

Seasonal dynamics of mosquitoes (Diptera: Culicidae) in Osijek (Croatia) for the period 1995–2004

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Abstract: The ten year research (1995–2004) of adult mosquitoes in the area of Osijek by dry ice baited CDC traps has provided significant information on mosquito species, species prevalence, their dynamics and seasonal activity. A total of 207,136 adult mosquitoes comprising twenty species belonging to the following genera: *Anopheles*, *Ochlerotatus*, *Aedes*, *Culex*, *Coquillettidia*, *Culiseta* and *Uranotaenia* were collected. The seven most common species collected during this study were: *Aedes vexans* (75.6%), *Ochlerotatus sticticus* (13.3%), *Culex pipiens* complex (5.9%), *Anopheles maculipennis* complex (1.9%), *Oc. cantans* (0.9%), *Oc. caspius* (0.6%) and *Oc. excrucians* (0.6%). The temporal changes in the relative abundance of the twenty mosquito species in the Osijek area were reflected in three peaks: in May, July and September. Seasonal population dynamics differed between years and between mosquito species in relation to wetland, urban and forest habitats and was influenced by water level of the Drava and the Danube rivers.

Key words: mosquitoes; seasonal dynamics; *Aedes vexans*; CDC trap; water level

Introduction

Spatial and temporal changes of mosquito population activity and the problems caused by mosquitoes can be evaluated in three ways: by larvae monitoring, by adult mosquito monitoring and by individual reports of citizens (AMCA 1990; Reisen et al. 1990). In general, the purpose of mosquito monitoring lies in faunal characteristics, the daily and seasonal dynamics and abundance, defining the areas with mosquito annoyance and determining potential vectors of diseases. Out of the total of 50 mosquito species recorded in Croatia (Merdić et al. 2004; Klobučar et al. 2006; Žitko & Merdić 2006), 20 have been found in the area of the city of Osijek (Merdić 1996; Merdić & Lovaković 2001).

In the Osijek area, the programme of mosquito control deals with invasions of primary floodwater mosquito species (*Aedes vexans* and *Ochlerotatus sticticus*), the abundance of which typically reflects floods in the wetlands of the Nature Park Kopački rit and inundations close to Osijek, and constitutes a major nuisance to local public (Merdić & Lovaković 2001; Sudarić Bogojević et al. 2007). There are also some other species hatching in stagnant waters and channels (the most numerous *Anopheles maculipennis* complex) and species having their breeding sites in barrels, cans, drains, septic tanks etc. (*Culex* spp.) (Merdić 1996).

The knowledge of the mosquito community structure and seasonal variations in mosquito abundance in the Osijek area, along with meteorological and eco-

logical parameters, is crucial for a precise prediction of mosquito invasions in urban environments and for efficient mosquito control strategies. The objective of this study was to describe temporal changes in adult mosquito abundance and species richness in the Osijek city area.

Material and methods

The study area is the city of Osijek (171 km²; 45°32' N, 18°44' E), which is located near the Nature Park Kopački rit in north-eastern Croatia. Kopački rit is a floodplain area of the Danube River, situated at the confluence of the Drava and Danube rivers, and it is one of the largest alluvial plains in Europe. Floods caused by the increase in water levels of the two rivers create numerous water bodies in inundations along the two rivers and the region of the Kopački rit, creating favourable conditions for mosquito development. The climate of Osijek is moderate-continental, with the rainfall of 700–800 mm/year and absence of very dry months. There are approximately 1800–1900 sunny hours, with 1290–1350 hours during the growing season. The average annual temperature is rather high (10.7°C), with the mean in July ranging between 19.5°C and 21.9°C, and the average temperature of –1.4°C in January, with a relative humidity varying from 70–80%.

The mosquito seasonal distribution and population abundance data were obtained through monthly trapping (on the 10th and 25th day in a month) from May to September in the period 1995–2004. Adult mosquitoes were collected using Centers for Disease Control (CDC) traps which

Table 1. Total number and relative abundance of all adult mosquitoes collected monthly with CDC baited CO₂ traps during the 10-year research in Osijek.

