

**Acta Scientifica Naturalis**

Former Annual of Konstantin Preslavsky University – Chemistry, Physics, Biology, Geography

Journal homepage: <http://www.spisanie.shu.bg>

Received: 30.11.2017

Accepted: 11.01.2018

**Seasonal activity of *Leptoiulus trilineatus* (C.L. Koch, 1847) and *Megaphyllum trassylvanicum* (Verhoeff, 1897) (Diplopoda: Julida: Julidae)****Darina Bachvarova, Aleksandar Doichinov, Rayme Abdulova**

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**Abstract:** The article presents the results of a study of the soil surface seasonal activity of two species of Julidae, widely spread in the Balkan Peninsula: *Leptoiulus trilineatus* (C.L. Koch, 1847) and *Megaphyllum trassylvanicum* (Verhoeff, 1897). The material was collected by means of pitfall traps between May 2007 and May 2009 in natural and urban habitats exposed to varying degrees of anthropogenic pressure. In the study period 1474 specimens of *L. trilineatus* and 618 specimens of *M. trassylvanicum* were collected. The impact of the soil and air temperature and humidity on the seasonal activity of both species was measured through statistical analysis. The statistical data processing was conducted using SPSS 9.0 and StatPlus 3.5.3 software packages.

*L. trilineatus* and *M. trassylvanicum* are polytopic, mesophilic and mesothermic species with year-round activity in the studied area. There is no statistically significant correlation between the degree of anthropogenic impact and the activity of the two species.

*Leptoiulus trilineatus* shows equal preference for both urban and natural habitats in the studied area. The species demonstrates the typical of all millipedes bimodal activity, which is the highest in spring and the beginning of winter – in the periods from March to May and from November to December. The coefficients of correlation dependence of *L. trilineatus* activity on the tested abiotic environmental factors are not statistically significant. The Pearson-Brave coefficient which measures the effect of soil humidity on species activity is 0.417, which shows a positive correlation. *M. trassylvanicum* has the highest frequency in urban biotops such as parks in the urban and suburban areas of Shumen and in the coniferous habitats on the Shumen Plateau. In this area the species demonstrates its highest activity in spring and summer (from February to July). The abiotic factors with statistically significant effect on the soil surface activity of *M. trassylvanicum* are the soil and air temperature – the values of the Pearson-Brave correlation coefficients are 0.708 and 0.586 respectively.

**Keywords:** Diplopoda, *Leptoiulus trilineatus*, *Megaphyllum trassylvanicum*, seasonal activity, analysis of variance.

## Introduction

The growth, successful development and reproduction of soil-living invertebrates are determined to a great extent by a complex of physical and chemical characteristics of the environment. The seasonal dynamics of the activity of these species depends first of all on their biological and ontogenetic features, their breeding cycles, as well as the trophic resources and the soil and climatic conditions of the environment.

The myriapods often live near moist environments, together with other forest invertebrates [1]. They inhabit the forest floor – the leaf litter, the litter/soil interface, the uppermost soil or deadwood, where humidity is sufficiently high to satisfy their ecological requirements [2]. The environmental factors which have the most significant effect on the distribution and activity of millipedes are temperature and humidity [3, 4, 5, 6]. There are two distinctive peaks in the millipedes' seasonal activity: a relatively high one in the spring and a lower one in autumn, while their activity is reduced in summer months most probably due to low humidity, and in winter due to low temperatures.

In studies conducted in oak forests in southern Belgium, Branquart et al. [7] recorded the largest catches of millipedes in April-May, which completely coincided with their breeding season [5, 8, 9]. During this period adult specimens become active in search of a partner or suitable hatching sites [10]. Despite this regularity, some specific differences in species activity have been observed: due to their phenology, the Chordeumatida species have the longest active period in the year, even in the winter months, while the active period of the crampetosomatids is strictly limited to autumn and winter. Julidae demonstrate bimodal activity with two peaks – in spring and autumn; polydesmids are active mainly in spring and summer, and less frequently in autumn, and glomerids are mostly active in spring [6, 7]. According to Haacker [11], the activity of millipedes, especially glomerids, is extremely restricted between July and mid-October when the surface soil layer becomes depleted of leaf material. During this period, glomerids and julidae can be found coiled in the mineral soil layers. Mediterranean millipedes are active only in wet periods - spring and winter, while during dry periods (from May to October) they go deep into the soil [12, 13] to avoid the adverse effects of high temperatures and low humidity on the surface of the substrate.

