# Mosquito (Diptera: Culicidae) fauna in an area endemic for West Nile virus

O. Šebesta[1], J. Halouzka[1], Z. Hubálek[1✉], Z. Juřicová[1], I. Rudolf[1], S. Šikutová[1], P. Svobodová[1], and P. Reiter[2]

[1] *Institute of Vertebrate Biology ASCR, Květná 8, 60365 Brno, Czech Republic*
[2] *Institut Pasteur, 25-28 rue du Dr Roux, 75015 Paris, France*

*Received 27 October 2009; Accepted 23 March 2010*

ABSTRACT: Mosquito collections with CDC light traps using dry ice and pigeon-baited traps were carried out in south Moravia (Czech Republic) from April to October in 2007 and 2008 at two study sites. In 2007, 11 two-day captures were carried out in two-week intervals, and 1,490 female mosquitoes of nine species were caught. In 2008, 15 two-day trappings of mosquitoes were carried out: 6,778 females of 22 species of mosquitoes were trapped. The results showed marked differences in abundance and species composition of mosquitoes between both study sites and between the trapping methods. In the floodplain forest ecosystem of the Soutok study area, *Aedes vexans* predominated. The species composition in the Nesyt study site was more varied and the most common species was *Culex pipiens*. At the latter study site, *Anopheles hyrcanus* (var. *pseudopictus*) and *Uranotaenia unguiculata,* mosquito species with largely southern Eurasian distribution, were repeatedly demonstrated. The largest capture of mosquitoes was in traps with $CO_2$ placed at a height 1 m above the ground. The capture of mosquitoes in the pigeon-baited traps as well as in the traps with $CO_2$ placed in the canopy of trees was markedly lower in both study sites, with the predominant species being *Culex pipiens*. ***Journal of Vector Ecology* 35 (1): 156-162. 2010.**

*Keyword Index: Anopheles, Aedes, Culex*, climate, ecology, mosquitoes.

## INTRODUCTION

Special attention has been directed toward mosquitoes in south Moravia within the Czech Republic as a result of the frequent flooding of their extensive breeding sites in flood-plain forests along the lower courses of the Morava and Dyje rivers (Kramář and Weiser 1951). Numerous entomological studies have been carried out here (Kramář 1958, Palička 1967, Vaňhara and Rettich 1998, Minář et al. 2001, Olejníček et al. 2004, Rettich et al. 2007). Regular monitoring of mosquito breeding sites, primarily focused on protecting the human population from this pestilent insect, has also taken place in the region since 1995 (Šebesta, unpublished data).

A second reason for the increased interest in mosquitoes of south Moravia is the relatively warm climate of this region. The lowlands near the lower courses of the Morava and Dyje form one of the warmest regions of the Czech Republic and, due to their position in proximity to the Pannonian lowlands and the lowlands of Lower Austria, serve as a gateway for thermophilic species of plants and animals. Several interesting species of mosquitoes have been discovered here. In the middle of the 20th century, for example, the occurrence of *Anopheles atroparvus* and *An. labranchiae* (Havlík and Rosický 1949, 1952, Rosický and Havlík 1951, Minář and Rosický 1975), the incidence of which was not reported in other parts of the Czech Republic, was recorded here. Other interesting findings arose in the 1970s and 1980s, when the incidence of *Ae. nigrinus* (Vaňhara 1987), and the Mediterranean species *Uranotaenia unguiculata* (Ryba et al. 1974) and *Culex martinii* (Vaňhara 1981, 1986), was noted here for the first time in the Czech Republic. The latest species found is *An. hyrcanus* (Votýpka et al. 2008, Šebesta et al. 2009). These species had also not been found previously at other locations in the Czech Republic. Of the 45 species of mosquitoes whose occurrence within the entire Czech Republic has been reported (Minář and Halgoš 1997, Országh et al. 2006, Rettich et al. 2007), 37 species in total have been detected in south Moravia (Vaňhara 1991, Vaňhara and Rettich 1998, Rettich et. al. 2007, Šebesta et al. 2009).

Great attention is also devoted to local mosquitoes as potential vectors of pathogens, from which the Tahyna, Batai, Lednice, and West Nile viruses have been recorded (Danielová et al. 1972, Rosický et al. 1980, Hubálek et al. 1998, 2000). Up until the middle of the last century, an endemic incidence of malaria was noted in this region (Havlík and Rosický 1952).

As part of the European project EDEN (2005–2009), mosquito fauna has been studied since 2006 in the south Moravian endemic region of the "Rabensburg" genomic lineage of West Nile virus (Hubálek et al. 1998, 2000, Bákonyi et al. 2005). The aim of this study was to analyze species composition of local mosquito fauna, compare it with previous reports of other authors, and collect material for subsequent arbovirological studies.