Species/Date 1995–2004	10.5.	20.5.	10.6.	25.6.	10.7.	25.7.	10.8.	25.8.	10.9.	25.9.	Σ	%
<i>Aedes vexans</i> Meigen, 1830	19135	27711	9731	8158	25222	20313	12537	3873	24578	5280	156538	75.57
<i>Ochlerotatus sticticus</i> Meigen, 1838	6986	3998	1090	2239	12004	787	410	18	99	7	27638	13.34
<i>Culex pipiens</i> c. L., 1758	238	342	1116	1845	2828	3677	1208	484	250	152	12140	5.86
<i>Anopheles maculipennis</i> c. Meigen, 1818	365	367	352	921	1016	254	485	180	84	7	4031	1.95
<i>Ochlerotatus cantans</i> Meigen, 1818	1344	195	122	41	137	0	0	0	0	0	1839	0.89
<i>Ochlerotatus caspius</i> Pallas, 1771	272	9	13	251	64	99	68	7	27	387	1197	0.58
<i>Ochlerotatus excrucians</i> Walker, 1856	514	323	296	15	9	11	0	0	0	0	1168	0.56
<i>Culex modestus</i> Ficalbi, 1889	3	9	13	80	140	72	295	79	16	3	710	0.34
<i>Coquillettidia richiardii</i> Ficalbi, 1889	0	9	175	171	171	43	75	25	4	2	675	0.33
<i>Aedes roccicus</i> Dolbeshkin, Gorickaja et Mitrofanova, 1830	98	135	32	1	37	24	1	3	0	7	338	0.16
<i>Aedes cinereus</i> Meigen, 1830	42	6	30	6	2	29	0	65	11	4	195	0.09
<i>Ochlerotatus rusticus</i> Rocci, 1790	128	7	0	0	0	0	27	1	0	0	163	0.08
<i>Culiseta annulata</i> Schrank, 1776	33	1	5	27	23	4	6	0	3	2	104	0.05
<i>Anopheles hyrcanus</i> Pallas, 1771	7	1	3	2	22	1	16	23	3	3	81	0.04
<i>Anopheles claviger</i> Meigen, 1804	0	1	7	10	48	0	0	2	0	1	69	0.03
<i>Ochlerotatus cataphylla</i> Dyar, 1916	12	0	0	0	0	0	0	0	0	0	12	0.01
<i>Anopheles plumbeus</i> Stephanus, 1828	0	0	0	1	0	0	0	0	0	0	1	0.00
<i>Culex territans</i> Walker, 1856	0	0	0	0	0	0	0	1	0	0	1	0.00
<i>Ochlerotatus leucomelas</i> Meigen, 1804	0	0	0	1	0	0	0	0	0	0	1	0.00
<i>Uranotaenia unguiculata</i> Edwards, 1913	0	0	0	0	0	0	0	0	1	0	1	0.00
Not identified	17	30	54	35	15	30	1	48	0	4	234	0.11
Σ	29194	33144	13039	13804	41738	25344	15129	4809	25078	5859	207136	

were baited with dry ice (CO₂), powered with 6V 4Ah batteries and put into the vegetation 1 m above the ground. This trap is a modification of the New Jersey Light Trap (Service 1976). Since this trap does not include a source of light that would attract male mosquitoes and other insects, the CDC trap collects only female mosquitoes. The traps were distributed throughout nine sites in the centre of the city of Osijek and were operated between the dusk and dawn (16.00–09.00 hours). Traps were always set on the same sites and in the same order.

The mosquitoes captured were killed by freezing and processed and identified to species according to the keys by Gutsevich et al. (1976) and Schaffner et al. (2001). The species with an unclear taxonomic status that needed further analysis and confirmation, were defined to the level of complex (*Anopheles maculipennis* complex, *Culex pipiens* complex), so they are listed in the paper in the same manner. Some species were unidentified, but all included in analysis.

Daily averages of the water levels of the Drava and the Danube rivers were calculated from hourly reports of the Hrvatske vode (Croatian Waters).

Correlation analysis was used to examine the relationship between mosquito abundance and species richness by season and by year, as well as mosquito abundance and water level variables.

Results

A total of 207,136 mosquitoes were captured from nine sampling sites in Osijek in 1995–2004. They belonged to 20 species, representing seven genera: *Anopheles*, *Ochlerotatus*, *Aedes*, *Culex*, *Coquillettidia*, *Culiseta* and *Uranotaenia*. Table 1 shows species numbers and relative abundances of each species collected in the study area expressed as a percentage of the total number of adult mosquitoes collected. The quantitative and qualitative analysis of mosquitoes indicates that the most

abundant species were *Ae. vexans*, comprising more than 75% of the total samples, followed by *Oc. sticticus* (13.3%), *Cx. pipiens* complex (5.9%), *Anopheles maculipennis* complex (1.9%), *Oc. cantans* (0.9%), *Oc. caspius* (0.6%) and *Oc. excrucians* (0.6%). The remaining 13 species comprised less than 1.3% of all mosquito species collected during this study (Table 1). Four of them were represented only with one specimen, while twelve *Oc. cataphylla* appeared only in the first seasonal counts in all ten years. Permanent eight species showed moderate population dynamics throughout the season.

