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Species Diversity of Amphibians and Reptiles in Relation to Habitat Diversity at a Natura 2000 Area in NW Bulgaria

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Abstract: Pastrina Protected Area, a medium-sized Natura 2000 protected area in NW Bulgaria, was chosen as a model site for studying the species diversity of amphibians and reptiles in relation to habitat diversity. The analyses encompass field data for a five-year period. The territory of the survey was divided into a 1 x 1 km grid. The number of species and the number of habitats were recorded for each grid cell. For open and agricultural habitats data were taken from a map with physical blocks. For forested territories, the national forest database was used. The grid cells with missing data were filled in using CORINE Land Cover. A list of threats was prepared in order to estimate their impact. A strong correlation between habitat and species diversity was found: the latter was clearly determined by the presence of certain habitats known to maintain high species diversity. Habitats such as the thermophilous deciduous forests and their surrounding territories were the most important habitats, with higher species diversity and a higher percentage of species of conservation priority, e.g. *Triturus cristatus, Bombina bombina, B. variegata* and *Testudo hermanni*. These species were found from only 11.7% of the territory of the protected area, that makes those species vulnerable, with an uncertain future status.

Key words: amphibians, reptiles, correlation, protected area, threats

Introduction

Reptile and amphibian populations are declining worldwide (Pechman et al. 1991, Wake 1991, Heyer et al. 1994, Gibbons et al. 2000), having more species at risk than either birds or mammals (IUCN 2018, Gardner et al. 2007). Reptiles and amphibians are recognised as a very important element of the food chain and as indicators for the health of the ecosystems. With this regard, it is important to determine habitat types that provide a refuge for reptiles and amphibians by hosting individuals escaping from logging areas or other land-use practices and also to provide recommendations for future monitoring and management practices with implications for the conservation of the species community (Hutchens & DePerno 2009). The species diversity, defined by measuring the species richness and the relative abundance of reptile and amphibian assemblages, was used for calculating community variables for protected habitats and the forest manageable area. The patterns of habitat use and abundance of the reptile community have been used to predict the effects of proposed management schemes (East et al. 1995). Areas protected for their high wildlife value invariably require a certain level of habitat management in order to maintain their value as refugees for certain species (Edgar et al. 2010). Worldwide, habitat loss and forest fragmentation are recognised as key factors for the global extinction of genetically distinct populations and species (Bierregaard et al. 1992, Hughes et

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al. 1997, Brooks et al. 1999, Stuart et al. 2004). Habitat loss and human pressure are among the main threats for amphibians and reptiles worldwide (Leache et al. 2006).

Reptiles have relatively limited dispersal abilities, a fact making them particularly vulnerable to the effects of habitat fragmentation. Generally, they are slow-moving animals and cannot cross large areas of unsuitable terrains to move to another, more suitable but distant habitat. This dispersal also increases the risk of predation. With some variation between species, reptiles prefer mid-successional habitats (Edgar et al. 2010). They need open areas for warmth, as well as more vegetated areas for shelter. Such conditions can be met relatively easily, though some management objectives are in favour of one extreme of the successional gradient (Edgar et al. 2010). Prior to landscape modification by humans, the attractiveness of habitats would change over larger periods of time, and reptile populations themselves could shift and fluctuate considerably both spatially and temporally. Reptiles are, therefore, particularly vulnerable to declining habitat quality and inappropriate habitat management (Edgar et al. 2010). Due to their low vagility, slow speed of movement and often narrow habitat tolerances, amphibians are also very susceptible to the negative effects of anthropogenic activities (Cushman 2006).

Extensive deforestation has resulted in a mosaic distribution of native vegetation set in a matrix of land under different types of human use, such as pasture and cultivated fields (Saunders et al. 1991). Several factors influence the richness, distribution and abundance of species within native vegetation fragments, including incident light, temperature and relative humidity (Murcia 1995, Harper et al. 2005). Deforestation usually modifies environmental factors, as well as interspecific interactions (e.g. predation, parasitism and competition), generating an edge effect at the interface between the forest and the surrounding matrix (Murcia 1995, Harper et al. 2005). Some species indicate a good quality of the interior forest habitat and their disappearance may serve as either an indication of habitat degradation within a fragment, or proof that the fragment is not large enough to exclude edge effects. Different responses to spatial and environmental gradients and different degrees of tolerance to microclimatic changes indicate that each species ensemble requires a different conservation strategy (Urbina-Cardona et al. 2006). Morrison et al. (1992) define habitat as a patch with a set of environmental conditions and resources promoting the occupancy, survival and reproduction by individuals of a given species. Damian et al. (2008) have demonstrated the great importance and ecological role of isolated small patch habitats for reptile conservation in fragmented agricultural landscapes.

