J. Entomol. Res. Soc., 20(2): 119-129, 2018 ISSN:1302-0250

Change Phenotypic Traits in Ground Beetles (Carabidae) Reflects Biotope Disturbance in Central Europe

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ABSTRACT

A trend of morphometric characteristic bioindication use and flight ability of the *Carabidae* is their valuation in relation to land use intensity. There are biotopes that are less disturbed, characterized by the presence of individuals that have a higher body average volume value, the so-called ellipsoid biovolume (EV), more than the presence of apterous and brachypterous species. We undertook a bioindication valuation in 7 types of biotopes in the Juhoslovenská kotlina basin and the Veporské vrchy Mts. In 2015-2016, we recorded 3605 individuals (1682 ♂♂, 1923 ♀♀) belonging to 54 species. Biotope heterogeneity and natural conditions are very important for locality selection. We tested 3 hypotheses: 1) the average body size value (EV) of ground beetles decreases with the increasing intensity of biotopes disturbance. 2) a higher number of apterous and brachypterous species appears in stable biotopes and continuous forest cover in the country, 3) the macropterous species predominates in unstable biotopes and isolated forest cover. We confirmed different EV and disturbed intensity by the level of significance pα=0,05. The apterous and brachypterous species dominated in undisturbed and stable biotopes. The macropterous species dominated in disturbed biotopes.

Key words: Bioindication, ellipsoid biovolume, flight ability, Carabidae, Slovakia.

INTRODUCTION

Approximately 80% of the human population of industrialized countries live in the towns. It is important to know what the impact of human activities is on cultural aspects, and to identify factors that influence the species biodiversity for biodiversity conservation in town surroundings (Pickett *et al*., 2001). It is also important to find out and test the indentificators of the biodiversity status, as well as its causes and changes. There is one key identificator for carabid beetles which determines environmental pollution level-the change in body size and vagility (distributing the species in a certain area) (McGeoch, 1998; Gaublomme *et al*., 2008; Eyre *et al*., 2013). Braun *et* *al*., 2004 used the concept of ellipsoid biovolume (EV) for specifying the body size (volume) of ground beetles that is measured by morphometric signs of individuals (length, height, width). The family *Carabidae* is suitable for EV calculation because of their relative body size, which is ellipsoidal. There are important detailed processed ecological characteristics of species that help to determine the environmental quality. The *Carabidae* react sensitively to influences which are caused by anthropogenic activities (Stork, 1990; Lagisz, 2008; Maleque *et al*., 2009; Avgin and Luff, 2010; Rusch *et al*., 2013). Szyszko, 1983 formulated and confirmed the hypothesis "the decrease of environmental disturbance allows for a bigger average body size" while he was studying ground beetles during the restoration of a pine forest. Šustek,1987 pointed to the decreasing body size of the *Carabidae* in areas that are under intensive anthropogenic disturbance. According to Niemelä *et al*., 2002 the urbanisation affects the biodiversity decrease of the *Carabidae* species (also diversity) and in most cases, increases the number of little species towards the city centre. Weller and Ganzhorn, 2006 found that the body size decrease is caused by urbanisation towards the city centre for the species *Carabus nemoralis.* Sukhodolskaya, 2013 also pointed to the same fact for the species *Carabus cancellatus* a *Carabus granulatus*. Magura *et al*., 2006 analyzed the body size changes of the *Carabidae* in the countryside, urban and suburban areas. They recorded the presence of many individuals of various species which are larger in size in the countryside than in urban and suburban areas. Lövei and Magura, 2006 found that the body size of carnivorous *Carabidae* species decreases in the surroundings of industrial zones. The change in body size wasn't recorded in a less polluted environment. Braun *et al*., 2004 pointed out the increase of the average body size due to the reduction in pollution in the countryside which is caused by the decommissioning of industrial areas.

