European Mosquito Bulletin ***28*** *(2010), 213-224*
Journal of the European Mosquito Control Association
ISSN 1460-6127; w.w.w.e-m-b.org
First published online 29 October 2010

Composition and seasonal activity patterns of mosquito communities collected with malaise traps at Etang de Virelles Nature Reserve (Virelles, Hainaut), a migratory bird sanctuary and possible site for arbovirus transmission in Belgium

W. Dekoninck[1,2], M. Pollet[1,2,3] & P. Grootaert[1]

[1] Royal Belgian Institute of Natural Sciences, Entomology Department, Vautierstraat 29, B-1000 Brussels, Belgium [2] Terrestrial ecology unit, Ghent University, K. L. Ledeganckstraat 35, B-9000 Ghent, Belgium [3] Research Institute for Nature and Forest, Kliniekstraat 25, B-1070 Brussels, Belgium

Abstract

The phenology of mosquito communities is important in the assessment of risks of transmission and control of several arboviruses, especially in situations where migratory and non-migrating bird species congregate, The Etang de Virelles Nature Reserve (Belgium) and its associated wetlands is a prime example of an area attracting large numbers of both resident and migratory birds. Malaise Traps set in four different marshland habitats in this nature reserve, collected nine species of mosquito during the spring and summer of 2006 and proved, both from the operational viewpoint and in the results obtained, to be a good adult Culicidae sampling technique, . When emptied each week they also enable us to construct seasonal activity patterns of the resident mosquitoes. The most abundant species, *Ochlerotatus cantans*, a potential vector of West Nile virus, and *Aedes cinereus/geminus* exhibited different seasonal activity patterns, even in adjacent habitats. Densities of the floodwater species, *Ae. cinereus/geminus*, were lower in more sheltered humid forest vegetation and willow carr than in more open reed and *Filipendula* marshland areas. Low numbers and postponed emergence of *Oc. cantans* was observed in three sites with fluctuating ground water levels. This species was significantly more abundant in the more stable ground water levels of the permanently saturated willow care. The mosquito community found in the willow car habitat consists only of species which overwinter as eggs. Species overwintering as adults or larvae were present in the other parts of the study area.

Key words: Culicidae/ phenology/ Malaise traps/ marshland/ habitat characteristics

Introduction

Mosquitoes are vectors of many pathogens responsible for human and animal morbidity and mortality world wide. They have been the subject of intensive population studies with the aim of facilitating prediction of outbreaks of malaria or of arboviruses affecting humans and/or livestock. Longitudinal field studies, including monitoring of seasonal abundance and activity of vector species enables efficient mosquito control and provides knowledge of when and where to take appropriate protective measures. Moreover, knowledge of the population dynamics of mosquitoes and their role in sylvatic disease transmission of concern to humans, companion animals and domesticated animals, is far from complete. Monitoring of mosquito populations in areas not or little affected by human activities, such as forests and nature reserves, is often carried out only after outbreaks of human or domestic animal diseases have occurred (Romi *et al*., 2004). Some well known examples of vector studies in the Mediterranean and Southern Europe are the large scale investigations of their role in the transmission of viruses such as West Nile virus (WNV), of mosquito communities in the Camargue and in Italy, where studies have additionally shown that the irritation caused by mosquito bites adversely affects sufferers of Human Herpes Virus 8/Kaposi's Sarcoma in regions where this condition is prevalent (Ascoli *et al*., 2006; Mouchet *et al*., 1970; Murgue *et al*., 2001, Ponçon *et al*., 2007).

Field studies of population dynamics and phenology have employed various types of trap to collect adult mosquitoes, and some types have proved to be effective surveillance tools for monitoring seasonal prevalence and species composition of mosquito populations (Campbell, 2003). Some are even reported to have reduced numbers of mosquitoes in certain situations (Dilling, 2004). Several designs of trap, varying greatly in effectiveness and usefulness, have been designed (Kline, 1999; Campbell, 2003) and commercial advertisements sometimes claim that traps can control mosquitoes. Most traps use attractants such as heat,, carbon dioxide, kairomones, or moisture, which mimic emanations produced by warm-blooded hosts (Kline & Mann, 1998). Traps used to collect mosquitoes in western Europe comprise several types of Malaise Trap (Korgankar *et al*., 2008), of which the Mosquito Magnet Liberty Plus (MMLP) is an example, the BG-Sentinal trap (Rose *et al*., 2006; BioGents, 2007; Williams *et al*., 2007) and the CDC trap (McNelly, 1989; Ascoli *et al*., 2006), all using stimuli, and several types of Gravid trap (Scott *et al*., 2001; Lee & Kokas, 2004) which collect ovipositing mosquitoes.

