowest values (Fig. 5A). In regards to the presence of grasses, both the populations from sites 3 and 5 differed significantly from each of the other populations (Table 3). The presence of grasses was the lowest in site 3 and was the highest in site 5 (Fig. 5A). In regards to the presence of stones/rocks, again the population in site 3 differed significantly from the other populations. The population from site 5 was significantly different from the other populations, with the exception of site 4, where the result was at the threshold of statistical significance ($P = 0.05$; Table 3). The presence of stones/rocks was the highest in site 3, while it was the lowest in site 5 (Fig. 5A).

Seasonal variations in microhabitat use

Seasonal variations in the characteristics of the microhabitats used by *V. ammodytes* were present only in sites 1 and 3. In site 1 the presence of all three components (trees/shrubs, grasses, and stones/rocks) varied across seasons (Fig. 5B). The presence of trees/shrubs was the lowest in summer and the results between summer and autumn were statistically significant (Table 4). The presence of grasses decreased in autumn and the results between summer and autumn were statistically significant (Table 4). The presence of stones/rocks in summer was slightly higher than in the other two seasons and statistically significant differences were present between summer and spring (Table 4). In site 3, the presence of both grasses and stones/rocks differed significantly in summer, compared to spring and autumn (Table 4). The presence of grasses increased in the summer microhabitats of vipers, in contrast to that of stones/rocks, which decreased during this season (Fig. 5D).

DISCUSSION

In the five study sites, *V. ammodytes* showed a very narrow spatial niche, exhibiting a preference for different types of stony and rocky habitats and microhabitats, covered with herbaceous and shrubby vegetation (Fig. 1). In contrast, the species clearly avoided bare habitats, dark deciduous forests, and agricultural habitats without or with very low presence of stones (Fig. 1). Our results are in agreement with the available literature about habitat use of *V. ammodytes* (Tuleshkov, 1959; Bruno, 1967; Beshkov, 1993; Stumpel and Hahn, 2001; Heckes et al., 2005; Plasinger et al., 2014; Mebert et al., 2015; Ghira, 2016).

Interpopulation variations in habitat and microhabitat use

Although there were some clear differences in habitat use and spatial niche breadth between the different populations in the current study, no latitude-based patterns were evident. Interestingly, it seems that the availability of suitable habitats was not the only factor to explain the interpopulation variations in habitat use. For instance, even though the OU habitat type H7 was the most abundant habitat in sites 1, 2, and 4, and its abundance was equal between the first two, in sites 1 and 4 (Fig. 2) this habitat was used much more often (and thus, was locally classified as OU), than in site 2, where it was classified as RU (Fig. 1). Similarly, the PR habitat type H2 was only slightly less abundant in site 1 compared to site 3 (Fig. 2). However, in site 1 this habitat was used much more rarely (and was classified as RU) than in site 3, where it was one of the most preferred habitats (Fig 1), at least in spring and autumn (see below). Therefore, it appears that habitat use does not depend solely on the availability of suitable habitats, but probably on a combination of factors. Such factors might be the local characteristics, habitat structure, and microclimatic conditions of the site (Reinert, 1984; Burger and Zappalorti, 1989; Kurek et al., 2018), population dynamics (Viitanen, 1967; Prestt, 1971; Luiselli et al., 1994; Charland and Gregory, 1995), or food abundance in the different habitats (Luiselli et al., 1994; Madsen and Shine, 1996; Luiselli, 2006).

