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Surveying Open Habitats in Natura 2000 Sites in Bulgaria to Assess Food Availability for the Marbled Polecat *Vormela peregusna* (Güldenstädt, 1770) and Steppe Polecat *Mustela eversmannii* (Lesson, 1827) (Carnivora: Mustelidae)

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Abstract: A methodology for monitoring the food resources of two threatened species of European mustelids was developed and successfully tested. It was based on abundance estimates of rodents considered the main prey of the Marbled and the Steppe polecats in Bulgaria: the European ground squirrel, the lesser blind mole-rat, and the voles. Most of the sampling sites were characterized by low abundance of all three investigated prey species. Differences in rodent species' abundance were observed among regions. Among the five examined regions, West Central Bulgaria, which exhibits the highest occurrence of the marbled polecat, is characterized by the most abundant prey, although the optimal prey was not present in the studied sites. Our results suggest that in the western part of its range the distribution of the marbled polecat may not be as reliant on the presence of the European ground squirrel as traditionally assumed. Being simple and unexpensive the proposed transect method for assessment of the polecats' prey abundance is suitable for conservation purposes and national or regional monitoring.

Key words: mustelids, *Spermophilus*, *Nannospalax*, *Microtus*, prey abundance

Introduction

The marbled polecat *Vormela peregusna* (Guldenstaedt, 1770) and the steppe polecat *Mustela eversmanii* (Lesson, 1827) are threatened species of mustelids, included in the Bern Convention (Annex II) and in the Habitats Directive 92/43/EEC (Annexes II and IV). Both species are listed in the Bulgarian Red Data Book as Vulnerable (Spassov & Spiridonov 2015) and protected by Biological Diversity Act of Bulgaria (Annexes II and III). Declines in steppe rodent species due to rodenticide use, habitat loss and degradation are among the main threats to both polecat species in Europe (Spassov 2007a, 2007b, Tikhonov et al. 2007, Šálek et al. 2013, Maran et al. 2016).

Although rare, the marbled polecat is widespread in most of the territory of Bulgaria (with the exception of the upper parts of the mountains and the vast forests), with highest density in its northeast-

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ern and western parts (Spassov et al. 2002, Spassov 2007b, Spassov & Spiridonov 2011, Zidarova 2022). The steppe polecat is found in Northern Bulgaria, considered the most common in the easternmost and central parts of the Danube Plain (Šálek et al. 2013). Both mustelid species are typical for the open habitats. The marbled polecat inhabits steppe habitats, secondary grasslands in plains and valleys, forest edges, rocky areas, vineyards, gardens and sometimes settlements (Spassov & Spiridonov 1993). The steppe polecat is found in pastures, steppes, wastelands, arable lands, meadows, riparian scrubs and groves, abandoned gardens, vineyards, etc. (Spassov 2007a, Tsingarska 2009).

Both species are important secondary consumers in the food chains of the grassland ecosystems. Their optimal prey are rodents, especially large colonial representatives of the order (ground squirrels and hamsters), but also voles, mice, water voles, blind mole-rats, etc. (Vásárhelyi 1926, Atanassov 1966, Abelentsev 1968, Heptner & Naumov 1988, Spassov et al. 2002, Gorsuch & Larivière 2005, Spassov 2007b, Ottlecz & Farago 2008). Sometimes they consume hares, birds, amphibians, reptiles, fish and carrion. In the absence or low abundance of large colonial rodents, *Microtus* species may become the most frequently preyed species by the steppe polecat (Heptner & Naumov 1988, Ottlecz & Farago 2008). It can be assumed that this also applies to the marbled polecat, although comprehensive studies on this aspect are currently lacking. For many predator species, prey is a resource of critical importance, shaping their distribution. The occurrence of both mustelid species is considered associated with that of the steppe rodents (Heptner & Naumov 1988, Spassov et al. 2002) in their European range: *Spermophilus citellus*, *Mesocricetus newtoni* and *Cricetus cricetus*. An investigation on *V. peregusna* in Bulgaria reveals that its distribution is not necessarily related to that of *S. citellus* (Zidarova et al. 2022). However, the spatial overlap between polecats and ground squirrels is mentioned as evident in other studies (Heptner & Naumov 1988, Spassov et al. 2002).

