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Species composition of mosquitoes (Diptera: Culicidae) in relation to climate conditions in South-Eastern Slovakia

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Abstract: In years 2010–2013, we collected mosquito larvae and adults at selected locations in Eastern Slovakia. We have identified 22 mosquito species belonging to 6 genera: *Aedes* (4 species), *Anopheles* (4 species), *Coquillettidia* (1 species), *Culiseta* (1 species), *Culex* (3 species) and *Ochlerotatus* (11 species), with eudominant representation of *Culex pipiens/Cx. torrentium* (42.36%), *Aedes vexans* (36.65%) and *Ochlerotatus sticticus* (12.15%). As for larvae, we collected 16 species belonging to 5 genera: *Aedes* (4 species), *Anopheles* (1 species), *Culex* (2 species), *Culiseta* (1 species) and *Ochlerotatus* (8 species). Out of the total number of 29,155 specimens of identified larvae, eudominant species were again *Cx. pipiens* (33.80%), *Ae. vexans* (19.27%) and *Oc. cantans* (15.99%). Seasonal changes in the population dynamics of individual species of larvae, as well as adults, reflect different climatic conditions of local sites.

Key words: fauna; mosquitoes; abundance; environmental data; seasonal changes

Introduction

Mosquitoes belong to the most important vectors of pathogenic organisms and with regard to health of humans and animals they are being paid adequate attention in Europe. Their role in the transmission of viruses and parasites is well known also in the territory of Slovakia (e.g., Ťahyňa virus, Čalovo virus, West Nile virus, *Dirofilaria* spp.) (Bárdoš et al. 1959; Bárdoš & Danielová 1959; Gratz 2006; Bocková et al. 2013b). External environmental conditions play an important role in the life cycle and transmission of infectious agents and in the spread of diseases. In the last decades, the impact of global warming has become more and more visible and is demonstrated in our territory, as it is in the rest of the world, by weather fluctuations. The most extreme demonstrations of these changes undoubtedly include the environment temperature increase, frequency of extreme and hazardous weather phenomena (storms, floods, windstorms, etc.), extension of areas regularly affected by drought and abnormal rainfall, decrease in the amount and allocation of permanent snow cover. For the short period of the last four years, the mean annual temperature in Slovakia has increased from 1.3 °C (2010–2011) – 1.6 °C (2012–2013), compared to the long-term average (1961–1990), and the rainfall totals were fluctuating from abnormal (year 2010, 132% of normal) to subnormal (years 2011–2012, 84.5 and 88.5% of normal) levels.

Modified bioclimatic conditions, as a result of climatic changes, have a negative impact on the entire ecosystem. The changes occur primarily with regard to the biological diversity and geographic distribution of organisms; in atypical climate zones there is a constantly growing occurrence of invasive species that not only impose the risk of the entire ecosystem disruption, but also the risks connected to the spread of exotic diseases.

Thorough knowledge of the species composition, ecology, and permanent monitoring of mosquitoes in the environment with the constantly changing climate have a durable significance for the real assessment of risks related to the spread of pathogens and for the planning of potential control measures.

Material and methods

Monitored territory

Monitored locations are geographically situated in the Košice Region territory. The region's area is 6,753 km^2 and it includes the areas of the Košice Basin which extends in the south-east part of Slovakia. From the climate point of view, the Basin belongs to the areas with warm, semi-arid climate, with the long-term mean annual temperature of 8.7 °C, the mean annual rainfall of 600–850 mm, and 60–70% air humidity. Mosquito larvae were collected at eight locations (Ťahanovce, Šebastovce, meanders of Hornád River, Paňovce, Rozhanovce, Košické Olšany, Medzev, Perín Chým), adults at seven locations (Ťahanovce, Šebastovce, meanders of Hornád River, Paňovce, Rozhanovce, Beniakovce, Košické Olšany) (Fig. 1).

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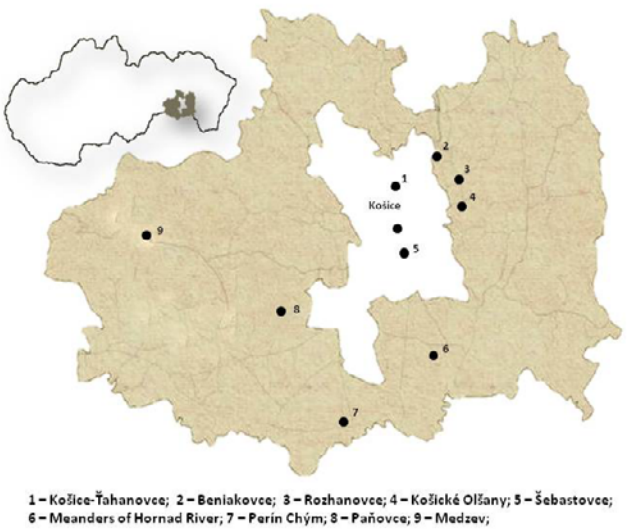

Fig. 1. Map of mosquito larvae and adults collection sites in the Košice Basin.

