Journal of the American Mosquito Control Association, 13(4):356–360, 1997

STUDY ON THE FLYING HEIGHT OF *AEDES CASPIUS* AND *CULEX PIPIENS* FEMALES IN THE PO DELTA AREA, ITALY[1]

ROMEO BELLINI, RODOLFO VERONESI, STEFANO DRAGHETTI AND MARCO CARRIERI

Centro Agricoltura e Ambiente, Via di Mezzo Levante 2233, 40014 Crevalcore, Italy

ABSTRACT. We have studied the vertical distribution of Culicidae in the "Ancona di Bellocchio" protected area (Regional Park of the Po Delta, Emilia-Romagna, Italy) by means of nonilluminated CDC traps baited with CO_2. Traps were placed at heights of 1.5, 3, 4, and 5 m from the ground in open areas and at 1.5, 3, 4, 5, 6, and 7 m in wooded areas. We calculated the average flying height of the species caught, i.e., in order of decreasing flying height, *Culex pipiens* Linnaeus *s.l.*, *Culex modestus* Ficalbi, *Coquillettidia richiardii* (Ficalbi), *Aedes detritus* (Haliday), *Aedes caspius* (Pallas). We also calculated the linear regression lines for both sites and found that 90% of *Ae. caspius* flew within a height of 1.64 m from the ground level, 95% within 2.68 m, and 99% at a height not exceeding 4 m; whereas 90% of *Cx. pipiens* moved within 3.45 m from the ground level, 95% within 4.02 m, and 99% within 4.76 m. The vertical distributions of *Ae. caspius* and *Cx. pipiens* did not vary significantly over the seasons and were not affected by the presence of trees or variations in temperature and wind velocity within the range of measurements obtained. The data obtained provide useful information for planning *Ae. caspius* control measures based on a mechanical barrier capable of preventing mosquitoes from moving toward residential settlements and tourist resorts bordering on the protected area as an alternative to aerial treatment with the larvicide *Bacillus thuringiensis* subsp. *israelensis.*

INTRODUCTION

The heights at which Culicidae fly have been studied in various environments and analyses made of the behavior of several species (Snow and Pickard 1956, Love et al. 1963, Davies et al. 1971, Service 1971, Pfuntner et al. 1988, Mian et al. 1990). Extensive studies have been carried out on the vertical distribution of sylvan yellow fever vectors in Africa (Haddow et al. 1947, Mattingly 1949, Goma 1965, Snow 1975, Gillies and Wilkes 1976).

However, no research appears to have been conducted on the vertical distribution of *Aedes caspius* (Pallas). A Palearctic species that is particularly well adapted to coastal environments, *Ae. caspius* tolerates varying levels of salinity in larval breeding sites, and larval development is linked to the alternating dry and flood regimes of areas where eggs are laid. Its marked anthropophily, dispersal, and population size cause infestations to be very annoying in the coastal tourist resorts around the Po Delta (Emilia-Romagna, Italy), so much so that they significantly damage the tourist economy of the area (Bellini and Veronesi 1994, Veronesi et al. 1995). It is one of the most important pests in the area and the focus of most mosquito control campaigns.

The main aim of this work was to study the flying height of mosquito species developing in the "Ancona di Bellocchio" protected area, where a large *Ae. caspius* breeding site exists, and control campaigns have so far been conducted by aerial application of *Bacillus thuringiensis* subsp. *israelensis* De Barjac. However, this approach to controlling *Ae. caspius* does not guarantee optimum control efficacy, and at certain times during the season, the airplanes used for spraying cause major disturbances among the birds that nest in the protected wetlands. Therefore, alternative control strategies need to be found, so as to improve the effectiveness of the anti-Culicidae campaign. Among these, the erection of a physical barrier to prevent females in a protected area from moving to an adjacent tourist resort may be viable. Understanding the composition of the local mosquito fauna and the vertical distribution of the adult mosquitoes is essential in order to assess the necessary parameters for erecting such a barrier.

MATERIALS AND METHODS

Two fixed stations, approximately 150 m apart, were selected for conducting this study, in order to adequately represent the landscape and vegetation of the area. Both sites bordered on the natural protected area: one was located in a wood consisting mainly of 10–13-m-high *Pinus pinea* L.; the other was located in an open area, with vegetation consisting of grass and shrubs up to 1.5 m in height.