The body size of the *Carabidae* is associated with flight ability- vagility. Majzlan and Frantzová,1995 mentioned that a larger than average body size correlates with the appearance of apterous and brachypterous Carabidae species. A lower than average body size indicates the appearance of macropterous species in a biotope. Porhajášová and Šustek, 2011 confirmed that the species that live in stable ecosystems have lost their flight ability. Flight ability endures within species that come from ecosystems that are exposed to cyclical changes. The predominance of apterous and brachpterous species indicates bigger environmental stability, macropterous species indicate a less stable environmental ecosystem. Shibuya *et al*., 2014 discovered that apterous species dominated in forest ecosystems and macropterous beetle species dominated in grass ecosystems. This result shows that winged form is related to ecosystem stability, mostly structure and vegetation type. Rouabah *et al*., 2015 analyzed the influence of vegetation structure on the species diversity, population density and spatial distribution of ground beetles in correlation to their body size. They confirmed that big-bodied species indicate high population density in forest covers, while small species prefer species-rich meadow vegetation.

Jelaska and Durbes, 2013 compared the flight ability and the body size of the *Carabidae* in an isolated and continuous forest. Isolated and smaller forests were occupied by smaller macropterous species, bigger apterous and brachypterous species dominated in continuous forest.

The aim of paper is to evaluate the flight ability changes and body size changes (EV) of ground beetles in 9 localities that represent 7 types of biotopes with different intensity of anthropogenous activities. We focused on evaluating working hypotheses which we set 1) the average body size value of the *Carabidae* (EV) decreases increases from anthropic low disturbed biotopes to more disturbed ones, 2) there is a higher number of apterous and brachypterous species in stable biotopes and continuous forest covers, 3) macropterous species predominate in unstable biotopes and isolated forest covers compared to continuous forest covers.

MATERIAL AND METHODS

Ground beetle research took place from 2015 to 2016 in 9 localities and 7 types of biotopes (according to Ružičková *et al*., 1996). We collected 3605 individuals (1682 ♂♂ and 1923 ♀♀) belonging to 54 species (see Appendix). We used pitfall traps (750 ml) (Novák *et al*., 1969). Five pitfall traps were arranged in 1 line per each locality and were 10 m away from each other. There were 45 pitfall traps in 9 localities altogether. As a fixation fluid, we used 4% saline. We identified the collected material; the nomenclature and flight ability was edited according to Hůrka (1996).

Study area

The study areas are in the southern part of Slovakia (central) in the surroundings of Lučenec town, Poltár town and the village of Utekáč. Location data and biotope characteristics of the localities are presented in Table 1.

Table 1. Location data of the study localities and their biotope characteristics.

Locality		C. a.	m.a.s.l	Biotope/Management	Geographic coordinates
1	Lichovo	Utekáč	518	Culture of Piceaabies/logging	48°36′27″N 19°48′23″E
2	Lichovo		556	meadow/random mowing	48°36′30″N 19°48′35″E
3	Farkaška		446	nitrophilous waterside vegetation /overgrowth	48°36′34″N 19°47′52″E
4	Kúpna hora	Poltár	300	Carpathian oak-hornbeam forest /logging	48°26′09″N 19°49′27″E
5	Prievranka		272	pasture/grazing	48°25′52″N 19°49′08″E
6	Pažiť		218	nitrophilous waterside vegetation /streamside vegetationtreatment	48°25′41″N 19°46′35″E
7	PriĽadove	Lučenec	258	Carpathian turkey oak forest /logging	48°19′08″N 19°37′48″E
8	Zajačiebrehy		208	fallow field /overgrowth	48°19′17″N 19°39′05″E
9	Ľadovo		207	nitrophilous waterside vegetation /streamside vegetation treatment	48°20′12″N 19°37′06″E

Explanatory notes: C.a.-cadastral area; m.a.s.l.-metres above sea level.

Determination of the Carabidae ellipsoid biovolume (EV)

According to Braun *et al.,* 2004, we measured morphometric signs for each individual by using a digital microscope (0,1mm accuracy): (i) the length-dorsal length between the anterior edge of the labrum and the terminal part of elytra, (ii) the width-dorsal length between the maximum width of elytra, and (iii) the thickness- the maximum dorsoventral thickness of the left side of the ground beetle body. Each parameter was measured 3x, the final value is the arithmetic average (this method was chosen to minimize measuring errors). Then, the EV from our measured morphometric signs was calculated as EV=(π/6)xLxHxW, where: L=individual length, H=individual height, W=individual width.

