

RESULTS OF TEN YEARS OF MOSQUITO (DIPTERA: CULICIDAE) MONITORING IN OSIJEK, CROATIA

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The monitoring of the mosquito population for the purpose of possible better mosquito control took place in Osijek from 1995-2004. For the period of ten years, the primary research method for monitoring the mosquitoes used was dry ice baited CDC-traps. This provided significant information on mosquito species, their dynamics and seasonal activity. In total, 207,136 mosquito specimens were collected. Among this number 20 species were identified, however, 7 species made up 99% of the total. From these 7, three ecological groups based on breeding sites were determined: flood water mosquitoes, container breeding mosquitoes, and permanent and semi-permanent water breeding mosquitoes. The seasonal dynamics of these three groups are shown in this paper. The average dynamics of all mosquito species was reflected in three peaks: at the end of May, in the middle of July (the biggest) and in the middle of September. The eudominant species was *Aedes vexans* which comprised 76 % of the total mosquito fauna in the investigated area. The flood water species highly correlated with the average dynamics of all the species in the area of Osijek as well as with the average water level of the Danube River.

Mosquitoes, seasonal dynamics, water level, *Aedes vexans*, monitoring, CDC-trap

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U programima kontrole komaraca odabiru se tehnike suzbijanja komaraca kojima prethodi uzorkovanje ličinki i odraslih komaraca različitim metodama kojima se procjenjuje veličina populacije. Dugogodišnje istraživanje odraslih komaraca, koje je predstavljeno u ovom radu, provedeno je u Osijeku metodom CDC-klopke sa suhim ledom kao atraktantom. To istraživanje dalo je važne rezultate o vrstama komaraca, dinamici njihova pojavljivanja i sezonskoj aktivnosti. U desetogodišnjem je razdoblju navedenom metodom ukupno

uhvaćeno 207 136 jedinki komaraca. Jedinke sedam od determiniranih 20 vrsta čine 99 % ukupnoga broja komaraca. Najdominantnija među njima jest vrsta *Aedes vexans* koja čini 76 % u ukupnoj fauni komaraca navedenoga područja. Sezonska dinamika komaraca različita je za vrste komaraca poplavnih, urbanih staništa i komaraca permanentnih vodenih staništa. Prosječna dinamika svih 20 vrsta komaraca ima tri vršne vrijednosti: krajem svibnja, sredinom srpnja i sredinom rujna. Dinamika poplavnih vrsta (*Ae. vexans* i *Oc. sticticus*) u visokoj je korelaciji s prosječnom dinamikom svih vrsta komaraca na području Osijeka, kao i s prosječnom visinom vodostaja Dunava. Prosječna sezonska dinamika urbanih komaraca i komaraca permanentnih voda znatno se razlikuje od poplavnih. Utjecaj vodostaja rijeka Drave i Dunava u direktnoj je vezi s brojem komaraca na istraživanom području.

komarci, sezonska dinamika, vodostaj, *Aedes vexans*, monitoring, CDC-klopka

Introduction

A well organized programme of mosquito control must comprise mosquito research, monitoring, treatments of larval and adult mosquitoes, and the control of these treatments. In Croatia the Programme for Obligatory Disinfestation, is given by law (“Narodne novine – Official Gazette of the Republic of Croatia”, No 60/92, 26/93, 29/94).

Mosquito control is not solely aimed at decreasing the number of mosquitoes to avoid the irritation caused by mosquito presence and bites, but also for the wider goal of mental and physical healthcare for the population. Apart from annoying man with their buzzing, mosquitoes are molesters (lat. *moleste* – to annoy) responsible for severe itching at bite spots related to allergic skin reactions. Mosquitoes also disturb people’s peace and health, and in a broader sense they distract people in everyday activities, leisure, and outdoor recreation. Furthermore, because of their role as potential vectors of diseases such as malaria, Yellow fever, Dengue, encephalitis etc, it is no surprise that mosquitoes are subject to numerous biological and epidemiological studies and are one of the most explored insect groups.

