Spread of the West Nile virus vector *Culex modestus* and the potential malaria vector *Anopheles hyrcanus* in central Europe

Jan Votýpka[1,2✉], Veronika Šeblová[1], and Jana Rádrová[1]

[1] *Faculty of Sciences, Charles University, Viničná 7, 12844 Prague, Czech Republic*
[2] *Biology Centre, Institute of Parasitology, Czech Academy of Sciences, Branišovská 31, 37005 České Budějovice, Czech Republic*

Received 14 February 2008; Accepted 22 August 2008

ABSTRACT: Mosquito faunal studies were carried out in five separate wetland regions in the Czech Republic during 2004-2007, sampling with dry ice-baited and sentinel host-baited CDC traps. A total of 79,245 adults was identified, representing 23 mosquito species that belonged to the genera *Anopheles, Culiseta, Coquillettidia*, *Aedes*, and *Culex*. Our findings reveal that the mosquito fauna is enriched by new elements in the Mediterranean region. Historical and CDC trap data suggest that the newly-emerging potential malaria vector, *Anopheles hyrcanus,* has reached the northern limit of its distribution in the Czech Republic, and the important West Nile virus (WNV) vector, *Culex modestus,* has widened its distribution in the Czech Republic. No significant differences were observed in a total number of mosquitoes collected by traps baited with either the sentinel animals or with CO_2, although species abundance differed. A relatively higher proportion of *Cx. modestus* was collected in the sentinel-baited traps, while the proportion of *Cx. pipiens* was higher in the CO_2-baited traps. ***Journal of Vector Ecology* 33 (2): 269-277. 2008.**

Keyword Index: Mosquitoes, WNV, Czech Republic, climate change.

INTRODUCTION

In recent years, several vector-borne diseases affecting both humans and domestic animals have re-emerged and spread in Europe with major health, ecological, socio-economical, and political consequences (Reiter 2001, Gubler 2002, Zell 2004, Rogers and Randolph 2006). Despite a temperate climate and high economic and hygiene standards, several mosquito-borne viruses appear to circulate in Central Europe (Hubálek and Halouzka 1996, 1999). Serological surveys and viral isolates from mosquitoes indicate that Sindbis (SINV), West Nile virus (WNV), Usutu virus (USUV), and partially Batai virus (BATV) are widespread and probably enzootic in many countries of the region (Hubálek and Halouzka 1996, Gratz 2004, Hubálek et al. 2005). WNV has emerged and re-emerged as has also been demonstrated in the United States (Garmendia et al. 2001). During the past 40 years, human and equine outbreaks of WNV were reported from many European countries, and human cases of West Nile fever also occurred in the Czech Republic (southern Moravia) in July 1997, after heavy rains caused extensive flooding along the Morava River (Hubálek and Halouzka 1999). Based on the abundance, feeding behavior, previous WNV isolations, and recent experimental transmission, several mosquito species were implicated as the main WNV vectors in the European WNV outbreak, including *Culex pipiens*, *Cx. modestus*, and *Coquillettidia richiardii* (Hubálek and Halouzka 1999, Balenghien et al. 2006).

Mosquito abundance is monitored world-wide and these insects serve as a suitable group for studying changes caused by trends in environmental conditions (e.g., Hemmerter et al. 2007). These types of studies were undertaken by several authors within the Czech and Slovak Republics. Since 1958, mosquito distribution was determined in just a few localities (Kramář 1958, Minář and Halgoš 1997, Országh et al. 2006). So far, 42 mosquito species have been recorded in the Czech Republic (37 in Bohemia and 37 in Moravia) (Országh et al. 2006), with some of them considered as rare (e.g., vectors of WNV – *Cx. modestus* and *Cq. richiardii* in Bohemia). However, species composition is not stable in time, and climate change during recent years resulted in several new records of Mediterranean mosquito species in Slovak territory, including *Culex theileri* (Halgoš and Petrus 1996) and *Anopheles hyrcanus* (Halgoš and Benková 2004). Both of these species are currently absent in the Czech Republic. Similarly in southern Moravia, two new thermophilic species were recorded during the last three decades: *Culex martinii* and *Uranotaenia unguiculata* (Vaňhara 1981; Minář and Halgoš 1997).

Our mosquito surveillance program focused on the distribution, vector capacity, and feeding behavior of mosquitoes with the following goals: 1) to monitor mosquito populations and changes in species composition over time at several localities in the Czech Republic (Bohemia and Moravia), 2) to detect feeding preferences and behavior as well as spatial distribution of the mosquito species involved in WNV transmission, and 3) to test the mosquitoes and wild and domestic birds for WNV in order to identify possible disease foci. In the present article, we provide a report on the first objective.

MATERIALS AND METHODS

Our study was conducted in 2004-2007 to determine mosquito diversity at five separate wetland areas in the Czech Republic. Sampling was initiated at the end of June and terminated in August. In one locality (Blatná), traps were placed weekly from the beginning of April and continuing through October.