The Natura 2000 Protected Area (SPA) Pastrina is one of the least studied areas in Bulgaria and there are only two publications about the amphibians and reptiles there (Slavchev et al. 2014, Tzankov & Slavchev 2016). The main goal of this study was to collect data for the habitat and species diversity and to analyse implement them in a functional, applicable management plan.

Materials and Methods

Pastrina is an isolated hill in NW Bulgaria, in the northern part of the Western Balkan Range (N 43.42 E 23.30). The territory is a part of the Natura 2000 protected network in Bulgaria; it covers 3551.58 ha (Natura 2000 Bulgaria 2007). Data were collected between 2008 and 2012, in periods of animal activity (March – October): 2008 (April, July and August); 2009 (March, April, May, June and July); 2010 (April, May and September); 2011 (March, April, September and October); 2012 (April, July and August). A total of 157 tracks (587km) were performed in the study area. On each survey, we carried out between one and four visits in the territory of Pastrina. Each research group consisted of three to five members who spent up to seven hours in the field.

A variety of methods was chosen in order to increase the efficiency of finding animals. Surveys like turning rocks and logs and excavating burrows were done during daytime, with some exceptions done in the late evening and at night – acoustic searching for frogs and setting funnel traps and dip netting (for newts and amphibian larvae) in the water bodies. For the amphibians and some of the reptiles, the study sites were chosen based on the presence of natural stagnant water ponds. The sizes of the ponds (n=7) were not equal (between 30 and 200 m^2). In two out of seven ponds dip netting were not done, because of their small size. In the other ponds, both methods were used. The funnel traps were with dimension 25/25/50 cm and the net for deep netting was 50 cm in diameter. The funnel traps were set in the ponds in the evening and collected in the morning. In the traps were placed empty plastic bottles as floats, which provided a camera over the surface of the water for breathing. For observation of pond terrapins, binocular Opticron 8x42 was used.

During these periods we marked 841 waypoints for 22 species of amphibians and reptiles using the transect method (Table 1). The geographic coordinates for each location were recorded in situ with a GPS receiver Garmin eTrex Vista (manufacturer specified accuracy ± 5 m). Coordinates were recorded as latitude and longitude in decimal degrees and referred to the WGS84 (World Geodetic System of 1984) datum. The survey territory was divided into a 1x1 km cell grid (MGRS), forming a total of 58 grid cells. The precise geographic locations for each unpublished field observation were associated with a respective habitat on a digital map. The map was a compilation of several digital vector layers: physical blocks (for open and agricultural habitats), forest database (for forest habitats) and CORINE Land Cover 2006 (for supplementing missing data). The land-use types corresponded to the CORINE Land Cover nomenclature. The detailed description of map generation was given in the reports for the reptile and amphibian species in the project "Mapping and identification of conservation status of natural habitats and species – Phase I" (the map was compiled by G. Popgeorgiev; available online at: http://natura2000. moew.government.bg/Home/Documents). Every identified type was later attributed to species or habitat classes in order to evaluate its impact.

In the next stage, all polygons were transferred into 12 new habitat categories (Appendix I). This was necessary because in many cases we found inconsistency between CORINE Land Cover data and the habitats recorded during the field observations. The Shannon Diversity Index (H') was calculated for every grid cell in respect to the species and habitats. The Shannon Index for Habitats was calculated for all grid cell, while for species – only where they were established. The evenness (J') was calculated for habitat communities. Both (H') and (J') were processed with Past 2.17 software (Hammer et al. 2001).

Chao's Jaccard Raw (uncorrected for unseen species) Abundance-based Similarity Index was calculated (Chao et al. 2005) with EstimateS software (Colwell 2006). Employing such a similarity index could ameliorate the failings of species richness and relative abundance (Hutchens & DePerno 2009). This approach has been shown to reduce substantially the negative bias that undermines the usefulness of traditional similarity indexes, especially with incomplete sampling of rich communities (Chao et al. 2005). Unweighted pair-group average (UPGMA) algorithm was used for matrix visualisation.