In some EU countries (France and Germany) mosquito control has achieved good results in (Becker, 1997) protecting urban areas from mosquitoes; however, mosquito control results in Croatia are patchy. The reason is that systematic mosquito control is not implemented in wider areas but only in major towns (Osijek, Zagreb). Systematic mosquito control on a professional basis began in the Osijek

area in 1978, but only since 1995 has it had scientific and professional support from the mosquito experts from the Department of Biology of Josip Juraj Strossmayer University of Osijek.

The mosquito control programme in Osijek deals with the nuisance of floodwater mosquitoes which breed in the nearby wetlands of Kopački rit and inundations close to Osijek. Problems caused by mosquitoes are only seen when humans and adult mosquitoes interact. Consequently, in order to issue a warrant for mosquito control, one or more methods for sampling adult mosquitoes are used in almost 90 % of programmes in the world. One of the methods includes an aspirator which captures mosquitoes directly from a person while another one is based on sampling by CDC-traps (Centre for Disease Control, 1978), originating from the New Jersey Light Trap (NJLT). These traps can be used for mosquito control, and 25 females caught during one night by NJLT is considered as standard in USA (Florida Coordinating Council on Mosquito Control, 1998; Ellis, 2004; <http://www.cityofgainesville.org>; <http://www.nature.nps.gov>). This standard is not applicable to CDC-traps. The Osijek area, where CDC-traps are also in use, cannot be assessed according to a tolerance threshold of 100 mosquitoes caught a night (<http://www.mda.state.md.us>) since that number is often exceeded.

The CDC-trap is the main tool in numerous programmes of adult mosquito monitoring due to the following reasons: it is designed to resist various field conditions and can be used for a certain period of time (up to 24h); it determines the qualitative and quantitative structure of mosquito species; enables sampling of a wide variety of mosquito species in a particular area; it enables an overview of the daily and seasonal activity of mosquitoes – which is a prerequisite for systematic adult control; it provides the determination of mosquito distribution in a larger area; enables checking the efficiency of adulticiding; and it collects living material that may be subject to further analyses (biological, chemical etc.).

The dry ice baited CDC traps were used in the Osijek mosquito control program as one of the essential components. Some results of the ten years of usage of these traps are presented in this paper.

Materials and methods

The investigation was carried out in the broader area of the city of Osijek figure 1, (north-eastern Croatia, UTM CR25 and CR26), during a period of 10 years. This study discusses the data obtained from 1995 to 2004. Battery-opera-

ted, dry ice-baited CDC traps were set in 9 locations (Donji grad N 45 33 41,2 E 18 43 37,4; Jug II N 45 33 7,2 E 18 42 34,3; Sjenjak N 45 33 7,2 E 18 42 34,3; Iktus N 45 33 44,7 E 18 41 46,7; Županijska N 45 33 20 E 18 40 49,8; Strma N 45 33 57,9 E 18 40 7,2; Retfala S N 45 34 2,5 E 18 39 20,3; Retfala J N 45 33 25,4 E 18 39 13,3 and Josipovac N 45 34 23 E 18 36 16,9) and operated twice a month from May to September each year. The traps were put in yards of houses 1 m from the ground and active for over 24 hours ($\pm 2h$), with a 9 kg dry ice cube. A total of 207,136 mosquitoes were collected in 900 samplings during this period.



Figure 1. Map of investigated area with sites: 1 Donji grad, 2 Jug II, 3 Sjenjak, 4. Iktus, 5 Županijska, 6. Strma, 7 Retfala S, 8 Retfala J, 9 Josipovac

The determination of species was performed according to the keys stated in Gutshevich et al, 1976, and Schaffner et al, 2000. The prepared mosquitoes are stored at the Department of Biology of the Josip Juraj Strossmayer University of Osijek.

The data of the water levels of the Drava and the Danube were obtained from Hrvatske vode.