The mosquito species richness varied during the ten years of investigation (average annual number of species was 13), as well as during each season. The relative abundance of mosquito populations showed large differences between the years. The greatest abundance was found in 2004 ($n = 46,213$), followed by 1995 ($n = 38,520$) and 2002 ($n = 38,370$), whereas it was lowest during the extremely dry year 2003 ($n = 568$), (Figs 1, 2). Monthly fluctuations in relative adult mosquito abundance changed also throughout the seasons and were displayed in three peaks: end of May, middle of July, middle of September. Seasonal peaks in average mosquito abundance were temporally asynchronous, with the total number of species ($r = 0.21$) (Fig. 3).

Seasonal dynamics of mosquito populations for every year from 1995–2004 with respect to their total numbers is presented in Fig. 2. The total abundance of adult mosquitoes increased every year in different periods of the season. Thereby, the highest peak in 1995 was at the beginning of the season, in 2004 in the middle of the season, and in 2002 at the end of the season, which was due to the water level of the

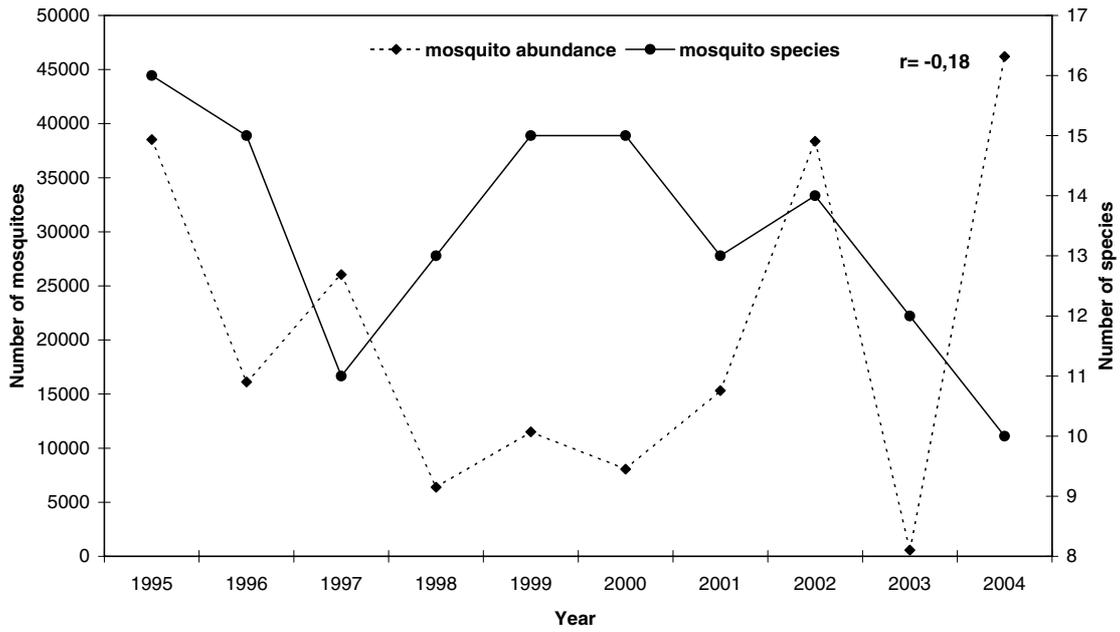


Fig. 1. Correlation between total number of mosquitoes and total number of species in 1995–2004.