Košice – Ťahanovce (224 m a.s.l., 48° 45′ N, 21° 15′ E). The locality lies in basin of the Hornád River that flows near the village Ťahanovce. Collection sites were small periodic pools and stagnant water biotopes left after inundation of the Hornád River. Mosquito larvae were also collected in arms of slowly flowing waters of brook on the woodland margin or in the area between village and woodland.

Košice – Šebastovce (209 m a.s.l., 48° 39′ S, 21° 16′ E). This locality in proximity of Šebastovce village is heavily agricultural area including cultivated and non-cultivated fields, vineyards, wind belts and cart ways for agronomic vehicles. Larvae were sampled in drainage channel alongside the fields and traces left by heavy machines, filled by rain water. After the floods also pools of water left on fields were inspected.

Beniakovce (203 m a.s.l., 48° 46′ N, 21° 19′ E). The mosquito trap was localized in ornamental garden (with fruit trees, ornamental grasses) of country house, near the garden lake.

Rozhanovce (215 m a.s.l., 48° 45′ N, 21° 21′ E). This is the locality about 1 km away from Rozhanovce village. Samples were collected in the brink of the Grey Lake grown with nut sedge, in a streamlet entering the lake, in shallow pools of water on the meadows, on the meadow-woodland space and in the forest floor.

Košické Olšany (202 m a.s.l., 48° 44′ S, 21° 20′ E). Larvae were collected in drainage channel, adults were collected in agriculturally used garden of country house.

Paňovce (241 m a.s.l., 48° 60′ S, 21° 06′ E). The locality is characterized by favorable temperature conditions and heterogonous biotopes. Sampling was performed in flooded meadow and woodland biotopes (mixed deciduous forest), in a forest streamlet and waterside zone of a lake grown with reeds.

Medzev (318 m a.s.l., 48° 42′ N, 20° 53′ E). The studied locality stretches in a narrow woodland zone between the main road and river, which is a temporal water biotope inundated after the floods.

Perín-Chým (250 m a.s.l., 48° 54′ N, 21° 19′ E). In the locality sampling was done in periodic plashes nearby fish ponds, adjacent water drain and voluminous shallow water pool left on fields after floods.

Meanders of the Hornád River (204 m a.s.l., 48° 34′ N, 21° 18′ E). The territory stretches from Trstené pri Hornáde village to the Milhosť village represent the last remnants of the riparian forest preserved throughout the Košice Basin.

Environmental data

During each collection in the field, we recorded selected ecological parameters: water temperature, water pH, air temperature, humidity and flow. Temperature measurements were carried out using the TOPCOM (T-110W) digital thermometer; water pH measurements were carried out using the MULTIMETER MFD 79. Air temperature, humidity and flow were measured using the Testo 625 digital scanner. Daily average, minimum and maximum values of air temperature, air humidity, wind flow velocity, and daily rainfall totals were obtained in seasons 2010 and 2011 from the Hydrometeorological Institute in Košice. In years 2012–2013, we obtained these parameters from the website *www.weather.org* (weather archive).

The collections were carried out between April and August, always in weekly intervals. Temperature and rainfall development on the monitored territory in the period 2010–2013 is shown in Figs 2 and 3.

Collection of mosquito larvae and adults

Larvae and pupae intended for direct identification

Mosquito larvae were collected applying a standard method (Kramář 1958), using a sieve with a small mesh size, by sliding on the water level or close underneath the water level, down to the depth of 15–20 cm, i.e., the depth where larvae dive when the water level is disturbed. Collected larvae were taken out of the sieve using a plastic pipette and carried to the prepared and designated small plastic pots containing 75% alcohol that not only kills the larvae, but also serves as the fixation solution. For the long-term storage of larvae we used the mixture of 70% alcohol and 1% glycerine that prevents larvae from drying (Labuda 1974).

For the purpose of more accurate identification, morphologically closely related species were casted into Canada balsam and subsequently identified using available keys (Kramář 1958; Becker et al. 2010).

In case of occurrence of larvae species which are difficult to identify according to typical morphological signs, to avoid species misidentification, some of the collected larvae and pupae were transported alive to the laboratory to be grown into adults. Larvae and pupae were kept in plastic buckets covered with a small-eye mesh and filled with water taken from original hatching sites. Hatched adults were collected using an electric exhaust fan and fixed by freezing (–18 °C). Identification of adults (males and females) facilitated easier and more precise identification of larvae.