## MATERIALS AND METHODS

The two selected study sites, where the presence of the West Nile virus had been documented, are about 20 km distant from one another. They constitute two distinct, yet


Figure 1. Map of study sites in the Czech Republic.

typical, habitats for the monitored region (Figure 1).

The Soutok area (48°37' – 48°44'N, 16°53' – 16°59'E; 151–153 m above sea level) is part of an extensive complex of floodplain forests and irregularly inundated meadows located between the Morava and Dyje Rivers and in close proximity to their confluence ("*soutok*" in Czech), relatively unaffected by human activities. Spring floods of various amounts are an almost yearly event and are caused by groundwater and seepage water. The region also often tends to be flooded by overflowing rivers. In the summer months, overflows occur irregularly and sometimes repeatedly in a single year. The main breeding sites of *Aedes* species are found here. In this location, two localities were selected. The results from both, however, are processed together. In each of the two locations, the West Nile virus (Rabensburg lineage) was recently isolated from *Cx. pipiens* mosquitoes (Hubálek et al. 1998, 2000, Bakonyi et al. 2005).

Nesyt Fishpond (48°47'N, 16°43'E, 176 m a.s.l.) is located near the village of Sedlec. It is part of a complex of five fishponds forming the Lednické Rybníky National Nature Reserve. Nesyt was established in 1418, and with an area of 322 ha, it is the largest Moravian fishpond. Its banks are bordered with a dense, almost impenetrable growth of reeds (*Phragmites communis*), which in some places reaches a width of several tens of meters. The mosquito trapping site is comprised of a cluster of trees (willows) and shrubs growing on the edge of the waterfront vegetation and is bordered by a meadow with the Slanisko Nature Reserve, characterized by the appearance of halophilic plants and insects (e.g., *Scorzonera parviflora, Tripolium pannonicum, Bucculatrix maritima, Coleophora halophilella)*. At Nesyt, circulation of the West Nile virus was documented indirectly years ago by the detection of specific antibodies in local domestic ducks (Juřicová and Halouzka 1993). The location is outside the flooded area, and the condition of the water there is stable.

The region of south Moravia is characterized by a relatively dry and warm climate with an average daily temperature of 9.3° C and an average annual precipitation of 490 mm. From a meteorological perspective, the conditions in the two years of this study (2007, 2008) were different. The winters were warm, and no snow cover was formed in south Moravia. The mean January 2007 temperature was +4.2° C (the warmest January within the last 50 years; difference from the average 1961-1990 is +6.1° C). In January 2008 the mean temperature was +2.1° C (difference +4.0° C from the average). Mean temperatures in February 2007 and 2008 were +4.4° C (difference +4.1° C) and 3.2° C (difference +2.9° C), respectively (Figure 2). Snowfall was low even in the mountains in the Czech Republic, and thus the spring floods did not arrive. The flow rate of water in the Morava and Dyje Rivers was below average for nearly the entire year, and thus neither river overflowed. The larval site at the Soutok location was inundated only by groundwater and seepage water for only a short period and to a small extent.

Trapping of mosquito adults was conducted from the beginning of April to the end of October. Two types of traps were used: CDC miniature light traps with $CO_2$ (BioQuip Products, Inc., Rancho Dominiquez, CA, U.S.A., supplemented with dry ice, and lard-can traps baited with a live pigeon (LePore et al. 2004, Deegan et al. 2005). Both types of traps were hung in parallel at heights of 1 m and