In contrast to habitat use, there were some latitude-based patterns in vipers' microhabitat utilization. In the populations from the northern and central parts of western Bulgaria (sites 1-4), vipers were found mainly in stony-rocky microhabitats with less presence of shrubs and grasses, a pattern also reported for populations from other parts of the species range (Mebert et al., 2015; Ghira, 2016). Going south, however, to the southernmost population (site 5), vipers were found in microhabitats with more shrub and grass presence, equal to that of the stones/rocks. The structure and conditions (e.g., vegetation, light exposure, temperature, humidity) of the different microhabitats within a particular habitat may differ (Connell, 1961; Lugo et al., 1999; Petren, 2001; Bailey, 2009; Keith et al., 2020) and this might be why latitude-based differences were evident in microhabitat but not in habitat use. The observed differences in microhabitat use might be due to one of several reasons, or to a combination of most or all of them. First of all, these patterns might be a consequence of the specific characteristics of the different studied areas. Sites 1-4 were located in karst terrains, while site 5 in the Kresna Gorge was situated in a grassy-shrubby area. Secondly, the thermal conditions of the environment might also affect these patterns. The valley of Struma River in south-western Bulgaria, in which the Kresna Gorge is located, falls into the continental-Mediterranean zone, which is characterized by overall higher temperatures (Kopralev, 2002). Ambient temperatures in the stony/rocky-dominated microhabitats in this area may become too high, causing vipers to select more grassy and shrubby areas that provide more suitable temperatures. It is important to state, however, that the karst terrains in northern Bulgaria (i.e., Karlukovo) are also characterized by overall high temperatures (Nedyalkov et al., 2024), so this hypothesis seems less plausible. Another possible reason for the observed geographic differences in vipers' microhabitat use could be the effect of interspecific competition. In the northern and central parts of western Bulgaria, snake species richness is lower (up to six different species coexisting in sympatry and/or sintopy) than that in south-western

Bulgaria, where up to 12 different species coexist in sympatry and/or sintopy (Beshkov, 1978, Petrov and Beshkov, 2001; Stojanov et al., 2011). It is possible that the stronger interspecific competition in this area, with species such as *Malpolon insignitus* (Geoffroy Saint-Hilaire, 1827), *Platyceps najadum* (Eichwald, 1831) and *Dolichophis caspius* (Gmelin, 1789), which all share similar habitats and have similar diet with *V. ammodytes* (Beshkov, 1978) may drive the latter to use suboptimal microhabitats. Microhabitat segregation is known to reduce competition between ecologically similar species and/or species with similar diets, which share the same habitat (Luiselli, 2006; Martínez-Freiría et al., 2010; Mebert et al., 2015; Dyugmedzhiev et al., 2019). Further studies are needed, however, to evaluate this hypothesis.

Seasonal variations in habitat and microhabitat use

Our results, showing some seasonal variations in habitat use of *V. ammodytes*, are in agreement with the results of Beshkov (1993). Such seasonal shifts in habitat use are well-documented for other European viper species (Duguy, 1963; Viitanen, 1967; Prestt, 1971; Saint Girons, 1980; Naulleau et al., 1998; Anderson, 2003; Wollesen and Schwartze, 2004; Graitson, 2008). Our results however suggest that these seasonal variations in habitat use are much more complex than the basic seasonal pattern described by Beshkov (1993) (see Introduction), and dependent on the local characteristics of the area, inhabited by a particular population. The seasonal variations were most prominent in sites 1 and 3. In site 1, vipers hibernating dens were usually located in habitat H12 (Dyugmedzhiev et al., 2020). Shortly after spring emergence, vipers moved from their dens to the adjacent, more open and sunny habitat H7, where they were found until mid-autumn. During summer, only pregnant females remained close to the hibernating areas, although they inhabited H7 and not H12. Around the beginning of October, all vipers again returned close to the hibernating areas, where they were usually found basking in H7, but near their hibernating dens in H12 (usually between 20-100 m). By the second part of October and the first half of November, vipers moved to their hibernating dens in H12, where they spent the warm parts of the days basking.

A similar pattern was evident in a different habitat type (H2) in site 3. In this site, vipers used a big abandoned old quarry as a hibernating area. Vipers inhabited this quarry from the period of spring emergence until the end of the mating period (usually around mid to late May (Dyugmedzhiev et al., 2020). After this period, only pregnant vipers as well as a few immature individuals were detected in this habitat, until the second half of September, when the rest of the vipers started to return (Dyugmedzhiev et al., 2020). As it appears, from late spring until autumn, most vipers migrate from the quarry to the adjacent habitats. However, because we were not able to detect a sufficient number of individuals in the vast area of those adjacent habitats, and none of the individuals captured there were identified as previously captured in the quarry, the true patterns and scale of this seasonal migration cannot be ascertained at this stage. Although it was evident that in site 2, habitats H1 and H7 were the vipers "preferred" areas during summer, our data is not comprehensive enough to point out the spring and autumn "preferred" habitats.

