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The flight distances of floodwater mosquitoes (Aedes vexans, Ochlerotatus sticticus and Ochlerotatus caspius) in Osijek, Eastern Croatia

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Abstract: In spring 2004, the mark–release–recapture study was conducted in the Osijek area, covering the total of 171 km², to describe dispersal pattern of three floodwater mosquito species (*Aedes vexans*, *Ochlerotatus sticticus* and *Oc. caspius*). Forty CO₂ baited Center for Disease Control and Prevention (CDC) traps were set at distances varying from 0.95 to 16.7 km from release site. Fifty thousand mosquitoes were released, and a total of 22 marked mosquitoes were recaptured in 12 traps, 82% of which were collected in the first six days after release. The maximum distance of recaptured mosquito (*Oc. sticticus*) was found at 11.68 km from the release site. During the study, the average dispersal rate per day for mosquitoes ranged from 0.96 km to 3.6 km in regard to different species.

Key words: floodwater mosquitoes; flight distances; mark-release-recapture method; Croatia

Introduction

The research on the dispersion of floodwater mosquitoes, which have very strong flying abilities, in general was conducted by a few authors (Smith et al. 1956; Brust 1980; Horsfall et al. 1973, 1975; Briegel et al. 2001). The dispersion of specific species of floodwater mosquitoes, Ochlerotatus sticticus (Meigen, 1838) and Aedes vexans (Meigen, 1830) was investigated by Stage et al. (1937), Gjullin et al. (1950), and Brust (1980), while the passive migration of Ae. vexans was investigated by Horsfall (1954). Previous research has identified that those species can cover significant distances from their breeding site, ranging from 620 m to more than 48 km, but at least 10 km (Ba et al. 2005; Becker et al. 2003; Brust 1980; Carpenter & LaCasse 1955; Gjullin et al. 1950; Headlee 1945; Mohrig 1969; Sparks et al. 1986). In this study we focus on flight distance of three floodwater mosquito species of Aedes and Ochlerotatus in the area of Osijek (population approximately 130,000) and the adjacent Nature Park Kopački rit. The fauna and the species abundance of the studied area is published by Merdić (1993), Merdić & Lovaković (2001), Merdić & Sudarić (2003) and Sudarić Bogojević et al. (2009). Geographical, hydrological and climatic characteristics of Kopački rit create ideal conditions for the development of large mosquito populations of the important pest species: Ae. vexans, Oc. sticticus and Oc. caspius (Pallas, 1771) which occur from April to October. Due to the high density of mosquito populations in this area, females in search of blood meal randomly move to the direction of forests, but also to urban areas close to their

breeding site (Horsfall et al. 1973). In our research the nearest residential area is the city of Osijek, the edge of which is just over 1 km southwest of the breeding sites in Kopački rit. The objective of the work was to study movement of floodwater mosquitoes from Kopački rit to Osijek, however, traps could not be set north of the marking site because of mine fields as a result of recent war activities. So it is unknown how many mosquitoes dispersed in these directions (Sudarić Bogojević et al. 2007). Information about the dispersal of mosquitoes from their breeding sites is useful when defining boundaries of area to be treated with insecticides to prevent the annoyance caused by mosquitoes in neighbouring communities. Knowledge of the flight behaviours of different mosquito species is also necessary for the monitoring and prevention of the spread of mosquito-borne diseases. All species in our study have been shown to be potential vectors of West Nile virus (Zeller & Schuffenecker 2004; Medlock et al. 2005) and Tahyna virus in Europe (Becker et al. 2003). Despite the importance of floodwater mosquitoes as pests and potential vectors of arboviruses, limited research has been addressed to the dispersal of these mosquitoes. The purpose of the present paper was to determine how far floodwater mosquitoes fly and in what direction from their breeding sites, in order to improve current surveillance and control practices in the Osijek area.