Until very recently, the distribution of mosquitoes in Belgium was poorly known and the latest checklist of the Belgian Culicidae names only the 24 species found in the collection held by the Royal Belgian Institute of Natural Sciences (Gosseries & Godderis, 1991). Because of the need for improved knowledge of both endemic and invading mosquito species, a four year project, named Modirisk, was started in 2007 (see www.modirisk.be). This project aims at surveying and mapping the mosquito populations present in Belgium, and focuses on both native and immigrant vector communities. From the start of the Modirisk project mosquitoes have been collected by use of Mosquito Magnet Liberty Plus (MMLP) Malaise Traps in more than 900 selected sites. In addition to the data gathered within the Modirisk project, these traps seem to be suitable for the screening of Culicidae throughout Belgium. The Entomology Department of the Royal Belgian Institute of Natural Sciences (RBINS) used Malaise Traps during the Site Quality Assessment (SQA) projects to catalogue Dipteran (Syrphidae, Dolichopodidae, Empididae) and Hymenopteran groups (e.g. Dekoninck *et al*., 2005). Malaise Traps capture large numbers and a diversity of flying insects including Hymenoptera and Diptera (Matthews & Matthews 1970; Darling & Packer, 1988; Grootaert *et al*., 1988; Noyes, 1989; Campbell & Hanula, 2007) and. are widely used in surveys of insect abundance and diversity. Malaise Traps emptied weekly over a complete season, give valuable information on the population dynamics and phenology of the species present.

The aims of this study were to:

- make an inventory of the Culicidae of the Etang de Virelles Nature Reserve during the active flight season, using Malaise Traps at sited in four different types of habitat;
- describe these mosquito communities and to study the population dynamics of each of the constituent species, with special attention paid to potential vectors of arboviruses;
- discuss the value and use of Malaise Traps as a collection method and as a means of studying mosquito communities.

Materials and Methods

Study area, sampling technique and identification

The Etang de Virelles Nature Reserve is the site of an artificial lake near the city of Chimay. Covering an area of 1.25 km², the Nature Reserve is well known as a resting place for many migratory birds each spring and autumn (Jacob, 1983; Dubois *et al.*, 1987; de Bellefroid *et al.*, 2008). Because of the huge number of migratory and non-migrating birds and the presence of a large network of mosquito breeding sites, this region can also be marked as a risk area for outbreaks of West Nile virus (Malkinson & Banet, 2002).

Four sites representing the most abundant habitat types near the lake (Site A - Reed marsh; Site B - *Filipendula* marsh; Site C - humid Alder Forest; Site D - Willow carr) were chosen. A description of the sites and vegetation can be found in Table 1. A single Malaise Trap fitted with a vial containing an 80% alcohol solution was placed in each site and operated from 5-V-2006 until 1-IX-2006. The traps were emptied each week and Culicidae were separated from the other material. All specimens were identified using the keys of Schaffner *et al.*, (2001). We were unable to differentiate between the sibling species *Ae. cinereus* and *Ae. geminus,* and therefore regard all specimens of both as *Aedes cinereus*/ *geminus*.

Weekly catches per species per site

In order to detect any differences in activity between sites of the two most abundant species in the traps (and hence in population dynamics), we recorded the numbers of individuals caught each week throughout the sampling season.

Seasonal relative densities

The number of mosquitoes emerging and foraging in an area during a sampling period depends on environmental conditions during that period. In order to be able to compare counts of mosquitoes collected in different periods we calculated the relative density of each mosquito species during each period according to the formula of Kocataş (1992) and Şimşek (2006), in which RD (relative density) = NA (number of all specimens of each species collected during each period)/N (the number of specimens of all species collected during each period) x 100. However, since our main interest in this paper is the relative seasonal density of each species, numbers caught in each of the four malaise traps have been lumped together.