According to Beshkov (1993), the seasonal shifts in habitat use of *V. ammodytes* could be explained by the search for optimal thermal and solar radiation conditions, water sources, shelters, etc. However, food availability in the different habitat types might also play a role in these patterns (Viitanen, 1967; Prestt, 1971; Luiselli et al., 1994; Madsen and Shine, 1996; Luiselli, 2006). It is possible that spring/autumn habitats might have a more limited food base, such as small rodents, lizards, and centipedes, which are the most common prey of *V. ammodytes* (Beshkov, 1977; Luiselli, 1996; Dyugmedzhiev, 2020; Anđelković et al., 2021; Tomović et al., 2022). The fact that most vipers rarely use those habitats during summer, which is the most active feeding period, especially for adult vipers (Dyugmedzhiev, 2020) might be considered as an argument in support of this hypothesis. Similarly to the current study, some seasonal differences in microhabitat utilization were also reported for *V. ammodyes* from Serbia, Montenegro, and North Macedonia (Crnobrnja-Isailović et al., 2007) as well as for the ecologically similar *Vipera latastai* (Brito, 2003). These variations are most likely to be a consequence of the respective seasonal changes in habitat use.

Study limitations

There are some issues, coming from the method, used to evaluate habitat preference, that need to be treated with caution. First of all, since the search effort was not even across each habitat type, it is possible that the use of some habitat types could be underestimated. Furthermore, the small overall areas of some habitat types, such as the abandoned buildings (H3) might cause an overestimation of the habitat preference compared to habitats with large areas (such as the rocky/stony areas overgrown with grass and shrubs, H7). Regarding the asphalt roads (H18), it is difficult to get a clear idea, based on the Ivlev's index values alone, since the only vipers that we found in this habitat type were dead ones (and they were excluded from the analyses). In any case, it appears that the roads are acting like a barrier, disrupting the vipers' home range.

CONCLUSIONS

Vipera ammodytes is a highly petrophilic species and in the studied areas showed a clear preference for various stony and rocky habitats and microhabitats, overgrown with herbaceous and shrub vegetation, while it avoided bare habitats, dark deciduous forests as well as cultivated agricultural lands. Habitat and microhabitat use seems to depend on a combination of many other factors such as season, locally specific characteristics like habitat structure and availability, population dynamics, food availability, physical and microclimatic conditions, and possibly the extent of the interspecific competition.

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SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found at <http://www-9.unipv.it/webshi/appendix/index.html> manuscript number 15928

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TABLES

Table 1. Description of the characteristics of the different habitat types in which vipers were searched for and the microhabitat characteristics, presented by the percentage of trees/shrubs, grasses, and stones/rocks in the places where vipers were observed in each habitat. Values are expressed as "means ± SD (Min-Max)" when n > 1, "[absolute value]" when n = 1, or "–" when n = 0.