*Leptoiulus trilineatus* (CL Koch, 1847) is a Eastern European species (EEU) found in Albania, Austria, Bosnia and Herzegovina, Bulgaria, Croatia, European part of Turkey, Switzerland, Serbia, Kosovo, Vojvodina and Montenegro [14]. In Bulgaria, it is found in the Balkan Mountains, Rhodope Mountains, Vitosha, Rila, Sredna Gora, the Northern lower part of the Balkans, Strandzha-Sakar, Lozen Mountain, Lyulin, Dervent Heights, the Danube Plain, Kamchia River and Struma River, Kresna Gorge, Black Sea Coast [15], Shumen and the Shumen Plateau [16] and the Madara Plateau [17]. *L. trilineatus* is a polytopic, mesophilic and mesothermic species reported in Bulgaria from altitudes up to 2200-2300 m from caves, open habitats (meadows), forest habitats – mainly deciduous forests consisting of *Quercus dalechampii*; *Fagus sylvatica*; *Q. petraea* and *Q. cerris*; *F. sylvatica* and *Q. petraea*; *Q. petraea* and *Carpinus orientalis*; *Q. frainetto*; *Quercus* sp.; *C. betulus* and mixed forests of *F. sylvatica* and *Pinus nigra* [15] as well as urban parks [18].

*Megaphyllum trassylvanicum* (Verhoeff, 1897) is also a Eastern European species (EEU) found in Bosnia and Herzegovina, Bulgaria, Croatia, European part of Turkey, Greece, Hungary, Macedonia, Romania, Ukraine, Serbia, Kosovo, Vojvodina and Montenegro [14]. In Bulgaria the species is found up to 1300 m above sea level and is registered in Stara Planina, Rhodope Mountains, the Northern lower part of the Balkans, Lozen mountain, Strandzha, Bakadzhik Heights, the Danube Plain, Black Sea coast [15], Shumen and the Shumen Plateau [16] and the Madara Plateau [17]. Similar to *L. trilineatus*, *M. trassylvanicum* is a polytopic, mesothermic and mesophilic species found in Bulgaria in urbanized habitats (urban parks), in caves, open habitats (pastures), deciduous, coniferous and mixed forests of *Quercus* sp.; *Carpinus betulus*; *Pinus* sp., *Fagus sylvatica* *L. ssp. moesiaca* [15, 18].