Six traps were positioned in the wooded area at 1.5, 3, 4, 5, 6, and 7 m from the ground, and 4 were placed in the open area at 1.5, 3, 4, and 5 m from the ground. We decided to sample up to a higher level in the wooded area in order to check for the presence of females hunting for blood meals in the higher foliage (Burgess and Haufe 1960). In the open area, the traps were stacked at varying heights on the same pole. In the wooded area, the traps were positioned on different trees within a radius of 15 m, with the 5-m and 7-m-high traps on the same tree. The traps used were nonilluminated CO_2-baited CDC traps, each containing 500

[1] Study conducted within the project, "Culicids Control Programme in the Coastal Tourist Resorts Within the Po Delta Area," financed by Emilia-Romagna Tourist Regional Bureau (Regional Law n.15/91) together with the municipalities of Comacchio and Ravenna.

Table 1. Culicidae species caught by CO_2 traps (females/trap/night) in the 2 stations located in the "Ancona di Bellocchio" area, Emilia-Romagna, Italy, 1996.

Species	Wooded area (6 traps × 13 nights)	Open area (4 traps × 13 nights)	Total specimens caught
Aedes caspius	8.1 (30.13%)	67.0 (72.50%)	4,117 (59.65%)
Aedes detritus	0.01 (0.05%)	0.3 (0.29%)	15 (0.22%)
Culex pipiens	17.6 (65.76%)	21.4 (23.11%)	2,488 (36.05%)
Culex modestus	1.1 (3.96%)	3.6 (3.91%)	271 (3.93%)
Culiseta annulata	0	0.04 (0.04%)	2 (0.03%)
Coquillettidia richiardii	0.02 (0.10%)	0.13 (0.15%)	9 (0.13%)
Total	26.8 (100%)	92.5 (100%)	6,902 (100%)

g solid CO_2 and provided with 2 holes in the bottom to ensure the emission of 200–300 ml/min during the catching period. These traps are widely used for monitoring Culicidae populations and are regarded as among the most reliable (Meyer et al. 1984, Reisen and Pfuntner 1987).

The experiment was conducted from May 22 to October 3, 1996, following some preliminary tests in 1995 to define procedures and select the most appropriate stations. Traps were placed in both stations one night a week, from at least 1 h before sunset until the following morning. In case of rain or strong wind, the catch was repeated during the same week. Metereological data on average temperature, rainfall, wind velocity, and wind direction for the nights during which the experiments were conducted were obtained from the Volano station, about 20 km away.

Any influence from weather conditions on flight behavior was assessed for *Ae. caspius* and *Culex pipiens* Linnaeus *s.l.*, the 2 species for which a sufficient number of specimens were caught. To this purpose, we investigated the weather conditions recorded in the course of the typical period of daily activity for both species (unpublished data), from 1900 to 2200 h and from 0500 to 0800 h the following day for *Ae. caspius,* and from 2000 to 0600 h of the following day for *Cx. pipiens.* Because of the distance of the weather station from the sampling site, we assumed that variations in temperature and wind velocity between the 2 sites would not be significant. The influence of the wind was assessed by comparing catches when wind velocity was less than 1 m/sec with catches when wind velocity exceeded 1 m/sec. Besides affecting flying ability (Bidlingmayer et al. 1995), the wind may cause CO_2, which tends to spread downward because it is heavier than air, to disperse horizontally. Similarly, we analyzed any influence of temperature on flying height by comparing the flying heights at temperatures above and below 18°C (Bidlingmayer et al. 1995).

The relation between the number of catches in the 2 stations was studied by analyzing data by means of a 2-way ANOVA, after the angular conversion of percentage results, followed by Duncan's test. To ensure homogeneity of criteria in comparing the open and wooded areas, only traps placed at the same height and specimens caught on the same dates were considered. Subsequently, average flying height was calculated using Tukey's test to process data obtained from both stations, having been previously analyzed with a one-way ANOVA. The linear regressions were obtained by the logarithmic conversion of heights (β) and percentage of catches (α) using model $\ln(1 + \alpha) = a + b \ln(\beta)$. Covariance analysis (ANCOVA) was used to compare regression lines.