We studied the occurrence of mosquitoes around fishponds in various localities in the Czech Republic from 2004 to 2007. The wet fishpond areas of southern Bohemia and the large river basin in southern Moravia host rich populations of migratory and resident birds. CDC traps were placed in five different regions (four in Bohemia and one in Moravia), represented by seven distant localities (four in southern and one in western, central, and eastern Bohemia, and southern Moravia) and 26 sites selected according to local conditions and distribution of water sources across the landscape (Table 1, Figure 1). Four localities were situated in southern Bohemia and included seven fishponds sites around Blatná city (Zadní Topič – 49°25'N, 13°53'E, 432 MSL; Kaneček – 49°26'N, 13°53'E, 445 MSL; Podskalák – 49°26'N, 13°52'E, 447 MSL; Lhotka – 49°24'N, 13°51'E, 508 MSL; Žabinec – 49°24'N, 13°48'E, 487 MSL; Hříbárna – 49°24'N, 13°52'E, 483 MSL; Lomnice – 49°25'N, 13°52'E, 437 MSL), four fishponds sites around Česke Budějovice city (Černíš – 49°0'N, 14°24'E, 384 MSL; Vrbenský – 49°0'N, 14°26'E, 391 MSL; Zadní Topole – 49°3'N, 14°22'E, 386 MSL), fishpond Blatec (49°6'N, 14°18'E, 393 MSL), and a protected landscape area, Řežabinec (49°15'N, 14°5'E, 380 MSL). In southern Moravia, traps were situated at five fishpond sites near Mikulov city (Nestyt – 48°46'N, 16°43'E, 178 MSL; Křivé – 48°50'N, 16°42'E, 290 MSL; Šibeník – 48°47'N, 16°37'E, 196 MSL; Nový – 48°47´N, 16°40'E, 192 MSL; Milovický forest – 48°50'N, 16°42'E, 290 MSL). As far as the weather permitted, traps were placed daily during testing periods, and in the majority of sites both types of CDC traps (CO_2-baited and sentinel–baited) were used (Table 1).

In addition to the above-mentioned places, we studied mosquito occurrence around fishponds in western Bohemia at four sites (Bezděkovský – 49°45'N, 12°42'E, 511 MSL; Borský – 49°45'N, 12°43'E, 495 MSL; Modrý – 49°44'N, 12°44'E, 481 MSL; Regent – 49°54'N, 12°44'E, 535 MSL), in central Bohemia around fishponds within Prague (V Pískovně – 50°5'N, 14°34'E, 240 MSL; Litožnice – 50°4'N, 14°36'E, 256 MSL), and in eastern Bohemia at three sites (Nový – 49°49'N, 15°27'E, 400 MSL; Rousínov – 49°50'N, 15°27'E, 346 MSL; Běstvina – 49°49'N, 15°36'E, 357 MSL). Most of these sites in western, central, and eastern Bohemia were visited only once or twice, and only CO_2–baited CDC traps were used. All of these sites were monitored to confirm the occurrence of *Cx. modestus* and other mosquito species that had yet to be identified. For that reason, all data presented in this study are only related to catches from southern Bohemia and southern Moravia, with the only exception related to the distribution of *Culex modestus* throughout the Czech Republic.

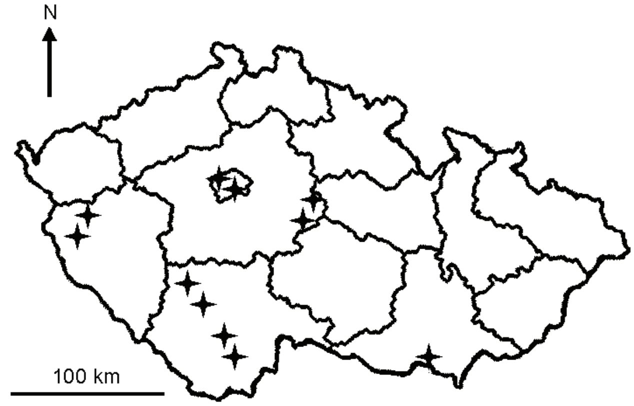

Figure 1. Map of all collecting localities in the Czech Republic where *Culex modestus* were found.

Dry ice–baited or sentinel host–baited CDC miniature light traps (Models 512 and 1012, John W. Hock Company, Gainesville, FL) without bulbs were placed near fishponds, open water pools overgrown with vegetation, and several other natural and artificial aquatic habitats in a variety of land-use areas. CDC traps baited with dry ice were set up in all studied sites for two to five consecutive nights. They were set out before dusk, and were picked up next morning. Chickens and Japanese quail were used as sentinel birds, while rabbits and guinea pigs were sentinel mammals. Animal hosts were placed in cages just before transporting them to field sites and returned within an hour after removing the insect traps the next morning. Birds had access to food and water during insect trapping. They were marked with colored tape on their legs and rotated among the trap locations. Because two CDC light traps were placed near each animal-baited cage, catches from both traps were pooled and considered as the catch of one trap. Animal-baited cages consisted of a double cage (inner cage: 50×40×30 cm, outer cage: 60×50×35 cm) with a Plexiglas® roof to allow visual orientation of vectors and to protect the birds from rain. In total, 323 trap nights were performed for dry ice–baited and 62 for sentinel host–baited traps (Table 1). Except for the Blatná locality, traps were run for a total of 95 nights (eight nights between 2 July and 30 July 2004; 30 nights between 4 July and 20 September 2005; 40 nights between 5 June and 30 September 2006; 17 nights between 24 April and 11 August 2007).