## Material and Methods

### Study area

The material was collected from 8 collecting sites in the area of Shumen and the Shumen Plateau, North-East Bulgaria, distinguished by their location, composition of plant formations and level of anthropogenic pressure (Fig. 1). Two of the sites: the **City park of Shumen (UI)** with geographic coordinates: N 43°16'260", E 26°56'403" and the **Park of Shumen University (UII)** (N 43°16'750", E 26°56'870") are located in urbanized areas, while the third collecting site, **Park Kioshkovete (SU)** is located in the suburban area of Shumen (N 43°15'927"; E 26°54'028"). The locations are semi-natural biotopes of the PmxFor type (Mixed plantations of park type). The plant formations in these biotops include: *Aesculus hippocastanum* Linnaeus, 1753, *Tilia tomentosa* Moench, *Platanus orientalis* L., 1753, *Acer campestre* L., *Juglans regia* L., (1753), *Cercis siliquastrum* L., *Robinia pseudoacacia* L., *Pinus nigra* J.F.Arnold 1785, etc. The other five collecting sites are natural habitats located in the Shumen Plateau Natural Park: **Bukaka reserve (RI)**, **beech forest** dominated by *Fagus sylvatica* L. ssp. *moesiaca* (K. Malý) Hjelmquist with some presence of *Carpinus betulus* L., 1753, *Quercus dalechampii* Ten., *Quercus frainetto* Ten. (1813), *Quercus cerris* L., 1753, and *T. tomentosa* (N 43°15'592", E 26°53'310"); **mixed deciduous-coniferous forest (RII)**, consisting of *Fagus sylvatica* L., 1753, *C. betulus*, *P. nigra*, and *Pinus sylvestris* (L., 1758) ( N 43°16'502", E 26°53'376"); **Hornbeam forest (RIII)**, dominated by *C. betulus* and *Quercus petraea* (Matt.) Liebl., 1784, with some presence of *F. sylvatica*, *C. orientalis* Mill, *Q. frainetto*, *Q. cerris* and *Populus tremula* L., 1753 (N 43°14'605", E 26°54'618"); **Pine forest (RIV)**, dominated by *P. nigra*. (N 43°16'791", E 26°53'562") and **Open meadow (RV)**, covered with *Chrysopogon gryllus* (L.) Trin., *Festuca valesiaca* Schleich, *Dichanthium ischaemum* (L.) Roberty and other xerophilic grass species (N 43°15'504", E 26°55'599").

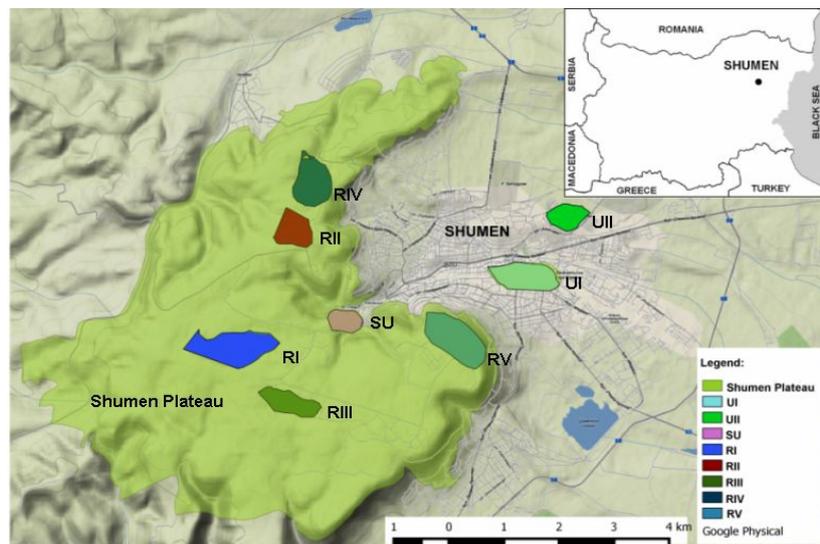


Fig. 1. Map of the study area showing the location of the collecting sites

The patterns of seasonal activity of *L. trilineatus* and *M. transsylvanicum* were identified by analysing material collected from pitfall traps [5, 19]. They traps contained only active specimens [5, 20]. Therefore, the number of captured individuals can be interpreted not only as an indicator of their density of distribution, but also as an indicator of species activity [7]. In each of the collecting sites, 10 pitfall traps were placed at a 10-metre distance in a straight line, and the results were reported monthly in the period from May 2007 to May 2009.

During the study period the average air temperature in the Shumen plateau was 8-10°C, while the temperature in the urban zone was 11-12°C. Temperatures were registered at the end of December 2007 and in the first half of January 2008 which was in contrast to the winter of 2008-2009 when there were no registered temperatures below 0°C (Fig. 2).