Table 2. Catches of *Aedes caspius* and *Culex pipiens* obtained at various sampling heights, Bellocchio, Emilia-Romagna, Italy, 1996.

Trap height (m)	*Aedes caspius*		*Culex pipiens*	
	Wooded area	Open area	Wooded area	Open area
1.5	577	2,954	646	805
3	21	407	199	148
4	7	98	104	82
5	15	27	136	76
6	7	—	148	—
7	4	—	144	—

RESULTS

The Culicidae species caught were *Ae. caspius, Aedes detritus* (Haliday), *Cx. pipiens, Culex modestus* Ficalbi, *Coquillettidia richiardii* (Ficalbi), and *Culiseta annulata* (Schrank).

The most numerous species found in both stations were *Ae. caspius* and *Cx. pipiens* (Table 1), which are known to be a major nuisance in the area around the Po Delta (Bellini and Veronesi 1994, Veronesi et al. 1995).

As regards the vertical distribution of catches, *Ae. caspius* was found to concentrate mainly at a height of 1.5 m in both sites; 91.4% of the total catches in the wooded area and 84.7% of the total in the open area were at this height. *Culex pipiens* catches were also concentrated in traps placed at 1.5 m, although percentages were significantly lower than those recorded for *Ae. caspius:* 46.9% in the wooded area and 72.4% in the open area (Table 2).

Table 3. Number of specimens/night obtained in both stations for the main species considering traps up to 5 m of height (4 traps), Bellocchio, Emilia-Romagna, Italy, 1996.

Species	No. collections	Wooded area (No. females ± SD)	Open area (No. females ± SD)	P[1]
Aedes caspius	13	47.7 ± 36.8	268.1 ± 480	0.04
Culex pipiens	13	83.5 ± 75.1	85.5 ± 70.1	0.98

[1] Duncan's test.

The average number of *Cx. pipiens* catches/night was not found to vary significantly between the 2 areas under study, whereas the number of *Ae. caspius* was significantly higher in the open area (Table 3).

Average flying height, compared with Tukey's test after analyzing data with one-way ANOVA ($P < 0.0001$) and obtained from the total number of catches in both stations, highlighted significant differences between the two species (Table 4).

Comparison of average monthly flying heights obtained for *Cx. pipiens* and *Ae. caspius* revealed no significant seasonal variation, even though a decreasing trend was recorded for *Ae. caspius,* starting from 1.83 m in May and reaching 1.57 m in October.

Culex pipiens was found to be the highest flying species, followed by *Cx. modestus, Cq. richiardii, Ae. detritus,* and *Ae. caspius,* which exhibited the lowest flying height. *Culiseta annulata* was not considered because of the insufficient number of catches (Table 4).

Covariance analysis did not highlight any significant differences between the regression lines obtained for the 2 sites, both for *Ae. caspius* (intercept $F = 0.069$ and $P = 0.79$; slope $F = 0.148$ and $P = 0.702$) and *Cx. pipiens* (intercept $F = 0.019$ and $P = 0.891$; slope $F = 1.363$ and $P = 0.246$; Table 5). Therefore, it was possible to calculate regression lines using data for all catches for both stations for *Ae. caspius* and *Cx. pipiens* (Fig. 1a, 1b). The comparison between the total regression lines highlights a significant difference in the vertical distributions of the 2 species (intercept $F = 0.185$ and $P = 0.667$; slope $F = 29.066$ and $P < 0.001$).

Because of the data available on weather conditions, no significant correlation was found between the weather parameters considered and average flying height. Indeed, no significant differences were recorded in the average flying height by comparing flying heights obtained at temperatures below or above 18°C, nor with wind velocity above or below 1 m/sec.

From the regression line between trap height and the arc sine of percentages of the seasonal average of overall catches for both stations, we can calculate that 90% of *Ae. caspius* fly no higher than 1.64 m from the ground, 95% fly within 2.68 m, and 99% within 4 m. As regards *Cx. pipiens,* however, 90% of females fly within 3.45 m from the ground, 95% within 4.02 m, and 99% within 4.76 m (Fig. 2).