Data analyses

The Statistica Cz. Ver. 7.0 (StatSoft, Inc., 2004) statistical program and the Canoco ver. 4.5 (Ter Brak and Šmilauer, 2002) for the statistical analysis of morphometric signs, EV and flight ability were used. Data were analyzed both by the methods of descriptive and inductive statistics: distribution normality (the Shapiro-Wilk´s W test), homogeneity of variance (the Levene's test), average compliance (the Kruskal-Wallis test), differences in average value of pairs using the Post hoc test (LSD) and correlation of biotope types to the *Carabidae* flight ability (the Monte Carlo permutation test). The data obtained was processed using the methods mentioned above. This data belongs to the types of data on the ratio scale, namely the discrete data (number of individuals and species) and the ratio data (EV within individuals). According to the level of significance $p_α = 0,05$ (if $p > pα$ we cannot reject H_0 according to the level of significance $p_α=0,05$) we tested H_0 hypotheses (i) a random selection comes from a set of normal distribution (ii) there is no difference in the average value of EV for both sexes, (iii) the types of biotopes do not influence on flight ability of the Carabidae.

RESULTS

During both years of the study the average values in morphometric signs for both sexes were decreased in forest localities (1, 4, 7), nitrophilous waterside vegetation localities (3, 6, 9) and biotope fallow field locality (8). On the contrary the average values in morphometric signs for both sexes were increased in meadow (2) and pasture (5) biotopes. The average values were close to the median values, meaningthat there was not a big difference in measured data. Variance and standard deviation didn't change significantly.

The summary EV of all trapped specimens was 2156267 mm^3. The highest EV was confirmed in localities 1=619909 mm^3, 4=491240 mm^3 and 7=355582 mm^3. The lowest EV was in localities 5=52430 mm^3, 6=64690 mm^3, and 9 = 91467 mm^3. We confirmed EV 124829 mm^3 in locality 2, then EV 144348 mm^3 in locality 3, EV 211768 mm^3 in locality 8. The EV average value was decreased in both sexes in forest localities (1, 4, 7) and in nitrophilous waterside vegetation localities (6, 9), but increased in both sexes in the biotope of meadow (2), pasture (5) fallow field (8), and nitrophilous waterside vegetation (3). The average values were close to the median values, meaning that there is no big difference between measured data. Variance and standard deviation didn't change significantly.

According to Shapiro-Wilk´s W-test the disruption of distribution normality data EV in each locality for both Carabidae sexes (p-value=0,00) was established. According to the Levene's test the EV homogeneity of variance for both sexes in each locality according to the level of significance (pα=0,05, homogeneity is if value p<0,05) was evaluated. During both years of the study the final EV for males was p-value=0,00 and the homogeneity of variance was confirmed. Females had p-value=0,19 for year 2015, and p-value=0,26 for year 2016, and the heterogeneity of variance was confirmed (caused by eggs inside the females). A non-parametric Kruskal-Wallis test (ANOVA) for testing H_0 hypothesis was used because of distribution normality data disruption. The EV value result of the average compliance is H_0 rejection (p-value=0,00) for both sexes in each locality. This means that the EV average values are statistically different (Fig. 1). The EV average decrease in the forests (1, 4, 7) and river bank vegetation localities (6, 9) was confirmed. The EV average increase in the biotopes in open areas (2, 5, 8) and river bank vegetation (3) was confirmed.

In rejecting the H_0 hypothesis of the Kruskal-Wallis test (ANOVA), we decided to choose the Post hoc (LSD) test (ANOVA). Using this tool, we identified which EV average values of pairs are different for both sexes in each locality by the level of statistical significance pα=0,05. Significant differences of the EV average value for both sexes during the years 2015 and 2016 showed a statistical decrease (Table 2).