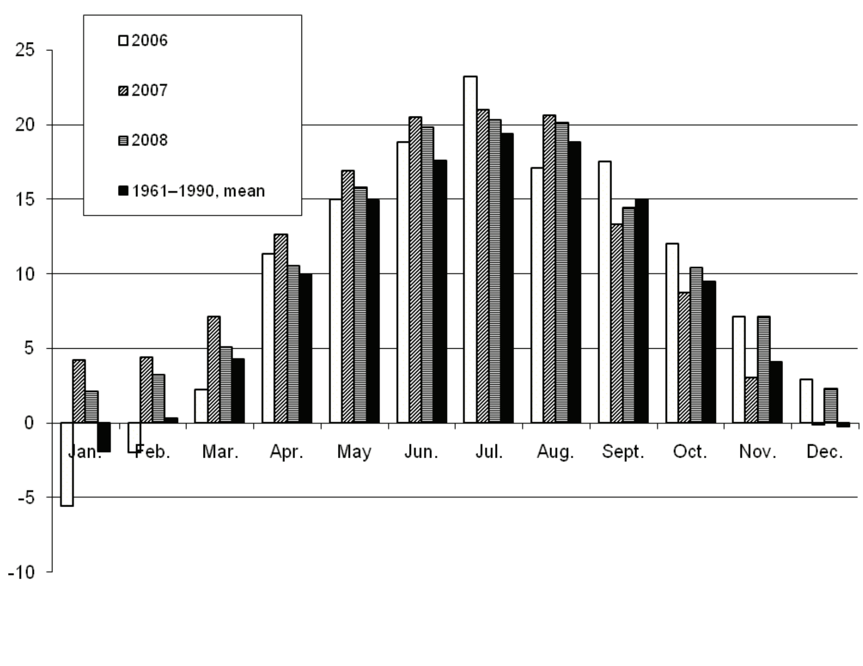


Figure 2. Mean monthly air temperature (° C) in the study area, 2006-2008, compared with a long-term average (Velké Pavlovice; data from Czech Hydrometeorological Institute in Brno).

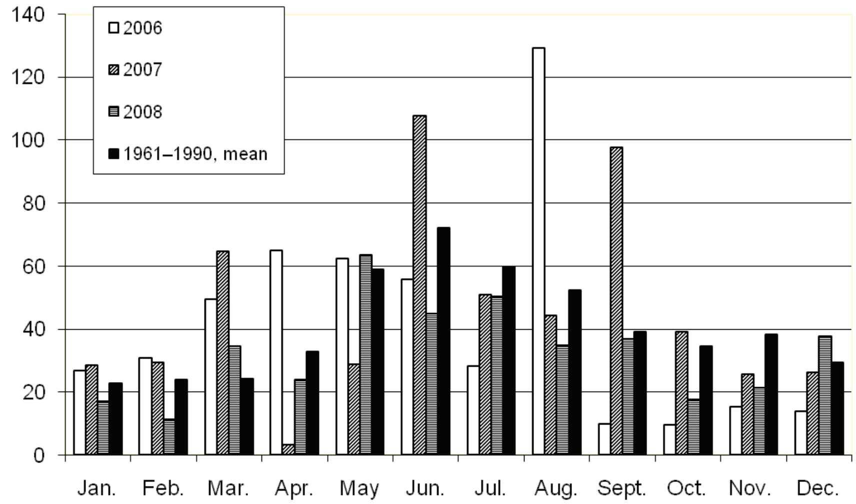


Figure 3. Monthly sum of precipitation (mm) in the study area, 2006-2008, compared with the long-term average (Velké Pavlovice; data from Czech Hydrometeorological Institute in Brno).

Table 1. Species composition of mosquitoes at study site Nesyt.

| Species | Number collected | Percent of collection |
|---|---|---|
| *Culex pipiens/torrentium* | 1,514 | 51.9 |
| *Aedes vexans* | 430 | 14.8 |
| *Aedes cantans* | 393 | 13.5 |
| *Aedes cinereus* | 83 | 2.8 |
| *Aedes sticticus* | 19 | 0.7 |
| *An. maculipennis s.l.* | 42 | 1.4 |
| *An.claviger* | 50 | 1.7 |
| *Cs. annulata* | 105 | 3.6 |
| *Cq. richiardii* | 29 | 1.0 |
| *Ae. flavescens* | 9 | 0.3 |
| *Ae. cataphylla* | 7 | 0.2 |
| *Cx. modestus* | 136 | 4.7 |
| *Cs. morsitans* | 2.0 | 0.1 |
| *An. hyrcanus* | 57 | 2 |
| Other species | 39 | 1.3 |
| Total | 2,915 | |

Table 2. Species composition of mosquitoes at study site Soutok.

| Species | Number collected | Percent of collection |
|---|---|---|
| *Ae. vexans* | 4,618 | 86.3 |
| *Ae. sticticus* | 337 | 6.3 |
| *Cx. pipiens/torrentium* | 239 | 4.5 |
| *An. maculipennis s.l.* | 34 | 0.6 |
| *An. plumbeus* | 45 | 0.8 |
| *Ae. cantans* | 19 | 0.4 |
| *Ae. rossicus* | 34 | 0.6 |
| Other species | 27 | 0.5 |
| Total | 5,353 | |

5 m; the horizontal distance between individual traps was about 25 m. The traps were distributed around 16:00 (EET) and were left exposed overnight. Mosquitoes were collected in the morning around 09:00, transported in a refrigerating bag (at about 0° C), and stored in the laboratory at –60° C until examination. Identification of females was conducted according to Kramář (1958) and Becker et al. (2003), and isolated males were not included in the overall results. In parallel with these trapping techniques, control collections were done of mosquito males (hypopygium morphology) and fed anopheline females (for oviposition) and larvae and pupae to make exact species identification of mosquitoes possible. A paired t-test was used to statistically compare the data.