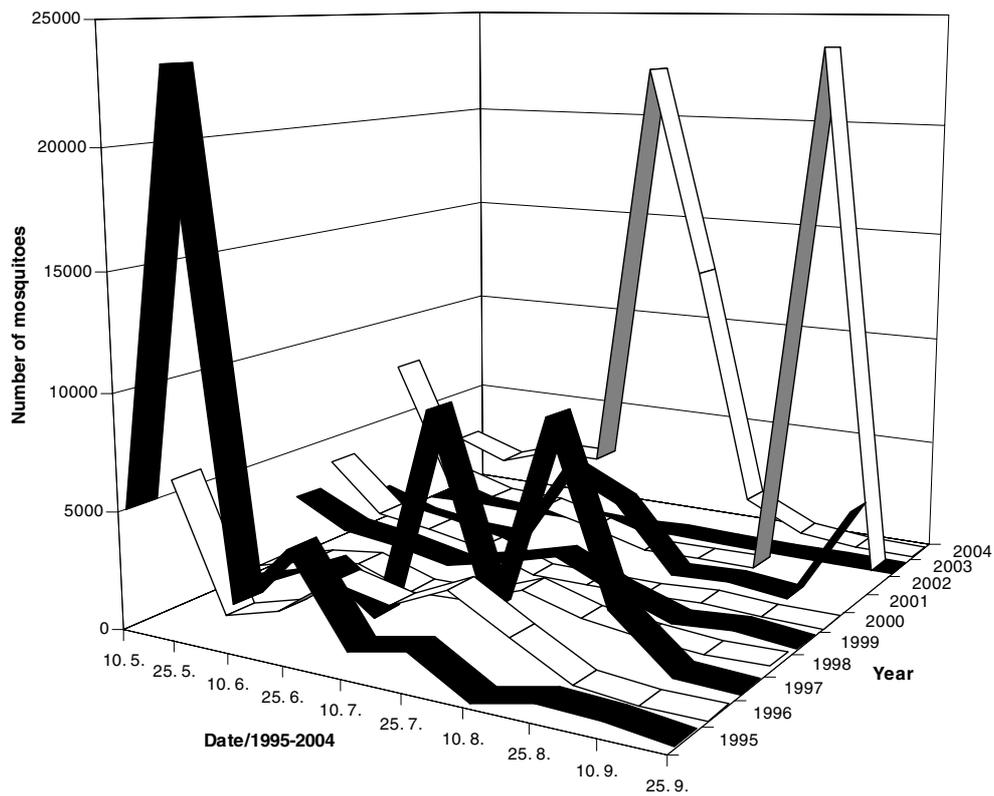


Fig. 2. Seasonal dynamics of mosquito populations with respect to their total number in 1995–2004.

Drava and Danube rivers. In 1997, two similar peaks of mosquito abundance occurred in July and August, while in the remaining six years three peaks (at the beginning, in the middle, and at the end of the season) were typical. In Fig. 3 (dotted line), the relative average seasonal dynamics in abundance of all twenty mosquito species is presented; it matches the peaks stated above. During the ten years research period,

only in two years the seasonal dynamics in abundance highly correlated with the relative average dynamics of mosquitoes: 1999 ($r = 0.63$) and 2004 ($r = 0.66$), while in two dry years, a very low correlation with the average dynamics was found: 1998 ($r = 0.09$) and 2003 ($r = 0.04$) as mosquito abundance decreased approximately almost to the level of disappearance of mosquito populations.

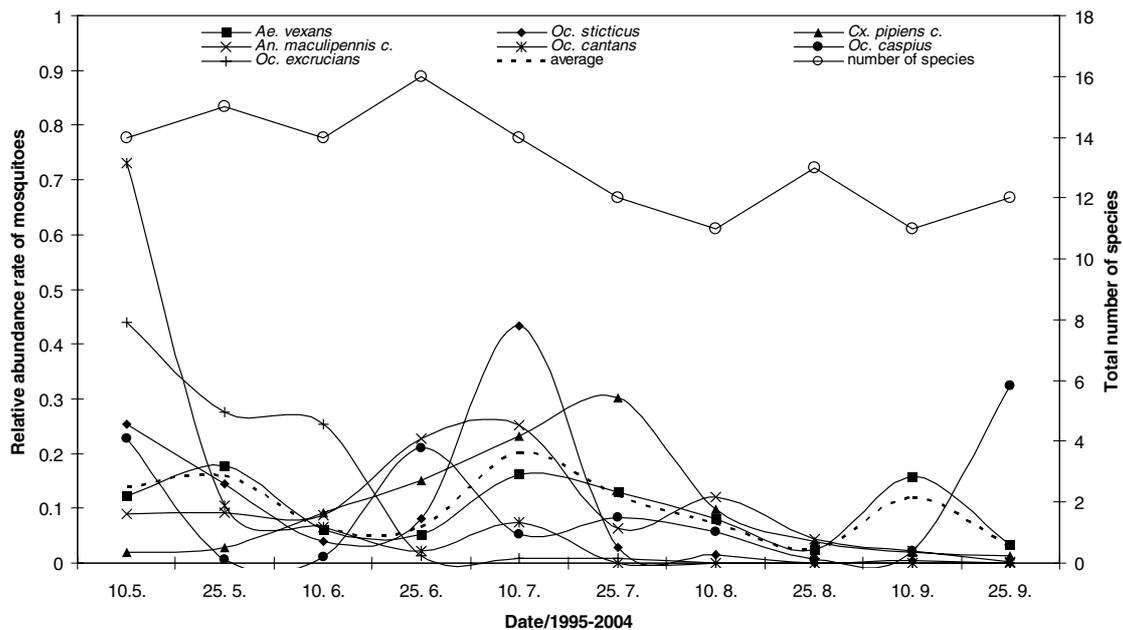


Fig. 3. Total number of mosquito species and seasonal dynamics of the seven most common species with respect to their relative abundance rate during the 10-year research in Osijek.