Collection of adult individuals

Adults were primarily collected using the CDC Miniature Light Traps with CO_2 baits in form of dry ice (2 kg of micro pellets). The traps were exposed at the height of approximately 150 cm above the ground late in the afternoon; they were activated always at about the same time of the day (5.00–6.30 p.m.) and deactivated at about 8.00–9.00 a.m. of the following day. As an additional collection method we used an entomological clap-net. Right in the field, all the collected mosquitoes were placed in the PVC boxes containing dry ice and transported to the laboratory, where all the individuals were morphologically identified under a stereomicroscope using identification keys by Kramář (1958) and Becker et al. (2010). Females were identified immediately after killing; in males (collected mostly using an entomological

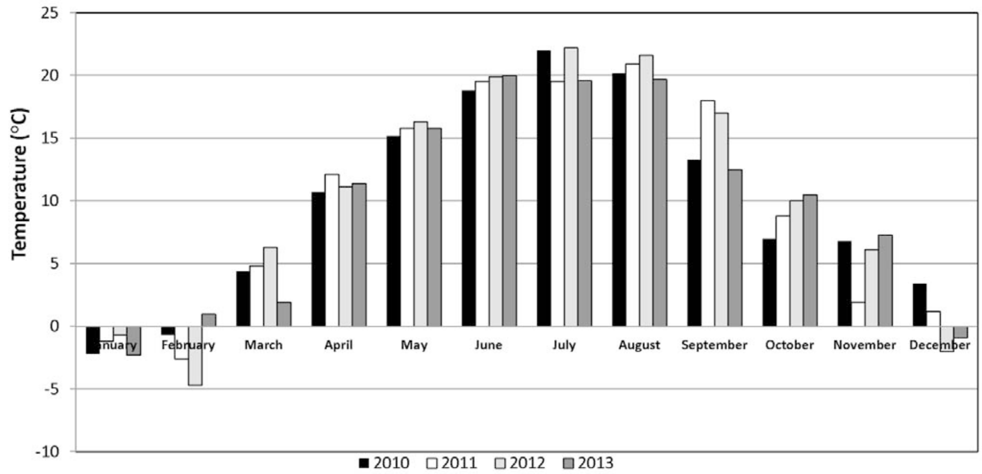

Fig. 2. Average monthly temperatures in the monitored territory during 2010–2013.

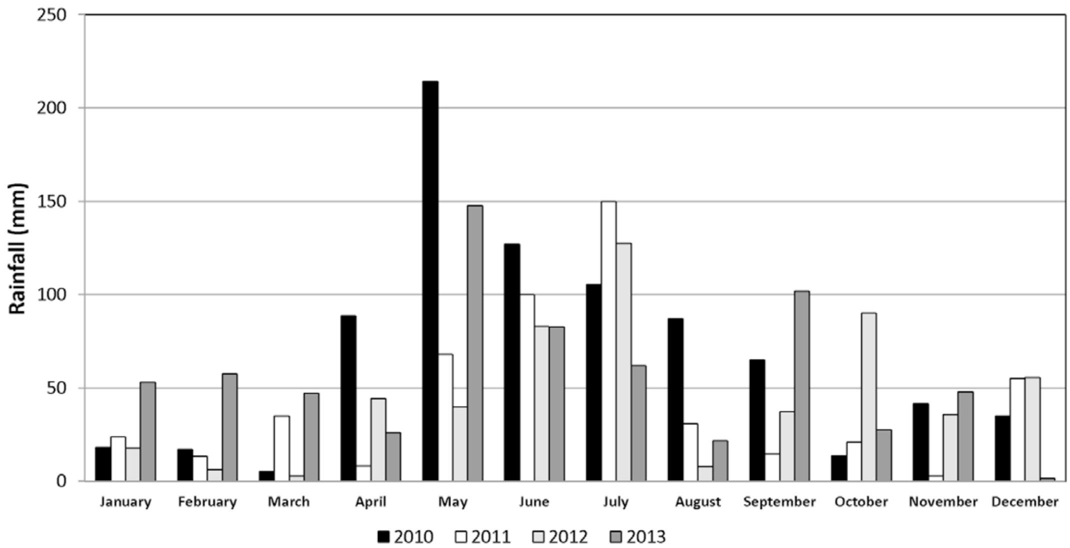

Fig. 3. Monthly rainfall totals in the monitored territory during 2010–2013.

clap-net), precise identification required a prior preparation of a microscopic hypopygium specimen, which was preceded by a 24-hour maceration in 96% alcohol, separation of abdomen with hypopygium, washing in distilled water, drying and casting in Canada balsam. Mosquito females of identified species were stored in the freezer at –18 °C for further use.

Statistical analyses
Statistical analysis was carried out using the FigurePad InStat version 3.01 (FigurePad Software, San Diego, CA). The results were processed using the regression analysis. The values were regarded as significant at $P < 0.05$.

Results

Mosquito larvae
Within the monitored period, 29,155 larvae of 16 species were collected. An eudominant portion of the larvae fauna (relative abundance > 10%) consisted of *Culex pipiens* (33.80%), *Aedes vexans* (19.27%), *Ochlerotatus cantans* (15.99%), and a dominant portion (relative abundance 5–10%) consisted of *Ae. cinereus* (5.94%), *Oc. sticticus* (5.54%) and *Oc. punctor* (5.05%).

In individual years, we observed different seasonal occurrences of larvae with regard to the species composition and abundance (Tables 1, 2) that corresponded to the meteorological situation at individual locations.