Habitat type	**Microhabitat characteristics**		
	Trees/Shrubs	Grass	Stones/Rocks
H1: Rocks / screes (natural) with scattered shrubs and trees growing on them	23.45 ± 8.57 (10 – 50)	18.97 ± 8.17 (0 – 40)	57.59 ± 8.72 (30 – 70)
H2: Abandoned quarries overgrown with a mixture of grasses, shrubs and scattered trees	26.38 ± 12.26 (0 – 50)	18.07 ± 13.73 (0 – 70)	55.71 ± 14.47 (20 – 90)
H3: Abandoned old buildings and ruins	13.33 ± 5.77 (10 – 20)	43.33 ± 25.17 (20 – 70)	43.33 ± 25.17 (20 – 70)
H4: Stone piles / stone walls (man-made) overgrown with grass, and with only scattered shrubs present	17.5 ± 13.88 (0 – 30)	41.25 ± 18.85 (20 – 70)	40 ± 10.69 (20 – 50)
H5: Stone piles / stone walls (man-made) entirely or almost entirely overgrown with shrubs	30 ± 9.29 (10 – 50)	33.65 ± 12.05 (10 – 70)	36.15 ± 8.44 (20 – 60)
H6: Stone piles / stone walls (man-made) entirely or almost entirely overgrown with a mixture of trees and shrubs	40.5 ± 8.87 (20 – 50)	21.5 ± 14.61 (0 – 50)	38 ± 19.56 (10 – 60)
H7: Rocky / stony areas (natural), overgrown with a mixture of grass and shrubs	24.02 ± 14.57 (0 – 70)	32.61 ± 14.66 (0 – 80)	43.01 ± 13.73 (0 – 80)
H8: Rocky / stony road scarps (man-made) overgrown with a mixture of grass and shrubs	22.67 ± 10.81 (0 – 40)	23 ± 12.64 (0 – 50)	32.33 ± 9.35 (20 – 60)
H9: Light highly sparse deciduous forests with shrub undergrowth, growing on rocky / stony areas	30 ± 11.55 (20 – 40)	32.5 ± 9.57 (20 – 40)	42.5 ± 5 (40 – 50)

H10: Rivers and streams	[10]	[0]	[30]
H11: Rocky / stony areas (natural) entirely or almost entirely overgrown with trees and shrubs	23.75 ± 15.98 (0 – 40)	25 ± 13.09 (10 – 40)	47.5 ± 19.82 (20 – 80)
H12: Light mediumly sparse deciduous forests with shrub undergrowth, growing on rocky / stony areas	37.35 ± 14.42 (10 – 80)	18.09 ± 12.73 (0 – 60)	44.56 ± 15.3 (10 – 80)
H13: Bare or almost bare rocks / screes with a very sparse grass vegetation growing on them	20 ± 15.19 (0 – 40)	23.57 ± 10.08 (10 – 50)	57.86 ± 17.62 (30 – 90)
H14: Dirt roads	10 ± 14.14 (0 – 20)	15 ± 7.07 (10 – 20)	20 ± 0 (20 – 20)
H15: Ecotone – bordering area between a forest and an open habitat, overgrown with mixture of trees, shrubs and grasses	40 ± 13.09 (10 – 50)	48.75 ± 14.58 (30 – 80)	11.25 ± 8.35 (0 – 30)
H16: Shrubbery area without or with very few stones /rocks	35 ± 21.21 (20 – 50)	30 ± 14.14 (20 – 40)	35 ± 7.07 (30 – 40)
H17: Meadows / pastures with scattered shrubs and no or very few stones /rocks on them	30.71 ± 13.28 (10 – 60)	45 ± 14.54 (30 – 70)	24.29 ± 11.58 (0 – 40)
H18: Asphalt roads	–	–	–
H19: Abandoned bare or almost bare quarries with a very scarce vegetation	–	–	–
H20: Dry ravines in thick and dark deciduous forests with shrub undergrowth	–	–	–
H21: Grassy road scarps (man-made) without or with very few rocks / stones	–	–	–
H22: Mud / dirt / muck areas without vegetation	–	–	–
H23: Bare sand screes without vegetation	–	–	–

H24: Abandoned old gardens / vineyards / pastures, which are not cultivated or planted anymore	–	–	–

Table 2. Results from the $\chi 2$ test between the number of observations of vipers during the different seasons in habitat types with sufficient sample size for each of the five populations. For abbreviations of the habitat types, see Table S1.

Population	Habitat type	$\chi 2$	df	P
Karlukovo	H7	10.78	2	0.005
	H12	11.51	2	0.003
Lakatnik	H1	14.33	2	0.0008
	H7	8.38	2	0.02
	H8	3.91	2	0.14
	H12	1.91	2	0.39
Balsha	H2	14.87	2	0.0006
	H7	1.99	2	0.37

Table 3. Results of Kruskal-Wallis H tests assessing differences in three microhabitat characteristics (Trees/Shrubs, Grasses, Stones/rocks) among the five different populations.