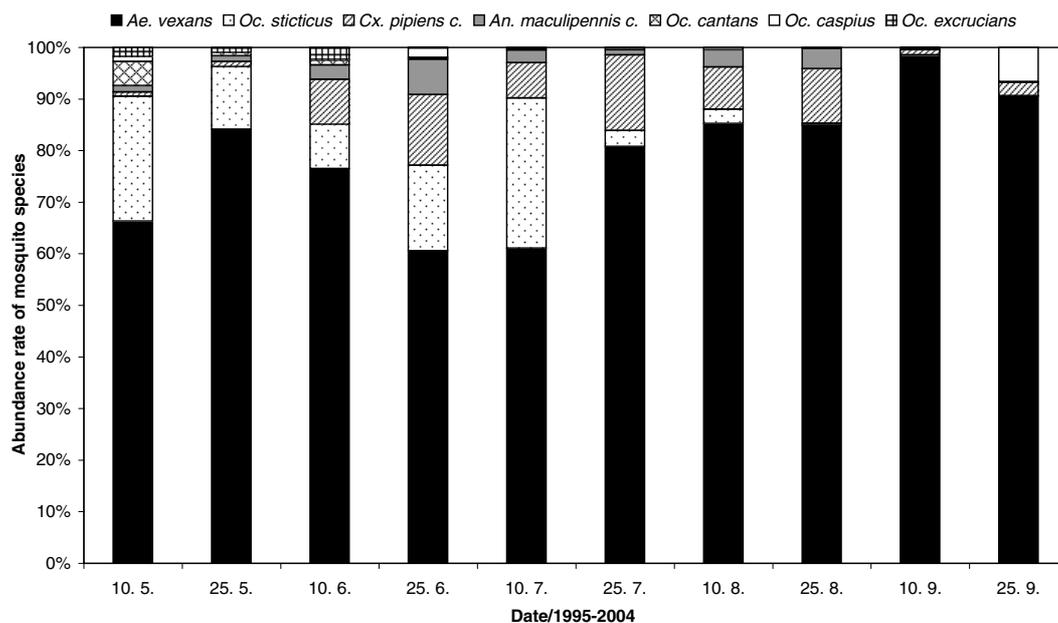


Fig. 4. Percentage of total mosquito numbers of seven mosquito species collected during the 10-year research in Osijek.

The seasonal distributions of seven dominant species reveal population fluctuations (Fig. 3). Because of the high population density of *Ae. vexans*, its seasonal population distribution followed the same general trend as for all twenty mosquito species ($r = 0.94$), (Fig. 3). The majority of *Ae. vexans* specimens was registered on May 25 ($n = 27,711$), which amounts to 83.6% of the total mosquito number caught on that day. It was followed by July 10 (60.4%) and September 10 (98%), (Table 1, Fig. 4). During the whole research period, the dominance of *Ae. vexans* over other mosquito species was evident in every month; its share in the total mosquito numbers

ranged from 59% (June 25) to 98% (September 10) (Fig. 4).

Ochlerotatus sticticus, as the second most abundant species, is characterized by two peaks: at the beginning and in the middle of the season (Fig. 3). The proportion of *Oc. sticticus* among the collected mosquitoes was high on May 10 (23.9%), it decreased by June 10 (8.4%), increased again to the highest average population densities by July 10 ($n = 12,004$; 28.8%), and finally decreased gradually to the end of the season (Table 1, Fig. 4). The seasonal dynamics of the species highly correlates with the average dynamics of the total mosquito population ($r = 0.79$).

