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Comparison of different trapping methods for surveillance of mosquito vectors of West Nile virus in Rhône Delta, France

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ABSTRACT: Five trapping methods were compared for monitoring potential vectors of the West Nile virus in four areas in the Camargue Plain of France: carbon dioxide traps, bird-baited traps, gravid traps, resting boxes, and human landing catches. A total of 73,721 specimens, representing 14 species, was trapped in 2006. Results showed significant differences in species and abundance between the type of traps. Many more specimens were collected using CO₂ traps than any other method, with an average of 212 specimens per night per trap ($p < 0.05$). *Culex pipiens* was the most abundant species collected (36.8% of total with CO₂ traps), followed by *Aedes caspius* (22.7%), *Anopheles hyrcanus* (18.3%), *Culex modestus* (18.3%), and *Aedes detritus* (3.2%). Bird-baited traps captured only eight specimens per night per trap on average, mainly *Cx. pipiens* (89.9%). The species collected and their abundance are influenced by the trap location, at ground or canopy level. *Culex pipiens* was twice as abundant in the canopy as on the ground, whereas it was the opposite for *Ae. caspius*, *An. hyrcanus*, and *Ae. detritus*. *Culex modestus* was equally abundant at both levels. Resting boxes and gravid traps were much less efficient, capturing around 0.3 specimens per night per trap. Results are discussed in relation to West Nile virus surveillance. *Journal of Vector Ecology* 37 (2): 269-275. 2012.

Keyword Index: Species composition, adult mosquito traps, southern France, West Nile virus, *Culex pipiens*, *Culex modestus*.

INTRODUCTION

The French Mediterranean coast is a very attractive area for economic and touristic interests. It is also a region where mosquito populations are very abundant. Suitable climate, high water table, and diversity of larval habitats combine to produce large populations of a number of mosquito species. In the Rhône Delta, the Camargue is known for its landscape of wetlands, ponds, marshes, and rice fields, and its richness of different bird species. This area is also a reserve for wildlife.

Mosquito monitoring was conducted to evaluate the diversity and dynamics of mosquito species which are potential vectors of diseases like the West Nile fever, which was detected several times around the Mediterranean basin during equine outbreaks in the 1960s, and more recently in 2000, 2003, 2004, and 2006 (Murgue et al. 2001, Jourdain et al. 2008). In southern France, West Nile virus is transmitted by *Culex pipiens* Linnaeus and *Culex modestus* Ficalbi (Balenghien et al. 2008).

Regular adult mosquito trappings were conducted for ten months in 2006 using different methods: bird and carbon dioxide (CO₂) baited traps placed at the ground level and in the canopy, resting boxes, gravid traps, and human landing catches. The main objective was to obtain functional information on the efficiency of different methods for mosquito monitoring, particularly in relation to the surveillance of the West Nile virus vectors.

MATERIALS AND METHODS

Study areas

The Rhône delta is marked by a Mediterranean climate; summers, and especially July and August, are warm and dry prior to important autumnal rainfalls in September and October and mild and wet winters (annual rainfalls: 433.5 mm in 2006). The temperature begins to increase in February (mean: 6.7 ± 2.2° C) reaching its maximum in July (27 ± 1.3° C) and decreasing progressively until the end of the mosquito season in December (8 ± 3.8° C).

The investigations were conducted in four study areas (SA1 to SA4) of the Rhône Delta (Figure 1). The sites were located along a west-east transect which increasingly consisted of wetlands (ponds, marshes, paddies, reed beds, swamps) (43°42'07" - 43°30'20"N, 4°00'33" - 4°47'29" E). The Sussargues site (SA1: 43°42'07" N, 4°00'33" E, elevation 50 m) contains Mediterranean forest, scrubland, and stone quarries, with few habitations; Tour Carbonnière (SA2: 43°36'28" N, 4°13'49" E, elev. 0 m) is located close to the village of Saint Laurent d'Aigouze where rice fields, ponds, and reed beds dominate; in Méjanès (SA3: 43°34'13" N, 4°30'02" E, elev. 3 m), rice fields, reed beds, and marshes with meadows and horses are abundant; finally, Marais du Vigueirat is a natural reserve (SA4: 43°30'20" N, 4°47'29" E, elev. 0 m) and is formed of marshes, swamps, paddies, and reed beds. Circulation of the West Nile virus has been reported in these areas since 2000 (Murgue et al. 2001, Jourdain et al. 2008).