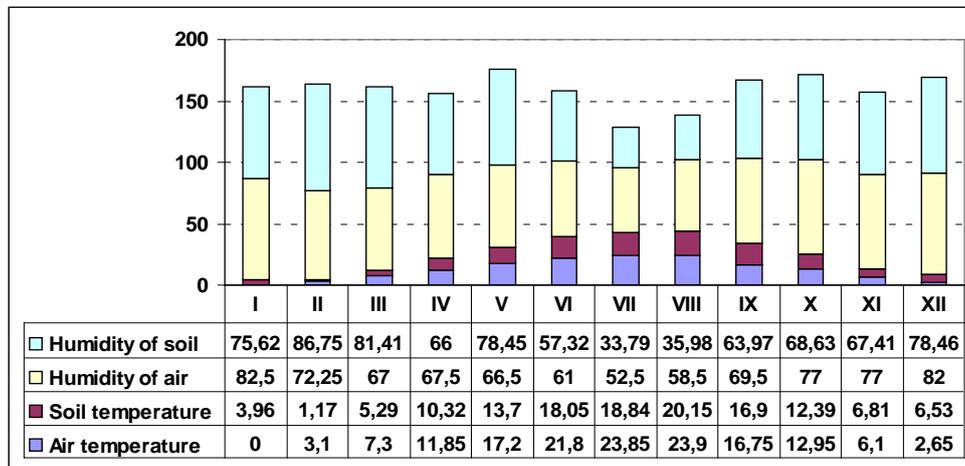


Fig. 2. Average monthly values of air and soil humidity (%) and air and soil temperature (°C) (based on data from the Meteorological station, Shumen)

For the main part of the study precipitation in the region was scarce – to 50l/m<sup>2</sup>, except in fall and winter (from the second half of August to the beginning of January) and a few weeks in May and June when it reached 100 l/m<sup>2</sup> (Fig. 3). In the period October-November due to heavy rains 150 l/m<sup>2</sup> were registered.

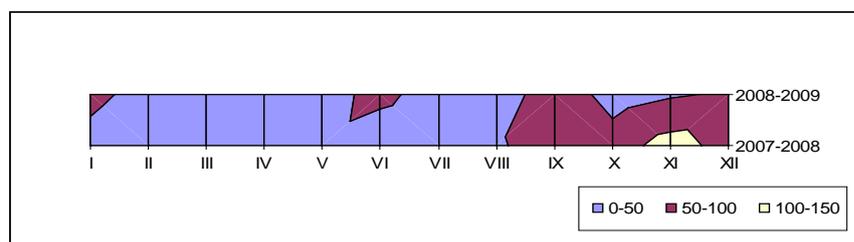


Fig. 3. Average monthly amount of precipitation for the period 2007-2009 in l/m<sup>2</sup> (based on data from the Meteorological station in Shumen)

Statistically significant differences in the distribution of *L. trilineatus* and *M. transsylvanicum* at different collecting sites were identified through a preliminary LSD (*Least Significant Difference*) analysis and the non-parametric ranking test of Kruskal-Wallis. The influence of basic environmental factors (temperature and humidity of soil and air) on the ground activity of *L. trilineatus* и *M. transsylvanicum* is measured by correlation analysis of the average values of the environment parameters by months and by the number of captured individuals establishing certain standardized coefficients of Pearson-Brave. The statistical processing of data was conducted with the software packages SPSS 9.0 and StatPlus 3.5.3.

## Result and Discussion

*L. trilineatus* and *M. transsylvanicum* were found both in the studied anthropogenically affected habitats and in the natural habitats in the region of Shumen and the Shumen Plateau. Regardless of the differences in their habitat distribution [18], the number of collected specimens of both species was significant: 1474 of *L. trilineatus* and 618 of *M. transsylvanicum*. *L. trilineatus* was not found only in the traps placed in the most urbanized central part of the city (the City Park of Shumen – UI), but the number of specimens in the other urban area (the Park of Shumen University – UII) and the Bukaka reserve (RI) is very large and similar – 479 and 495 specimens respectively. For *M. transsylvanicum*, the largest number of specimens was reported in the UII (290). This species was not registered in two of the forest habitats of the plateau – the hornbeam forest (RIII) and the Bukaka reserve (RI). For both species, Preliminary LSD analysis and dispersion homogeneity tests revealed statistically significant differences with respect to their distribution across sites. As the data does not allow normalization, Kruskal-Wallis nonparametric rank test (Tables 1 and 2) was used to check the true variation in the number of individuals of each kind in the sites.