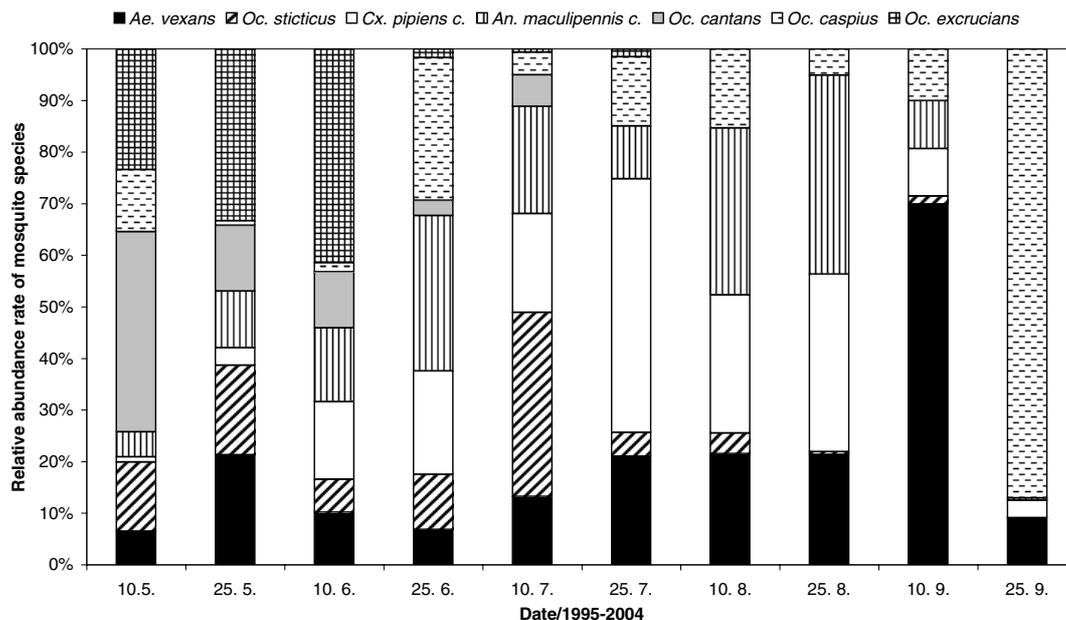


Fig. 5. Relative abundance rate of the seven mosquito species in the total mosquito fauna of Osijek.

The abundance of the *Culex pipiens* complex increased from May 10 ($n = 238$) to July 25 ($n = 3,677$). Towards the end of the season, the population density sharply decreased to only 15 specimens collected during the last two months (Table 1, Figs 3, 4).

Anopheles maculipennis complex displayed a bimodal peak: on July 10 ($n = 1,016$) and August 10 ($n = 485$), (Table 1, Fig. 3). The abundance rate varied from 0.1% at the end of the season to 6.7% on June 25, when the greatest relative abundance rate was recorded (Fig. 4).

The peaks in abundance of the *Oc. caspius* populations occurred on May 10 (0.9%), June 25 (1.8%), September 25 (6.6%) and a smaller peak on July 25 (0.4%). The population dynamics of *Oc. caspius* was negatively correlated with the average dynamics of the total number of mosquitoes ($r = -0.25$) (Table 1, Figs 3, 4).

The five species stated above appeared during the entire year while the species *Oc. cantans* and *Oc. excrucians* were only present in the first half of the season. *Ochlerotatus cantans* occurred from May to July 10 and showed the highest population density at the beginning of May (4.6%) (Table 1, Figs 3, 4). The seasonal distribution pattern of *Oc. excrucians* was quite similar to that of *Oc. cantans*, but with a smaller total number. During the study period, the peak in abundance was observed in May ($n = 837$) and the lowest numbers in July ($n = 20$), (Table 1, Figs 3, 4).

The seasonal distribution in the representation of the seven most abundant mosquito species in the Osijek area expressed as a relative abundance rate is shown in Fig. 5.

The population dynamics of mosquitoes moderately correlated with changes in the water level of the Drava ($r = 0.44$) and the Danube ($r = 0.52$) rivers, as shown in Fig. 6 based on fluctuations in the average

water level of the rivers within the 10-year period of investigation.

Discussion

During the ten years of research various changes in the mosquito fauna and seasonal dynamics of mosquito abundance were documented in the city of Osijek. Temporal variation in the mosquito population dynamic is linked with environmental conditions in the breeding habitats, such as weather and hydrological data (Reisen & Reeves 1990; Reisen et al. 2007). The number of mosquito species varied in every year of investigation, whereas mosquito abundance showed marked differences between years.

The annual temporal pattern in the relative mosquito abundance closely paralleled the water level dynamics in the Drava and Danube rivers, especially in 1999 and 2004. In 1999, the water level in Kopački rit was stable for a longer period, but quite small numbers of mosquitoes were collected. One of the possible reasons for that could be the fact that the water level of the Danube River was very high in September and October 1998. Therefore, the summer floodwater mosquitoes laid their eggs at a distant ground, which resulted in the absence of spring population's peak in the following year. Another reason was the fact that ecological conditions caused by the high water level in the flooded area resulted in a different composition and lower abundance of the mosquito fauna in comparison with that recorded when the water level in the flooded area fluctuated (i.e. in 1995, 1997, 2002 and 2004). At the same time, in 2004 the number of occurring species decreased to a minimum (10). The observed changes obviously represented a part of long-term fluctuations in water dynamics as culmination of a warmer and dry season was also recorded in 2003.