Study area

The location of the study was the city of Osijek, including

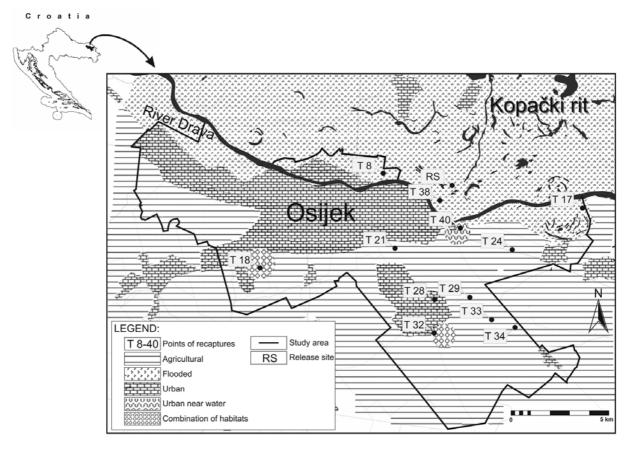


Fig. 1. Distribution of recapture sites in the study area of Osijek in relation to release site.

11 city districts, with the release site in the southern part of the Kopački rit (45°33′31″ N; 18°46′27″ E). The floodplain area of Kopački rit is situated north-east of the city of Osijek in the Baranja region, in a triangle formed by the rivers Drava and Danube, and it is described in detail in a previous paper (Sudarić Bogojević et al. 2007). The sampling was conducted in five different environments: flooded (5 stations), urban (8 stations), urban near water - Drava River (4), agricultural – crops or orchards (17 stations), and combinations of urban and agricultural habitats (6 stations). Certain environmental data (wind, temperature, humidity and rainfall) were obtained from the official meteorological station at the Osijek Airport, 20 km southeast from the release site. The average values of the daily measures were used in the analysis presented in this paper. The direction and velocity of wind were calculated based on ten-minute recordings during 24 hours for every day of sampling. These observations were obtained for the time period 16:00–16:00 h due to sampling schedule.

Material and methods

Mark-release-recapture method

On 27 April, 2004, 23 $\rm CO_2$ baited CDC traps (manufactured) without light were set up in an area of about 2 km². Next morning, the captured mosquitoes were placed in a transparent box ($60 \times 60 \times 60$ cm), photographed, and counted using the photos (approximately 50,000 mosquitoes). We presumed that females were mostly unfed. A sample of 200 mosquitoes was used to determine the mosquito species and the figures were then multiplied to estimate the total number of mosquitoes per trap per

night in the investigated area. Among the sample, Ae. vexans comprised 52%, Oc. sticticus 46%, and Oc. caspius only 2%. An atomizer was used to add some fluorescent powder (flame-orange B-735, Shannon Luminous Materials Inc. Santa Ana, CA) through an opening on the box. Mosquitoes were released on 28 April, 2004. On 29th April, 2004 forty CO₂ baited CDC-traps were set up within the city of Osijek area at regular distances (0.95 to 16.7 km) from the release site. The traps were operated continuously for 11 days and the nets, dry ice and batteries were changed every 24 hours. On 3 May, 2004, due to technical difficulties, the traps worked without the attractant, so the sampling results of that day were excluded from the statistical analysis. However, in order to calculate the average distance travelled per day, the meteorological results of that day were included as the fifth day of investigation. Trap collections were transported to the laboratory each day and examined under ultraviolet light (30 W) to detect fluorescent markings. All marked mosquitoes were identified to species (Gutsevich et al. 1976; Schaffner et al. 2001). The mark-release-recapture method was used to measure the flight distance of a single mosquito species. The distance between particular stations, as well as the distance of a certain station from the release point was calculated using the GIS package ILWIS (Unit Geo Software Development 2001; http://www.itc.nl/ilwis/).

Results

During the investigation, only 22 marked mosquitoes were recaptured (0.044% of total released) in 12 of 40 trap sites. Twelve of these mosquitoes were Oc. sticticus (54%), 7 were Ae. vexans (32%) and 3 were Oc. caspius

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Table I Mar	xımıım observe	i distances d	ot recaptured	l mosanitoes	from release si	t.e