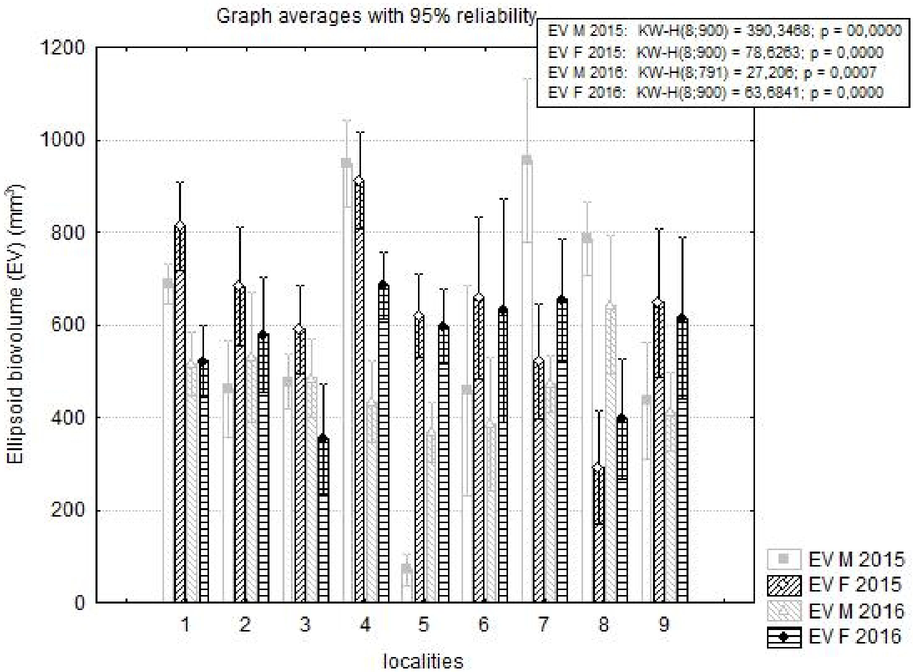

Fig. 1. Variance analysis (Kruskal-Wallis test (ANOVA)) of Carabidae EV average values in each locality by reliability 95 %. Explanatory notes: M-males, F-females.

A larger percentage in the presence of apterous and brachypterous species in forest biotopes (1, 4, 7) was found dividing the species and their flight ability. Macropterous species had the highest percentage of representation in the open areas biotopes (2, 5, 8) (Table 3). There was the predominace of apterous species in nitrophilous waterside vegetation (3), which is caused by a connection to forest vegetation and its enrichments of apterous ones. On the other hand, nitrophilous waterside vegetation (loc. 6 and 9) consisted mostly of macropterous species. These were adjacent to fields and pastures that influenced the flight of smaller macropterous species. The predominance of apterous species indicated its higher stability in the biotope (1, 3, 4, 7), macropterous species confirmed slower environmental stability (2, 5, 8, 6, 9), in this case it was caused by the effects of intensive agriculture.

Table 2. Differences of ellipsoid biovolume average values for males and females in localities.