Table 4. Average flight height in meters, calculated on the basis of the overall number of catches, Bellocchio, Emilia-Romagna, Italy, 1996.

Species	No. collections	Flight height (m, mean ± SD)	Tukey's test
Culex pipiens	25	2.66 ± 0.90	b[1]
Culex modestus	16	2.26 ± 0.57	ab
Coquillettidia richiardii	4	2.00 ± 1.00	ab
Aedes detritus	4	1.98 ± 0.71	ab
Aedes caspius	26	1.74 ± 0.24	a

[1] Unshared letters denote significant differences.

DISCUSSION

The survey conducted confirmed data previously collected in adjacent areas (Bellini and Veronesi 1994, Veronesi et al. 1995), i.e., that the most represented species in the Po Delta natural area is *Ae. caspius* (Table 1).

Indeed, the presence of *Ae. detritus,* another anthropophile species with good flying ability, was found to be limited to 2 generations: a spring cohort (last female trapped on 23 May, 1996) and an autumn cohort, which reaches the emergence stage toward the end of September (first trapped on 24 September, 1996).

Cx. pipiens was found in considerable numbers in both stations, and *Cx. modestus,* a rather vexing species active in the central summer months, was present only in small numbers. Neither species is capable of flying from the natural wetlands to the inhabited settlements in such numbers as to constitute a nuisance for tourists.

Other noxious Culicidae species, such as *Cq. richiardii* and *Cs. annulata,* were caught only sporadically.

In view of these results, it is clear that efforts to find means of preventing the flight of mosquitoes from the natural protected areas to tourist resorts must focus on *Ae. caspius.* The number of specimens of this species caught in the open area (85%) was markedly greater than the number of those caught in the wooded area (15%). The great disparity between the numbers observed clearly indicates that *Ae. caspius* prefers open areas with no tree vegetation when searching for a blood meal

Table 5. Parameter of linear regression among the percentage of catches (ln[1 + α]) and trap heights (ln[β]), Bellocchio, Emilia-Romagna, Italy, 1996.

Species	Trap station	a ± SE	b ± SE	R^2	*P*
Aedes caspius	Wooded area	0.81 ± 0.43	−0.55 ± 0.04	0.83	<0.00
	Open area	0.79 ± 0.03	−0.54 ± 0.03	0.88	<0.00
	Pooled	0.80 ± 0.03	−0.54 ± 0.02	0.85	<0.00
Culex pipiens	Wooded area	0.53 ± 0.04	−0.30 ± 0.04	0.57	<0.00
	Open area	0.62 ± 0.06	−0.38 ± 0.05	0.54	<0.00
	Pooled	0.57 ± 0.04	−0.34 ± 0.03	0.54	<0.00

and when moving from the breeding site to the coastal tourist resorts.

As regards the vertical distribution, the findings of previous works that detected a marked difference between the flying heights of mammophilic and ornithophilic species (Service 1971, Gillies and Wilkes 1976) were largely confirmed. Contrary to this, Love et al. (1963) obtained more catches of both *Culex* and *Aedes* from the highest traps placed at about 12 m from the ground in a wooded area of Georgia. Their results were obtained using New Jersey light traps and involved different species of mosquitoes.

Snow (1975), working in the Gambia in open savannah, found that most species fly near the ground, with only some *Culex* species caught in large numbers at higher levels.

In the area under examination, the average flying height of *Cx. pipiens* known primarily as an ornithophilic species, was found to be significantly different from that of *Ae. caspius,* a markedly mammophilic species (2.66 m vs. 1.74 m, respectively).

In West Africa, Gillies and Wilkes (1976) were able to identify 3 different groups clearly defined in terms of their vertical distribution, which included species with markedly different behaviors. On the contrary, we were not able to classify the species trapped into well-defined groups because the largest number of catches for each species was obtained with the trap placed at the lowest level (1.5 m from the ground).