Results

During 10-year period a total of 207,136 mosquitoes belonging to 7 genera and 20 species were caught in the town of Osijek. The seven prevalent species are the following: *Aedes vexans*, *Ochlerotatus sticticus*, *Culex pipiens* complex,

Anopheles maculipennis complex, *Ochlerotatus cantans*, *Ochlerotatus caspius* and *Ochlerotatus excrucians*, and together make up 99 % of the captured species.

During all those years the highest number of mosquitoes was reported in the middle of the season, in July, which is confirmed by 67082 caught specimens, being 32 % of the total mosquito number. Slightly fewer mosquitoes were caught in May (62338), while half as many mosquitoes were captured in September (30935). Then June (26843) and August (19938) were found to be the months with decreased mosquito numbers. Each of the three significant months has its peak of mosquitoes caught in traps with regard to the entire research period: 20 % of the total number of mosquitoes was caught on July 10, 16 % on May 25, and 12 % on September 10. Figure 2 presents the three values which match the peaks stated above and, at the same time, represents the dynamics of mosquito numbers throughout the season. The relative, average dynamic of mosquitoes is shown in figure 3 (dotted line) where three mosquito “invasions” can be seen as well: at the end of May, in the middle of July and in the middle of September.

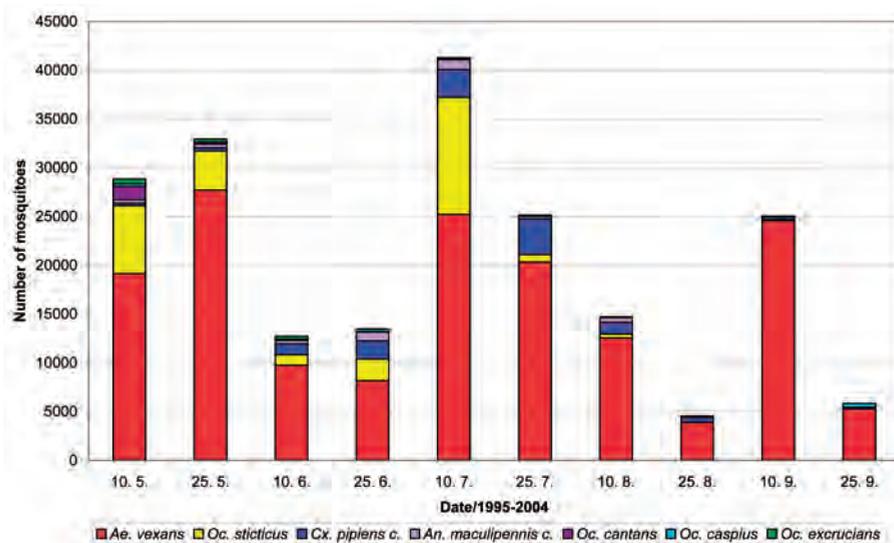


Figure 2. Total number of mosquito per season during the 10-year research in Osijek

The analysis of mosquitoes indicates that two floodwater species are the most numerous. *Ae. vexans* is eudominant with a share of 76 % in the mosquito fauna of the city of Osijek. The number of specimens fluctuates, and biggest number was registered at the end of May (27711), which amounts to 84 %. Two other peaks were registered, the first at the beginning of July and the second at the beginning of September. The biggest percent (98 %) of *Ae. vexans* over other mosquito species during the entire research period was most evident at the beginning of September (figure 2).

The second most numerous species is also a floodwater species, *Oc. sticticus*, with a total share of 13 %. The largest number of specimens (12004) of that species was registered at the beginning of July when its share in the total mosquito fauna amounted to 29 % (figure 3)

Furthermore, the container-breeding species *Cx. pipiens* complex is connected with urban areas only, with a share of 6 % in the Osijek mosquito fauna. This species was most numerous during July (6505; 10 %). The relative share of this species reached its peak at the end of July.

The permanent and semi permanent water breeding species (*An. maculipes* complex) had its own dynamics. The relative share of this species reached its peak in July (figure. 3).