Year	Sex	Statistically Significant Differences Between Localities
2015	♂	loc.1 with loc.2, 3, 4, 5, 6, 7 and 9 (p-value = 0,00); loc. 2 with loc. 4, 5, 7 and 8 (p-value = 0,00); loc.3 with loc.4, 5, 7 and 8 (p-value = 0,00); loc.4 with loc.5, 6, 9 (p-value = 0,00) and 8 (p-value = 0,02); loc.5 with loc.6, 7, 8 and 9 (p-value = 0,00); loc.6 with 7 and 8 (p-value = 0,00); loc.7 with loc.8 (p-value = 0,02) and 9 (p-value = 0,00); loc.8 with loc.9 (p-value = 0,00)
2016	♂	loc.1 with loc.5 (p-value = 0,00); loc.2 with loc.5 (p-value = 0,02); loc.3 with loc.8 (p-value = 0,04); loc.4 with loc.8 (p-value = 0,00); loc.5 with loc. 8 (p-value = 0,00); loc.6 with loc.8 (p-value = 0,01); loc.7 with loc. 8 (p-value = 0,03); loc.8 with loc.9 (p-value = 0,00)
2015	♀	loc.1 with loc. 3, 5, 7 and 8 (p-value = 0,00); loc.2 with loc.4 (p-value = 0,01) and 8 (p-value = 0,00); loc.3 with loc.4 and 8 (p-value = 0,00); loc.4 with loc.5, 7, 8, 9 (p-value = 0,00) and 6 (p-value = 0,02); loc.5 with loc. 8 (p-value = 0,00); loc.6 with loc.8 (p-value = 0,00); loc.7 with loc.8 (p-value = 0,01); loc.8 with loc.9 (p-value = 0,00)
2016	♀	loc.1 with loc.3 (p-value = 0,01) 4 (p-value = 0,00) and 7 (p-value = 0,04); loc.2 with loc.3 (p-value = 0,01); loc.3 with loc.4, 5, 7, 9 (p-value = 0,00) and 6 (p-value = 0,01); loc.4 with loc.8 (p-value = 0,00); loc.5 with loc.8 (p-value = 0,01); loc.6 with loc.8 (p-value = 0,04); loc.7 with loc.8 (p-value = 0,00); loc.8 with loc.9 (p-value = 0,03)

Table 3. The percentage representation of apterous (A), brachypterous (B) and macropterous (M) Carabidae species in study localities.

Locality	Year 2015			Rok 2016			Standard Errors
	A %	B %	M %	A %	B %	M %	
1	64	29	7	56	38	6	1,48
2	33	20	47	26	26	48	0,84
3	47	29	24	42	29	29	0,80
4	45	36	18	36	43	21	0,60
5	25	8	67	17	11	72	1,88
6	17	25	58	8	31	62	1,14
7	50	31	19	31	31	38	0,67
8	42	0	58	24	12	64	1,58
9	15	31	54	36	29	36	0,67

The difference of the *Carabidae* representation between localities was evaluated based on the flight ability and EV using the redundancy analysis (RDA) (Fig. 2). All species dataset with EV values and a matrix of flight ability of species were used (1A-apterous species per year 2015, 2A-apterous species per year 2016, 1B-brachypterous species per year 2015, 2B -brachypterousspecies per year 2016, 1M -macropterous species per year 2015, 2M-macropterous species per year 2016). As a first step, the length of the gradient using the 1st ordination axis (lengths of gradient=SD) of a detrended correspondence analysis (DCA) was analysed. Using the linear redundancy analysis (RDA) the highest value in the 1st ordination axis was SD=2,733. The RDA explains 36,7% of data species variability, then there is 43,6% of data species variability which is explained by the environmental variables in the 1st ordination axis. Using the Monte Carlo permutation test (Canoco) H_0 hypothesis (p-value=0,00) for macropterous species (per year 2015) was rejected. Other flight ability forms of the species accorded with the H_0 hypothesis. The values were: apterous species in 2015 (p-value=0,51), in 2016 (p-value=0,50); brachypterous in 2015 (p-value = 0,56), in 2016 (p-value=0,06); macropterous species in 2016 (p-value=0,43). The maximum inflation factor value in 2016 for “macropterous species was 14,63 and they correlated to the variable” brachypterous species in 2015.

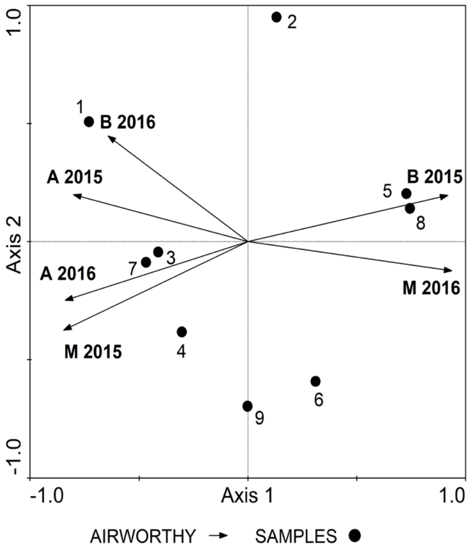

Fig. 2. Flight ability of Carabiade in localities during years 2015 and 2016. Explanatory notes: A-apterous, B-brachpterous, M-macropterous.