Mosquitoes were killed with CO_2 and transported to the laboratory on dry ice. Specimens were sorted under a stereomicroscope and the numbers of unengorged and engorged females and males were recorded. They were identified to species and stored at -70° C for further investigations. Females separated by species were placed in pools of one to 50 specimens for subsequent testing for WNV by using a specific polymerase chain reaction (PCR) assay. These samples were stored at -70° C until tested. Blood meals of engorged females were extracted and preserved on filter papers and the source of blood was detected by sequencing. Results from the PCR tests will be reported separately. Adults were identified using morphological characters under a stereomicroscope. Males

Table 1. Mosquitoes collected at 26 trap sites separated by regions, localities, years, and type of CDC trap bait. T/N – Number of trap-nights per site; Mean – Mean number of mosquitoes per one trap-night ± SE.

		2004		2005				2006				2007	
		CO_2		CO_2		SENTINEL		CO_2		SENTINEL		CO_2	
Region	Locality	T/N	Mean ± SE	T/N	Mean ± SE	T/N	Mean ± SE	T/N	Mean ± SE	T/N	Mean ± SE	T/N	Mean ± SE
Southern B.	Blatná			21	75.8 ± 19.2			29	110.4 ± 33.1			17	47.6 ± 13.2
	Č. Budějovice			14	30.9 ± 6.6	6	13.5 ± 2.7	16	123.6 ± 13.5	16	36.1 ± 4.9		
	Blatec	5	175.2±68.2	6	26.7 ± 6.9	2	9.3 ± 5.7	19	306.0 ± 31.1	14	232.1 ± 98.4	14	36.3 ± 5.9
	Řežabinec	3	674.7±321.7	20	61.3 ± 8.7	6	35.8 ± 6.7	7	1033.6 ± 146.6	6	271.1 ± 61.9	12	11.9 ± 3.2
Southern M.	Mikulov			39	109.5 ± 17.3			32	541.0 ± 171.4	12	660.1 ± 140.1	33	129.3 ± 17.8
Western B.								6	75.4 ± 19.6			6	45.2 ± 6.8
Central B.	Prague			6	29.5 ± 9.1			6	82.6 ± 15.9				
Eastern B.				6	16.6 ± 5.6			6	168.5 ± 29.0				

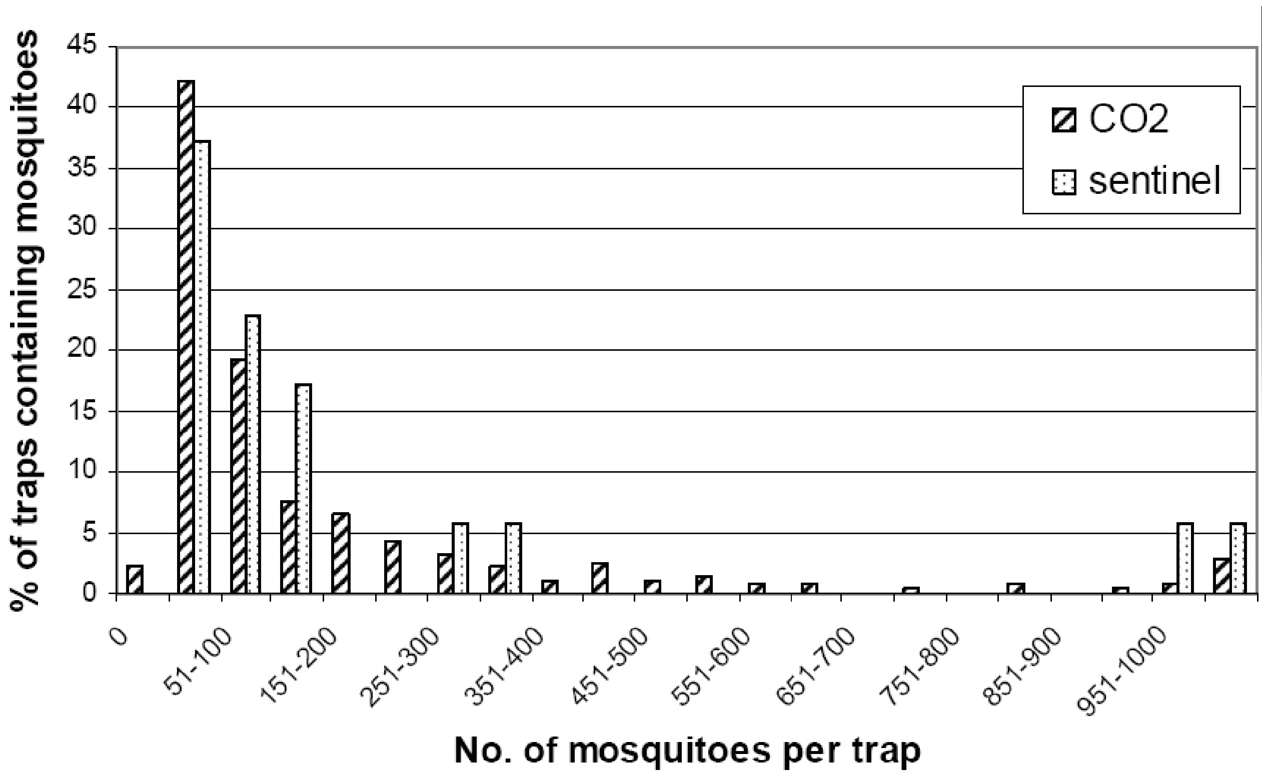

Figure 2. Number of mosquitoes collected in each of 323 CO_2-baited and 62 sentinel-baited trap nights over the study.