Table 1. Mean and final results from the non-parametric disperse analysis of *L. trilineatus*

<i>Ranks</i>			
<i>Total number of specimens</i>	<i>Collecting Sites</i>	<i>N</i>	<i>Mean Rank</i>
0	UI	24	39.50
479	UII	24	139.46
82	SU	24	95.35
495	RI	24	142.56
202	RII	24	110.98
70	RIII	24	88.48
62	RIV	24	72.13
84	RV	24	83.54
1474	Total	192	

**Test Statistics<sup>a,b</sup>**

	Total number of specimens
<i>Chi-Square</i>	68.866
<i>df</i>	7
<i>Asymp. Sig.</i>	<b>0.000</b>

- a. Kruskal Wallis Test
- b. Grouping Variable: collecting si

Table 2. Mean and final results from the non-parametric disperse analysis of *M. transsylvanicum*

<i>Ranks</i>			
<i>Total number of specimens</i>	<i>Collecting sites</i>	<i>N</i>	<i>Mean Rank</i>
1	UI	24	65.88
290	UII	24	158.35
205	SU	24	153.88
0	RI	24	63.00
1	RII	24	65.88
0	RIII	24	63.00
118	RIV	24	133.27
3	RV	24	68.75
618	Total	192	

**Test Statistics<sup>a,b</sup>**

	Total number of specimens
<i>Chi-Square</i>	143.503
<i>df</i>	7
<i>Asymp. Sig.</i>	<b>0.000</b>

- a. Kruskal Wallis Test
- b. Grouping Variable: collecting site

A correlation analysis of the mean values of tested environmental parameters by month in relation to the number of collected specimen (Tables 3 and 4) was used to study the influence of the

environmental factors (temperature and humidity of air and soil) on the ground surface activity of the species.

The results show that correlation coefficients in *L. trilineatus* are not statistically significant. It is interesting, however, to note the negative correlation between the species activity and the soil temperature (-0.459) and a positive correlation between the species activity and soil humidity level (0.417) (Table 3).

Table 3. Correlation coefficients of Pearson-Brave for *L. trilineatus*

\*\* Correlation is significant at the 0.01 level (2-tailed). \* Correlation is significant at the 0.05 level (2-tailed)

Factor	Correlations	Air temperature (°C)	Soil temperature (°C)	Humidity of air (%)	Humidity of soil (%)	Total number of specimens
Air temperature (°C)	PearsonCorrelation Sig. (2-tailed) N	1 . 12	0.953** 0.000 12	-0.713** 0.009 12	-0.760** 0.004 12	-0.321 0.309 12
Soil temperature (°C)	PearsonCorrelation Sig. (2-tailed) N	0.953** 0.000 12	1 . 12	-0.617* 0.033 12	-0.793** 0.002 12	<b>-0.459</b> 0.133 12
Humidity of air (%)	PearsonCorrelation Sig. (2-tailed) N	-0.713** 0.009 12	-0.617* 0.033 12	1 . 12	0.740** 0.006 12	-0.107 0.740 12
Humidity of soil (%)	PearsonCorrelation Sig. (2-tailed) N	-0.760** 0.004 12	-0.793** 0.002 12	0.740** 0.006 12	1 . 12	<b>0.417</b> 0.178 12
Total number of specimens	PearsonCorrelation Sig. (2-tailed) N	-0.321 0.309 12	-0.459 0.133 12	-0.107 0.740 12	0.417 0.178 12	1 . 12

In *M. transsylvanicum*, a positive statistically significant dependence of the activity of individuals on air temperature (0.708) and soil temperature (0.586) was reported. A negative statistically significant dependence on air humidity (-0.713) was also recoded (Table 4).