Although decreasing in many areas, *S. citellus* is still widespread in the country (Koshev & Kocheva 2008, Koshev 2022). The two hamster species, however, are rare and display low densities (Peshev et al. 2004, Popov 2015a, 2015b). Actually, the most common rodents in the open habitats in most of the country are *Microtus* sp. and *N. leucodon* (Minkova & Popov 2002, Peshev et al. 2004, Popov 2007). Therefore, we assumed that the most important potential prey of the marbled and the steppe polecats in Bulgaria are the European ground squirrel, the Blind mole rat, and voles. Consequently, our research focuses on their abundance in the polecats' habitats.

According to the Natura 2000 database (https://eunis.eea.europa.eu), the European ground squirrel is present in approximately 68% and the Romanian hamster in approximately 32% of the protected sites in Bulgaria where both or one of the polecat species are present. Prey rodents such as voles and molerats aren't listed in the Habitats Directive (92/43/EEC), respectively their status is not monitored. Thus, this database cannot provide a comprehensive view of the food resources of the polecats within Natura 2000 network.

The marbled polecat is included in the standard forms of 181 and the steppe polecat – of 26 sites of the Natura 2000 network in Bulgaria (https://natura2000.egov.bg). In the studies conducted for the purposes of Article 17 reporting period 2007-2012 the food supply of the marbled polecat was assessed as "Favourable" in approximately 36%, "Unfavourable – Inadequate" – in approximately 63%, and as "Unfavourable - Bad" – in less than 1% of the sites. The approximate share of estimates of the food resources of the steppe polecat was as follows: "Favourable" – 60%, "Unfavourable - Inadequate" – 36%, and "Unfavourable - Bad" – 4% of the sites. Although these evaluations are based on expert opinion and are not supported by quantitative data on polecats' potential prey, they give us a warning. The significant proportion of unfavourable estimates indicates a need for collection of quantitative data and an outline of trends in the state of the food resources of the two polecat species.

Considering the importance of *S. citellus*, *N. leucodon* and *Microtus* sp. in maintaining the populations of the open habitat carnivores, we aimed to estimate the abundance of these rodents as a primary food resource for the two threatened species of mustelids, the marbled polecat and the steppe polecat, in model Natura 2000 sites.

Materials and Methods

The field methods used for estimating the abundance of *N. leucodon* and *S. citellus* were based on the approaches laid down in the methodologies for monitoring of these species developed for the purposes of the National Biodiversity Monitoring System (NBMS) (https://bit.ly/3NcK8sQ, Zidarova & Kostova 2021). The abundance estimates of voles were made by means of modification of a method standing on presence/absence of vole activity signs (Delattre et al. 1996, Quéré et al. 2000, Jareño et al. 2014).