Vectors of arboviruses

Information on medical and veterinary importance of each of the species collected and its vector potential with regard to several arboviruses was obtained from Schaffner *et al*., (2001) and Hubálek (2008). In this study we focus on vectors of WNV.

Results

General results

We collected a total of 1,277 specimens belonging to nine species during the course of this investigation (Table 1). One *Culex* specimen and 19 *Ochlerotatus* specimens could not be identified to species level because most of the discriminating characteristics were lost during collection. *Aedes cinereus* s.l. comprised 47% and *Oc. cantans* 46% of all mosquitoes collected. Females made up 79.6% of the total catch, but we collected ten times more females than males of *Ae. cinereus/ geminus.*

Total numbers of all species found at each of the four collection sites are presented in Table 2 and relative densities of each species during each sampling period are given in Table 3. Almost all (97%) of the *Oc. cantans* collected were from the traps at the edge of *Molinia* grassland in the Willow carr, and represent 68% of all *Oc. cantans* collected during this study. *Aedes cinereus* s.l. was the most abundant species in the Reed marsh and the *Filipendula* marsh (respectively 83% and 66% of all mosquitoes at those sites, and respectively 51% and 40% of the total number of *Ae. cinereus* s.l. collected during this study). *Culiseta morsitans* was found in all sites except in the Willow carr. Somewhat surprisingly, neither *Anopheles* spp. nor *Coquillettidia richiardii* were collected during this study.

We collected more mosquito species in the two open (Reed marsh and *Filipendia* marsh) sites than in the two closed (Humid forest and Willow carr) sites and collected fewer individuals (n = 128) in the Humid forest site than in any of the other three sites. All mosquitoes found in the Willow carr area belong to species which overwinter as eggs (Fig. 1).

Population dynamics of the most abundant species

Because the malaise traps were emptied each week, seasonal population dynamics of the two most abundant species, *Oc. cantans* and *Ae. cinereus/geminus* could be studied (Figure 4). Though low numbers of the univoltine *Oc. cantans* were still present at all sites into September, this species was only abundant at one site (Willow carr) and then only in the first half of the year. In the Reed marsh, the *Filipendula* marsh and the Humid forest this species was found at fluctuating densities of lower than 20 and usually less than 10 per site, making it one of the scarcest species in these three sites.

Aedes cinereus s.l. is a multivoltine mosquito (Schaffner *et al*., 2001; Wegner, 2009), It was not found at the Willow carr site, but main activity peaks in other three sites occurred at the end of June/beginning of July and were followed by smaller peaks during the later sampling periods in July, August and September. Similarly, *Culiseta morsitans,* a univoltine species which overwinters in the larval stages (Schaffner *et al*., 2001), was not present in the Willow carr site, though it was found in almost equal numbers in each of the other sites, where we collected 87% of the specimens between August, 5th and the beginning of September.

Vectors of West Nile Virus

Several potential vectors of arboviruses were collected. *Ochlerotatus cantans* was the most abundant, but low numbers of *Oc. punctor*, *Da. geniculata*, *Cx. pipiens* and *Cx. territans* were also found.

Discussion

The use of Malaise Traps proved to be a good sampling technique for Culicidae, and collected large numbers of several species, some, such as *Culex territans* and *Culiseta morsitans,* regionally rare, However, *Anopheles* spp. were not caught in these traps, though the presence of *An. plumbeus* was detected by net sweeping along the lake. Further trials of the suitability of these traps for sampling *Anopheles* populations appear to be necessary. In contrast to mosquito traps such as MMLP Traps or Gravid Traps which depend upon an attractant, Malaise Traps operate passively and only collect specimens that are actively flying. These passive traps collect more females than males, as also do the CO_2 baited traps, probably because males do not need to seek bloodmeals away from emergence and swarming areas. During this study we collected more than four times as many female as male mosquitoes.