Results

Totally, 22 species were detected (nine amphibians and 13 reptiles; Table 1). There was no statistically significant correlation between habitat diversity and species diversity when all grid cells were included (Pearson's r=0.32, p>0.05). After removing the two cells with extreme values, the relation became significant (Pearson's r=0.68, p<0.001). The first cell was with the lowest species diversity (H'=0.056) but with a relatively high habitat diversity (H'=1.621). The second one was with the highest species diversity (H'=2.022) and a medium-high habitat diversity (H'=1.153; Table 1). The analysis relating to the links between the diversity of species and habitats was based on 26 UTM grids 1 x 1 km, which was about 60% of the grids in the surveyed area (n = 58). The species richness was unevenly distributed in the study area. This could be explained by increased habitat fragmentation and the resulting mosaic character of the distribution of most species. Significant influence might also have the fact that the level of study was not the same in all UTM grids – some grids with greater species richness were visited more frequently, while others located in the periphery were less frequently examined.

Habitats with higher (H') included P, SH, CF and OD. These with lower (J') included OS, PO and IS (Table 1). Geographically, both species and habitat diversity tend to be higher toward the peripheral part of the studied territory (Figs. 1A, 1B). In contrast, habitat evenness tends to be with heterogeneous distribution (Fig. 1C).

The tree based on Chao's Jaccard Raw similarity index grouped together habitat types with higher values of (H'): CF, P, OD, TC, OS and SH (Fig. 2). The next cluster was formed by PF and UA. Water bodies formed a separated cluster (IS and IW).

Totally, ten types of threats were identified in the Pastrina SPA (Appendix II).

Discussion

Our research confirms the usefulness of the similarity indices together with diversity indices when defining priority areas. The total number of detected species has indicated a faunistically rich region. Habitats with higher Shannon Index include 96% of the species and 92% of the specimens found, which determines their highest conservation priority. In those habitats the threats with stronger impact were established.

The good habitat quality of the forest interior and its disappearance may be an indication of habi-

Table 1. Number of specimens encountered in the habitats and the values of the Shannon index in respect to the species and habitats and Evenness in respect to the habitats. For habitats abbreviation codes see Appendix 1.

	P	SH	CF	OD	OS	TC	IW	UA	PO	IS	AL	RR	Total specimens	Total habitats	Shanon index (H')
Testudo hermanni	7	13	3	1	4				4		1		31	7	1.64
Podarcis tauricus	19	7	2	8	15	1						4	***56***	***7***	1.64
Lacerta viridis	38	35	32	4	130	1		4	20	5			269	9	1.56
Ablepharus kitaibelii	23	14	13	6	1	2							59	6	1.46
Dolichophis caspius	5	2	1		3				1				12	5	1.42
Podarcis muralis		6		3	3					8			20	4	1.30
Vipera ammodytes	3	1	6	1	12								23	5	1.23
Zamenis longissimus	2	1	2										5	3	1.06
Pelophylax ridibundus	16	155			12		2			100			285	5	1.03
Bombina variegata	4	1	3										8	3	0.97
Hyla orientalis		4	1		4								9	3	0.97
Rana dalmatina	16	290	3	1	45		2	4					361	7	0.71
Coronella austriaca			1		1								2	2	0.69
Triturus cristatus		112			213								325	2	0.64
Natrix natrix			2							1			3	2	0.64
Bufotes viridis		32	1	1	3								37	4	0.52
Lissotriton vulgaris	2	102			16								120	3	0.48
Bufo bufo	4	103			4								111	3	0.31
Bombina bombina	2	143	1		3								149	4	0.21
Darevskia praticola				1									1	1	0.00
Emys orbicularis		1											1	1	0.00
Natrix tessellata										1			1	1	0.00
Total specimens	141	1022	71	26	469	4	4	8	25	115	1	4	1890		
Total species	14	19	15	9	17	3	2	2	4	5	1	1			
Shanon index (H')	2.15	2.09	1.88	1.87	1.63	1.04	0.69	0.69	0.60	0.53	0.00	0.00			
Evenness (J')	0.84	0.72	0.71	0.85	0.59	0.95	1.00	1.00	0.55	0.33	0.00	0.00			

tat degradation within a fragment, or a proof that the fragment is not large enough to exclude edge effects. Different responses to spatial and environmental gradients and different degrees of tolerance to microclimatic changes indicate that each assemblage requires a different conservation strategy. Therefore, the specific threats referring to each of the habitats need to be determined.

The top five species with higher conservation status (*Testudo hermanni, Triturus cristatus, Emys orbicularis, Bombina bombina, B. variegata*) are mostly confined to the most important habitats for herpetofauna protection (as defined in this paper), namely pastures (P), scrub and/or herbaceous vegetation associations (SH), *Carpinus* and *Fraxinus* forests (CF), other deciduous forests (OD) and open spaces with little or no vegetation (OS). Inland waters (IS) and inland wetlands (IW) are of importance when amphibians are examined separately.