In 2010, the highest numbers of larvae were collected in the end of the season, in July (33.5%) and August (28.5%), i.e. in the period when extreme precipitation was finishing. In the collected group of larvae, the so-called calamitous species prevailed, i.e. *Ae. vexans* (n = 3655) and *Oc. sticticus* (n = 602), as well as species with the tendency of outbreaks after floods, e.g., *Cx. pipiens* (n = 4757). When assessing the impact of ambient temperature, rainfall amount, and relative air humidity on the larvae abundance at individual locations, we observed a relationship with the temper-

Table 1. Comparison of mosquito species composition and abundance of larvae collected in 2010–2013.

Species	2010		2011		2012		2013		Total
	A	%	A	%	A	%	A	%	
Aedes cinereus	146	1.47	**924**	**10.37**	**578**	**10.73**	86	1.73	**1734**
Ae. vexans	**3655**	**36.83**	0	0.00	234	4.34	**1732**	**35.01**	**5621**
Anopheles maculipennis	273	2.75	0	0.00	74	1.40	38	0.80	**385**
Culiseta annulata	9	0.09	**1159**	**13.01**	94	1.74	17	0.34	**1279**
Culex pipiens	**4757**	**47.93**	524	5.90	**2897**	**53.80**	**1679**	**33.94**	**9857**
Cx. territans	0	0.00	6	0.06	0	0.00	0	0.00	**6**
Ochlerotatus cantans	454	4.57	**3110**	**34.93**	**625**	**11.60**	474	9.60	**4663**
Oc. cataphylla	8	0.08	**1076**	**12.10**	302	5.60	50	1.01	**1436**
Oc. communis	0	0.00	151	1.70	167	3.10	90	1.81	**408**
Oc. flavescens	0	0.00	364	4.10	46	0.90	2	0.04	**412**
Oc. geniculatus	6	0.06	0	0.00	0	0.00	9	0.20	**15**
Oc. leucomelas	0	0.00	244	2.74	0	0.00	0	0.00	**244**
Oc. punctor	13	0.13	**1148**	**12.90**	232	4.30	81	1.63	**1474**
Oc. refiki	0	0.00	2	0.02	0	0.00	0	0.00	**2**
Oc. rusticus	0	0.00	1	0.01	0	0.00	0	0.00	**1**
Oc. sticticus	602	6.10	193	2.16	135	2.50	**688**	**13.90**	**1618**
Total species	10		13		11		12		16
Total no. of specimens	9923		8902		5384		4946		29155

Explanations: Eudominant species A > 10% (in bold); dominant species A = 5–10%; satelite species A < 5%.

Table 2. Mosquito larvae species collected on each sampling sites, including number of individuals (A – abundance) and relative abundance (%).

Species	Ťahanovce		Šebastovce		Meanders		Rozhanovce		Paňovce		Košické Olšany		Perín Chým		Medzev	
	A	%	A	%	A	%	A	%	A	%	A	%	A	%	A	%
Aedes cinereus	34	1.20	167	2.00	17	0.50	2	0.07	**1486**	**14.30**	26	4.70	2	0.30	0	0.00
Ae. vexans	60	2.20	**3314**	**38.90**	**1037**	**31.60**	27	1.02	321	3.10	**120**	**21.70**	**742**	**99.20**	0	0.00
Anopheles maculipennis	41	1.50	198	2.30	44	1.30	57	2.17	7	0.10	37	6.70	1	0.10	0	0.00
Culiseta annulata	4	0.10	88	1.03	**1312**	**40.00**	9	0.30	56	0.50	1	0.20	0	0.00	0	0.00
Culex pipiens	**2426**	**87.30**	**2647**	**31.00**	**677**	**20.60**	**2410**	**91.90**	**1461**	**14.10**	**233**	**42.10**	3	0.40	0	0.00
Cx. territans	0	0.00	0	0.00	6	0.20	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Ochlerotatus cantans	21	0.80	707	8.30	1	0.03	19	0.70	**3371**	**32.50**	**85**	**15.30**	0	0.00	268	97.80
Oc. cataphylla	0	0.00	303	3.60	30	0.90	0	0.00	**1095**	**10.60**	2	0.40	0	0.00	6	2.20
Oc. communis	7	0.30	19	0.22	27	0.80	0	0.00	349	3.40	6	1.10	0	0.00	0	0.00
Oc. flavescens	0	0.00	149	1.74	0	0.00	0	0.00	263	2.50	0	0.00	0	0.00	0	0.00
Oc. geniculatus	1	0.03	1	0.01	1	0.03	12	0.50	0	0.00	0	0.00	0	0.00	0	0.00
Oc. leucomelas	0	0.00	0	0.00	0		0	0.00	244	2.30	0	0.00	0	0.00	0	0.00
Oc. punctor	0	0.00	26	0.30	32	1.00	86	3.30	**1412**	**13.60**	4	0.70	0	0.00	0	0.00
Oc. refiki	0	0.00	2	0.02	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Oc. rusticus	0	0.00	0	0.00	0	0.00	0	0.00	1	< 0.01	0	0.00	0	0.00	0	0.00
Oc. sticticus	183	6.60	**905**	**10.60**	97	3.00	0	0.00	307	3.00	40	7.20	0	0.00	0	0.00
Total species	9		13		11		8		13		11		4		2	
Total specimens	**2777**		**8526**		**3281**		**2622**		**10373**		**554**		**748**		**274**	