Site	Distance (km)	Direction	$Oc.\ sticticus$	$Ae.\ vexans$	Oc. caspius
T - 18	11.68	WSW	3^{rd} day		
T - 34	8.01	SSE	v		$4^{\mathrm{th}} \mathrm{day}$
T-32	7.70	ssw	$3^{\rm rd} {\rm day}$		v
	7.70		Ţ.	$3^{\rm rd} {\rm day}$	
T - 33	7.25	SSE	$1^{st} day$	·	
T - 17	6.81	E-ESE		$10^{\rm th}~{ m day}$	
T - 28	5.87	S- SSW	$3^{\rm rd} {\rm day}$	Ť	
	5.87		Ţ.	$3^{\rm rd} {\rm day}$	
	5.87		$4^{\mathrm{th}} \mathrm{day}$	·	
T - 29	5.57	S-SSE	$3^{\rm rd}$ day		
T-24	4.59	SE	$2^{\rm nd} {\rm day}$		
	4.59		$3^{\rm rd} {\rm day}$		
	4.59		Ţ.	$7^{ m th}~{ m day}$	
T-21	4.40	sw		v	1^{st} day
	4.40				$1^{st} day$
T-8	3.54	W-WNW	$1^{st} day$		·
	3.54		$1^{st} day$		
	3.54		$6^{\mathrm{th}} \mathrm{day}$		
	3.54		·	$6^{\mathrm{th}} \mathrm{day}$	
T-40	2.21	SSE	3^{rd} day		
T - 38	0.95	SW	- day	$7^{\mathrm{th}} \mathrm{day}$	
	0.95			$7^{\rm th} {\rm day}$	
Total			12	7	3

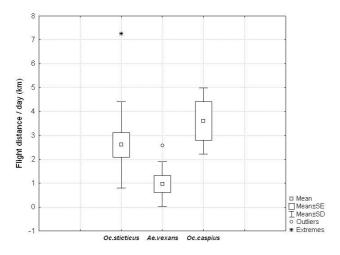


Fig. 2. Range of average distance travelled per day for each mosquito species.

(14%). These percentages differed from the percentages of each species released (see materials and methods), but our sample size was too small to conduct a statistical significant. Among 400 trap collections, a total of 16 contained one to two marked mosquitoes, and the ratio of marked to unmarked for all mosquitoes varied from 0.66% to 0.01%. Most marked mosquitoes were recaptured at 2 to 8 km from the release site (Table 1, Fig. 1). The dispersion pattern with distance for all recaptures was unimodal with a peak at 4 to 6 km distance from the release site. The maximum recorded dispersal was 11.68 km by Oc. sticticus that was recaptured on the third day after release (1 May) at T-18 site and had flown to the west-southwest of the release site. The av-

erage distance travelled per day of this species varied from 0.59 to 7.25 km, which is also the maximum distance covered in one day by a single mosquito. The average dispersal rate per day for Oc. sticticus was 2.6 km (Fig. 2). The second most abundant recaptures were the individuals of Ae. vexans. Minimum distance covered by an individual of this species was 0.95 km, and the maximum distance was 7.7 km south of the release site. However, the average dispersal rate per day for Ae. vexans was 0.96 km (0.14-2.57 km/day) (Fig. 2). Three marked Oc. caspius mosquitoes were recorded at two trap sites at the distance of 4.4 km, and 8.01 km south-southeast of the release site (average 3.6 km/day). Although being the least abundant, this species was the fastest to cover certain distances (Fig. 2). The highest numbers of recaptures were recaptured in traps located within or next to agricultural type of habitat and in the flooded area, rather than in urban areas. In total recaptures, Oc. sticticus was found in all five types of habitat, predominantly in agricultural (33% of all Oc. sticticus recaptures) and flooded area (25%). Ae. vexans was recaptured in all but urban habitat near water, and it was the most dominant in flooded type of habitat (57%). Oc. caspius was found only in agricultural habitats. In the first six days after release 82% of all recaptured mosquitoes were collected, with the most being captured on the third day (n =8). During the final days of the investigation 4 marked mosquitoes were recaptured, all of which were Ae. vexans. The last mosquito was recaptured on the tenth day after release in a trap located 6.81 km east-southeast of the release site. No marked mosquitoes were recaptured on the eight day (6 May) when the largest number of unmarked mosquitoes (115,558) was captured indicat-

Table 2. Average daily values of meteorological factors in the study area of Osijek.