	Lakatnik	Balsha	Bosnek	Kresna
Trees/Shrubs: Kruskal-Wallis H test: H = 20.56, P = 0.004, n = 648				
Karlukovo	1	1	0.99	0.02
Lakatnik	–	0.11	0.12	0.0009
Balsha	0.11	–	1	0.48
Bosnek	0.12	1	–	1
Grasses: Kruskal-Wallis H test: H = 58.06, P < 0.0001, n = 648				
	Lakatnik	Balsha	Bosnek	Kresna
Karlukovo	1	< 0.00001	1	0.005
Lakatnik	–	0.0003	1	0.0009
Balsha	0.0003	–	0.03	< 0.00001
Bosnek	1	0.03	–	0.008
Stones/rocks: Kruskal-Wallis H test: H = 75.62, P < 0.0001, n = 648				
	Lakatnik	Balsha	Bosnek	Kresna
Karlukovo	1	0.003	0.21	< 0.00001
Lakatnik	–	0.004	0.37	< 0.00001
Balsha	0.004	–	0.00003	< 0.00001
Bosnek	0.37	0.00003	–	0.05

Table 4. Results of Kruskal-Wallis H tests between the microhabitat characteristics in the places of observations of *V. ammodytes* during the different seasons in the population around Karlukovo (second row) and Balsha (sixth row). The p-values from the post-hoc tests testing for differences in the presence of the three microhabitat components between the different seasons in the two populations are presented in rows 3-5 and 8-10, respectively. Sp – spring; Su – summer; Au – autumn.

Karlukovo								
Shrubs: Kruskal-Wallis H test: H = 15.58, P = 0.0004, n = 220			Grasses: Kruskal-Wallis H test: H = 12.04, P = 0.002, n = 220			Stones/rocks: Kruskal-Wallis H test: H = 8.55, P = 0.01, n = 220		
	Su	Au		Su	Au		Su	Au
Sp	0.09	0.55	Sp	1	0.07	Sp	0.03	1
Su	–	0.00004	Su	–	0.003	Su	–	0.08
Balsha								
Shrubs: Kruskal-Wallis H test: H = 3.99, P = 0.14, n = 147			Grasses: Kruskal-Wallis H test: H = 23.33, P < 0.00001, n = 147			Stones/rocks: Kruskal-Wallis H test: H = 19.99, P < 0.00001, n = 147		
	Su	Au		Su	Au		Su	Au
Sp	0.19	0.56	Sp	0.00002	1	Sp	0.0004	1
Su	–	1	Su	–	0.0002	Su	–	0.0001

CAPTIONS TO FIGURES

Fig. 1. General and local habitat preference of *V. ammodytes* based on the values of the Ivlev's index. A) Categorization of the different habitat types, based on the values for general preference of the Ivlev's index, calculated with the combined data from all five populations; categorization of the different habitat types, based on the values for local preference of the Ivlev's index, calculated for Karlukovo (B), Lakatnik (C), Balsha (D), Bosnek (E) and Kresna Gorge (F). Different preference categories are presented with different colors: green bars – preferred habitat types, PR; blue bars – often used habitat types, OU; orange bars – rarely used habitat types, RU; red bars – avoided habitat types, AV. For abbreviations of the habitat types, see Table 1.

Fig. 2. Area of the different habitat type categories in the different study sites, based on the values for the general habitat preference of the Ivlev's index, calculated with the combined data from all five populations. The most abundant habitat types from each preference category are presented within the bar, except those from the AV category, which are presented combined. Habitat types with very small areas are presented combined as "Other". For abbreviations and exact size of the habitat types, see Table 1 and S1, respectively.