## RESULTS

A total of 8,268 female mosquitoes of 22 species, belonging to six genera, was caught in the traps during 2007–2008 (Tables 1 and 2). One additional species (*Ae. dorsalis*) was collected only with an entomological net.

At the Nesyt location, a total of 2,915 female mosquitoes was caught during the two years. The relative overall abundance was 14.0 females/trap/night. The mosquitoes belonged to 17 species, the dominant being *Cx. pipiens*, with 1,514 captured females, representing 51.9% of the total number of captured specimens. A summary of the mosquitoes captured at Nesyt is shown in Table 1. The collection of five females of *Ur. unguiculata* and, in particular, 57 females of *An. hyrcanus* (var. *pseudopictus*) (2.0%) is interesting. At the Nesyt location, this species was the most abundant of the *Anopheles* genus (Šebesta et al. 2009). In the Soutok region, 5,353 females were caught in 2007 and 2008 combined. The relative overall abundance of mosquitoes was 12.87 females/trap/night. The mosquitoes belonged to 13 species, the dominant being *Ae. vexans* with 4,618 females (86.3%). A summary of species captured at this location is shown in Table 2.

In both years, capture in CDC light–$CO_2$ traps was

Table 3. Total female mosquitoes captured in different traps, 2007-2008.

| | CDC light–$CO_2$ traps | | Pigeon-baited traps | | Total |
|---|---|---|---|---|---|
| | 1 m height | 5 m height | 1 m height | 5 m height | |
| *Anopheles claviger* | 53 | 0 | 0 | 0 | 53 |
| *An. hyrcanus* | 56 | 1 | 0 | 0 | 57 |
| *An. maculipennis s. l.*[1] | 74 | 2 | 0 | 0 | 76 |
| *An. plumbeus* | 36 | 9 | 3 | 0 | 48 |
| *Aedes cinereus* | 82 | 1 | 0 | 0 | 83 |
| *Ae. rossicus* | 34 | 3 | 0 | 0 | 37 |
| *Ae. vexans* | 4,988 | 60 | 2 | 1 | 5,051 |
| *Ae. cantans*[2] | 398 | 9 | 1 | 4 | 412 |
| *Ae. caspius* | 25 | 3 | 0 | 0 | 28 |
| *Ae. cataphylla* | 15 | 0 | 0 | 0 | 15 |
| *Ae. excrucians* | 4 | 0 | 0 | 0 | 4 |
| *Ae. flavescens* | 8 | 1 | 0 | 0 | 9 |
| *Ae. sticticus* | 350 | 4 | 2 | 0 | 356 |
| *Culex modestus* | 126 | 10 | 2 | 0 | 138 |
| *Cx. pipiens*[3] | 451 | 863 | 198 | 241 | 1,753 |
| *Culiseta annulata* | 102 | 6 | 0 | 0 | 108 |
| *Cs. morsitans* | 1 | 1 | 0 | 0 | 2 |
| *Coquillettidia richiardi* | 29 | 3 | 1 | 0 | 33 |
| *Uranotaenia unguiculata* | 5 | 0 | 0 | 0 | 5 |
| Total | 6,837 | 976 | 209 | 246 | 8,268 |

[1] *An. maculipennis* and *An. messeae*.
[2] Together with *Ae. annulipes.*
[3] Together with Cx. *torrentium.*

considerably higher (25.3 per trap per night) and all detected species were found therein (Table 3). *Ae. vexans* was the most represented, while *Cx. pipiens, Ae. cantans*, *Ae. sticticus*, and *Cx. modestus* were markedly less so and other species represented fewer than 1% of the total number of captured females. Only 455 specimens (1.4 females/trap/night) of eight mosquito species were caught in pigeon-baited traps, with *Cx. pipiens* the dominant species collected (Table 3).