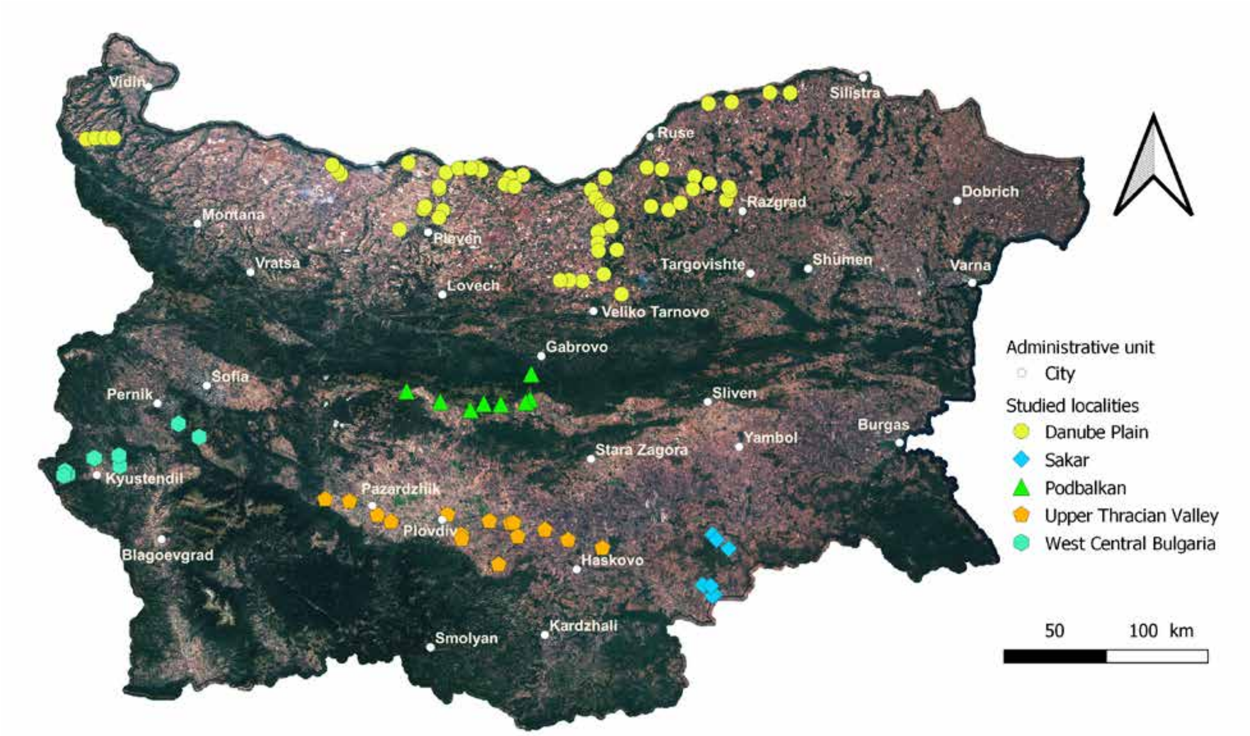

Fig. 1. A map of regions of observation and transects location (based on a satellite map of Bulgaria from Sentinel 2 by Geopolymorphic Cloud, 2017).

Our purpose was to elaborate an easy to use and inexpensive unified method for collecting quantitative data for the three selected rodent species in parallel. We made transects in suitable for the marbled polecat and the steppe polecat habitats (predominantly pastures, meadows, and wastelands) in five regions in Bulgaria: Danube plain, Sakar, Podbalkan, Upper Tracian Valley, and West Central Bulgaria (Fig. 1). They were largely chosen based on the habitat suitability models of the two polecat species developed for the purposes of project "Lot 4 'Mapping and determining conservation status of mammals without bats', activity 4, project DIR – 59318-1-2 'Mapping and identification of conservation status of natural habitats and species - Phase I', MoEW, Bulgaria (2011-2013)" and according to expert opinion. Transects' length depended on the specific local terrain conditions. Each transect was divided into 100 m intervals and data on three parameters were collected for every interval, at 2.5 m on each side of the transect line (an area usually visible even in dense vegetation). The recorded parameters were: (1) Number of active burrows of *S. citellus*; (2) Presence/absence of *N. leucodon* (piles of soil); (3) Presence/absence of *Microtus* sp. (vole activity signs).

According to the classical concepts, the typical mounds of *N. leucodon* are relatively large and arranged in a row. However, these criteria are not always valid, as the features of digged soil depand on a number of factors. Therefore, additional characteristics described by Gusztáv (2010) were examined, i.e. presence of root and stalk remains that are sharply bitten in acute angle. In some more questionable cases, the diameter of the tunnel was also considered (more than 5 cm in *N. leucodon*).