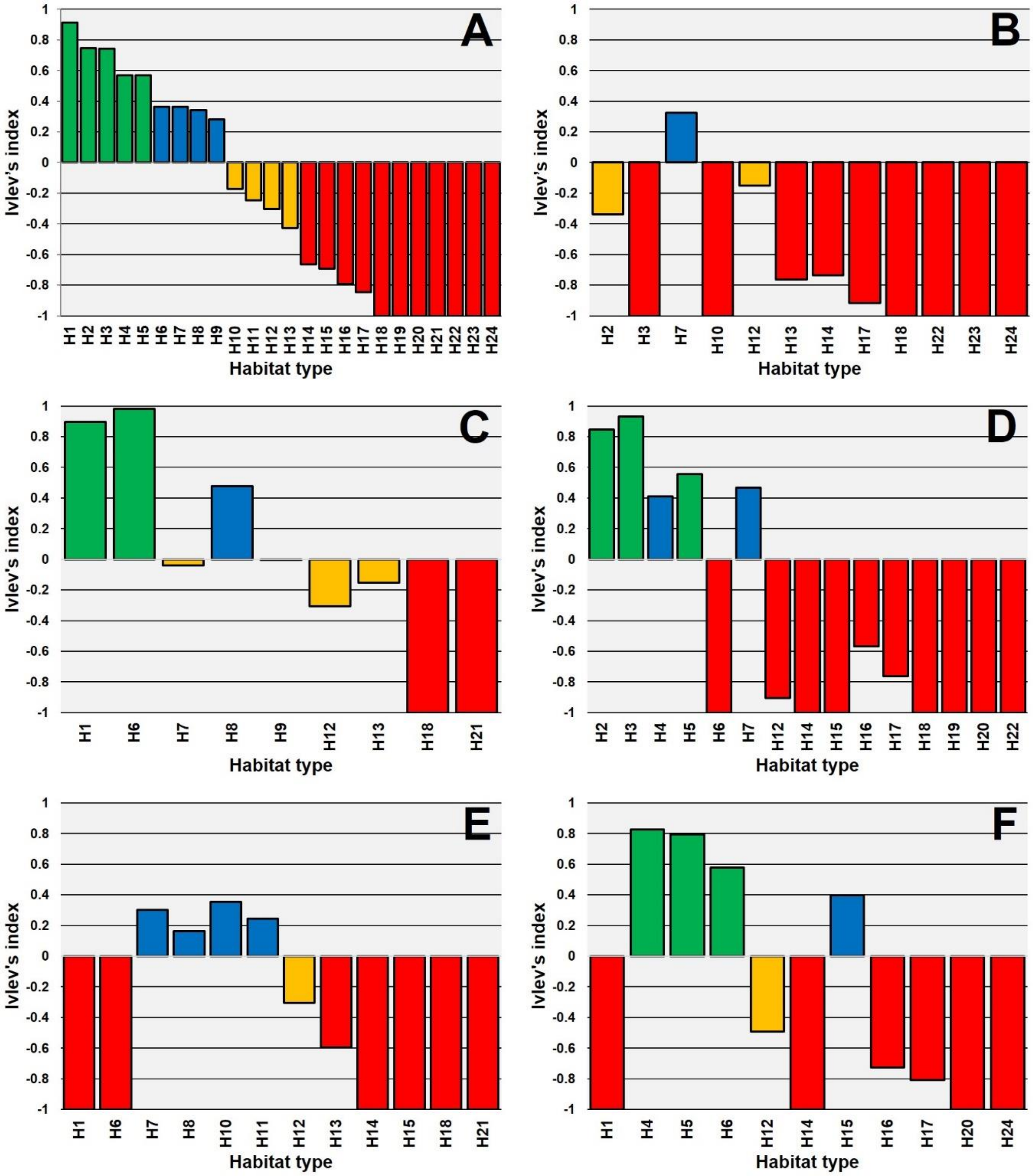
Fig. 3. Similarity in habitat use of *V. ammodytes* between the five studied populations, based on the Morisita index.

Fig. 4. Grouping between habitat types used by *V. ammodytes* and seasons, based on the results from the first two dimensions of the correspondence analysis. For abbreviations of the habitat types, see Table 1.

Fig. 5. Comparison of the microhabitat characteristics, presented as the percentage of trees/shrubs (grey), grasses (white), and stones/rocks (black) in the places of observations of *V. ammodytes*. A)

Five studied sites with the combined data from the different seasons; different seasons for Karlukovo (B), Lakatnik (C), and Balsha (D). Bosnek and Kresna Gorge are not presented, due to the insufficient sample size for these populations.