Explanations: Eudominant species A > 10% (in bold); dominant species A = 5–10%; satelite species A < 5%.

ature in Paňovce ($P = 0.001$). At other locations, no relationship was statistically confirmed for any of the three monitored meteorological parameters. In 2011, a dominant amount of larvae (78%) was collected at the beginning of the season, in April and May (13%). Although the beginning of the season was relatively dry, the larvae benefited from water accumulated from the melting snow. Collected group of larvae included primarily larvae of spring mosquito species, e.g. *Oc. cantans* ($n = 3110$), *Oc. punctor* ($n = 1148$) and *Oc. cataphylla* ($n = 1076$). Weather in summer months was mostly very warm and dry, which caused that water from majority of hatching sites evaporated and the amount of collected larvae significantly decreased. A correlation between the ambient temperature and the larvae abundance was observed in Paňovce ($P = 0.002$) and in Meanders of the Hornád River ($P = 0.03$); a relationship with the humidity was observed in Meanders of the Hornád River ($P = 0.04$). High temperatures, time and spatial unevenness of the rainfall in the 2012 season caused that the most numerous collections of larvae were trapped in the middle of the season, in May (41.8%) and June (26.1%), after the first more intensive rains of the season. A dominant portion of larvae consisted of *Cx. pipiens* ($n = 2897$; 53.8%). A correlation between the selected environmen-

Table 3. Adult mosquito species collected on each sampling sites, including number of individuals (A – abundance) and relative abundance (%).

Species	Ťahanovce		Šebastovce		Meanders of Hornad River		Rozhanovce		Košické Olšany		Beniakovce		Paňovce	
	A	%	A	%	A	%	A	%	A	%	A	%	A	%
Aedes albopictus	0	0.00	4	0.03	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Ae. cinereus	121	1.40	72	0.60	50	0.50	5	1.20	17	0.30	1	0.02	**1196**	**6.40**
Ae. rossicus	0	0.00	38	0.30	302	2.80	0	0.00	5	0.08	1	0.02	117	0.60
Ae. vexans	**1362**	**15.60**	**7582**	**61.80**	**5110**	**47.00**	**545**	**27.10**	**4406**	**77.20**	**2575**	**60.10**	**987**	**5.30**
Anopheles claviger	9	0.01	13	0.10	6	0.05	1	0.04	21	0.40	2	0.04	0	0.00
An. maculipennis s.l.	63	0.70	71	0.60	207	1.90	89	4.40	36	0.60	51	1.20	42	0.20
An. plumbeus	3	0.03	0	0.00	1	< 0.01	0	0.00	0	0.00	0	0.00	1	< 0.01
Coquillettidia richiardii	0	0.00	0	0.00	257	2.40	0	0.00	2	0.03	2	0.04	1	< 0.01
Culiseta annulata	15	0.20	5	0.04	119	1.09	8	0.40	4	0.07	0	0.00	16	0.08
Culex modestus	9	0.10	0	0.00	3	0.02	4	0.20	3	0.05	1	0.02	1	< 0.01
Cx. pipiens/Cx. torrentium	**6060**	**69.50**	**1954**	**15.90**	**2906**	**26.70**	**1268**	**63.10**	**756**	**13.20**	**1105**	**25.80**	**12721**	**68.10**
Cx. territans	0	0.00	8	0.06	0	0.00	0	0.00	0	0.00	0	0.00	98	0.50
Ochlerotatu annulipes	0	0.00	3	0.02	2	0.01	0	0.00	0	0.00	0	0.00	1	< 0.01
Oc. flavescens	1	0.01	0	0,00	2	0.01	0	0.00	0	0.00	0	0.00	0	0.00
Oc. communis	0	0.00	0	0,00	0	0.00	0	0.00	0	0.00	0	0.00	6	0.03
Oc. cantans	11	0.12	476	3,90	200	1.80	8	0.40	25	0.40	0	0.00	665	3.60
Oc. caspius	2	0.02	119	0.97	7	0.06	23	1.10	230	4.00	**478**	**11.20**	12	0.06
Oc. cataphylla	0	0.00	13	0.10	2	0.01	0	0.00	0	0.00	0	0.00	0	0.00
Oc. geniculatus	2	0.02	4	0.03	15	0.10	3	0.10	15	0.30	8	0.20	19	0.10
Oc. punctor	0	0.00	13	0.10	0	0.00	0	0.00	0	0.00	0	0.00	24	0.10
Oc. sticticus	**1067**	**12.20**	**1890**	**15.40**	**1677**	**15.40**	54	2.70	187	3.30	60	1.40	**2773**	**14.80**
Total specimens	8725		12265		10866		2008		5707		4284		18680	
Total species	13		16		17		11		13		11		17	

Explanations: Eudominant species A > 10% (in bold); dominant species A = 5–10%; satelite species A < 5%.