For every rodent species, three categories of abundance representing its potential importance as a food resource for the polecats were assumed as described in Table 1:

Category I: Low abundance: the rodent species is absent or of negligible abundance, i. e. has little or no importance as a food resource for polecats;

Category II: Medium abundance: The species is comparatively frequent, which determines its relative importance as a food resource for polecats;

Category III: High abundance: The species exhibit high abundance, which determines its high importance as a food resource for polecats.

Descriptive statistics were calculated using Microsoft Excel. Confidence intervals for the frequency parameter (proportions) were calculated using the exact interval method (Clopper & Pearson 1934). Confidence intervals for the mean abundance of *S. citellus* in Sakar and Upper Thracian Valley are not shown due to the highly asymmetric distribution of the data (prevalence of zero values), resulting in

Table 1. Ranges of the investigated parameters for each species and category. N – total number of 100 m intervals in a site, n - number of 100 m intervals with species' signs presence in a site. The mean number of holes in a 100 m interval for a given transect and the occurrence parameter - n/N give indirect information on the presence and abundance of species and are a good tool for comparison in space and time.

Abundance / Species	Unit	I /Low/	II /Medium/	III /High/
S. citellus	Mean number of burrows/100 m transect	0-1.99	2-3.99	More than 4
N. leucodon	n/N	0-0.33	0.33-0.66	0.66-1
Microtus sp.	n/N	0-0.33	0.33-0.66	0.66-1