Figure 1.



A
Ivlev's index
1 0.8 0.6 0.4 0.2 0 -0.2 -0.4 -0.6 -0.8 -1
H1 H2 H3 H4 H5 H6 H7 H8 H9 H10 H11 H12 H13 H14 H15 H16 H17 H18 H19 H20 H21 H22 H23 H24
Habitat type
B
Ivlev's index
H2 H3 H7 H10 H12 H13 H14 H17 H18 H22 H23 H24
Habitat type
C
Ivlev's index
H1 H6 H7 H8 H9 H12 H13 H18 H21
Habitat type
D
Ivlev's index
H2 H3 H4 H5 H6 H7 H12 H14 H15 H16 H17 H18 H19 H20 H22
Habitat type
E
Ivlev's index
H1 H6 H7 H8 H10 H11 H12 H13 H14 H15 H18 H21
Habitat type
F
Ivlev's index
H1 H4 H5 H6 H12 H14 H15 H16 H17 H20 H24
Habitat type

Figure 2.

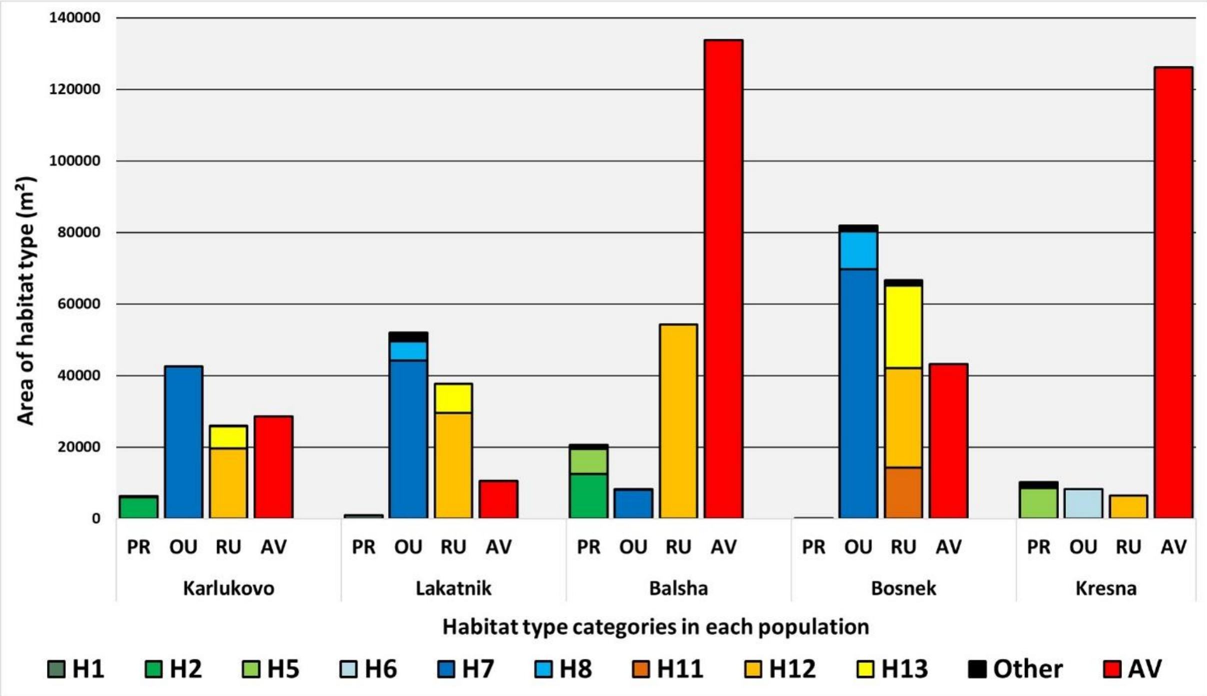


Figure 3.