When suitably sited and regularly emptied, Malaise Traps can give valuable information on mosquito seasonal activity patterns and allow assessment of population structure throughout the season, including bird migration periods. One of the most abundant species during this study, *Oc. cantans*, shows different seasonal activity patterns at different habitats along the lake. This can probably be explained by varying canopy characteristics and ground water fluctuations in the study area. In some cases, particularly when and where larval sites are belatedly flooded, emergence and densities of *Oc. cantans* can be adversely affected (Schaffner *et al*., 2001). This species over-winters in the egg stage and larvae only appear at the end of the winter (Schaffner *et al*., 2001). In this study the soil in the Willow carr site was almost continuously saturated with ground water, with early hatching resulting in an early springtime adult activity peaks. In the other sites the soils were only occasionally flooded and much hatching was probably postponed. In these sites the adults appeared later, without marked peaks of abundance but persisted until the end of the summer. Indeed, fluctuations in ground water levels have an important effect on the dynamics of mosquito communities. Other studies around rivers or lakes where water levels fluctuate, have reported that large numbers of floodwater species such as *Ae. vexans* and *Oc. sticticus,* appear as biting adults suddenly and their appearance depends on these water fluctuations (Becker *et al.*, 2003).

This study has demonstrated that Malaise Traps are very effective as a surveillance tool to monitor seasonal prevalence and species composition of potential mosquito vectors of WNV. Although not conducted during this study, information about the location and periodicity of larval sites used by the resident mosquito species in relation to weather patterns would greatly add to environmental knowledge, and would be invaluable the event of an arbovirus outbreak.

Conclusion

Species known to be capable of transmitting several arboviruses, including WNV, were found to be abundant during spring and summer in and around the Etang de Virelles Nature Reserve. Abundance of one, *Oc. cantans,* seems to be influenced by microclimate, particularly fluctuating levels of ground water and the nature of emergent and bordering vegetation at several locations in the Etang de Virelles Nature Reserve. In some sites this species seems to emerge earlier than in other sites. This is important information as this species could be an important sylvatic and bridge vector of various viruses, or even be directly involved in outbreaks affecting humans. This could also be said of other mosquito species in other regions and Nature Reserves. Identification of seasonal trends in abundance of mosquito populations is essential for development of appropriate disease prevention and control methodology. Mosquito population density variations are closely linked to rainfall and temperature (Zyzak *et al*., 2002; Crowley, 2003). However, other variable factors should also be taken into consideration as this study has highlighted. Knowledge of the biology and ecology of the mosquito is essential for predicting outbreaks of disease and, if necessary, control of pest mosquitoes (Dilling, 2004).

Acknowledgements

We thank Les Amis de Virelles, and Sébastien Pierre in particular, for authorising and assisting with the sampling campaign near the Etang de Virelles. We are grateful to Francis Schaffner for the confirmation of the identifications of *Culiseta morsitans* and *Culex territans*. This research was partly supported by the Belgium government (Belspo; SD/BD/04A, Modirisk-project).

References

Ascoli, V., Facchinelli, L., Valerio, L., Zucchetto, A., Dal Maso, L. & Coluzzi, M. (2006) Distribution of mosquito species in areas with high and low incidence of classic Kaposi's sarcoma and seroprevalence for HHV-8. *Medical and Veterinary Entomology* **20,** 198-208.

Becker, N., Petrić, D., Zgomba, M., Boase, C., Dahl, C., Lane, J. & Kaiser, A. (2003) Mosquitoes and their control. Kluwer Academic/ Plenum Publishers, New York.

BioGents (2007) Manual for the BG-Sentinel: an innovative trapping system for mosquitoes and other blood-sucking insects. Regensburg, Germany BioGents AG (Available from: http://www.bg-sentinel.com/bilder/Manual_BG-Sentinel.pdf).

Campbell, C.B. (2003) Evaluation of five mosquito traps and a horse for West Nile vectors on a north Florida equine facility. Thesis, University of Florida, Gainesville, Florida.

Campbell, J.W. & Hanula, J.L. (2007) Efficieny of Malaise traps and colored pan traps for collecting flower visiting insects from forested ecosystems. *Journal of Insect Conservation* **11**, 399-408.

Crowley, J.C. (2003) Determining seasonality of nuisance flies and evaluating stable fly pests on horses at an equine facility in North Central Florida. Thesis, University of Florida, Gainesville, Florida.