The negative correlation of macropterous species was confirmed in forest localities 4 and 7 for the first year, which indicated the presence of apteorus species. Then the negative correlation of apterous species (due to intensive logging) was confirmed for the second year, causing a decrease of these species. The forest locality 1 confirmed the positive correlation of apterous species in the first year, but the logging caused a closer correlation to smaller brachypterous species in the second year. Moreover, the EV decrease which was caused by logging in forest localities was confirmed. Locality 1 presented continuous forest vegetation, locality 4 and 7 were isolated localities surrounded by fields and pasture. This fact also influenced the representation of the species on the basis of flight ability. If we compare it to locality 1: a higher percentage representation of macropterous species and a lower representation of apterous ones in locality 4 and 7, was influenced by flying macropterous species from surrounding fields and pasture (caused by discontinuous forest vegetation cover). Nitrophilous waterside vegetation (6, 9) and pasture (2) are placed further from vectors in an ordination graph. The biotope of nitrophilous waterside vegetation (3) is close to axis 2 between the positive correlation of apterous species in 2015 and the negative correlation of apterous species in 2016. This fact shows a slight flight ability fluctuation of the species in localities 3, 6, 2, moreover the EV fluctuation was slight in these localities. In 2015, pasture (5) and fallow field (8) correlated to the brachypterous species. The predominance of macropterous species is not outlined by a vector because of negative correlation to forest biotopes. There is an increase of brachypterous and macropterous species in the second year, indicating the correlation of these vectors. We assume that we confirmed the EV increase in these localities which was caused by the influence of succession and decrease of disturbed pressure.