The main threats and benefits to amphibians and reptiles related to land use have been classified by Edgar et al. (2010). They define grazing to have a positive effect for reptiles by limiting the development of scrub and, thus, preventing a site from becoming too shaded. Reptiles can bask close to cover in areas of low-growing vegetation amongst denser habitat. Uncontrolled grazing, however, may have a negative effect over the populations of amphib-

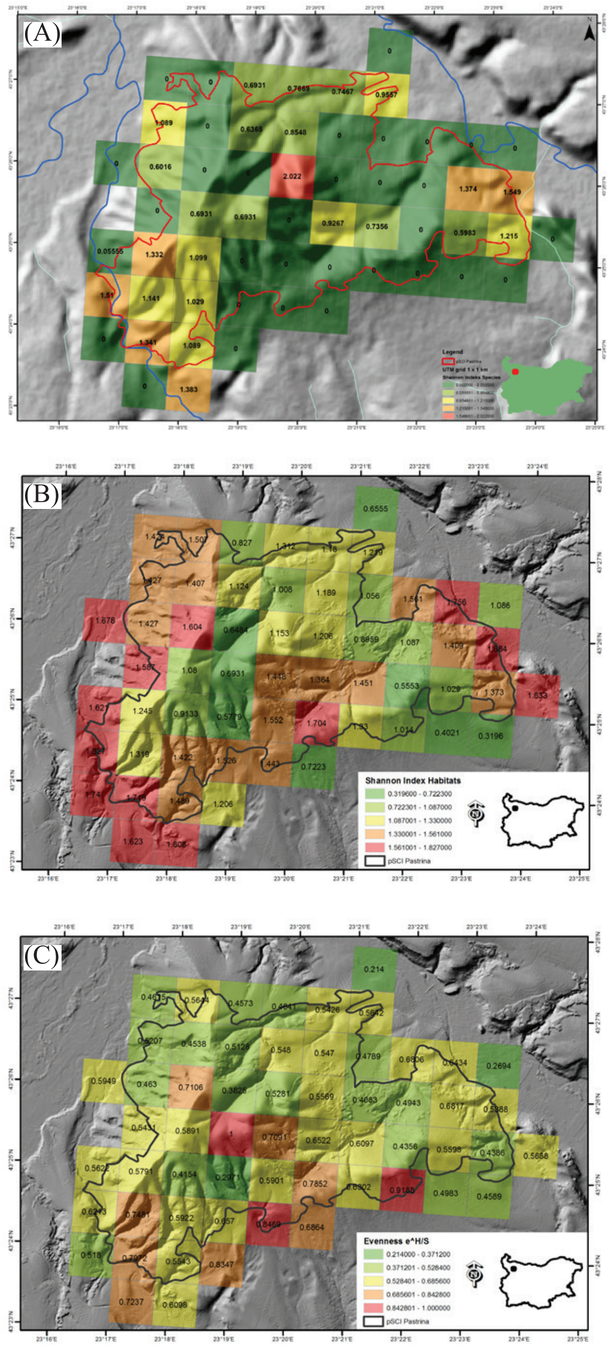

Fig. 1. Shannon index values for species (A), for habitats (B) and Evenness values (C) for habitats in UTM 1 x 1 km grid cells.

ians and reptiles. Overgrazing, in fact, reduces the suitable shelters for these groups. This threat affects mostly pastures and scrub and/or herbaceous vegetation associations. Vast territories on the southern slopes (12 grids) of the Pastrina Hill were covered with sparse vegetation due to overgrazing in the near past; they are highly unsuitable for the studied objects. At least a 20-year period is needed for the vegetation to recover and to reach the complexity preferred by reptiles (Edgar et al. 2010). Uncontrolled