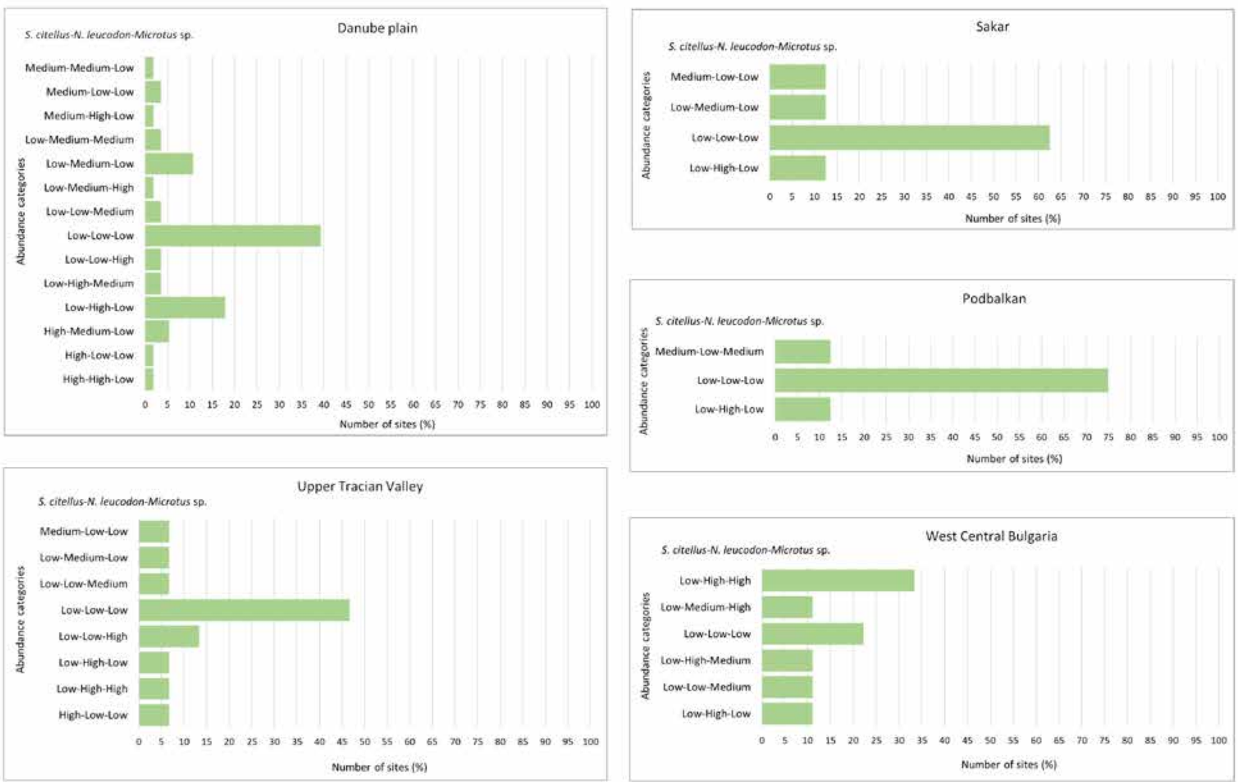

Fig. 2. Shares (in %) of the observed combinations of abundance categories of the three rodent species in all studied regions.

negative lower limits of the intervals and lack of biological meaning. Abundance of *S. citellus*, *N. leucodon*, and *Microtus* sp. was calculated for each site and then assigned to the appropriate category (or class) The number of sites (as a percentage of total number) with each of the abundance class combinations available for the three species in a given region is shown graphically. For each of the species *S. citellus*, *N. leucodon* and *Microtus* sp., the percentage of sites with a particular abundance category in each of the study regions was also calculated. Considering species' habitat preferences, it can be assumed that the observed voles' signs in the majority of the sampling sites refer to *Microtus arvalis* ex. gr. However, due to the difficulty of distinguishing individual species based on activity signs and the possibility of presence of *Microtus subterraneus* in sites with higher humidity, we considered all the voles in our study as *Microtus* sp.

Results

The share of the observed combinations of abundance categories of the three rodent species in all five studied regions is presented in Fig. 2.

The sampling sites with low abundance of all three investigated rodent species have a predominant share in all regions with the exception of West Central

Table 2. Descriptors of the rodent samples from the studied regions.

Region	N sites	Unit	Min	Max	Mean	Std Dev	Std. Error	95%CI
S. citellus								
Danube Plain	56	N burrows / 100 m	0	6.19	0.83	1.50	0.20	0.43-1.23
Sakar	8		0	2.24	0.33	0.78	0.28	-
Podbalkan	8		0	2	0.79	0.75	0.27	0.16-1.42
Upper Tracian Valley	15		0	4.80	0.74	1.45	0.38	-
West Central Bulgaria	9		0	0	0	0	0	0
N. leucodon								
Danube Plain	56	n100 m presence / N100 m total	0	1	0.36	-	-	0.30-0.38
Sakar	8		0	0.71	0.16	-	-	0.06-0.23
Podbalkan	8		0	1	0.13	-	-	0.02-0.18
Upper Tracian Valley	15		0	0.91	0.27	-	-	0.24-0.37
West Central Bulgaria	9		0	1	0.59	-	-	0.42-0.65
Microtus sp.								
Danube Plain	56	n100 m presence / N100 m total	0	1	0.16	-	-	0.12-0.18
Sakar	8		0	0.14	0.03	-	-	0.003-0.10
Podbalkan	8		0	0.57	0.09	-	-	0.03-0.21
Upper Tracian Valley	15		0	0.89	0.25	-	-	0.28-0.42
West Central Bulgaria	9		0	1	0.53	-	-	0.42-0.65

Table 3. Sites with a given abundance category by species.

Region	N sites	Sites with an abundance category (%)		
		Low	Medium	High
S. citellus				
Danube Plain	56	83.93	7.14	8.93
Sakar	8	87.50	12.50	0.00
Podbalkan	8	87.50	12.50	0.00
Upper Tracian Valley	15	86.67	6.67	6.67
West Central Bulgaria	9	100	0	0
N. leucodon				
Danube Plain	56	51.79	23.21	25.00
Sakar	8	75.00	12.50	12.50
Podbalkan	8	87.50	0	12.50
Upper Tracian Valley	15	80.00	6.67	13.33
West Central Bulgaria	9	33.33	11.11	55.56
Microtus sp.				
Danube Plain	56	83.93	10.71	5.36
Sakar	8	100	0	0
Podbalkan	8	87.50	12.50	0.00
Upper Tracian Valley	15	73.33	6.67	20.00
West Central Bulgaria	9	33.33	22.22	44.44

Bulgaria where habitats characterized by high abundance of mole-rats and voles predominate (Table 2, 3, Fig. 2). The share of habitats with high abundance of voles and low abundance of the other two rodent species is relatively large in Upper Thracian Valley while sites characterized by mole-rats' high abundance tend to be more common in Danube plain.