Table 4. Correlation coefficients of Pearson-Bravefor *M. transsylvanicum*

\*\* Correlation is significant at the 0.01 level (2-tailed). \*Correlation is significant at the 0.05 level (2-tailed)

Factor	Correlations	Air temperature (°C)	Soil temperature (°C)	Humidity of air (%)	Humidity of soil (%)	Total number of specimens
Air temperature (°C)	PearsonCorrelation Sig. (2-tailed) N	1 . 12	0.953** 0.000 12	- 0.713** 0.009 12	-0.760** 0.004 12	<b>0.708*</b> 0.010 12
Soil temperature (°C)	PearsonCorrelation Sig. (2-tailed) N	0.953** 0.000 12	1 . 12	-0.617* 0.033 12	-0.793** 0.002 12	<b>0.586*</b> 0.045 12
Humidity of air (%)	PearsonCorrelation Sig. (2-tailed) N	-0.713** 0.009 12	-0.617* 0.033 12	1 . 12	0.740** 0.006 12	<b>-0.713**</b> 0.009 12
Humidity of soil (%)	PearsonCorrelation Sig. (2-tailed) N	-0.760** 0.004 12	-0.793** 0.002 12	0.740** 0.006 12	1 . 12	-0.410 0.186 12
Total	PearsonCorrelation	0.708*	0.586*	-	-0.410	1

number of specimens	Sig. (2-tailed) N	0.010 12	0.045 12	0.713** 0.009 12	0.186 12	. 12
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To analyze the ground surface activity of both species ( $Y$ ) in the the months during the observation period ( $X$ ), a regression model presented in the equation  $Y = b_0 + b_1X + b_2X^2 + b_3X^3$  was used. The empirical and theoretical values of  $F$  confirm that the models used are adequate ( $Sign.F < \alpha = 0.05$ ), and the coefficients of determination  $R^2$  are statistically significant (Table 5).

Table 5. Summarised results of the regression analysis of *L. trilineatus* and *M. transsylvanicum*

<i>Leptoiulus trilineatus</i>				
Factor	Regression equation	Coefficients		Sign. F
		Regression coefficient	Coefficient of determination	
Month	$Y = b_0 + b_1X + b_2X^2 + b_3X^3$ const. $b_0 = -85.24$ const. $b_1 = 172.24$ const. $b_2 = -34.28$ const. $b_3 = 1.84$	R = 0.79	R <sup>2</sup> = 0.62	Sign. F = 0.0429
<i>Megaphyllum transsylvanicum</i>				
Month	$Y = b_0 + b_1X + b_2X^2 + b_3X^3$ const. $b_0 = -72.94$ const. $b_1 = 70.26$ const. $b_2 = -9.84$ const. $b_3 = 0.39$	R = 0.88	R <sup>2</sup> = 0.78	Sign. F = 0.0054

The regression coefficients  $R = 0.79$  (for *L. trilineatus*) and  $R = 0.88$  (for *M. transsylvanicum*) and the corresponding coefficients of determination –  $R^2 = 0.62$  and  $R^2 = 0.78$  indicate that the variation in relative frequencies (69.0% and 78.0%) is mostly due to regression, while the rest of it is due to factors not included in the models. Since the Sig.T values in both models are less than 0.05 (except for the  $b_0$  constant for *L. trilineatus*  $0.3893 > \alpha = 0.05$ ), the coefficients in the regression equations are statistically significant (Table 6, column (6)).