Collection methods

Mosquitoes were trapped from February to December in 2006. Five different trapping methods were used: (1) carbon dioxide (CO₂)-baited traps, (2) bird-baited traps, (3) resting boxes, (4) gravid traps, and (5) human landing catches. CO₂-baited traps were modified from CDC Miniature Light Traps Model 512 - without light source, baited with ±1 kg dry ice. Two traps were set up in each study area, one placed close to the ground, the other in the canopy at a height of between 5 and 10 m, depending on the vegetation height. They were used overnight (from 18:00 to 10:00) in a place protected from wind exposure.

Bird-baited traps are cylinders divided into five removable parts, the central part containing a pigeon. These traps, whose development was inspired by cylindrical lard-can traps (Bellamy and Reeves 1952), were designed by P. Reiter from the Pasteur Institute, Paris, and are quite similar to those used in previous studies (Deegan et al. 2005, Dabro and Harrington 2006). Two traps were utilized overnight (from 18:00 to 10:00) at each site; one close to the ground and the other in the canopy. Both were hung on trees for protection against sunlight and wind exposure.

Resting boxes are passive artificial resting sites made of five wood panels (30.5 x 30.5 cm) and an open side (Morris 1981). The exterior of each box was painted in black, as suggested in Crans (1989), to improve their attraction, with green spots to make them less conspicuous to passers-by. Four boxes were placed on the ground in each study area in February, in sunlight-protected locations close to breeding sites. Boxes were not removed between trapping sessions. Mosquitoes resting in boxes were collected between 10:00 and 14:00 with a mouth aspirator.

Two CDC Gravid Traps Model 1712 (Reiter 1983) were used in each study area to collect *Culex* females. The traps placed on the ground were filled with a decoction of water in which hay had fermented for three days (<http://www.johnwhock.com/products/1712.htm>).

Human landing catches were performed at each site during one night, before and around dusk (from 20:00 to 24:00) and dawn (from 4:00 to 8:00). Mosquitoes landing on legs of human volunteers were collected with a mouth aspirator. All volunteers involved in these experiments are authors of this article; they agreed to participate and gave their written informed consent.

All collections were made within a 65 m radius, the median distance between CO₂- and bird-baited traps was 50 m, with small variations depending on the physical configuration of the study area. Traps were kept in the same collection sites during the campaign.

Trapping frequency, mosquito processing, and statistical analyses

CO₂ and bird-baited traps, as well as resting boxes, were used every fortnight, for two consecutive days, over a 10-month period in 2006, from February 20th (Week 8) to December 1st (Week 48). Gravid traps were used in the same way, but only until July (Week 28) because of their low yield (see results) and logistic constraints. Human landing catches

were conducted for eight nights during the summer.

Mosquitoes were identified using morphological characteristics (Schaffner et al. 2001). Average numbers of mosquitoes collected per trap per week were used for comparisons between trapping methods (traps, baits, or elevation) and locations. Differences between these results were analyzed using Generalized Linear Models (with a Poisson distribution), and the influences of the different parameters on the obtained models were compared with ANOVA.

Results were considered significant when $p < 0.05$. All data analyses were performed using the R statistical package.

RESULTS

Table 1 shows the total number of specimens, the average number per night-trap, and the relative abundance of each mosquito collected by each trapping technique. A total of 73,721 mosquitoes belonging to 14 species were collected in the four study areas in 2006. *Cx. pipiens* (38.34%), *Ae. caspius* (Pallas) (21.76%), *An. hyrcanus* (Pallas) (17.90%), *Cx. modestus* Ficalbi (17.82%), and *Ae. detritus* s.l. (including *Aedes detritus* Haliday and *Aedes coluzzii* Rioux, Guilvard and Pasteur) (3.04 %) were the most collected species. The number of collected specimens increased significantly from east to west (4.5%, 14.8%, 32.5% and 48.5% from SA1 to SA4, respectively). The total number of specimens and the average number per night-trap broken down by location and trapping method for the four most collected species are presented in Table 2.