A markedly higher capture of mosquitoes was recorded in traps placed at a height of 1 m. In total, 7,046 females were caught (22.3 trap/night). The dominant species was *Ae. vexans*, with lesser numbers of *Cx. pipiens*. At a height of 5 m ("canopy"), the occurrence of 1,222 female mosquitoes (3.9 per trap per night) was recorded, with *Cx. pipiens* dominant and lesser numbers of *Ae. vexans* (Table 3). We tested the statistical significance of differences in mosquito yields with traps situated at the two levels (1 m and 5 m), using a paired t-test and omitting those collection days when no mosquitoes were caught in the compared pair of traps. The light-$CO_2$ traps at ground level caught an overall average (both years, all study sites) of 88.07 mosquitoes, while those in the canopy captured only 7.93 individuals, a highly significant ($P$ = 0.0004) difference. However, the average number of *Cx. pipens* was 5.38 at ground level, but 10.14 at the canopy level, a significant ($P$ = 0.005) difference. It was also significant when both years 2007 and 2008 were treated separately ($P$ = 0.017 and $P$ = 0.05, respectively). In the pigeon-baited traps, the overall average was 3.53 at ground level, and 4.07 at canopy level for all mosquitoes (both values do not differ significantly, $P$ = 0.20), and for *Cx. pipiens* the averages were 3.36 and 4.10, respectively. This difference is also not statistically significant ($P$ = 0.12).

## DISCUSSION

The recent emergence of a few important mosquito-borne viruses in Europe (West Nile *Flavivirus*, Chikungunya *Alphavirus*) has increased the interest of medical entomologists in monitoring mosquitoes in endemic areas. Most European teams have used trapping methods similar to those in the present study, especially the CDC miniature light traps with $CO_2$ and bird-baited traps (Savage et al. 1999, Esteves et al. 2004, Romi et al. 2004, Balenghien et al. 2006, Ponçon et al. 2007, Aranda et al. 2009).

Forty-five species of mosquitoes were recorded in the Czech Republic, and 37 of them were also found in southern Moravia (Vaňhara 1991, Minář and Halgoš 1997, Vaňhara

Table 4. List of all mosquito species found in the Czech Republic, with their previous reports from South Moravia and the present study.

| Species | S. Moravia | This study | Species | S. Moravia | This study |
|---|---|---|---|---|---|
| *Anopheles atroparvus* van Thiel | +[1] | | *Ae. pullatus* (Coquillett) | | |
| *An. claviger* (Meigen) | + | + | *Ae. punctor* (Kirby) | + | |
| *An. hyrcanus* (Pallas) | +[1] | + | *Ae. refiki* (Medschid) | + | |
| *An. labranchiae* Falleroni | +[1] | | *Ae. riparius* (Dyar & Knab) | | |
| *An. maculipennis* (Meigen) | + | + | *Ae. rossicus* Dolbeskin, Gorickaja & Mitrofanova | + | + |
| *An. messeae* Falleroni | + | + | *Ae. rusticus* (Rossi) | | |
| *An. plumbeus* Stephens | + | + | *Ae. sticticus* (Meigen) | + | + |
| *Aedes annulipes* (Meigen) | + | + | *Ae. vexans* (Meigen) | + | + |
| *Ae. cantans* (Meigen) | + | + | *Coquillettidia richiardii* (Ficalbi) | + | + |
| *Ae. caspius* (Pallas) | + | + | *Culex hortensis* Ficalbi | | |
| *Ae. cataphylla* (Dyar) | + | + | *Cx. martinii* Medschid | +[1] | |
| *Ae. cinereus* Meigen | + | + | *Cx. modestus* Ficalbi | + | + |
| *Ae. communis* (De Geer) | + | | *Cx. pipiens* Linnaeus | + | + |
| *Ae. diantaeus* (Howard, Dyar & Knab) | | | *Cx. territans* Walker | + | |
| *Ae. dorsalis* (Meigen) | + | + | *Cx. torrentium* Martini | + | + |
| *Ae. excrucians* (Walker) | + | + | *Culiseta alaskaensis* (Ludlow) | + | |
| *Ae. flavescens* (Muller) | + | + | *Cs. annulata* (Schrank) | + | + |
| *Ae. geminus* Peus | + | | *Cs. glaphyroptera* (Schiner) | | |
| *Ae. geniculatus* Olivier | + | | *Cs. morsitans* (Theobald) | + | + |
| *Ae. intrudens* (Dyar) | + | | *Cs. ochroptera* (Peus) | | |
| *Ae. leucomelas* (Meigen) | + | | *Cs. subochrea* (Edwards) | + | |
| *Ae. nigrinus* (Eckstein) | +[1] | | *Uranotaenia unguiculata* Edwards | +[1] | + |
| *Ae. pulcritarsis* (Rondani) | | | | | |

[1]Within the Czech Republic, only reported from southern Moravia.

and Rettich 1998, Országh et al. 2006, Rettich at al. 2007, Šebesta et al. 2009) (Table 4). In this study, 23 species were recorded. Females of *Ae. cantans* and *Ae. annulipes,* and *Cx. pipiens* and *Cx. torrentium,* were not always distinguishable with certainty and were regarded tentatively in this study as being either *Ae. cantans* or *Cx. pipiens*. Using the oviposition identification technique, *Anopheles maculipennis* s.l. was represented by two species, *viz An. messeae* and *An. maculipennis* s.s. *Aedes dorsalis* was only collected with entomologic nets.