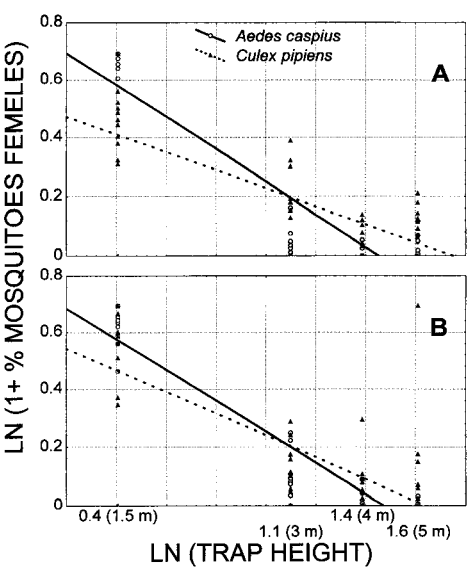

Fig. 1. Correlation between trap height (lnβ) and percentage of female mosquito catches (ln[1 + α]) A. Wooded area. B. Open area.

It must be noted that, because of the size of the area considered, it is possible that the *Cx. pipiens* population may be in constant genetic exchange with the *Cx. pipiens* autogenous populations from adjacent urban areas, which may account for the relative decline in ornithophilia and the consequent decrease in flying height. To test this hypothesis, comparative studies should be conducted on the flying height of *Cx. pipiens* populations scattered over wide natural and urban areas.

Wind is the weather parameter that previous studies found to have the greatest effect on the vertical distribution of Culicidae (Bidlingmayer et al. 1995). In our study, perhaps because of the distance of the weather station that provided data and the continuous variations in wind velocity that made it difficult to process data, it was not possible to detect any effect.

Our study led to findings comparable with those obtained by Burgess and Haufe (1960), who studied the vertical distribution of Culicidae in open areas and forests. However, within the forest, Burgess and Haufe found more mosquitoes in the tree foliage, whereas we found the highest number in the undergrowth.

Information obtained about the flying height of *Ae. caspius* females is encouraging with respect to the possible use of a physical barrier treated with insecticides to stop them from spreading to the tourist resorts.

The extensive wetlands (about 1,000 ha) at the mouth of the Reno River are formed by various areas containing a combination of *Salicornia* spp. and *Pucciniella* spp., which, because of rainfall and high tides, frequently become breeding sites for *Ae. caspius.* In recent years, approximately 3–4 floodings occurred in the spring–summer period, each of which caused a large number of eggs to hatch. Aerial treatment with *B. t. israelensis* does not achieve satisfactory mortality rates because of the thick grass vegetation and is also extremely expensive.

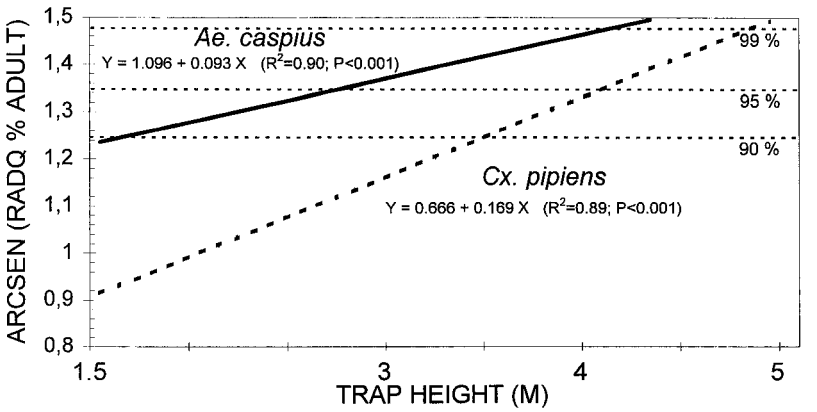

Fig. 2. Linear regression between CO_2 trap height and arc sine of cumulative percentage of females catches.

The wetlands are also an important nesting area for water birds which are disturbed greatly by the plane flying overhead 3–4 times/year in the spring.

The erection of a physical barrier approximately 2,700 m long and 3–4 m high separating the natural protected area from the residential and tourist areas might eliminate mosquitoes from inhabited areas without having to resort to larvicide or adulticide treatment.