There were statistically significant differences in the attractiveness of the five types of traps to mosquito species, as well as the trap elevation ($p < 0.05$). The average number of insects collected per night was 212 for CO₂-baited traps, 82 for human landing catches during the summer, eight for bird-baited traps, and less than 0.2 for resting boxes and gravid traps (Table 1).

CO₂-baited traps not only captured two-thirds of all mosquitoes, whatever the method, but also sampled all of the 14 species identified in this study. On average, 78 *Cx. pipiens*, 48 *Ae. caspius*, 39 *An. hyrcanus*, and 38 *Cx. modestus*, the four most abundant species, were captured per trap per night (Table 1). The bird-baited traps (2,564 specimens from nine species) collected mainly *Cx. pipiens* (89.9%) and, to a much lesser extent, *Cx. modestus* (4.2%). Human landing catches provided an average of 82 mosquitoes per volunteer per night, but this is partially overestimated because the method was used only over a short period during the summer when mosquitoes are abundant (eight trapping nights as compared to 332 and 328 for CO₂- and bird-baited traps, respectively). The four most abundant species collected were *An. hyrcanus* (42.96%), *Cx. modestus* (40.67%), *Ae. caspius* (9.17%), and *Cx. pipiens* (3.87%). Resting boxes (336 nights) and gravid traps (176 night traps) captured only 36 and 15 mosquitoes, respectively.

From the data obtained concerning the trap position, it was revealed that overall more mosquitoes were trapped close to the ground than in the canopy ($p < 0.05$) (Table 3).

Table 1. Total number of mosquitoes collected by different trapping methods. Numbers in italics correspond to the average number of mosquitoes per night trapping.

Trapping methods:	CO ₂ baited traps		Bird baited traps		Resting boxes		Gravid traps		Human landing catches		TOTAL	
	332 night traps	%	328 night traps	%	336 night traps	%	176 night traps	%	8 night traps	%		%
<i>Species</i>												
<i>Culex pipiens</i> Linnaeus	25,917	36.79	2,304	7.02	1	>0.00	15	0.1	25	3.13	28,262	38.34
<i>Aedes caspius</i> (Pallas)	15,968	22.67	17	0.05					60	7.50	16,045	21.76
<i>Anopheles hyrcanus</i> (Pallas)	12,911	18.33	2	0.01					281	35.1	13,194	17.90
<i>Culex modestus</i> Ficalbi	12,761	18.11	108	0.33					266	33.3	13,135	17.82
<i>Aedes detritus</i> s.l.	2,237	3.18	7	0.02							2,244	3.04
<i>Anopheles maculipennis</i> s.l.	387	0.55	120	0.37	35	0.10			5	0.63	547	0.74
<i>Culiseta annulata</i> (Schrank)	86	0.12	1	0.00							87	0.12
<i>Aedes vexans</i> (Meigen)	73	0.10	2	0.01					6	0.75	81	0.11
<i>Aedes rusticus</i> (Rossi)	36	0.05									36	0.05
<i>Coquillettidia richiardii</i> (Ficalbi)	36	0.05	3	0.02					9	1.13	48	0.07
<i>Anopheles algeriensis</i> Theobald	34	0.05							2	0.25	36	0.05
<i>Culiseta longiareolata</i> (Macquart)	3	0.00									3	0.00
<i>Aedes geniculatus</i> (Olivier)	2	0.01									2	0.00
<i>Culiseta subochrea</i> (Edwards)	1	>0.00									1	0.00
Total	70,452		2,564	7.8	36	0.1	15	0.1	654	81.8	73,721	100.00

Table 2. Total number of the four main mosquito species collected by different trapping methods, per study area (SA). Numbers in italics correspond to the average number of mosquitoes per night trapping.