We have not found any mosquito of the species *Ae. communis* that was reported by other authors from South Moravia. On the other hand, *Ur. unguiculata* was rarely reported by previous authors (Ryba et al. 1974), while it appeared in three samplings in this study. *An. hyrcanus* was first found here in 2005 (Votýpka et al. 2008). That study, however, was not published until the end of 2008 and thus was not known to us at the time of our study (Šebesta et al. 2009). The nearest finding of *An. hyrcanus* until this time was reported in Slovakia (Halgoš and Benková 2004).

The results of this study were affected by unusual meteorological conditions in the years 2007-2008, i.e., warm winter weather without snow cover associated with the resulting absence of floods. In this regard, it is interesting to compare the incidence of mosquitoes in this year with the results of a study from 2006 (Rettich et al. 2007), when, in addition to destructive spring floods, two local floods in June and August also affected the Soutok area. All of these events were followed by mosquito calamities. In that year, an extensive study of mosquito larvae was conducted: during April floods a large amount of larvae of spring species, especially *Ae. cataphylla* (20.4% of all collected larvae), *Ae. cantans/annulipes* (19.6%), and *Ae. intrudens* (7.1%) was discovered. At almost the same time as larvae of the spring species, larvae of species more typical of the summer season also appeared (*Ae. sticticus:* 39.0%, *Ae. vexans:* 8.3%, *Ae. cinereus/rossicus:* 4.2%). During the June and August floods of 2006, *Ae. vexans* (38.2% in June and 57.4 % in August), *Ae. sticticus* (30.3% and 34.6%, respectively), and *Ae. cinereus/rossicus* (26.1% and 7.9%, respectively) larvae predominated. In our study, the occurrence of female mosquitoes of spring species was detected only rarely; mosquito activity increased over the course of June, and the dominant species was *Ae. vexans*. It is also interesting that mosquitoes collected with entomological nets in the same habitats in 2006 yielded two arbovirus strains (Tahyna and West Nile), while no virus was recovered from mosquitoes collected during the present study in 2007 and 2008 (Hubálek et al. 2010).

A marked difference was recorded between the two study sites in the species composition of mosquito fauna. Fewer mosquito species were detected in the Soutok area and there was dominance of flood-water species *Ae. vexans* and *Ae. sticticus*, while at the Nesyt location the species composition was more varied. The results of mosquito collections were affected in both years by weather, resulting in a low incidence of pest mosquito species. This was reflected particularly in the Soutok area, where the number and size of periodic pools was markedly reduced as compared to normal.

The capture of mosquitoes in both types of traps varied greatly in terms of both quantity and species composition. Capture yield was markedly higher in CDC mini-light traps with $CO_2$, and all species of mosquitoes detected in this study were recorded in these traps (except for *Ae. dorsalis*). The capture efficiency of mosquitoes in pigeon-baited traps was very low but selective: *Cx. pipiens* was dominant in these traps. The height of the trap also had a crucial impact on the capture of mosquitoes. The entry of mosquitoes in traps placed 1 m above ground was almost seven times greater than into traps placed in the canopy 5 m above ground. The difference in species composition also was remarkable. At 1 m, all species of mosquitoes were detected in a composition corresponding with their incidence (with the exception of *Cx. pipiens*), while at 5 m *Cx. pipiens* clearly predominated and was caught significantly more frequently than in the ground traps. With pigeon-baited traps, we did not find significant differences in the all-mosquito or *Cx. pipiens* yield between the traps situated at different levels.

In conclusion, the study confirmed species richness of mosquito fauna in South Moravia, the region of occurrence of mosquito-borne diseases of humans including Ťahyňa bunyavirus and occasionally West Nile flavivirus infections (Rosický et al. 1980, Hubálek et al. 2000). In addition, two species of mosquitoes not occurring elsewhere in Czechland (a short geographic term for the Czech Republic) were repeatedly detected: *An. hyrcanus* and *Ur. unguiculata,* both southern faunistic elements. We also found that *Cx. pipiens* predominated at the canopy level with no difference between the trap type.

### *Acknowledgments*

We are grateful to Juraj Peško for his excellent technical assistance in the field, and to Dr. František Rettich and Dr Jan Minář for their help with mosquito identification. The study was supported by the EU integrated project GOCE-2003-010284 EDEN (it is catalogued by the EDEN Steering Committee as EDEN0206 (http://www.eden-fp6project.net).