Darling, D.C. & Packer, L. (1988) Effectiveness of malaise traps in collecting Hymenoptera: the influence of trap design, mesh size, and location. *Canadian Entomologist* **120**, 787-796.

de Bellefroid, F., Delflorenne, P., Paquet, J.-Y. & Pierret, S. (2008) Nidification de la Sterne pierregarin (*Sterna hirundo*) à l'étang de Virelles, une première pour la Wallonie. *Aves* **45**, 81-92.

Dekoninck, W., Desender, K. & Grootaert, P. (2005) Faunistische evaluatie en vergelijking van bosuitbreiding via bebossing, spontane verbossing, en extensieve begrazing van open terreinen: een studie in de Voerstreek. Rapport ENT.2005.01 in opdracht van Aminal afdeling Bos & Groen, project AMINAL/B&G/30/2002, p. 189.

Dilling, S.C. (2004) Evaluation of mosquito trapping efficiency and determination of seasonality for mosquitoes at the University of florida horse teaching unit. Unpublished Masters Thesis at the University of Florida, 121pp.

Dudbois, B., Hallet, C., Lambert, M. & Philippart, B. (1987) Virelles. Un étang, des poissons et des oiseaux. *Réserves Naturelles* **1987**, 118-119.

Gosseries, J. & Goddeeris, B. (1991) Culicidae. In Grootaert, P, De Bruyn, L & De Meyer, M, Catalogue of the Diptera of Belgium. Studiedocumenten van het KBIN **70**, 338.

Grootaert, P., Verlinden, L., Meuffels, H., Haghebaert, G., Pollet, M., Leclercq, M., De Meyer, M. & Magis, N. (1988) Diptères de la réserve naturelle de l'étang de Virelles en Belgique. *Bulletin et Annales de la Société royale belge d'Entomologie* **124**, 320-324.

Hubálek, Z. (2008) Mosquito-borne diseases in Europe. *Parasitology Research* **103** Suppl 1, S29-43.

Jacob, J.-P. (1983) L'intérêt ornithologique de l'étang de Virelles. *Natura Mosana* **37**, 28-32.

Kline, D.L. & Mann, M.O. (1998) Evaluation of butanone, carbon dioxide, and 1-octen-3-ol as attractants for mosquitoes associated with north central Florida bay and cypress swamps. *Journal of the American Mosquito Control Association* **14**, 289-297.

Kline, D.L. (1999) Comparison of two American Biophysics mosquito traps: the professional and a new counterflow geometry trap. *Journal of the American Mosquito Control Association* **15**, 276-282.

Kocataş, A. (1992) Ekoloji-Çevre Biyolojisi, Ege Üniversitesi Matbaasi, Izmir.

Korgaonkar N.S., Kumar, A., Yadav, R.S., Kabadi, D., Dash, A.P. (2008) Sampling of Adult Mosquito Vectors with Mosquito Magnet™ Pro in Panaji, Goa, India. *Journal of the American Mosquito Control Association* **24**, 604-607.

Lee, J.-H. & Kokas, J.E. (2004) Field evaluation of CDC gravid trap attractants to primary West Nile virus vectors, *Culex* mosquitoes in New York State. *Journal of the American Mosquito Control Association* **20**, 248-253.

Malkinson, M. & Banet, C. (2002) The role of birds in the ecology of West Nile virus in Europe and Africa. *Current Topics in Microbiology and Immunology* **267**, 309-322.

Matthews, R.W. & Matthews, J.R. (1970) Malaise trap studies of flying insects in a New York mesic forest. I. Ordinal composition and seasonal abundance. *Journal of the New York Entomological Society* **78**, 52–59.

McNelly, J.R. (1989) The CDC trap as a special monitoring tool. *Proceedings of the Seventy-Sixth Annual Meeting of the New Jersey Mosquito Control Association, Inc.* Lindenworld, New Jersey, 26-33.

Mouchet, J., Rageau, J., Laumond, C., Hannon, C., Beytout, D., Oudar, J., Corniou, B. & Chippaux, A. (1970) Epidemiologie du virus West Nile: etude d'un foyer en Camargue. V Le vecteur: *Culex modestus* Ficalbi Diptera: Culicidae. *Annales de l'Institut Pasteur* **118**, 839-855.