Table 6. Values and statistical significance of the coefficients in the studied regression models

Variable (1)	B (2)	SE B (3)	Beta (4)	T (5)	Sig. T (6)
<i>Leptoiulus trilineatus</i>					
X	172.24	59.882	7.846311	2.876	0.0206
X <sup>2</sup>	-34.28	10.488	-20.8553	-3.269	0.0114
X <sup>3</sup>	1.84	0.531	13.384	3.457	0.0086
constant	-85.24	93.646351		-0.910	<b>0.3893</b>
<i>Megaphyllum transsylvanicum</i>					
X	70.26	17.926	8.158	3.919	0.0044
X <sup>2</sup>	-9.84	3.1397	-15.266	-3.135	0.0139
X <sup>3</sup>	0.39	0.159	7.273	2.462	0.0392
constant	-72.94	28.033		-2.602	0.0315

The results of the analysis of the seasonal dynamics of the number of specimens show that *L. trilineatus* exhibit higher ground surface activity from mid-March to mid-May, with a second, lower

activity peak in November and December (Figure 4A). In these periods, the air and soil temperatures are positive and range from 3 to 17°C, and the humidity is significant (60-80%). The relatively low activity of the species during the summer months is due to high temperatures and low humidity.

*M. transsylvanicum* demonstrates increased activity from the end of February to mid-July, which is due to the positive statistically significant correlation with the air and soil temperature during the observation period (Figure 4B).

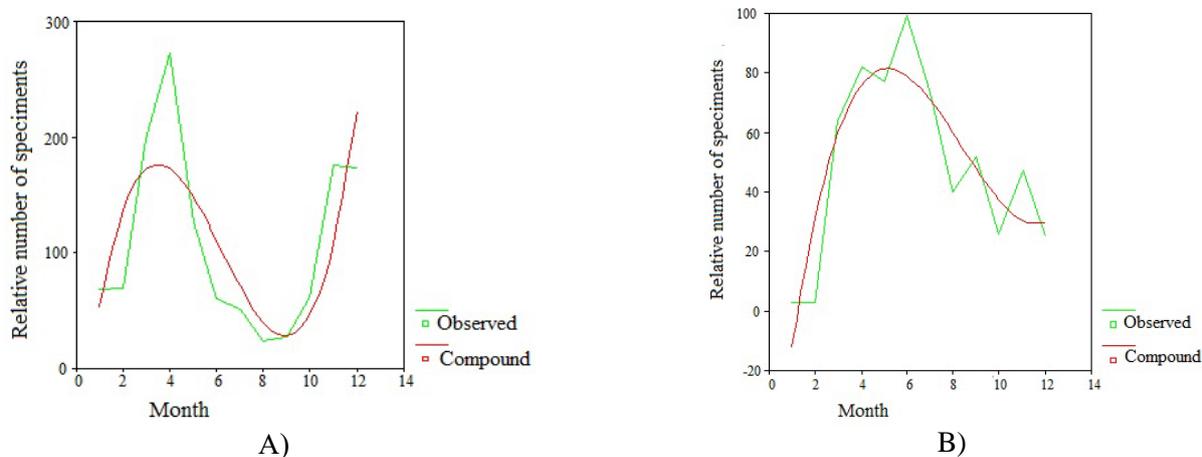


Fig. 4. Empirical (green) and compound (red) densities of distribution of *L. trilineatus* (A) and *M. transsylvanicum* (B) by month

### Conclusion:

*L. trilineatus* and *M. transsylvanicum* are species with year-round activity in the study area. No statistically significant correlation was found between the degree of anthropogenic impact and the activity of the two species.

*L. trilineatus* exhibits bimodal activity characteristic of July with peaks in activity in the spring (March-May) and early winter (November-December). The coefficients of correlation dependence of the activity of *L. trilineatus* on the tested abiotic factors of the environment – humidity and temperature of air and soil are not statistically significant. Nevertheless, Pearson-Brave has the highest absolute value, taking into account the negative correlation dependence of the influence of the soil temperature (-0.459). The coefficient of soil humidity impact on activity is 0.417 and reflects the presence of a positive correlation.

*M. transsylvanicum* demonstrates the highest ground-surface activity in spring and summer (from February to July). The abiotic factors of the environment with the most significant influence on the ground activity of *M. transsylvanicum* are air and soil temperature, with Pearson-Brave correlation coefficients being 0.708 and 0.586 respectively.

### Acknowledgements

The study has been financially supported by project RD-08-125/06.02.2017, funded by Konstantin Preslavsky University of Shumen, Department of Biology.

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