DISCUSSION

Anthropogenic (logging, maintenance of coastal vegetation) and natural (succession) influence on the Carabidae was evaluated. The EV and flight ability reached a more complete bioindication evaluation of localities. The volume of the Carabidae (EV) was counted according to Braun *et al.,* 2004. Species were divided up by flight ability according to Hůrka,1996; Majzlan and Frantzová,1995; Porhajášová and Šustek, 2011. The decrease to the average value, morphometric signs and EV in 2015, 2016 were confirmed using the descriptive statistics. This decrease was recorded in localities 1 (Culture of Picea abies), 4 (Carpathian oak-hornbeam forest), 7 (Carpathian turkey oak forest) and the stream side vegetation treatment of watercourse in the localities 6 and 9 (nitrophilous waterside vegetation). During 2015 and 2016, the increase of the average value of morphometric signs and EV was identified in localities 2 (meadow) and 5 (pasture). We took in to consideration the fact that it indicated the decrease of disturbed pressure and continuous ecological succession in localities. In 2015 and 2016, there was a decrease in the morphometric signs average, but an EV increase was found in localities 3 (nitrophilous waterside vegetation) and 8 (fallow field). The result shows that the decrease of anthropogenic pressure in localities can be considered, but not at a level such as in localities 2, 5. There were no big significant differences between the median and average, indicating the similarity between measured data values. The variance and standard deviation didn’t change a lot; it was taken into consideration that the variance didn’t change in the matrix. It was confirmed using the Kruskal-Wallis test (ANOVA) and descriptive statistics. It confirmed the different EV average values in each locality. Šustek,1987 also found a decrease in Carabidae body size in areas that are under going intensive anthropogenic disturbance. Lövei and Magura, 2006 referred to the decreasing trend in the body size of carnivorous species in the surroundings of industrial zones. Braun *et al.*, 2004 confirmed that the average body size increased with decreasing pollution in surrounding industrial areas. Szyszko, 1983 pointed to the increase in the average body size while a pine forest was recovering. The Post hoc (LSD) test (ANOVA) confirmed the decrease of statistical significant differences of the EV average values for both sexes in each locality during 2015 and 2016. The EV average value decrease in the direction of the village of Utekáč to Poltár town and Lučenec town was corroborated; they are open area localities: 2, 5, 8. The decrease was not detected for forest localities (1, 4, 7) and localities of streamside vegetation treatment of water course (3, 6, 9). Magura *et al.*, 2006 confirmed the decrease difference of the Carabidae body size from villages to urban and suburban areas. Weller and Ganzhorn, 2006 discovered the same trend for the species Carabus nemoralis and Sukhodolskaya (2013) for the species Carabus cancellatus and Carabus granulatus. The same results were recorded by Niemelä *et al.*, 2002 who confirmed this based on their study. The predominance of apterous species from a percentage representation on the basis of flight ability in forest biotopes was confirmed (1, 4, 7), which indicates a more ecological stable ecosystem. The macropterous species predominated in open area biotopes (2, 5, 8) which indicates the lowest environmental stability caused by the influence of developed agriculture. Shibuya *et al.*, 2014 provided the same result: the presence of flight ability species indicates environmental stability, in the analysis of forest, grasslands and herbaceous ecosystems. Rouabah *et al.*, 2015 found that the apterous species predominates in forest covers, and the predominance of macropterous speciesare are in meadow. Porhajášová and Šustek, 2011 noted that apterous and brachypterous species predominate in the floodplain forest; they indicated greater environmental stability. During both years, the decrease of the apterous species in forest localities, and the decrease of EV average value was confirmed using the RDA analysis. Forest localities 4 and 7 had a higher percentage representation of macropterous and a lower representation of apterous species compared it to locality 1. This fact is caused by the macropterous species flying from the surroundings fields and pasture around localities 4 and 7. Locality 1 has continuous vegetation cover which is a reason why there was a higher representation of apterous species. Jelaska and Durbes, 2013 affirmed the same fact. Nitrophilous waterside vegetation (3, 6, 9) and pasture (2) only fluctuated slightly on an EV average value, as did species flight ability. During the years studied, in the biotopes of pasture (5) and fallow field (8), the number of brachypterous and macropterous species increased in EV average value. The H0 hypothesis was rejected: types of biotopes do not influence flight ability of the Carabidae using the Monte Carlo permutation test (Canoco) for macropterous species from year 2015. The H0 hypothesis wasn't accepted for other flight ability forms of species. A high inflation factor value (14,63) was confirmed for macropterous species from year 2016 that correlated to brachypterous species from year 2015 (5, 8). This indicates a change to the intensity of anthropogenic disturbance influence. Several authors: Stork, 1990; McGeoch, 1998; Gaublomme *et al.*, 2008; Lagisz, 2008; Maleque *et al.*, 2009; Avgin and Luff, 2010; Eyre *et al*., 2013; Rusch *et al.*, 2013, pointed out that the family Carabidae is a suitable tool for indicating the biotopes distortion level and their ecological stability. The valuation of ecological stability is considered to be the basis for the evaluation of the current status, such as future alternatives of land use. It is a very important aspect of landscape planning documents in Slovakia (e.g. development of environmental systems). Bioindication evaluation using EV and flight ability from the family Carabidae can be used as one of the assessment approaches to the environmental quality and the intensity of anthropogenic impact on the landscape. We have statistically confirmed that the EV average value has decreased and there has been an intensive increase of anthropogenous influence (logging, treatment stream side vegetation, agriculture) for apterous species. The EV average value increase and the number of brachypterous species is caused by the decrease of anthropogenous activities. Isolated forest localities 4 and 7 had a higher number of macropterous species and a lower number of apterous species, if we compare them to forest locality 1, which has continuous vegetation cover. By a combination of 2 valuation methods, which were evaluated separately, we reached a more complete way of valuating anthropogenic interference intensity to the country.

ACKNOWLEDGEMENTS

This work was supported by grant KEGA 025UKF-4/2016 Animals in anthropogenic environment-academic text, e-learning.

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Received: June 07, 2017 *Accepted: April 05, 2018*