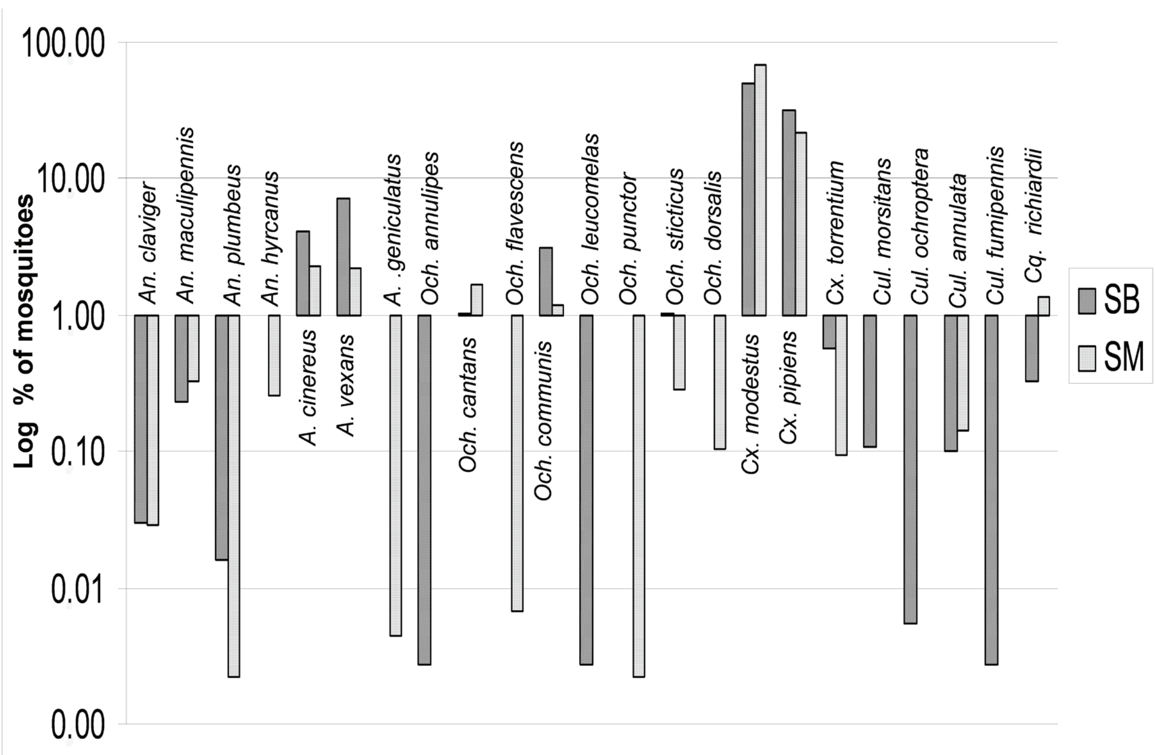

Figure 3. Relative proportion of all mosquito species captured in fishponds sites of southern Bohemia (SB) and southern Moravia (SM) during 2004–2007.

Table 2. List of mosquitoes collected near fishponds in the Czech Republic (2004–2007) separated by regions (SB – southern Bohemia, SM – southern Moravia) and type of CDC trap bait (CO_2 vs sentinel).

Species	Southern Bohemia		Southern Moravia	
	CO2 No. mosquitoes (% of total)	SENTINEL No. mosquitoes (% of total)	CO2 No. mosquitoes (% of total)	SENTINEL No. mosquitoes (% of total)
Anopheles claviger	8 (0.03)	3 (0.04)	13 (0.05)	
Anopheles maculipennis	82 (0.32)	4 (0.06)	145 (0.56)	1 (0.00)
Anopheles plumbeus	6 (0.02)		1 (0.00)	
Anopheles hyrcanus			92 (0.36)	15 (0.07)
Aedes cinereus	1,054 (4.06)	559 (7.83)	871 (3.37)	139 (0.69)
Aedes vexans	2,237 (8.61)	340 (4.76)	926 (3.58)	50 (0.25)
Aedes geniculatus				1 (0.00)
Ochlerotatus annulipes	1 (0.00)			
Ochlerotatus cantans	370 (1.42)	5 (0.07)	754 (2.92)	7 (0.03)
Ochlerotatus flavescens			3 (0.01)	
Ochlerotatus communis	1,151 (4.43)	18 (0.25)	534 (2.07)	
Ochlerotatus leucomelas		1 (0.01)		
Ochlerotatus punctor				1 (0.00)
Ochlerotatus sticticus	320 (1.23)	58 (0.81)	122 (0.47)	6 (0.03)
Ochlerotatus dorsalis			47 (0.18)	
Culex modestus	12,253 (47.16)	3,809 (53.25)	14,005 (57.18)	16,286 (80.34)
Culex pipiens	8,116 (31.23)	2,323 (32.54)	7,634 (29.53)	3,750 (18.50)
Culex torrentium	214 (0.82)		42 (0.16)	
Culiseta morsitans	39 (0.15)	1 (0.01)		
Culiseta ochroptera	2 (0.01)			
Culiseta annulata	28 (0.11)	1 (0.01)	51 (0.20)	14 (0.07)
Culiseta fumipennis	1 (0.00)			
Coquillettidia richiardii	102 (0.39)	17 (0.24)	610 (2.36)	2 (0.01)
Total	25 984	7 139	25 850	20 272

were identified by observing their hypopygia on microscope slides in CMCP-10 mounting medium. Identifications were made with keys contained in Kramář (1958) as well as comparisons with a synoptic specimen collection at the Department of Parasitology, Charles University, Prague.