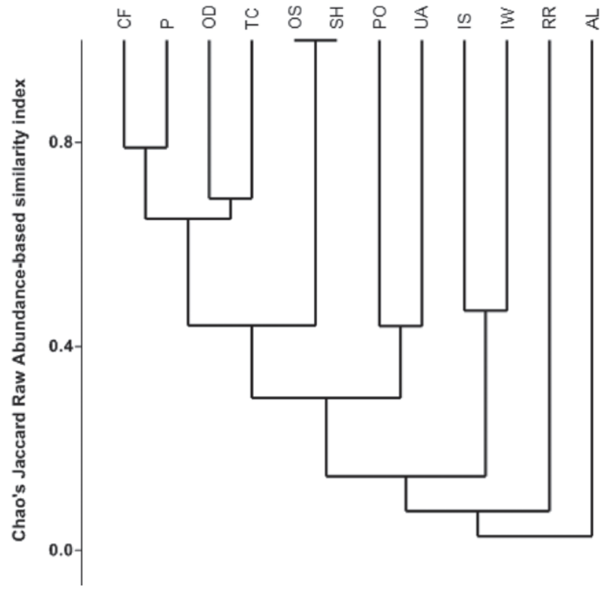

Fig. 2. Chao-Jaccard Similarity between habitats.

burning is another key factor. The territory of the survey suffers from annual uncontrolled burning. A great fire in 2011 affected 69% of the territory of Pastrina SPA, most of it (40 grids) in highly suitable habitats for tortoises (Slavchev et al. 2014).

On many sites scrub and tree management is needed in order to maintain mid-successional stage habitats. The root systems of living and dead scrub and trees provide refuge and hibernation sites (Edgar et al. 2010). Illegal logging and the removal of living and dead scrubs and trees by loggers is a problem which still exists in the area. This is a systematic act which affects the dead trees, as well as the lesser damaged trees. All these factors are responsible for the negative impact on habitats and herpetofauna itself.

According to Gibbs (1998), Ross et al. (2000), Russell et al. (2004) and Patrick et al. (2006), frogs and toads tend to be more tolerant to canopy removal and the subsequent ground temperatures elevation than salamanders. We found newts in two out of the seven explored inland water territories. The newts have not been found in water bodies, probably because they are not suitable for them, owing to habitat fragmentation, predation by fish or a complex of reasons that have not been surveyed yet. Tadpoles of some frog species are better survivors and may develop faster and survive better in ponds within clearcuts. Some amphibian species are likely to be attracted to the higher coverage of herbaceous vegetation around ponds in open environments but response to canopy removal around breeding ponds differs among species (Semlitsch et al. 2009, Felix et al. 2010). Hocking & Semlitsch (2007) have reported that grey tree frogs (*Hyla versicolor*) oviposited more eggs in ponds in clearcuts close to the forest edge than in ponds 50 m into the clearcuts, because adult tree frogs require mature trees for foraging (Johnson et al. 2007, 2008). Microclimatic conditions seem to be important for the post-breeding migration ability of some anuran species. Timber harvest also affects anurans, especially juveniles, by increasing predation or desiccation risks. Species response to the creation of young forests may vary regionally as species choose different microhabitats conditions (Patrick et al. 2006, Rittenhouse & Semlitsch 2009). Most of the amphibian species that had been found were presented in habitats with high degree of naturalness. However, these habitats have been negatively modified by different human activities, such as fires and different forestry activities. Those activities cause future threats like erosion, drying, etc. Timber harvest was postulated to be detrimental to amphibians and to benefit many reptiles (Greenberg 2002, Adams et al. 1996). This statement was related to reptile requirements of warm temperatures associated with higher light levels for egg incubation and successful development of hatchlings (Goin & Goin 1971, Deeming & Ferguson 1991). That is relevant for large forest patches. In small territories with highly fragmented habitats, forests patches are of essential importance for reptiles. Most of the encountered reptile specimens were located in scrub and/or herbaceous vegetation associations and open spaces with little or no vegetation but most commonly in close proximity to forest categories (*Carpinus* and *Fraxinus* and other deciduous forests). The numbers of lizards generally increase following canopy reduction (McLeod & Gates 1998, Greenberg 2002, Renken et al. 2004), a fact that was observed also in the surveyed territory. Some forest-dwelling reptile species may decline following timber harvest (Russell et al. 2004). Species like *Darevskia praticola, Anguis colchica* and *Zamenis longissimus* confined to forest habitats are detected extremely rarely.

Habitat diversity and heterogeneity correspond with species diversity. Although spatial heterogeneity is encouraged by the small-scale activities (circle-shaped clear-cut, thinning or site preparation) as they reduce major land-use effects, edge area and patchiness (Hunter 1990), the amphibian and reptile species response could be significant.

The use of multiple sampling techniques and methodologies is of great importance for deriving accurate inventory statistics and determining species richness. This practice is very important for future monitoring plans and could assist to mitigate the effects to individual species (Gardner et al. 2007a, Hutchens & DePerno 2009, 2009a). In conclusion, the unauthorised and improper use of resources in Natura 2000 site Pastrina can be a threat and could destroy habitats and the species associated with them.