Murgue, B., Murri, S., Zientara, S., Durand, B., Durand, J.P. & Zeller, H. (2001) West Nile outbreak in horses in Southern France, 2000: the return after 25 years. *Emerging Infectious Diseases* **7**, 692-696.

Noyes, J.S. (1989) The diversity of hymenoptera in the tropics with special reference to parasitica in Sulawesi. *Ecological Entomology* **14**, 197–207.

Poncon, N., Toty, C., Ambert, G.L., Le Goff, G., Brengues, C., Schaffner, F. & Fontenille, D. (2007) Population dynamics of pest mosquitoes and potential malaria and West Nile virus vectors in relation to climatic factors and human activities in the Camargue, France. *Medical and Veterinary Entomology* **21(4)**, 350-357.

Romi, R., Pontuale, G., Ciufolini, M.G., Fiorentini, G., Marchi, A., Nicoletti, L., Cocchi, M. & Tamburro, A. (2004) Potential vectors of West Nile Virus following an equine disease outbreak in Italy. *Medical and Veterinary Entomology* **18**, 14-19.

Rose, A., Kröckel, U., Bergbauer, R., Geier, M. & Eiras, Á.E. (2006) Der BGSentinel, eine neuartige Stechmückenfalle für Forschung und Überwachung. The BG-Sentinel, a novel mosquito trap for research and surveillance. *Mitteilungen der Deutschen Gesellschaft für allgemeine und angewandte Entomologie* **15**, 345-348.

Schaffner, F., Angel, G., Geoffroy, B., Hervy, J-P., Rhaiem, A. & Brunhes, J. (2001) *The Mosquitoes of Europe, identification and training program.* Montepellier.

Scott, J.J., Scott, C.C. & Crans, W.J. (2001) Use of an infusion-baited gravid trap to collect adult *Ochlerotatus japonicus*. *Journal of the American Mosquito Control Association* **17**, 142-143.

Şimşek, F.M. (2006) Seasonal frequency and relative density of larval populations of mosquito species (Diptera: Culicidae) in Şanhurfa Province, Turkey. *Turkish Journal of Zoology* **30**, 383-392.

Wegner, E. (2009) A study of mosquito fauna (Diptera: Culicidae) and the phenology of the species recorded in Wilanów (Warsaw, Poland). *European Mosquito Bulletin* **27**, 23-32.

Williams, C.R., Long, S.A., Webb, C.E., Bitzhenner, M., Geier, M., Russel, R.C. & Ritchi, S.A. (2007) *Aedes aegypti* population sampling using BG-sentinel traps in north Queensland Australia: statistical consideration for trap deployment and sampling. *Journal of Medical Entomology* **44**, 345-350.

Zyzak, M., Loyless, T., Cope, S., Wooster, M. & Day, J. (2002) Seasonal abundance of *Culex nigripalpus* Theobald and *Culex salinarius* Coquillett in north Florida, USA. *Journal of Vector Ecology* **21**, 155-162.

Table 1. General characteristics of the sites sampled.

	Site A	Site B	Site C	Site D
Description of the site	Reedmarsh bordering the lake	Humid *Filipendula* marshland	Humid deciduous forests (carr); alder forest	Willow car and *Molinia* grassland with shallow fen
Dominant vegetation	*Phragmites australis* 2.5m, only very few herbs: *Lycopus europaeus.* Some *Salix sp.* +/- 5 m in a row of 20 m.	*Filipendula ulmaria* dominant, also *Lythrum salicaria*, *Angelica sylvestris*, *Juncus* sp., *Carex* sp. +/- 1 m. One side bordered with *Alnus glutinosa* +/- 10-20 m. Other side bordered with *Salix* sp. +/- 5-20 m. Other side bordered with *Phragmites australis* (+/- 20 m²) +/-2 m	*Alnus glutinosa* +/-20 m with undergrow of *Fraximus excelsior* and *Crataegus monogyna* +/- 3 m. *Carex sp.*, *Filipendula ulmaria* and *Rubus fruticosus*.	*Salix sp.* +/- 10 m. *Carex* sp. and *Caltha palustris.* One side reed marsh, other side *Filipendula ulmaria*, *Mentha aquatica* and *Phragmithes.* Other side *Alnus glutinosa* (+/- 20 m) and *Molinia caerulea.*
Humidity and water level	Reed marsh often and quickly dry in spring and summer when a few days without rain	Sometimes dry in spring and summer when a few days without rain, but with many ditches (a network of +/- 30 m)	Often 10 cm water, but however quickly dry when a few days without rain in spring and summer.	Most humid sites with 20 cm of water and almost continuously humid.