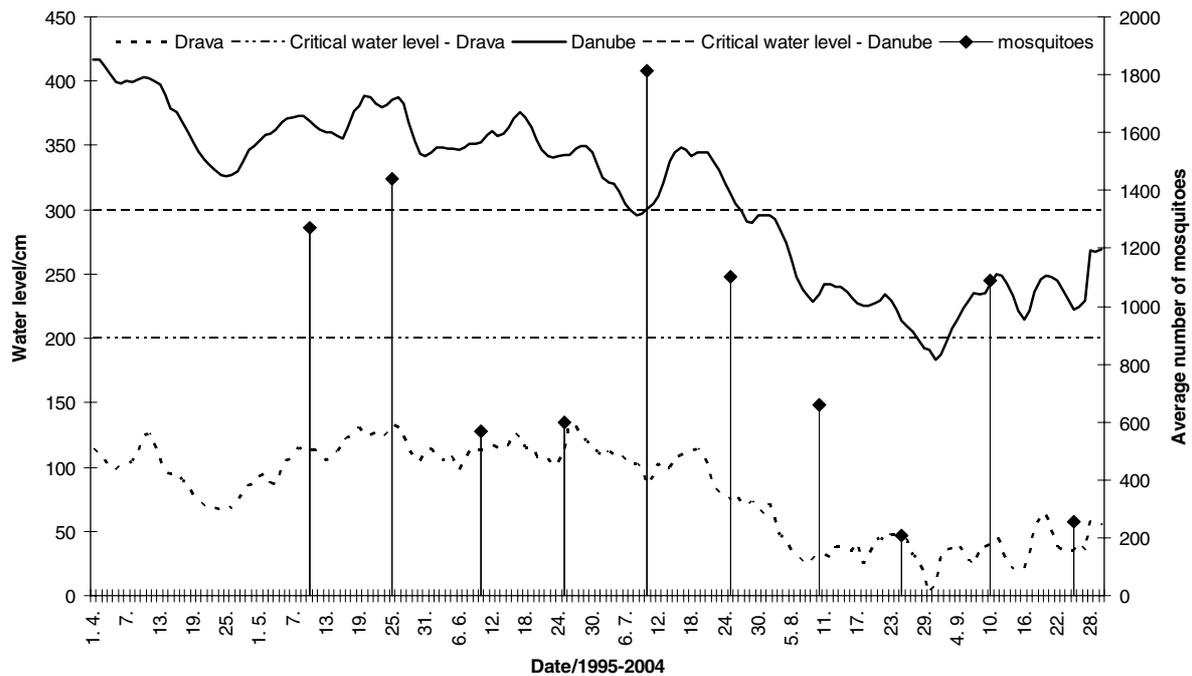


Fig. 6. Water level fluctuations of the Danube and the Drava rivers from April to October 1995–2004, in relation to the average mosquito abundance. The critical water levels of the Danube (+300 cm near Apatin) and the Drava (+200 cm near Osijek) means the thresholds of water inflow in the inundation along the rivers).

The composition and abundance of mosquito fauna are often influenced by predominant species (Reisen et al. 1983; Merdić & Lovaković 2001; Schäfer & Ludström 2001; Rydzanicz & Lonc 2003; Jackson & Paulson 2006; Schäfer et al. 2008). This research also discovered the prevalence of *Ae. vexans* (75.6%) as well as its satellite *Oc. sticticus* (13.3%) in the Osijek area as well as in the Kopački rit area (Merdić 1993). Both species belong to floodwater mosquitoes which develop in wetlands and inundations (40% wetlands in the Osijek-Baranja County are inundations along the Drava River, and 60% inundations along the Danube River) and invade the city of Osijek as a result of an increased water level of the Danube and the Drava rivers (e.g., due to snow and ice melting in the Alps), heavy precipitation and optimal abiotic factors in breeding sites. This research has proved that the dynamics of the water level of rivers directly affects the number of generations of floodwater mosquito species and their abundance rate in the respective period (Merdić & Lovaković 2001). In a study in Senegal, Mondet et al. (2005) detected the proportional influence of rainfall patterns on the number of generations and abundance of the *Ae. vexans arabiensis* population. Many scientists also found a strong relationship between the river runoff and mosquito abundance (Wegbreit & Reisen 2000; Minář et al. 2001; Shaman et al. 2002; Kim et al. 2006, Schäfer et al. 2008), though the impact of flooding water dynamic on reproduction breeding sites is not identical for all mosquito species (Porphyre et al. 2005).