ACKNOWLEDGMENTS

We thank the Ferrara Provincial Service for Agriculture and Nutrition for providing the climatic data and the Orsi Mangelli Animal-Hunting Farm for kindly granting us access to the study area.

REFERENCES CITED

Bellini, R. and R. Veronesi. 1994. Il programma di lotta ai Culicidi nelle località costiere della Regione Emilia-Romagna inserite nel Parco del Delta del Po. Atti XVII Congr. Naz. Ital. di Entomol., Udine 13–18 Giugno 1994, pp. 795–798.

Bidlingmayer, W. L., J. F. Day and D. G. Evans. 1995. Effect of wind velocity on suction trap catches of some Florida mosquitoes. J. Am. Mosq. Control Assoc. 11: 295–301.

Burgess, L. and W. O. Haufe. 1960. Stratification of some prairie and forest mosquitoes in the lower air. Mosq. News 20:341–346.

Davies, J. B., P. S. Corbet, M. T. Gillies and A. W. R. McCrae. 1971. Parous rates in some Amazonian mosquitoes collected by three different methods. Bull. Entomol. Res. 61:125–132.

Gillies, M. T. and T. J. Wilkes. 1976. The vertical distribution of some West African mosquitoes (Diptera, Culicidae) over open farmland in a freshwater area of the Gambia. Bull. Entomol. Res. 66:5–15.

Goma, L. K. H. 1965. The flight activity of some East African mosquitoes (Diptera, Culicidae). I. Studies on a high steel tower in Zika forest, Uganda. Bull. Entomol. Res. 56:17–35.

Haddow, A. J., J. D. Gillett and R. B. Highton. 1947. The mosquitoes of Bwamba County, Uganda. V. The vertical distribution and biting-cycle of mosquitoes in rain-forest, with further observations on microclimate. Bull. Entomol. Res. 37:301–330.

Love, G. J., R. B. Platt and M. H. Goodwin, Jr. 1963. Observations on the spatial distribution of mosquitoes in southwestern Georgia. Mosq. News 23:13–22.

Mattingly, P. F. 1949. Studies on West African forest mosquitos. Part II. The less commonly occurring species. Bull. Entomol. Res. 40:387–402.

Meyer, R. P., R. K. Washino, T. L. McKenzie and C. K. Fukushima. 1984. Comparison of three methods for collecting adult mosquitoes associated with ricefield and irrigated pasture habitats in northern California. Mosq. News 44:315–320.

Mian, L. S., M. S. Mulla, H. Axelrod, J. C. Chaney and M. S. Dhillon. 1990. Studies on the bioecological aspects of adult mosquitoes in the Prado Basin of southern California. J. Am. Mosq. Control Assoc. 6:64–71.

Pfuntner, A. R., W. K. Reisen and M. S. Dhillon. 1988. Vertical distribution and response of *Culex* mosquitoes to differing concentrations of carbon dioxide. Proc. Pap. Annu. Conf. Calif. Mosq. Vector Control Assoc. Inc. 56:69–74.

Reisen, W. K. and A. R. Pfuntner. 1987. Effectiveness of five methods for sampling adult *Culex* mosquitoes in rural and urban habitats in San Bernardino County, California. J. Am. Mosq. Control Assoc. 3:601–606.

Service, M. W. 1971. Flight periodicities and vertical distribution of *Aedes cantans* (Mg.), *Ae. geniculatus* (Ol.), *Anopheles plumbeus* Steph. and *Culex pipiens* L. (Dipt., Culicidae) in southern England. Bull. Entomol. Res. 60: 639–651.

Snow, W. E. and E. Pickard. 1956. Correlation of vertical and horizontal flight activity of *Mansonia perturbans* with reference to marked changes in light intensity (Diptera, Culicidae). Ann. Entomol. Soc. Am. 50:306–311.

Snow, W. F. 1975. The vertical distribution of flying mosquitoes (Diptera, Culicidae) in West African savanna. Bull. Entomol. Res. 65:269–277.

Veronesi, R., L. Donati and R. Bellini. 1995. Studio sulle specie di zanzare nocive nell'area del Delta del Po e sul loro contenimento. Quad. Staz. Ecol. Civ. Mus. St. Nat. Ferrara 9:261–273.