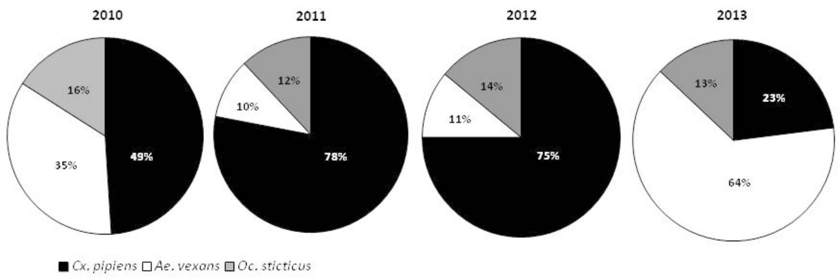

Fig. 4. Percentage of eudominant mosquito species in years 2010–2013.

tal factors (temperature, rainfall totals, ambient humidity) was observed only at the Paňovce location ($P = 0.0008$; $P = 0.001$; $P = 0.001$). In 2013, 4,946 larvae were collected. The highest number of larvae were collected in July (25.2%) and June (24.8%). Species with the highest abundance included *Ae. vexans* ($n = 1732$) and *Cx. pipiens* ($n = 1679$). A positive impact of climatic factors on the resulting count of larvae was confirmed in connection with the rainfall totals in Šebastovce ($P = 0.049$), Ťahanovce ($P = 0.01$), and with the ambient temperature in Paňovce ($P = 0.04$).

Mosquito adults

Within the period of 4 years, the total number of collected mosquito adults was 63,434, belonging to 22 species (Table 3). The species with the most frequent occurrence were *Cx. pipiens/Cx. torrentium* (42.3%), *Ae. vexans* (36.6%) and *Oc. sticticus* (12.1%). Changes in the percentages of these species during the four monitored years are shown in Figure 4. Three species (*Oc. cataphylla*, *Oc. communis* and *Oc. punctor*) were collected only using an entomological cap-net.

The highest number of mosquitoes was collected in June ($n = 17723$) and July ($n = 25487$), which represented altogether 68.1% of the total number of all collected mosquitoes. Mild decrease in their numbers was observed in August ($n = 14155$; 22.3%). The lowest numbers were collected in April ($n = 1362$; 2.2%) and May ($n = 4697$; 7.4%).

In general, survival of adults was not affected by weather conditions as significantly as it was in case of pre-imago stages. Nevertheless, in individual time periods we observed different monthly, as well as seasonal fluctuations. The maximum occurrence values for 2010 were observed in June (49.1%) and August (33.2%); for 2011 in May (53.4%) and July (21.5%); for 2012–2013 the highest frequency of adults was observed in June (32.5%; 30.1%) and July (34.4%; 44.2%).

The most frequent species of the *Aedes* genera was *Aedes vexans* (23252 ind.; 36.6%). Their highest numbers were collected in years 2013 ($n = 20036$) and 2012 ($n = 3034$). The abundance in years 2010 and 2011, with regard to the above mentioned extreme weather conditions, was incomparably lower ($n = 152$; $n = 20$). A significant portion of these mosquitoes were collected at locations in Šebastovce (32.6%), Meanders of the Hornád River (21.9%) and Košické Olšany (18.9%).

As for the rainfall, year 2011 was subnormal and supernormal in terms of temperatures; the number of hatching sites with water was minimal at the monitored locations; development of larvae into adults was strongly limited (*Ae. vexans* $n = 20$; *Oc. sticticus* $n = 24$; *Cx. pipiens/Cx. torrentium* $n = 158$). In the assessment of the impact of selected environmental factors (temperature, humidity and rainfall totals) on the abundance of the above listed mosquito species, statistical relation was not confirmed. On the other hand, years 2012 and 2013 were supernormal in terms of temperatures; rainfall allocation was uneven, but the amount of water was sufficient for larvae to complete their development (2012: *Ae. vexans* $n = 3034$; *Oc. sticticus* $n = 3574$; *Cx. pipiens/Cx. torrentium* $n = 19454$; 2013: *Ae. vexans* $n = 20036$; *Oc. sticticus* $n = 4045$; *Cx. pipiens/Cx. torrentium* $n = 7046$). The maximum occurrences of these species were reached mostly in June and July; in case of *Cx. pipiens/Cx. torrentium,* the second peak occurrence was observed in August, except for year 2013.