Species	Carbon dioxide ice traps (n=83 per SA)							
	SA1		SA2		SA3		SA4	
<i>Culex pipiens</i> Linnaeus	2,992	36	5,487	66	7,133	86	10,305	124
<i>Aedes caspius</i> (Pallas)	87	1	186	2	11,667	141	4,028	49
<i>Anopheles hyrcanus</i> (Pallas)	42	1	409	5	1,953	24	10,507	127
<i>Culex modestus</i> Ficalbi	14	0	4,297	52	155	2	8,295	100
TOTAL	3,135	38	10,379	125	20,908	252	33,135	399

Species	Bird baited traps (n=82 per SA)							
	SA1		SA2		SA3		SA4	
<i>Culex pipiens</i> Linnaeus	219	2.67	904	11.02	827	10.09	354	4.27
<i>Aedes caspius</i> (Pallas)	0	0.00	2	0.02	13	0.16	2	0.02
<i>Anopheles hyrcanus</i> (Pallas)	0	0.00	1	0.01	0	0.00	1	0.01
<i>Culex modestus</i> Ficalbi	10	0.12	57	0.70	22	0.27	19	0.23
TOTAL	229	2.79	964	11.76	862	10.51	376	4.53

Species	Human landing catches (n=1 (SA1),1(SA2),2(SA3),3(SA4))							
	SA1 (1)		SA2 (1)		SA3 (2)		SA4 (4)	
<i>Culex pipiens</i> Linnaeus	0	0	1	1	13	6.5	11	2.75
<i>Aedes caspius</i> (Pallas)	0	0	0	0	19	9.5	41	10.25
<i>Anopheles hyrcanus</i> (Pallas)	0	0	54	54	20	10	207	51.75
<i>Culex modestus</i> Ficalbi	0	0	41	41	41	20.5	184	46
TOTAL	0	0	96	96	93	46.5	443	110.8

Species	Total			
	CO ₂	BBT	H	Global
<i>Culex pipiens</i> Linnaeus	25,917	2,304	25	28,246
<i>Aedes caspius</i> (Pallas)	15,968	17	60	16,045
<i>Anopheles hyrcanus</i> (Pallas)	12,911	2	281	13,194
<i>Culex modestus</i> Ficalbi	12,761	108	266	13,135
TOTAL	67,557	2,431	632	70,620

However, the relative abundance of the captured species at both positions displayed important differences: trappings in the canopy provided a larger number of *Cx. pipiens* for both CO₂- and bird-baited traps ($p < 0.05$), whereas *Ae. caspius* and *An. hyrcanus* were found to be at least three times more abundant in traps at lower heights (Table 3). In contrast, *Cx. modestus* was similarly distributed at the different heights (ratio canopy/ground = 1).

Table 4 compares the average number of *Cx. pipiens* captured monthly per night with bird-baited traps and CO₂ traps. The number of specimens captured with bird-baited traps was fairly constant between April and October, while the number captured with CO₂-baited traps increased massively between April and July and then decreased to October. Considering the overall collection, CO₂-baited traps captured 89% of mosquitoes while 10% of specimens were collected by bird-baited traps.

DISCUSSION

In this study, five methods for trapping female mosquitoes were tested and compared at four locations in the Rhône delta plain. Two methods were based on traps mimicking the physical environment (artificial resting boxes and gravid traps), the others were based on baiting with CO₂, birds, or humans. Differences were observed among areas. The quantity of mosquitoes captured increased along the west-east transect in conjunction with the surfaces of breeding sites (Table 2).

Comparisons between traps were usually made on random collection sites, with trap rotations. However, with the Latin square method, it would not have been possible to collect mosquitoes in the four study areas during the same weeks due to logistic constraints. Thus, the trap rotation method was sacrificed, assuming that sampling mosquitoes within a 65 m radius (close enough to minimize the site effects but far enough to limit interactions between traps) and repeating the sampling effort two consecutive nights during

almost a whole year would neutralize these potential biases.

In Sussargues (SA1), located in the driest area, the majority of mosquitoes collected in both the CO₂- and bird-baited traps were *Culex pipiens*. *Culex modestus* represented only 0.1% of the *Culex* collected because its larval sites (rice fields, reed beds) are absent in this area.

In all three other stations situated in wetland areas: Tour Carbonnière (SA2), Méjanès (SA3), and Marais du Vigueirat (SA4), the bird-baited traps captured mainly *Cx. pipiens* (94%, 97%, and 95%, respectively), rather than *Cx. modestus*, demonstrating the ornithophilic behaviour of *Cx. pipiens*. Conversely, more *Cx. modestus* than *Cx. pipiens* were captured in human landing collections in all three areas, demonstrating its anthropophilic tendencies. While *Cx. pipiens* was abundant in CO₂-baited traps in all three areas, *Cx. modestus* was rarely collected by this method in Méjanès (SA3), unlike *Ae. caspius*, for unidentified reasons. The most abundant species captured in CO₂ traps in Marais du Vigueirat (SA4) was *An. hyrcanus*.