Spermophilus citellus, the optimal prey of the marbled polecat, has high abundance in some open habitats in Upper Tracian Valley and Danube plain (Tables 2, 3). The highest variation in its abundance is in Danube plain. In this species, medium abundance is registered most often in the low mountainous landscapes in Sakar and Podbalkan regions. It is absent in the studied open habitats in West Central Bulgaria.

According to our data, *N. leucodon* has more even distribution on the territory of the country in comparison with the other two species. High abundance was most often recorded in West Central Bulgaria and Danube plain, while low abundance was observed predominantly in Podbalkan (found only in one of the studied localities there but in maximum frequency), Sakar (found only in two of the studied localities there) and Upper Thracian Valley regions.

Microtus sp. is numerous in a significant portion of the sampling sites in West Central Bulgaria and Upper Thracian Valley. Our investigation reveals low densities of this rodent in most of the grasslands in Sakar, but also in the regions of Podbalkan and Danube plain.

Discussion

The proposed methodology is based on the assumption that the European ground squirrel, the lesser blind mole-rat, and the voles are the most significant prey in the optimal habitats of *V. peregusna* and *M. eversmanii* in Bulgaria. At the locality level, it is possible that in some cases other species are more important as food supply for these carnivores. However, we claim that in the majority of habitats occupied by them, the status of the selected rodent species provides a good basis for an assessment of their food resources.

Four out of five investigated areas are characterised by the prevalence of sites with a low abundance of *S. citellus*, *N. leucodon*, and *Microtus* sp. (Fig. 2, Table 3). West Central Bulgaria is the only studied region where the majority of the sampling sites has a high abundance of rodents (molerats and voles). This can be interpreted as a better state of the food base in this region compared to the other four, although the most optimal prey, the European ground squirrel, was not present in the sampling sites. Perhaps the high density of *N. leucodon* and *Microtus* sp. may explain the greater occurrence of the marbled polecat in this area, observed both in the 1990s (Spassov & Spiridonov 1993, Spassov et al. 2002) and nowadays (Zidarova 2022). Dobrudzha, the other area considered densely inhabited by *V. peregusna* (Spassov & Spiridonov 1993, Spassov et al. 2002) was not the subject of our study. West Central Bulgaria but also Upper Tracian Valley are characterized by great number of sites where high abundance of voles was recorded. According to a recent study (Zidarova at al. 2022) the habitat suitability of the marbled polecat is high in both areas (to a greater extent in the West Central Bulgaria) and a comparatively large amount of the species' records refer to these regions. Thus, our results suggest a weaker dependence of the *Vormela*'s distribution on that of its traditionally considered optimal prey, the ground squirrel, which is in accordance with the results of Zidarova at al. (2022) indicating much broader spatial niche of the marbled polecat in comparison with that of *S. citellus*. This probably applies largely to Bulgaria and territories with similar climate and landscape characteristics and to a lesser extent to the poorer ecosystems in large parts of *Vormela*'s Asian range. The alfa diversity of small mammal communities in dry semi-desert and desert habitats for instance is expected to be low and a strong predominance of one or two micromammalian species can be assumed (Kelt et al. 1996, 1999). This can make a small carnivore highly dependent on a particular species of rodent (such as a large colonial rodent) as a food resource. Therefore, the traditional perception that the distribution of the marbled polecat is associated with that of the large colonial rodents (Heptner & Naumov 1988, Spassov et al. 2002) should be reconsidered in the context of the significant differences in habitat characteristics in the different parts of the species' extensive range.