A correlation between the environmental factors and their impact on the number of individual mosquito species were confirmed in 2012 at Ťahanovce location, with regard to ambient humidity for *Ae. vexans* ($P = 0.02$), with regard to the ambient temperature for *Cx. pipiens/Cx. torrentium* and *Oc. sticticus* ($P = 0.04$); in Meanders of the Hornád River with regard to the ambient temperature for *Ae. vexans* ($P = 0.004$), and humidity for *Cx. pipiens/Cx. torrentium* ($P = 0.01$), and in Košické Olšany with regard to the ambient temperature for *Ae. vexans* ($P = 0.002$). In 2013, we observed a relationship with the ambient temperature for *Ae. vexans* ($P = 0.04$) and *Cx. pipiens/Cx. torrentium* ($P = 0.02$) in Šebastovce; for *Cx. pipiens/Cx. torrentium* ($P = 0.005$) and *Oc. sticticus* ($P = 0.01$) in Beniakovce, for *Cx. pipiens/Cx. torrentium* ($P = 0.01$) in Meanders of the Hornád River; for *Oc. sticticus* ($P = 0.04$) in Paňovce; for *Ae. vexans* ($P = 0.007$) and *Oc. sticticus* ($P = 0.01$) in Ťahanovce; for *Oc. sticticus* a relationship with the ambient temperature ($P = 0.01$) as well as humidity ($P = 0.04$) was observed.

The resulting numbers of collected mosquitoes at individual locations were changing, depending on the biotope type. The largest numbers of mosquitoes were collected in the suburban environment which included forests and a permanent water source (Paňovce, Šebastovce, Meanders of the Hornád River).

Discussion

Recently, Eastern Slovakia has an exceptional position in terms of mosquito occurrence. Significant mosquito outbreaks causing local calamities are related to constantly more frequent and more intensive floods. Characteristic climatic conditions of this territory, in terms of temperature, semi-arid climate, and favourable annual rainfall totals oTptimise the survival rate of the vector mosquitoes as such and positively affect life cycles of pathogens and facilitate their transmission by the vectors

Existing research on the monitored territory confirmed the presence of 24 mosquito species (*Ae. albopictus, Ae. cinereus, Ae. rossicus, Ae. vexans, An. claviger, An. hyrcanus, An. maculipennis* s.l., *An. plumbeus, Cq. richiardii, Cs. annulata, Cx. modestus, Cx. territans, Cx. pipiens/Cx. torrentium, Oc. communis, Oc. cantans, Oc. caspius, Oc. cataphylla, Oc. geniculatus, Oc. flavescens, Oc. leucomelas, Oc. punctor, Oc. rusticus, Oc. refiki, and Oc. sticticus)* (Bocková 2013).

Our work indicates that the species with the most frequent occurrence include *Cx. pipiens/Cx. torrentium, Oc. sticticus* and *Ae. vexans.* These species, in case of adults, represented 91%, in case of larvae 58.5%, of the collected mosquito individuals.

Cx. pipiens belongs to the mosquito species that are able to adapt well to urban as well as suburban environment. Larvae are present in all types of natural or artificial water biotopes, in still waters, but also flowing waters, as well as moors. They also survive well in fresh waters, brackish, clean as well as polluted waters. At suitable temperatures (30 °C), embryogenesis can occur as soon as on the 1^{st} day after the eggs are laid, the entire development is rather short (3–10 days), which means that larvae are able to make use of even a short presence of water to complete their development. This fact was confirmed at locations in Paňovce and Ťahanovce, when in 2012 the summer was very hot with a minimum rainfall. In this period, we collected very low numbers of mosquitoes, while the prevailing species included *Cx. pipiens/Cx. torrentium* (their larvae cannot be reliably distinguished, therefore we state them together) larvae of which were able to use the residual water in the drainage canal. In spite of the fact that *Cx. pipiens* is generally regarded as strictly ornithophilic mosquito species in Europe, including Slovakia, this species sucks blood also from other vertebrates. Its epidemiological role has been growing in the last years, as it belongs to the most important vectors of the West Nile virus, in north-eastern Italy it was also confirmed as the vector of the nematodes *Dirofilaria repens* and *D. immitis* (Latrofa et al. 2012).

Ae. vexans is the most common mosquito, it is a calamitous species with extensive geographical distribution and mass occurrence in summer months after heavy rains or floods. Similarly to *Cx. pipiens,* at suitable ambient temperature it is able to complete its development within 1–3 weeks. It is capable of transmitting a wide range of pathogens (*Dirofilaria* spp., Ťahyňa virus) (Gratz 2006; Bocková et al. 2013b).

Oc. sticticus belongs to calamitous species, as well. It is a typical mosquito of flooded forests, sporadically occurring also in meadows (Kramář 1958). After floods, it occurs in massive numbers and bothers animals and humans by unpleasant attacks during the day but also at night.

Within the monitored period (2010–2013), we observed differences in the seasonal dynamics, especially in larvae that were very sensitive to changes in climatic factors. Statistical analyses confirmed that the larvae abundance depended mainly on the ambient temperature. Recently, the average temperature in some months is as much as 1–2 °C above the long-term average and significant temperature fluctuations are typical mainly for spring and summer seasons. This phenomenon relates to the changes within the time horizon of larvae occurrence and the resulting larvae abundance. Research carried out in years 2005–2007 in the Czech Republic and in Slovakia in the inundated basin of the Morava River (Minář et al. 2007) indicateed that the first larval instar of early spring mosquito species *Oc. cataphylla* and *Oc. leucomelas* was observed, compared to the data existing at that time, as early as in the third week of March. The larvae overcome rather fast development at relatively high spring temperatures. The fourth instar was reached in the middle of April and in late April adults were observed, contradictory to, e.g., Kramář (1958) and Becker et al. (2010) who published data on adults of these species occurring from the end of April or beginning of May. In our research, we did not observe such remarkable differences in the onset of early spring species, despite warm weather; however, there was a strong correlation between the abundance and the ambient temperature. Direct relationship between the number of larvae and the rainfall totals was not statistically confirmed. We believe that this result does not correlate to the real situation, as the rainfall affects the water level of permanent and temporary hatching sites and subsequently the overall occurrence of larvae. In adults, the most significant differences in the seasonal activity were observed among the calamitous mosquito species *Ae. vexans*, *Oc. sticticus* and *Cx. pipiens*. Occurrence, and mainly abundance of these species is strongly bound to the presence and amount of water. Despite this fact, the relationship with the rainfall totals was not statistically confirmed.