## REFERENCES CITED

Aranda, C., M. P. Sánchez-Seco, F. Cáceres, R. Escosa, J. C. Gálvez, M. Masià, E. Marqués, S. Ruíz, A. Alba, N. Busquets, A. Vázquez, J. Castellà, and A. Tenorio. 2009. Detection and monitoring of mosquito flaviviruses in Spain between 2001 and 2005. Vector-Borne Zoonot. Dis. 9: 171-178.

Bakonyi, T., Z. Hubálek, I. Rudolf, and N. Nowotny. 2005. Novel flavivirus or new lineage of West Nile virus, Central Europe. Emerg. Infect. Dis. 11: 225–231.

Balenghien, T., F. Fouque, P. Sabatier, and D. J. Bicout. 2006. Horse-, bird-, and human-seeking behavior and seasonal abundance of mosquitoes in a West Nile virus focus of southern France. J. Med. Entomol. 43: 936-946.

Becker, N., D. Petrič, C. Boase, J. Lane, M. Zgomba, C.H. Dahl, and A. Kaiser. 2003. *Mosquitoes and Their Control*. Kluwer Academic/Plenum Publishers, Dordrecht. 518 pp.

Danielová, V., Z. Hájková, J. Minář, and J. Ryba. 1972. Virological investigation of mosquitoes in different seasons of the year at the natural focus of the Ťahyňa virus in southern Moravia. Folia Parasitol. 19: 25–31.

Deegan, C.S., J.E. Burns, M. Huguenin, E.Y. Steinhaus, N.A. Panella, S. Beckett, and N. Komar. 2005. Sentinel pigeon surveillance for West Nile virus by using lard-can traps at differing elevations and canopy cover classes. J. Med. Entomol. 42: 1039–1044.

Esteves, A., A. P. G. Almeida, R. P. Galão, R. Parreira, J. Piedade, J. C. Rodrigues, C. A. Sousa, and M. T. Novo. 2004. West Nile virus in southern Portugal. Vector-Borne Zoonot. Dis. 5: 410-413.

Halgoš, J. and I. Benková. 2004. First record of *Anopheles hyrcanus* (Dipera: Culicidae) from Slovakia. Biológia, 59, Suppl.: 15, 68.

Havlík, O. and B. Rosický. 1949. Key to the determination of central European members of the genus *Anopheles* (In Czech). Entomol. Listy 12: 98–102.

Havlík, O. and B. Rosický. 1952. Malaria in south Moravia after the Second World War (in Czech). Čas. Lék. Čes. 91: 888–893.

Hubálek, Z., J. Halouzka, Z. Juřicová, and O. Šebesta. 1998. First isolation of mosquito-borne West Nile virus in the Czech Republic. Acta Virol. 42: 119–120.

Hubálek, Z., I. Rudolf, T. Bakonyi, K. Kazdová, J. Halouzka, O. Šebesta, S. Šikutová, Z. Juřicová, N. Nowotny. 2010. Mosquito (*Diptera: Culicidae*) surveillance for arboviruses in an area endemic for West Nile (lineage Rabensburg) and Ťahyňa viruses in central Europe. J. Med. Entomol. 47, in press.

Hubálek, Z., H.M. Savage, J. Halouzka, Z. Juřicová, Y.O.Sanogo, and S. Lusk. 2000. West Nile virus investigations in South Moravia, Czechland. Viral Immunol. 13: 427–433.

Juřicová, Z. and J. Halouzka. 1993. Serological examination of domestic ducks in southern Moravia for antibodies against arboviruses of the groups A, B, California and

Bunyamwera (in Czech). Biologia (Bratisl.) 48: 481–484.

Kramář, J. 1958. Bitting mosquitoes – *Culicidae* (in Czech). Fauna ČSR, vol. 13. Nakladatelství ČSAV, Praha. 286 pp.

Kramář, J. and J.Weiser. 1951. Flood-water mosquitoes on the Lower Morava River (In Czech). Entomol. Listy 14: 170–177.

LePore, T.J., R. J. Pollack, A. Spielman, and P. Reiter. 2004. A readily constructed lard can trap for sampling host-seeking mosquitoes. J. Am. Mosq. Contr. Assoc. 20: 321–322.

Minář, J., I. Gelbič, and J. Olejníček. 2001. The effect of floods on the delelopment of mosquito populations in the middle and lower river Morava regions. Acta Univ. Carolinae, Biologica 45: 139–146.

Minář, J. and J. Halgoš. 1997. *Culicidae*. In: M. Chvála (ed.), *Check List of Diptera (Insecta) of the Czech and Slovak Republics*. Karolinum, Prague. 34 pp.