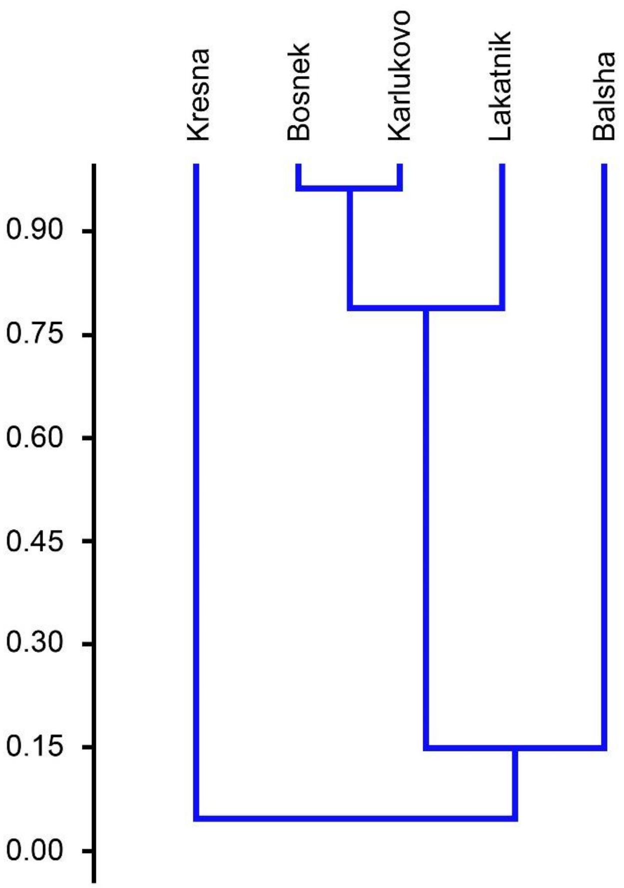


Figure 4.

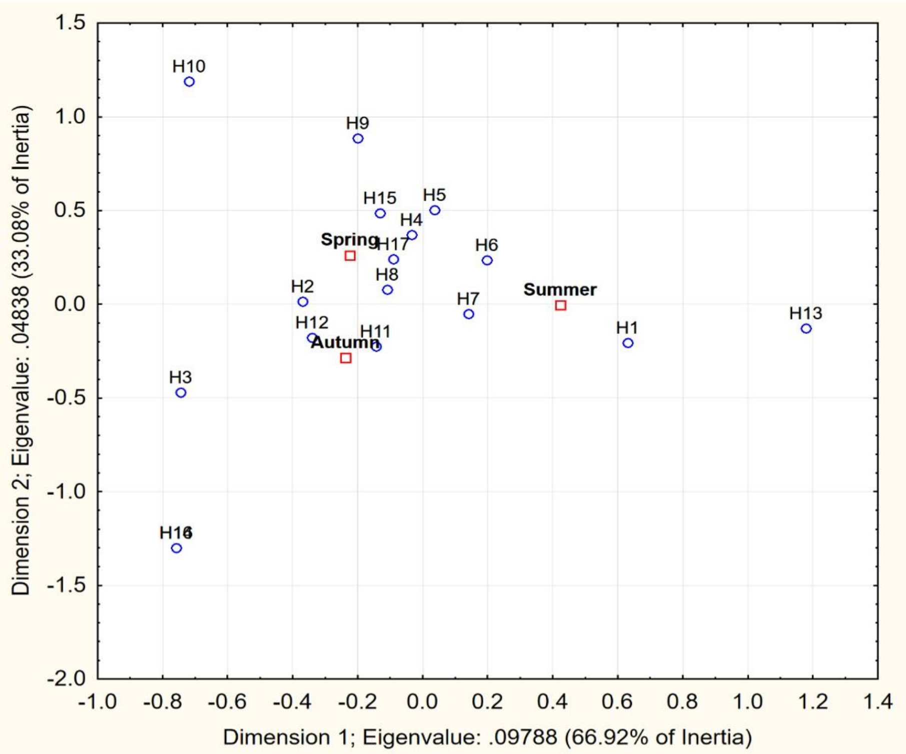


Figure 5.