Date / 2004	29 Apr.	30 Apr.	1 May	2 May	3 May	4 May	5 May	6 May	7 May	8 May	9 May	Average
Wind speed (m s ⁻¹) Wind direction Air temperature (°C) Relative humidity (%) Rainfall	3.2 E 17.7 54.4	2.3 NE 16.5 66.7	2.8 SW 18.5 62.6	3.5 SSW 17.6 58.7	4.4 ESE 17.6 58.7	6.3 SSE 18.1 60.7 light	6.2 SE 16.0 70.0 brief	4.1 WSW 14.4 71.5 brief	2.9 WNW 10.0 85.0 heavy	2.8 S 13.3 72.4 light	3.7 WSW 12.9 68.3	3.8 SSE 15.7 66.3

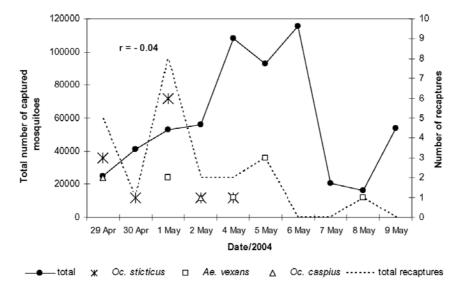


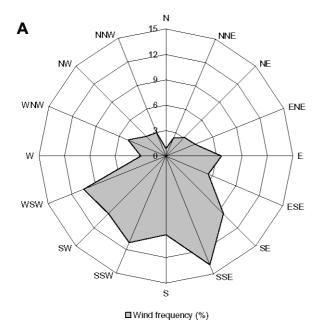
Fig. 3. Correlation between the total number of captured mosquitoes (marked and unmarked) and the number of recaptures (qualitative and quantitative composition).

ing a negative correlation between the total number of the captured mosquitoes and the number of recaptures (Fig. 3). Table 2 shows averages of wind speed and direction, temperature, humidity and rainfall. The average daily wind speeds ranged between 2.3 m s^{-1} , and 6.3 m s⁻¹. The overall mean wind speed for the period of the 11 days record was 3.8 m s^{-1} , which is a gentle breeze. South-easterly and south-westerly winds were the predominant direction during the study, with average velocities in the 3.7 m s^{-1} to 5.2 m s^{-1} range (Figs 4A, B). Winds from a north-easterly or northwesterly direction occurred much less frequently than from other directions, but these were prevailing winds at dusk for the period of trap activity. However, the predominant flight direction was to the south (SSW to SSE), with the prevailing same directions winds. Fortyfive percent of mosquitoes were recaptured at traps in south-westerly directions; 5, 4, and 1 to the SSW, SW and WSW, respectively. Thirty-six percent of the recaptured mosquitoes dispersed in south-easterly directions (4, 3 and 1 to the SSE, SE, and ESE, respectively) were from the six traps located between 2.21 and 8.01 km from the release site. The site with the largest number (n = 4) of all recaptures, was the trap T-8 (3.54 km west-northwest of the release site).

Discussion

The total of 22 recaptures or 0.044% of the total re-

leased mosquitoes is a respectful number for a large dispersion area, but it is not large enough for statistical analyses. A similar figure (0.061%) was found by Brust (1980). A great problem which we faced in the field work were breeding sites located in mined or potentially mined areas as a result of recent war activities. Such breeding sites are located outside the limits of the city of Osijek and cover the area of about 100 km². Therefore, as well as because 20% of the study area is potentially mined, we could not use the method of circular sampling. Meteorological factors, especially the wind, may have contributed to mosquito dispersion. Wind speeds in the study area may be reduced slightly by the sheltering effect of local topography, but the direction should be much the same. It is known that only when the wind speed is less than the flight speed of mosquitoes ($\sim 1 \text{ m s}^{-1}$) the mosquitoes fly opposite to the wind direction (Snow 1976; Service 1980, 1997). Due to the high values of wind velocities during the study $(2.3 \text{ to } 6.3 \text{ m s}^{-1})$ the expected inverse relationship between wind direction and flight direction was not found. Winds from a north-easterly or northwesterly direction prevailed at dusk for the period of trap activity and that could be the reason for direction of flying mosquitoes to the south of the study area. Because the wind speed was over 2 m s⁻¹, it can be assumed that the wind carried the mosquitoes. We also presumed that the wind speed and direction (2.29 m s⁻¹; NE) at dusk on 30 April had caused mosquitoes