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Appendix I:

Species list in systematic order: *Lissotriton vulgaris* (Lv), *Triturus cristatus* (Tc), *Bombina bombina* (Bbo), *Bombina variegata* (Bva), *Bufo bufo* (Bb), *Bufotes viridis* (Bv), *Hyla orientalis* (Ho), *Rana dalmatina* (Rd), *Pelophylax ridibundus* (Pr), *Emys orbicularis* (Eo), *Testudo hermanni* (Th), *Ablepharus kitaibelii* (Ak), *Darevskia praticola* (Dp), *Lacerta viridis* (Lvi), *Podarcis muralis* (Pm), *Podarcis tauricus* (Pt), *Dolichophis caspius* (Dc), *Zamenis longissimus* (Zl), *Natrix natrix* (Nn), *Natrix tessellata* (Nt), *Coronella austiaca* (Ca), *Vipera ammodytes* (Va)
Habitat list in alphabetic order: Arable land – AL, *Carpinus* and *Fraxinus* forests – CF, Inland waters – IS, Inland wetlands – IW, Open spaces with little or no vegetation – OS, Other deciduous forests – OD, Pastures – P, Predominantly oak forests – PF, Road and rail networks and associated land – RR, Scrub and/or herbaceous vegetation associations – SH, Tree crops – TC, Urban area – UA

Appendix II:

Threats registered at Pastrina Hill according to the list of threats of European Environment Agency (Available from https://www.eea.europa.eu/)

Code	Threats	Activities	N affected habitats	N affected species
A03.01	Intensive mowing or intensification	Assuring an ecotone stripes in the periphery of the arable land patches	7 (AL, OS, OD, P, PF, SH, TC)	21 (Lv, Tc, Bbo, Bva, Bb, Bv, Ho, Rd, Pr, Eo, Th, Ac, Lvi, Pt, Ak, Ca, Dc, Nn, Nt, Zl, Va)
A04.01.01	Intensive cattle grazing	Implementation of agro ecological measures for planned pasturing	3 (OS, P, SH)	8 (Th, Ac, Lv, Pt, Ak, Ca, Dc, Zl)
B01.02	Artificial planting on open ground (non-native trees)	Planning of planting with native trees species, measures to support the natural regeneration of forest habitats	3 (TC, OD, SH)	8 (Th, Ac, Dp, Lvi, Pt, Ak, Dc, Zl)
B02.01.02	Forest replanting (non-native trees)	Same	3 (OD, TC, CF)	11 (Ho, Rd, Th, Ac, Dp, Lvi, Ak, Ca, Nn, Nt, Zl)
B02.02	Forestry clearance	Implementation of forest-environment measures for restoration of the naturalness of forests, their various age composition, undergrowth and species diversity	3 (PF, TC, CF)	11 (Ho, Rd, Th, Ac, Dp, Lvi, Ak, Ca, Nn, Nt, Zl)
B02.03	Removal of forest undergrowth	Ban towards this practice	3 (PF, TC, CF)	11 (Ho, Rd, Th, Ac, Dp, Lvi, Ak, Ca, Nn, Nt, Zl)
H05.01	Garbage and solid waste	Control of the implementation of the already existing regulation	2 (UA, RR)	10 (Bb, Bv, Ho, Pr, Eo, Th, Ac, Lvi, Pm, Dc)
J01	Fire and fire suppression	Preventing fires in the protected Natura 2000 sites by applying penalties for any arson	8 (AL, OS, OD, P, PF, SH, TC, CF)	23 (Lv, Tc, Bbo, Bva, Bb, Bv, Ho, Rd, Pr, Eo, Th, Ac, Dp, Lvi, Pm, Pt, Ak, Ca, Dc, Nn, Nt, Zl, Va)
K01.01	Erosion	Control of overgrazing and deforestation and measures toward replanting with native tree species	7 (AL, OS, OD, P, PF, TC, CF)	15 (Bbo, Bva, Ho, Rd, Eo, Th, Ac, Dp, Lvi, Pm, Pt, Ak, Ca, Dc, Zl)
K01.03	Drying out	Activities for protection and long term sustainable measures for swampy areas, wetlands, small water bodies for watering, water fountain beds and nearby small swampy areas	2 (IS, IW)	10 (Lv, Tc, Bbo, Bva, Bb, Bv, Ho, Rd, Pr, Eo)