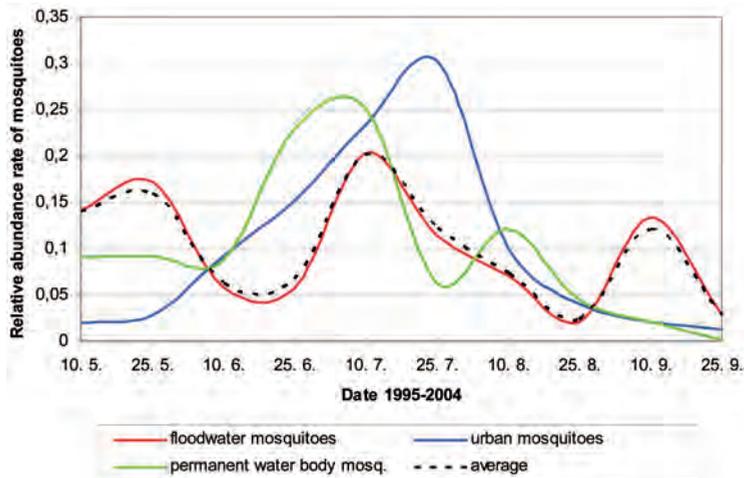


Figure 3. Seasonal dynamics of flood water, permanent water body and urban mosquitoes species in respect to their relative abundance rate

Five out of the seven most numerous species appear during the entire year while the species *Oc. cantans* and *Oc. excrucians* are only present in the beginning of the season with a significant share and disappear by the middle of the season.

The average seasonal dynamics of all twenty mosquito species overlaps with the dynamics of the two floodwater species (dotted line on figure. 3), ($r=0.985$, $p<0.001$). the container-breeding species of *Cx. pipiens* complex is most common during the summer months and its presence culminates at the end of July. The permanent and semi permanent water breeding species *An. maculipennis* complex features two peaks: the first one at the end of June and the beginning of July, and the second one in the first half of August.

The critical water levels with inundations of the Drava River and water inflow to Kopački rit from the Danube, are the following: for the Drava +200 cm in Osijek and for the Danube +300 cm in Apatin (Mikuska, 1979). Figure 4 outlines the average water level of the rivers within the 10-year period. The water level highly correlates with the average dynamics of mosquitoes in the same period.

Discussion

The mass nuisance of floodwater mosquitoes occurs in spring, April and May in particular, as a consequence of an increase in the water level (snow and ice melting in the Alps) of the two big rivers – the Danube and the Drava. After flooding, the development of great mosquito populations starts in inundations along the two rivers and the wetland of the Kopački rit Nature Park. Depending on the dynamics of water inflow and outflow, floodwater mosquito generations appear in the area each season. Previous research into mosquitoes has shown that the first generation of mosquitoes is usually the largest due to frequent overlapping with the highest water wave (Merdić & Lovaković 2001).

The geographical, hydrological and climatologic features of Kopački rit provide ideal conditions for developing large mosquito populations. Although more than 90 % of mosquitoes in the Osijek area belong to floodwater mosquito species, it is vital to mention that there are some other species that breed in containers and permanent water bodies.

The knowledge of the biology and the life cycle of mosquitoes enables timely mosquito control in the adult and/or larva stage.



Figure 4. Average water levels of the Danube and the Drava in the period from February to October 1995 – 2004

Changes in the fauna and seasonal dynamics of mosquitoes were recorded during the ten years of monitoring. The long-term adult mosquito monitoring has determined the dominant species and those which are present during the whole season, as well as, those that only appear in the beginning of the season.

A total of 32127.62 ha in the Osijek-Baranja County (Hrvatske vode data) are wetlands, out of which 40 % are riparian zones along the Drava river, and 60 % riparian zones along the Danube. Of the total wetlands of the county, 37 % belong to Kopački rit, which depends on the water level of the two rivers. Due to favourable biotic and abiotic factors (Gjullin et al, 1950), the primary breeding site of floodwater mosquito species in the area of Osijek in a broader sense is Kopački rit.