The absolute and relative effectiveness of two trapping methods (CO_2 vs sentinel animal) was evaluated by an analysis of variance (Statistica v. 6.0, main effect ANOVA) for all species together as well as for each of the species individually with respect to collection locality and season. As two CDC light traps were placed near each animal-baited cage, computation of trap effectiveness and selectiveness was achieved by analyzing the mean numbers of female mosquitoes collected per one trap.

RESULTS

We collected 79,245 mosquitoes during 385 trap-nights. Numbers of traps, mean numbers of mosquitoes in all regions, and numbers of each mosquito species collected by both type of traps (CO_2 and sentinel) in two well-studied regions (southern Bohemia and southern Moravia) are presented in Tables 1 and 2. A total of 51,834 mosquitoes from dry ice–baited CDC traps, representing 23 mosquito species belonging to five genera (*Anopheles, Culex, Culiseta, Coquillettidia*, and *Aedes*) and 27,411 mosquitoes from sentinel host–baited traps were collected (Table 2). The mean number of mosquitoes collected in each trap ranged from nine per trap night (sentinel trap) to a maximum of 1,033 per trap night (CO_2 trap) (Table 1). The number of mosquitoes collected in a single trap over one night ranged from 0 to 4,375 and 1,343 in CO_2 and sentinel–baited traps, respectively. Among all the traps, 1.4% (six) contained no

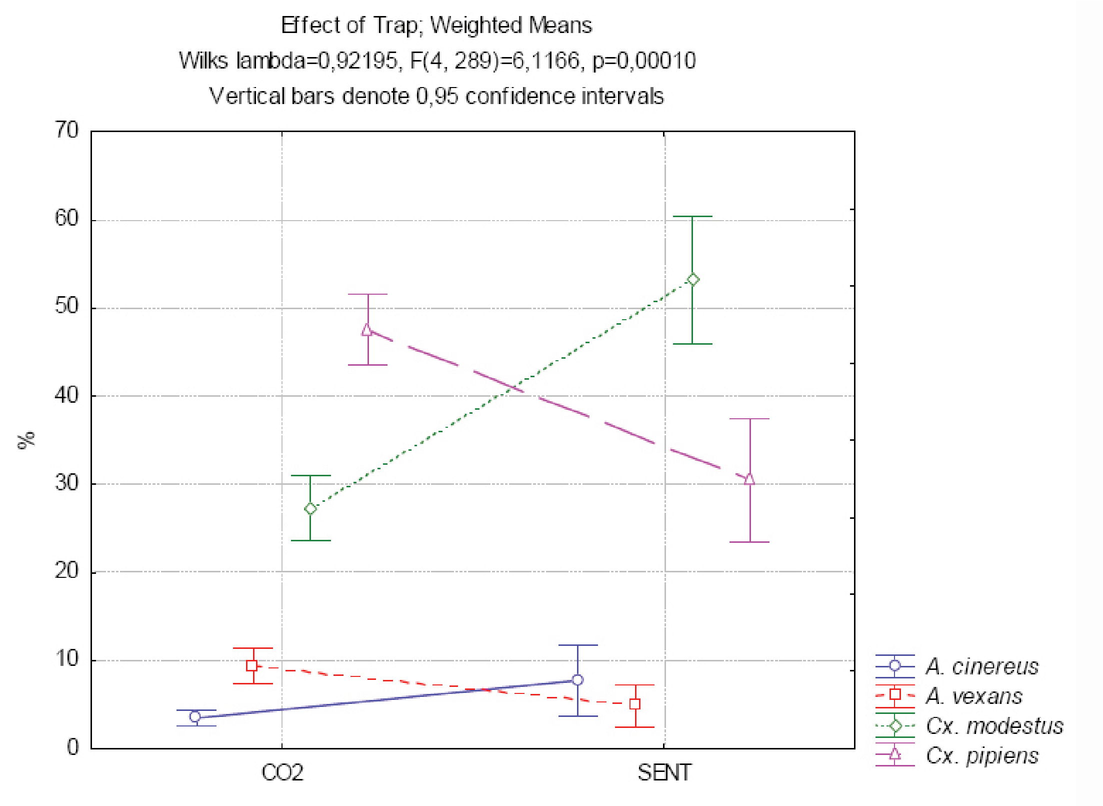

Figure 4. Relative proportion of *Cx. modestus* and *Cx. pipiens* according to trapping methods (CO2- baited vs sentinel-baited traps) in localities where both types of traps were set up together in 2005–2006.