Being listed in Annex II of Directive 92/43/EEC and in the National System for Environmental Monitoring of Bulgaria, the marbled and the steppe polecats are required to be periodically reported at national and regional level. Experience gained in recent years shows that collecting quantitative population data for these species requires undue effort in terms of time and resources. Camera trapping and transect methods, widely used in ecological and conservation studies of mammals and carnivores in particular, often do not give satisfactory results when it comes to rare, cryptic and/or nocturnal species (Steinbeiser et al. 2019). Within a monitoring survey, it is possible to collect data on species' presence at most, but not on other parameters such as population number, sex and age structure. In such cases, it is appropriate to monitor parameters that are easy to measure and at the same time indicative of environmental conditions for the target species. Such a parameter can be the food base. Although exhibiting opportunistic feeding habits, the two polecat species are obligate carnivores. Density or availability of prey is considered the main factor driving densities of obligate carnivores (Mitchell & Hebblewhite 2012). In this sense, *V. peregusna* and *M. eversmanii* are expected to be quite sensitive to the abundance of their optimal prey. Being specialized to hunt on large colonial rodents, these mustelids are expected to be particularly vulnerable in areas with strong anthropogenic impact causing prey depletion and/or high density of larger carnivores that are dominant competitors and/or predators on polecats. Steppe rodents' decline is considered one of the threats to the marbled and the steppe polecats (Schreiber et al. 1989, Šálek et al. 2013). Moreover, these mustelids share habitats with a number of larger carnivores such as jackals, foxes, stone martens, feral dogs, etc. In some cases, their local populations reach high densities, especially in the case of jackals and feral dogs (Markov & Lanszki 2012). This could be a precondition for interspecific competition, direct conflicts and mortality. In this regard maintaining high prey abundance is necessary to minimize competition and antagonistic interactions.

Intensive agriculture, the accompanying use of chemicals, degradation and loss of grassland habitats are considered the primary factors leading to population declines throughout the polecats' ranges (Spassov et al. 2002, Tikhonov et al. 2007, Šálek et al. 2013). Some of the threats have an indirect negative effect on polecats by reducing the abundance of their prey. Many of the significant recent changes in agricultural practices in a number of countries, including Bulgaria, have contributed to the unfavourable trend for the species. Reliable estimation of prey abundance based on cheap and simple methods would be of great benefit for the purposes of conservation and management of carnivore species. Transect method using surface indices is considered a good alternative of the classical "capture" methods (mark–recapture and single capture) for large-scale investigations or monitoring of the rodent species of our interest such as voles, ground squirrels and mole rats (Delattre et al. 1996, Quéré et al. 2000, Jareño et al. 2014, Koshev et al. 2016, Zidarova et al. 2016 a,b, Zidarova & Kostova 2021). The approach applied by us covers all the three species. It is non-invasive, inexpensive and easy-to-use, which makes it applicable to collecting data on threatened species (such as ground squirrels) as well as on hard-to-capture subterranean species (such as the mole-rat) over a relatively wide geographical range and in short time frames. Thus, the proposed methodology is applicable to assessing and monitoring the habitat quality of the protected polecat species *V. peregusna* and *M. evermanii*. It could be improved by recording the voles' presence at 10-meters intervals. This approach was used in studies on voles (Delattre et al. 1996, Quéré et al. 2000) and gave precise data on vole abundance. However, it takes more field efforts, which makes it less appropriate for the purposes of monitoring studies on large geographical scale such as within Natura 2000 network. Moreover, as population density fluctuates between years and seasons, overall population trends are more indicative for our purposes than an accurate estimate of current population status. In this regard, the proposed method is accurate and reliable enough to be used for a number of purposes, including quantifying changes in rodents' abundance over time. We recommend the approach used in our investigation for assessing the current state of the food resources of the polecats and/or tracking their changes over time – a priority task that should be considered when preparing conservation strategies, management or action plans for the protected polecat species, as well as for their monitoring.

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