The five trapping methods showed significantly different levels of efficiency in terms of trapping capacity. The performance of artificial resting boxes and gravid traps were found to be inefficient in the study area, although they have been used with great success in other parts of the world (Morris et al. 1980, Morris 1981, Reiter et al. 1986, Crans 1989, Di Menna et al. 2006, White et al. 2009). The poor results obtained with resting boxes may be explained by the presence of a large number of natural resting sites provided by the abundant vegetation that covers the ground in all study sites. Additional or larger units (like walk-in red boxes proposed by Service 1993, Reisen and Pfuntner 1987) should be used for further evaluation of this method as a mosquito surveillance tool in southern France. Similarly, there may be too many natural laying sites for gravid traps to have a probability of being colonized in the environmental conditions of the Camargue plain. Such low efficiency of the

gravid traps observed in this study in the Rhône Delta was also previously reported in California by Reisen and Pfuntner (1987), where it was especially noticeable when collections were conducted in the rural area close to larval habitat. These traps have the advantage of collecting gravid females potentially infected by viruses but, unfortunately, they are not suitable for the Camargue environment. In contrast, trapping using CO₂-baiting with birds or humans enable the capture of a significant number of female mosquitoes (Table 1). Carbon dioxide- and bird-baited traps were found to be the most “practical” methods for collecting the West Nile virus vectors in the field.

Carbon dioxide traps baited with dry ice were by far the most efficient wherever the location, enabling the collection of the largest diversity of species and number of females, regardless of their host preferences. In our study, the CO₂-baited trap was the only method that collected both West Nile vector species, *Cx. pipiens* and *Cx. modestus*, in large quantities. These traps were found to be around ten to 100 times more efficient for these two species than host-baited traps, depending on the location. Although, as with any other type of trap, they may be subject to bias, their high productivity in terms of species variety and number of specimens suggests that they give the best estimate of the diversity and abundance of mosquito fauna in the Camargue.

With regard to ornithophilic species, bird-baited traps captured overall a much lower number of mosquitoes than CO₂-baited traps, and there were large differences depending on location and the proportions of each species present. Compared to the results for CO₂ traps, fewer mosquitoes than expected were captured by bird-baited traps in SA4 (Marais du Vigueirat). This may be due to a “dilution effect” in this bird reserve area where avian hosts are very abundant.

Aedes caspius, *An. hyrcanus*, and *Ae. detritus s.l.*, abundantly captured with CO₂ traps and on human bait,

Table 3. Influence of trap positions on efficiency of CO₂ and bird-baited traps with average numbers of specimens collected per night per sort of trap.

Species	Ground			Canopy		
	CO ₂	Bird	Average	CO ₂	Bird	Average
<i>Culex pipiens</i> Linnaeus	48.17	7.09	27.63	107.96	6.96	57.46
<i>Culex modestus</i> Ficalbi	37.85	0.37	19.11	39.08	0.29	19.69
<i>Aedes caspius</i> (Pallas)	76.52	0.09	38.30	19.11	0.02	9.56
<i>Anopheles hyrcanus</i> (Pallas)	60.14	0.01	30.07	17.51	0.01	8.76
<i>Aedes detritus s.l.</i>	10.46	0.02	5.24	3.00	0.02	1.51
<i>Anopheles maculipennis s.l.</i>	2.19	0.07	1.13	0.14	0.51	0.32
<i>Coquillettidia richiardii</i> (Ficalbi)	0.07	0.01	0.04	0.14	0.01	0.07
<i>Culiseta annulata</i> (Schrank)	0.39	0.01	0.20	0.11	0.00	0.05
<i>Aedes vexans</i> (Meigen)	0.37	0.01	0.19	0.07	0.01	0.04
<i>Anopheles algeriensis</i> Theobald	0.16	>0.00	0.16	0.04	0.00	0.02
<i>Culiseta longiareolata</i> (Macquart)	>0.00	>0.00	>0.00	0.02	0.00	0.01
<i>Aedes geniculatus</i> (Olivier)	0.01	>0.00	0.01	0.01	>0.00	0.01
<i>Culiseta subochrea</i> (Edwards)	>0.00	>0.00	>0.00	0.01	>0.00	0.01
<i>Aedes rusticus</i> (Rossi)	0.22	>0.00	0.22	>0.00	>0.00	>0.00

Table 4. Average number of *Culex pipiens* per month, per night trapping and ratio in bird baited traps. Ratio = (Bird/(CO₂+1)).