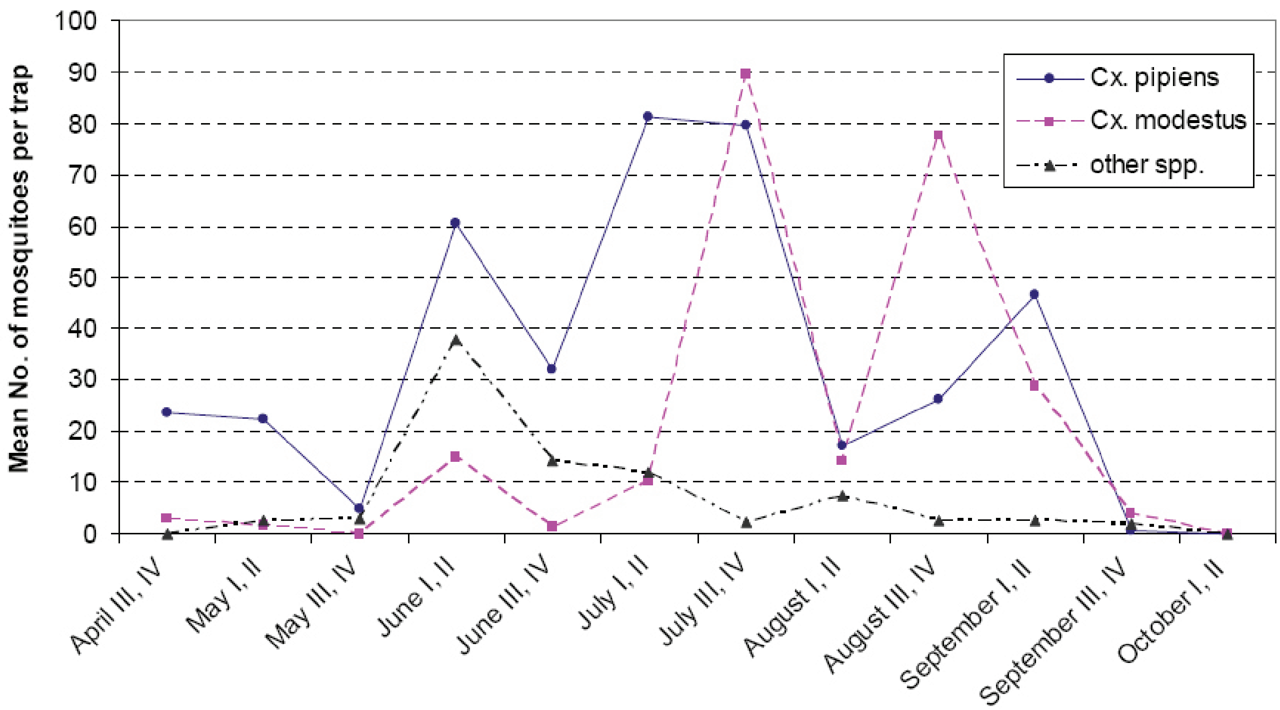

Figure 5. Temporal distribution of mosquitoes around seven fishponds in Blatná. Data represent collections made from April to October in 2005–2007.

mosquitoes at all and 37.6% (157) of the traps contained more than 100 (Figure 2).

Mosquitoes in the genera *Anopheles*, *Culex*, *Culiseta*, and *Coquillettidia* were identified to species, whereas *Aedes* were identified to species or species complex (e.g., *Aedes communis*). In 2004, 2,935 mosquitoes were collected over the course of eight trap nights. The most abundant species were *Cx. modestus* (62.3%), *Cx. pipiens* (14.4%), and *Ae. vexans* (12.3%). In 2005, 8,307 mosquitoes were collected over the course of 126 trap nights and the most abundant species were *Cx. pipiens* (53.7%), *Cx. modestus* (33.1%), and *Aedes vexans* (6.8%). In 2006, 62,276 mosquitoes were collected over the course of 169 trap nights and the most abundant species were *Cx. modestus* (65.4%), *Cx. pipiens* (21.6%), and *Ae. vexans* (3.6%). In 2007, 5,727 mosquitoes were collected over the course of 82 trap nights. The most abundant species were *Cx. pipiens* (61.3%), *Cx. modestus* (18.8%), and *Coquillettidia richiardii* (9.8%). The total mosquito collection data for all species captured in CO_2 and sentinel-baited-traps located in the wetlands areas of southern Bohemia and southern Moravia for all the years together are summarized in Table 2. Differences in distribution of all mosquito species between southern Bohemia and southern Moravia are presented in Figure 3.

The dominant species collected using both type of CDC traps during 2004–2007 in southern Bohemia were *Cx. modestus* (48.4%), *Cx. pipiens* (31.4%), and *Aedes vexans* (7.8%). The most common species collected in southern Moravia were *Cx. modestus* (65.3%), *Cx. pipiens* (24.6%), and *Aedes vexans* (2.2%). Among all mosquito species, *Cx. modestus* was the most dominant (58.5%), followed by *Cx. pipiens* (27.5%) and *Ae. vexans* (4.5%). With reference to genera, *Culex* spp. comprised 86.4%, *Aedes* spp. 12.1%, *Cq. richiardii* 0.9%, *Anopheles* 0.5%, and *Culiseta* spp. 0.2% of the mosquito fauna.

Occurrence of dominant species was similar in both well-studied regions, but the composition of less abundant species significantly differed (Figure 3). *Culiseta fumipennis*, *Culiseta ochroptera*, and *Culiseta morsitans* were captured only in southern Bohemia, whereas more prevalent *Anopheles hyrcanus* and *Aedes dorsalis* and a few specimens of *Aedes punctor, Aedes flavescens*, and *Aedes geniculatus* occurred only in southern Moravia. *An. hyrcanus* was repeatedly found during 2005–2007 at all studied sites in southern Moravia. Traps from western, central, and eastern Bohemia were specifically analyzed for the presence of *Cx. modestus*. This species was found at all studied sites (Figure 1) in relative high abundance, from 8% (western Bohemia) to 85% (eastern Bohemia).