In terms of species composition, the spectrum of 24 species collected during this study is lower, compared to western Slovakia, where in the Morava River basin 28 species were observed by Strelková & Halgoš (2012). In an unpublished report from the mosquito monitoring carried out in years 2007–2009, Jalili (Report on the mosquitoes monitoring in south-eastern and south-western Slovakia in 2007-2009, personal communication) mentioned 23 species from the area of south-eastern and south-western Slovakia. However, within the aforesaid research in eastern Slovakia, in the region of Trebišov (Boťany), the author identified only 13 mosquito species, compared to 20 species (*Aedes* and *Ochlerotatus* genera) that were recorded in the eastern Slovakia almost half a century ago (Vostál 1963). During our study we have collected, unlike in western Slovakia, species like *Ae. geminus, Oc. excrucians, Oc. intrudens, Cx. hortensis, Cs. morsitans* and *Uranotaenia unguiculata* are absent. From among the species known from this territory in early 1950s, they are *Oc. dorsalis, Oc. diantaeus, Oc. nigrinus, Oc. pulcritarsis* and *Oc. pullatus*. Out of the mosquitoes of the *Anopheles* genus that were confirmed in the 1960s (Vostál 1960), the species *An. messeae, An. atroparvus* and *An. labranchiae* were not present during our study.

The current climatic changes, affected primarily by global warming, resulted in the change of biodiversity and enables faster spread of non-indigenous mosquito species, as well as causative agents of diseases. In 2004, the first case of the Euro-Asian species *An. hyrcanus* was reported in Slovakia (Halgoš & Benková 2004), for which the original, northern border of occurrence extended to Lake Balaton. Another species, new for the Slovak mosquito fauna, is *Ae. (Stegomia) albopictus*, originally an Asian mosquito, for which significant expansion was recorded for the last 20 years and which belongs to the fastest spreading animals in the world (Benedict et al. 2007). Its occurrence in Central Europe was recorded almost concurrently with reports from the Czech Republic (Šebesta et al. 2012), Austria (Novak, personal information), and Slovakia (Bocková et al. 2013a). The ability of this mosquito to adapt to the mild climate results from its significant ecological tolerance and flexibility. The spread of exotic species to Central Europe brings potential risks related to the transmission of causative agents of diseases untypical for this climatic zone, such as dengue fever, West Nile fever, dirofilariosis, etc. Currently, the fastest spreading insect-borne disease in Europe is the subcutaneous (nodular) form of dirofilariosis (Genchi et al. 2009), evoked by *Dirofilaria repens*. Prevalence of canine dirofilariosis in endemic areas of Slovakia (Borská Lowland, Danube Lowland and East-Slovakia Lowland) ranges at the level of 30% (Iglódyová 2014). Based on examinations of mosquitoes that are intermediate hosts and also vectors of dirofilaria, by applying molecular methods, out of 8 species tested until now in Slovakia, *Aedes vexans* was identified as the vector (Bocková et al. 2013b). Similar results were observed in the Czech Republic (Rudolf et al. 2014). With regard to the climatic temperature changes as well as developed tourism, the question of the spread of pathogens of diseases regarded as eradicated (e.g., malaria) still remains a topical issue.

For the period until 2075, climatologic analyses assume further increases of the air temperature by 2–4 °C, changes in the annual rainfall sequence, i.e., their increase in winter and decrease in summer, higher frequency and longer duration of dry seasons and higher probability of shorter periods with extreme rainfall totals (Šťastný 2005). In general, this disturbed – intensified hydrological cycle, together with higher air temperatures, will lead to warmer and more humid climate.

Conclusion

Our research confirmed the presence of 24 mosquito species in Eastern Slovakia. The presence of species like *Ae. cinereus, Ae. vexans, Ae. (Stegomyia) albopictus, Oc. sticticus, Oc. caspius, Oc. cantans, An. maculipennis* s.l., *An. hyrcanus, Cx. pipiens/Cx. torrentium, Cx. modestus, Cq. richiardii* in the monitored territories represents a potential health risks. With regard to specific conditions of eastern Slovakia as well as the changing climate, continuous monitoring of the mosquito fauna composition is required, with focusing on the detection of invasive species, as well as pathogens they are able to transmit.

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