Month	Bait		Ratio
	CO ₂	Bird	
February	0.00	0.13	0.13
March	4.66	3.03	0.54
April	16.44	10.29	0.59
May	120.19	8.97	0.07
June	263.96	13.53	0.05
July	248.38	8.89	0.04
August	90.09	10.03	0.11
September	94.56	11.44	0.12
October	4.56	8.06	1.45
November	0.06	0.03	0.03

were poorly attracted by bird-baited traps, indicating marked behavioral dispositions and preference for feeding on mammals (Ponçon et al. 2007).

Both *An. hyrcanus* and *Cx. modestus* were abundant in collections on humans compared to other species, indicating their anthropophilic tendencies. Despite the high level of efficiency of this method, its significant constraints restrict its use over long periods of time. Moreover, these catches should be limited for ethical and sanitary considerations.

Placing both CO₂- and bird-baited traps in the canopy, rather than on the ground, increased the abundance of *Cx. pipiens* significantly ($p < 0.05$) because its flight range is higher than other species, matching its preference to bite birds (Novak et al. 1981, Anderson et al. 2004, Deegan et al. 2005, Lee et al. 2006). The abundance of *Culex modestus* in the canopy (Table 3) is also consistent with its opportunistic feeding behavior (Balenghien et al. 2006). Thus, as shown in other countries, the trap height influences the collections both qualitatively and quantitatively (Reisen et al. 1990, Anderson et al. 2004, Drummond et al. 2006).

West Nile virus transmission requires competent vectors, receptive hosts, and environmental parameters which allow contact between the vector and its different hosts. In France, *Culex* species are competent vectors for the West Nile virus, the experimental rate of infection being higher in *Cx. modestus* than that of *Cx. pipiens* (Balenghien et al. 2008). Comparison of trapping results confirmed that *Cx. pipiens* is indeed the main bird-feeder and therefore may be the primary species that amplifies and disseminates the West Nile virus the most among birds. *Culex modestus*, which is attracted by humans, may be an important bridge vector for dissemination among mammals. Several studies have reported seasonal shifts in host-feeding pattern (Hayes et al. 1973, Ritchie and Rowley 1981, Bertsch and Norment 1983). More recently, Kilpatrick et al. (2006) pointed out that seasonal variations in bird abundance could lead mosquitoes to bite and potentially infect other hosts, like horses or humans, and consequently start an outbreak of transmission. In the present study, *Cx.*

pipiens dynamics observed with bird baits did not vary strongly between April and October (see Table 4), but those observed with CO₂ baits showed large variations. Host-baited collections are under the cross-influence of mosquito and host densities (Service 1993) and the number of trapped mosquitoes on the hosts is a direct and accurate estimation of the host-vector contact rate related to both abundances. These evolutions of bird-vector contact rates will need to be considered in the West Nile virus epidemiological assessment in the Camargue, known for being a crossroads for migratory birds, and will be the subject of a future study.

In conclusion, because *Culex pipiens* and *Culex modestus* are the most likely candidates for West Nile transmission, it is important to sample them adequately. According to the results of our study, using CO₂-baited traps is the best method for unspecific mosquito surveillance or to determine seasonal dynamics. These traps captured both vector species in abundance compared to other methods. Trapping in the canopy is more efficient for the specific task of monitoring the West Nile virus vectors in the Rhone Delta by increasing the abundance of *Culex pipiens* without lowering those of *Culex modestus*. Host-baited methods or artificial sites provide fewer mosquitoes and are under the influence of the environment's composition (host abundances and/or natural sites). The attractiveness of traps to mosquitoes compared to the natural environment remains a decisive parameter which must be taken into consideration before starting a trapping campaign and during the interpretation of the results.

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