Minář, J. and B. Rosický. 1975. Evolution of anophelism in South Moravia (in Czech). Českoslov. Epidemiol. Mikrobiol. Imunol. 24: 40–46.

Olejníček, J., J. Minář, and I. Gelbič. 2004. Changes in biodiversity of mosquitoes in the years 2002-2003 caused by climatic changes in the Morava river basin. Acta Facult. Ecol. 12, Suppl 1: 115–121.

Országh, I., J. Minář, and J. Halgoš. 2006. Culicidae Meigen. In: L. Jedlička, V. Stloukalová, and M. Kúdela (eds.) *Checklist of Diptera of the Czech Republic and Slovakia*. Available at http://zoology.fns.uniba.sk/diptera/Culicidae.htm.

Palička, P. 1967. Contribution to the study of mosquitoes (Diptera, Culicidae) occurring in some areas of central and south Moravia. Acta Entomol. Bohemoslov. 64: 69-78.

Ponçon, N., C. Toty, G. L'Ambert, G. le Goff, C. Brengues, F. Schaffner, and D. Fontenille. 2007. Population dynamics of pest mosquitoes and potential malaria and West Nile virus vectors in relation to climatic factors and human activities in the Camargue, France. Med. Vet. Entomol. 21: 350-357.

Rettich, F., K. Imrichová, and O. Šebesta. 2007. Seasonal comparisons of the mosquito fauna in the flood plains of Bohemia and Moravia, Czech Republic. Europ. Mosq. Bull. 22: 10–16.

Romi, R., G. Pontuale, M. G. Ciufolini, G. Fiorentini, A. Marchi, L. Nicoletti, M. Cocchi, and A. Tamburro. 2004. Potential vectors of West Nile Virus following an equine disease outbreak in Italy. Med. Vet. Entomol. 18: 14-19.

Rosický, B. and O. Havlík. 1951. Anophelism of southern Moravia (in Czech). Entomol. Listy 14: 119–130.

Rosický, B., D. Málková, V. Danielová, Z. Hájková, J. Holubová, J. M. Kolman, Z. Marhoul, J. Minář, and A. Šimková. 1980. Ťahyňa virus. Natural focus in southern Moravia. Rozpravy ČSAV, Řada matematicko-přírodních věd 90: 1–107.

Ryba, J., Z. Hájková, and M. Kaftan. 1974. Occurence of *Uranotaenia unguiculata* Edwards, 1913 (*Diptera, Culicidae*) in Czechoslovakia. Folia Parasitol. 21: 142.

Savage, H.M., C. Ceianu, G. Nicolescu, N. Karabatsos, R. Lanciotti, A. Vladimirescu, L. Laiv, A. Ungureanu, C. Romanca, and T. F. Tsai. 1999. Entomologic and avian investigations of an epidemic of West Nile fever in Romania in 1996, with serologic and molecular characterization of a virus isolate from mosquitoes. Am. J. Trop. Med. Hyg. 61: 600-611.

Šebesta, O., F. Rettich, J. Minář, J. Halouzka, Z. Hubálek, Z. Juřicová, I. Rudolf, S. Šikutová, I. Gelbič, and P. Reiter. 2009. Presence of the mosquito *Anopheles hyrcanus* in South Moravia, Czech Republic. Med. Vet. Entomol. 23: 284-286.

Vaňhara, J. 1981. *Culex (Neoculex) martinii* Med., 1930. Faunistic records from Czechoslovakia. Acta Entomol. Bohemoslov. 78: 317.

Vaňhara, J. 1986. Establishment of *Culex martini (Diptera)* in the lowland forest ecosystem of southern Czechoslovakia. Acta Entomol. Bohemoslov. 83: 192–196.

Vaňhara, J. 1987. Faunistic records from Czechoslovakia. *Diptera, Culicidae*. Acta Entomol. Bohemoslov. 84: 68.

Vaňhara, J. 1991. A floodplain forest mosquito community after man-made moisture changes (*Culicidae, Diptera*). Regulated Rivers: Res. Manag. 6: 341–348.

Vaňhara, J. and F. Rettich. 1998. *Culicidae*. Diptera of the Pálava Biosphere Reserve of UNESCO. Folia Facult. Scient. Natur. Univ. Purkynianae Brunensis, Biologia 99: 97–105.

Votýpka, J., V. Šeblová, and J. Rádrová. 2008. Spread of the West Nile virus vector *Culex modestus* and the potential malaria vector *Anopheles hyrcanus* in central Europe. J. Vector Ecol. 33: 269–277.