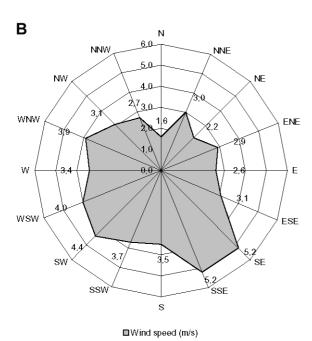


Fig. 4. A – The wind rose diagram for Osijek between 29 April and 9 May 2004. The direction is that from which the wind is blowing; radial distance represents the wind frequency (%). B – The wind speed at Osijek between 29 April and 9 May 2004.

initially to move towards the city and, eventually, to the trap site T-18 (11.68 km WSW from the release site) where an individual (*Oc. sticticus*) was recaptured on the third day. Actively or passively, this individual travelled approximately 3.89 km per day. Possibly, cross winds may have carried dispersing mosquitoes out of the route. Relative to the weather station, 20 km away and on flat terrain, the data on the wind velocity and direction may not be precise for all capture sites. Bellini et al. (1997) had similar difficulties in their work on the flying height of *Ae. caspius* and *Culex pipiens* (L., 1758). The mark-release-recapture study documented

a maximum flight distance of 11.68 km for Oc. sticticus. Although Oc. sticticus may have the potential to fly farther than was observed in this study, the greatest number of Oc. sticticus was found in traps ≤ 6 km from the release site. The same dispersal distance was documented for Ae. vexans and Oc. caspius. Even though the species Oc. caspius was the least abundant in the marked sample (2%) and in the number of recaptures (3), the distance it covered was the greatest. It was only found in agricultural trap sites which indicates the same conclusion in Bellini et al. (1997) that Oc. caspius prefers areas with no tree vegetation when searching for a blood meal and moving to the urban area. It is interesting that three and one recaptures of Ae. vexans found on the seventh and tenth day, respectively, in the traps closest to the Kopački rit which may indicate that these females were parous and returning to the breeding sites for oviposition. Although the main urban area of Osijek produces higher levels of CO₂ (man and industry), and much more brightness than the city areas south and northeast from the release site, there were more mosquitoes in total (unmarked) (Sudarić Bogojević et al. 2007). Since changes in mosquito flight direction can be caused by changes in wind direction (Bidlingmayer 1985), the light at dusk and high relative humidity (Bidlingmayer 1974) as well as the height of buildings – barriers (Bidlingmayer & Evans 1987), it is possible that the houses, apartment blocks and other tall buildings prevented the mosquitoes from moving towards the west. The lack of tall buildings, the large number of animal farms and large flat areas over which the mosquitoes fly at night may be the reasons why the traps set in the eastern part of the study area captured more females, and all but one recaptures. Moreover, the eastern part of the city has more open spaces making it possible for mosquitoes to fly without interruption during night when females set off on their appetitive flight (Bidlingmayer et al. 1974; Bidlingmayer & Hem 1981). This part is mostly agricultural (or used to be cultivated fields), with family farms where people keep cattle, the smell of which may have attracted female mosquitoes to the area and the traps set in the vicinity, which was in accordance with our data of recaptures. Brust (1980) showed that the smell of pigs and cows was an attractant for mosquitoes: four traps located near pig and cow farms (and far from rivers of floodplains) caught 72% of female mosquitoes, i.e., 46% of marked female mosquitoes. The results of this study provide insights into the distances that floodwater mosquitoes can cover, the average distance travelled per day of particular mosquito species and the flight direction. Our data indicates that the dispersion of mosquitoes was influenced by the species, speed and direction of the wind, as well as the micro and macro habitat of the area of distribution. Additionally, all these values underestimate the original flight of the mosquitoes because they show linear distances from the release site to the recapture site, hence mosquitoes possibly follow indirect routes in order to overcome physical and/or geographical barriers. Our future analysis on the dispersal of floodwater mosquitoes also needs to take into consideration the height and density of buildings over which the mosquitoes fly. The results of this study indicate similar dispersal behaviour in three floodwater mosquito species from Kopački rit and will be useful for efficient planning and implementation of mosquito control in the Osijek city area especially in cases of possible arbovirus epidemics.

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