The species *Ae. vexans* is most numerous in May, which is a consequence of the first spring water wave which is usually the highest, so it floods a great area comprising the mosquito breeding site. Although the

dynamics of this species varied throughout the season, its dominance was not endangered at all. Inter-annual variation in abundance of *Ae. vexans* strongly correlated with the average dynamics of the other mosquito species ($r = 0.94$), as could be expected due the high representation in the total mosquito fauna (75.6%). Petrić (1989) found a similar prevalence of *Ae. vexans* (55%) in Vojvodina. *Ae. vexans* can move to a distance up to 32 km from the breeding site (Brust 1980; Carpenter & LaCasse 1955; Headlee 1945; Sparks et al. 1986). In a research on mosquito migration in the Osijek area, the species has been observed 7.7 km from the breeding site (Sudarić Bogojević et al. 2007). This indicates that host-seeking females of *Ae. vexans* could disperse to Osijek and its surroundings from Kopački rit and cause severe nuisance for humans and domestic animals.

There are three other species relevant for the long-term population monitoring because they make 21% of the total mosquito fauna. These are: *Oc. sticticus*, *Cx. pipiens* complex which is represented in the explored area by two infraspecific forms, *Cx. p. pipiens* f. *pipiens* and *Cx. p. pipiens* f. *molestus* (Merdić 1996; Vinogradova 2007), whereas the *An. maculipennis* complex is represented by the species *An. messeae* and *An. maculipennis* (Merdić & Boca 2004).

Ochlerotatus sticticus is associated with *Ae. vexans* with larvae in habitats such as marshes, floodplains and inundation sites and one of them is often predominant (Schäfer & Lundström 2006). High abundance of both species is influenced by the extent of flooding, while climatic conditions after the floods might influence which species dominates in the adult mosquito fauna. Monthly population patterns of *Oc.*

sticticus include the first peak at the end of May, second peak during the second week in July, and third peak at the end of the season. The same seasonal dynamics of the species was reported in Slavonski Brod in 2001 (Merdić et al. 2003). *Ochlerotatus sticticus* is widespread and a nuisance mosquito that can fly up to 11.7 km from the breeding site (Sudarić Bogojević et al. 2007).

Nevertheless, the cumulative effect of breeding sites on mosquito abundance and species richness indicates the need for systematic mosquito surveillance activities in order to prevent the development of new potential breeding sites and treat the existing ones.

Determining the temporal changes in population abundance of urban mosquito species is a crucial step towards understanding the life cycle of potential vector species and optimizing vector control programs. The *Cx. pipiens* complex was predominant only in 2003 in very dry conditions suggesting that the population dynamics of these species is strongly connected with urbanization and they use every potential stagnant water as breeding sites (Vinogradova 2000; Su et al. 2003). Seasonally, spring floods and precipitation formed countless potential habitats that increased the abundance of the *Cx. pipiens* complex which started to create the population peak in May and reached maximum abundance in the middle of summer. Due to the fact that the life cycle of the species is much more intensive and progressive than the sampling itself (only twice a month) it might happen that particular generations were missed so that the seasonal dynamics is not realistic. More extensive and frequent sampling, beginning earlier in the season is needed to determine the seasonal fluctuations of this species. However, the dynamics analysis of the other six species was carried out under the same conditions, so the comparisons can be considered valid. A further study on the ecology of these species is warranted because some research indicated that temporal parameters were more important in identifying vector abundance than were the spatial variables (Bolling et al. 2005; Lampman et al. 2006).

In relation to population abundance, the following species do not create a large nuisance problem in the Osijek area, but their roles in pathogen transmission has been confirmed by many authors (Lundström 1999; Gaunt et al. 2001; Bakonyi et al. 2005; Gratz 2006; Hubálek 2008).

The population dynamics of the *An. maculipennis* complex is bimodal (Merdić & Boca 2004). *Ochlerotatus caspius* is characterized by three to four seasonal peaks, which are included in the description of the species biology (Merdić 1996; Bellini et al. 1997). Mosquito species belonging to the *Oc. cantans* group breed in forest habitats and only one generation per season is usually reported. This fact results from disappearing suitable habitats under prolonged dry conditions. The temporal pattern of *Oc. excrucians* is reflected in two peaks out of which the first one is much bigger.

The seasonal prevalence of twenty mosquito species varied: some were constant during the investigation,

some were sporadic, and some were associated with a specific period of the season. The species that should be considered medically important vectors of West Nile virus and can be found in the city of Osijek are *Cx. pipiens* and *Cx. p. pipiens* f. *molestus*, *Coquillettidia richiardii* and *Cx. modestus* as key vectors in Europe, followed by *Oc. caspius*, *Oc. cantans*, *Oc. sticticus*, *Ae. vexans*, *Ae. cinereus*, *Culiseta annulata* and *An. plumbeus* (Zeller & Schuffenecker 2004; Medlock et al. 2005). Further research on the mosquito population ecology would facilitate the planning of effective control strategies in the city of Osijek.

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