To compare relative effectiveness and species selectiveness of both trapping methods (CO_2 vs sentinel), the average of the relative abundances of each species was evaluated with respect to the trapping localities (only localities of southern Bohemia and Moravia where both types of traps were set up together) and seasons (2005 and 2006). The effectiveness of CO_2-baited traps (effect of season and locality were controlled) was significantly higher ($F_{(1, 342)} = 6.8942$, $p<0.01$). Hence, relative proportions of appropriate mosquito species were used for further analyses. Four of the most abundant species collected over all tested localities during 2005–2006 were *Cx. modestus* (average of relative abundance was 31.1% and 54.4%), *Cx. pipiens* (45.5% and 31.4%), *Ae. vexans* (6.5% and 5.4%), and *Ae. cinereus* (4.1% and 6.8%) in accordance with CO_2-baited and sentinel-baited traps, respectively. The difference in the percentage proportion of these four species was highly significant ($F_{(4, 289)} = 6.1166$, $p<0.001$), and the relative abundance of the two most dominant species (*Cx. pipiens* and *Cx. modestus*) is presented in Figure 4.

The temporal abundance of mosquitoes collected by CO_2-baited traps in several sites within Blatná is presented in Figure 5 (combined results from 2005–2007). The numbers of mosquito species (*Cx. modestus* and *Cx. pipiens*) were in both cases low in April and May, started increasing in June, were highest from July until August, and decreased rapidly in late September. The first specimens of *Cx. pipiens* and *Cx. modestus* were observed on April 27th (in 2007) and the last was caught on September 24th (in 2006).

DISCUSSION

One of the most important factors limiting mosquito distribution is temperature, and a change of climate could influence mosquito populations in several ways, as has been demonstrated in America and southern Europe. On the other hand, only a few studies have focused on this topic in central Europe (Halgoš and Petrus 1996, Olejníček et al. 2004). The Czech Republic was affected by several catastrophic floods (1997 and 2002) that significantly influenced species composition, as well as the occurrence of mosquito-borne diseases (Olejníček et al. 2004, Rettich 2004, Hubálek et al. 1998, 2005), e.g., WNV in which transmission from birds to mammalian hosts occurred due to bridge vectors like *Cx. pipiens* (form *molestus*), *Cx. modestus*, and *Cq. richiardii*.

These European primary vectors of WNV have already been recorded from the Czech Republic, but only *Cx. pipiens* are repeatedly reported to be abundant, whereas *Cq. richiardii* is mentioned as a rare species occurring in lowlands. Our data unambiguously confirmed this mention, even though in some sites the density of *Cq. richiardii* was relatively high (up to 22.1%, 2007, southern Moravia, Mušlov), particularly in 2007, most likely due to the mild winter temperatures in 2006 and 2007. Despite high anthropophily, the low density of *Cq. richiardii* in both parts of the Czech Republic does not justify involving this species as a primary vector of WNV in central Europe.

The Mediterranean thermophilic species *Cx.* (*Barraudius*) *modestus* was reported to be common in Slovakia (Minář and Halgoš 1997, Jalili et al. 2000), medium-abundant in Moravia (Minář 1969, Vaňhara and Rettich 1998, Minář et al. 2001), and rare to very rare in Bohemia. In the last comprehensive monograph focused on mosquito fauna in the Czech Republic (Kramář 1958), only two specimens are mentioned (Velký Tisí fishpond in southern Bohemia in 1954). A later study (Rettich et al. 1978) described only two more localities for *Cx. modestus*

(Majdalena and Lomnice nad Lužnicí, both in southern Bohemia) and authors mentioned this species as "rare." Although a number of studies that focused on the mosquito fauna of several localities within Bohemia were published, no additional specimens were recorded (Rettich et al. 1978, Rettich 1982, 2004). Based on our findings, we can confirm that thermophilic *Cx. modestus* became common in wetland areas in southern, eastern, western, and central Bohemia during recent years and it constitutes the major species of mosquito fauna in many places. As a consequence of the recent spread of *Cx. modestus* within the Czech Republic and its willingness to feed on both avian and mammalian hosts, this species appears to be the more appropriate emerging vector of WNV in the Czech Republic.

The greater effectiveness of CO_2-baited CDC traps can be explained by the overall amount of carbon dioxide released from dry ice in comparison to the concentration of CO_2 in the breath of sentinel animals. *Culex pipiens* occurred in both types of traps in relatively similar proportions, whereas *Cx. modestus* was more abundant in sentinel–baited traps. Since both birds and mammals were used as bait, our findings correspond with an assumption of higher mammalophily of *Cx. modestus* and confirm its role as an appropriate WNV bridge vector.

Although several European species of anopheline mosquitoes are important vectors of human malaria (e.g., *Anopheles atroparvus, An. plumbeus, An. sacharovi,* and *An. hyrcanus*), there is virtually no risk of the endemic malaria that was eradicated in the middle of the last century in central Europe, and *Anopheles* surveillance and control programs have been discontinued in many countries. All together, eighteen species of *Anopheles* are recognized in Europe, mainly in the subgenus *Anopheles* (Ramsdale and Snow 2000). *Anopheles* (*Anopheles*) *hyrcanus* (Pallas, 1771), belonging to the *hyrcanus* group, has a wide Palearctic distribution from Spain to China, covering the southern half of Europe, the Mediterranean area, and central Asia. It is typically associated with rice fields that create prolific larval sites, and large populations are frequently associated with rice-growing areas. The Old World *Anopheles hyrcanus* group consists of about 30 known species (Ramsdale and Snow 2000), and some species of this group are important for human health as vectors of malarial parasites and other mosquito-borne diseases in the Oriental and Palearctic regions. The European distribution of the western forms of *An. hyrcanus* includes several southern European countries (Ramsdale and Snow 2000) and was recently described from Slovakia (Halgoš and Benková 2004). New records from southern Moravia represent the northern point of *An. hyrcanus* occurrence in Europe. Our findings correspond with the previous findings of Halgoš and Benková (2004) in Slovakia and clearly demonstrate further spreading of this Mediterranean species involving malaria transmission within the temperate zone of central Europe. During the last three years, *An. hyrcanus* was repeatedly found in relatively high densities in all studied sites in southern Moravia and it is capable of becoming established in new territories.