The seasonal dynamics of the eudominant species, *Ae. vexans*, is almost identical to the average dynamics of all the mosquito species ($r=0.935$, $p<0.001$), which is understandable since the *Ae. vexans* species make up 76 % of the total mosquito fauna in the research area. A similar situation was registered in Vojvo-

dina with *Ae. vexans* as the prevalent species, having a share of 55 % in the total mosquito fauna (Petrić, 1989)

Since the Kopački rit is situated next to the town, it is to be expected that female mosquitoes in search of food (blood) migrate toward Osijek and other populated areas with a higher concentration of food and CO₂ (Horsfall et al., 1973). Some specimens of *Ae. vexans* can fly up to 32 km from the breeding site (Brust, 1980; Carpenter & LaCasse, 1955; Headlee, 1945; Sparks et al., 1986), so with respect to monitoring they are seen as a big issue when they fly away from the breeding site, across the wetlands, and into the urban area. It has proved that they migrate up to 7.7 km to Osijek from the breeding site in the Kopački rit (Sudarić Bogojević et al., 2007).

The species *Oc. sticticus* is a floodwater species too, with the possibility of reaching great numbers under special conditions. In the middle of July it reaches its peak in number and proportion with respect to the total mosquito population. The same status of the species was reported in Slavonski Brod in 2001 (Merdić et al., 2003). Unlike *Ae. vexans*, *Oc. sticticus* has a third peak at the end of the season. The species is aggressive, anthropophilic and very skilful in the air. It is noted that some specimens can fly up to 11.7 km from the breeding site (Sudarić Bogojević, 2005).

Besides the wetlands, there are other breeding sites, which are not so significant in the number and species they produce. The *Cx. pipiens* complex and *An. maculipennis* complex are characterized by such breeding sites and they appear in a great number during pluvial periods. The domestic, urban mosquito *Cx. pipiens* complex is a species that uses every potential stagnant water source (channels, depressions filled with water, barrels, cans, flower pots etc.) for its breeding sites (Vinogradova, 2000).

As many as 50 mosquito species have been registered in Croatia (Merdić et al., 2004, Klobučar et al., 2006, Žitko & Merdić, 2006), and 20 were recorded within this work. Some of these species are molestants only but other species are potential vectors. The potential vectors of the West Nile Virus are the following species: *Cx. pipiens*, *Ae. vexans* and *Oc. sticticus* (<http://www.cdc.gov/ncidod/dvbid/westnile/mosquitoSpecies.htm>), and they all appear in Croatia, i.e. Osijek (Merdić, 1996; Merdić & Lovaković, 2001; Merdić & Sudarić Bogojević, 2005), thus the justification for monitoring.

The container-breeding species, *Cx. pipiens* is most numerous in summer and has one seasonal peak only. Moreover, it starts to multiply in May and keeps on increasing in number until the end of July when its number began to go down slowly. The number of generations varies depending on the season (from one to ten) and it is chiefly connected with the availability (precipitation, human factor) and constancy (outflow, evaporation and etc.) of stagnant water, i.e. the source of water needs to be stable at least for a couple of days during summer in order to provide a fertile habitat for the development of mosquitoes.

The *An. maculipennis* complex breeds in stagnant waters (ponds, water tanks, channels) and also in the wetlands of Kopački rit while its first peak overlaps with the turn of June into July and the second smaller one with the middle of August, which was confirmed by Merdić and Boca (2004). The investigations by Merdić (1990) show that this complex includes two species, *An. messeae* and *An. maculipennis*, recorded in the Osijek area.

The wetlands along the Danube and the Drava are the main source of mosquitoes in the city of Osijek and the Osijek-Baranja County, so the treatments of mosquito larvae with highly selective biological insecticides (based on *Bti*, Becker, 1997) need to be aimed at that region in order to decrease the number of mosquitoes. Due to the fact that decisions on mosquito control have to be made according to realistic and precise monitoring data, every wrong assessment on the area and time of treatments (larval and/or adult) will harm the environment, and involve an economic loss.

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