Our findings support the view that the spread of newly-emerging Mediterranean mosquito species through temperate central Europe could be responsible for vector-borne disease. In southern Moravia, we have identified a new Mediterranean mosquito species, *An. hyrcanus,* and using historical data we demonstrated the substantial spreading of another Mediterranean species, *Culex modestus*, into the Czech Republic.

Acknowledgments

We thank Petr Volf for discussions on trap design. The work was supported by the Grant Agency of the Czech Republic (206/05/P045) and Ministry of Education (MSMT0021620828, LC06009).

REFERENCES CITED

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.

Garmendia, A.E., H.J. Van Kruiningen, and R.A. French. 2001. The West Nile virus: its recent emergence in North America. Microbes Infect. 3: 223–229.

Gratz, N.G. 2004. The vector-borne human infections of Europe – their distribution and burden on public health. WHO Regional Office for Europe, Geneva 154 pp.

Gubler, D.J. 2002. The global emergence/resurgence of arboviral diseases as public health problems. Arch. Med. Res. 33: 330–342.

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

Halgoš, J. and O. Petrus. 1996. First record of *Culex theileri* (Diptera, Culicidae) in Slovakia. Biologia Bratislava 51: 190.

Hemmerter, S., J. Šlapeta, A.F. Hurk, R.D. Cooper, P.I. Whelan, R.C. Russell, C.A. Johansen, and N.W. Berne. 2007. A curious coincidence: mosquito biodiversity and the limits of the Japanese encephalitis virus in Australasia. BMC Evol. Biol. 7: 100.

Hubálek, Z. and J. Halouzka. 1996. Arthropod-borne viruses of vertebrates in Europe, Acta Sc. Nat. Brno. 30: 1–95.

Hubálek, Z. and J. Halouzka. 1999. West Nile fever—a reemerging mosquito-borne viral disease in Europe. Emerg. Infect. Dis. 5: 643–650.

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., P. Zeman, J. Halouzka, Z. Juřicová, E. Štovíčková, H. Bálková, S. Šikutová, and I. Rudolf. 2005. Mosquito-borne viruses, Czech Republic, 2002. Emerg. Infect. Dis. 11: 116–117.

Jalili, N., I. Országh, J. Halgoš, and M. Labuda. 2000. Mosquitoes (Diptera, Culicidae) of Slovakia. Eur. Mosq. Bull. 6: 20–26.

Kramář, J. 1958. Biting mosquitoes – Culicinae (Diptera).

Fauna ČSR 13. Nakladatelství Československé akademie věd, Praha, 287 pp. (In Czech).

Minář, J. 1969. A contribution to the bionomy of *Culex modestus* Fic. (Diptera, Culicidae) in southern Moravia. Folia Parasitol. 16: 93–96.

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

Minář, J. and J. Halgoš. 1997. Zoogeographically significant mosquitoes in the territory of Bohemia and Slovakia. Dipterologica Bohemoslovaca 8: 129–132.

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. Dipterologica Bohemoslovaca 12: 115–121.

Országh, I., J. Minář, and J. Halgoš. 2006. Culicidae Meigen, 1818. In: L. Jedlička, V. Stloukalová, and M. Kúdela (eds.) Checklist of Diptera of the Czech Republic and Slovakia. Comenius University, Bratislava, Slovakia.

Ramsdale, C. and K. Snow. 2000. Distribution of the genus *Anopheles* in Europe. Eur. Mosq. Bull. 7: 1–26.

Reiter, P. 2001. Climate change and mosquito-borne disease. Environmental Health Perspectives 109 (Suppl. 1): 141–161.

Rettich, F. 1982. Mosquitoes of the Mělnik area (central Bohemia). Fac. Sci. Nat. Univ. Purkyn. Brun.-Biol. 74: 111–116.

Rettich, F. 2004. Unusual occurrence of mosquitoes (Diptera, Culicidae) after catastrophic floods in the Mělník region (Central Bohemia) in the year 2002. Folia Fac. Sci. Nat. Univ. Masaryk. Brun. 119: 239–245.

Rettich, F., J. Albert, and K. Kadlcik. 1978. A study of the mosquitoes of the Trebon Basin. Dipterologica Bohemoslovaca 1: 225–241.

Rogers, D.J. and S.E. Randolph. 2006. Climate change and vector-borne diseases. Adv. Parasitol. 62: 345–381.

Vaňhara, J. and F. Rettich. 1998. Culicidae. In: R. Rozkošný and J. Vaňhara (eds.) Diptera of the Palava Biosphere Reserve of UNESCO, I. Folia Fac. Sci. Nat. Univ. Masaryk. Brun. Biol. 99: 97–105.

Zell, R. 2004. Global climate change and the emergence/re-emergence of infectious diseases. Int. J. Med. Microbiol. 293 (Supp. 37): 16–26.