Table 2. Number of specimen and species collected at each site. In parentheses, the stage in which the species overwinters: E = species overwinters as egg, L = species overwinters as larva and A = species overwinters as adult, according to Schaffner *et al*. (2001).

Species	**Open vegetation: marshes**		**Closed vegetation: forests**		**Total**
	Site A Reed marsh	**Site B *Filipendula* marsh**	**Site C Humid forest**	**Site D Willow carr**	
	Female/Male	Female/Male	Female/Male	Female/Male	
Aedes cinereus/geminus (E)	289/20	222/21	31/9	4/5	601
Culex pipiens (A)	2/1	0/2			5
Culex territans (L)	1/0	2/0	3/0		6
Culiseta annulata (A)	3/0	2/0	1/0		6
Culiseta morsitans (L)	4/0	21/2	14/0		41
Dahliana geniculata (E)	2/0			1/0	3
Ochlerotatus cantans (E)	42/0	49/33	61/3	236/161	585
Ochlerotatus punctor (E)		0/1		1/0	2
Ochlerotatus rusticus (E)	1/0	3/0	3/0	1/0	8
Ochlerotatus sp.	6/0	7/2	2/0	2/0	19
Culex sp.			1		1
Number of specimens	371	367	128	411	1277
Number of species	8	8	6	4	9

Table 3. Seasonal relative densities of all mosquito species collected at the four sites and for each sampling period with (AED CIN = *Aedes cinereus/geminus*, CLX PIP = *Culex pipiens*, CLX TER = *Culex territans*, CUL ANN = *Culiseta annulata*, CUL MOR = *Culiseta morsitans*, DAH GEN = *Dahliana geniculata*, OCH CAN = *Ochlerotatus cantans*, OCH PUN = *Ochlerotatus punctor*, OCH RUS = *Ochlerotatus rusticus*).

Date	**AED CIN**	**CLX PIP**	**CLX TER**	**CUL ANN**	**CUL MOR**	**OCH CAN**	**DAH GEN**	**OCH PUN**	**OCH RUS**
5/05/2006	2.38	0.00	1.19	0.00	3.57	90.48	0.00	2.38	0.00
12/05/2006	7.07	0.00	1.01	0.00	0.00	91.92	0.00	0.00	0.00
19/05/2006	13.21	0.00	0.00	0.00	0.00	86.79	0.00	0.00	0.00
26/05/2006	4.27	0.00	0.00	0.00	0.00	94.87	0.00	0.00	0.90
2/06/2006	10.17	0.00	0.00	0.00	0.00	87.29	0.00	0.00	2.54
9/06/2006	71.43	0.00	0.00	2.86	0.00	25.71	0.00	0.00	0.00
16/06/2006	89.94	0.00	0.56	0.00	0.00	9.50	0.00	0.00	0.00
23/06/2006	81.61	1.15	0.57	0.57	0.00	15.52	0.00	0.00	0.57
30/06/2006	75.76	0.00	0.00	0.00	0.00	22.22	0.00	0.00	2.02
7/07/2006	50.00	0.00	0.00	0.00	0.00	50.00	0.00	0.00	0.00
14/07/2006	56.67	0.00	1.11	0.00	0.00	42.22	0.00	0.00	0.00
21/07/2006	64.71	0.00	1.96	1.96	0.00	31.37	0.00	0.00	0.00
28/07/2006	75.00	0.00	0.00	5.56	0.00	13.89	5.56	0.00	0.00
5/08/2006	63.64	0.00	0.00	4.55	9.09	22.73	0.00	0.00	0.00
11/08/2006	18.18	18.18	0.00	0.00	36.36	18.18	0.00	0.00	9.09
18/08/2006	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00
25/08/2006	56.00	2.00	0.00	0.00	28.00	14.00	0.00	0.00	0.00

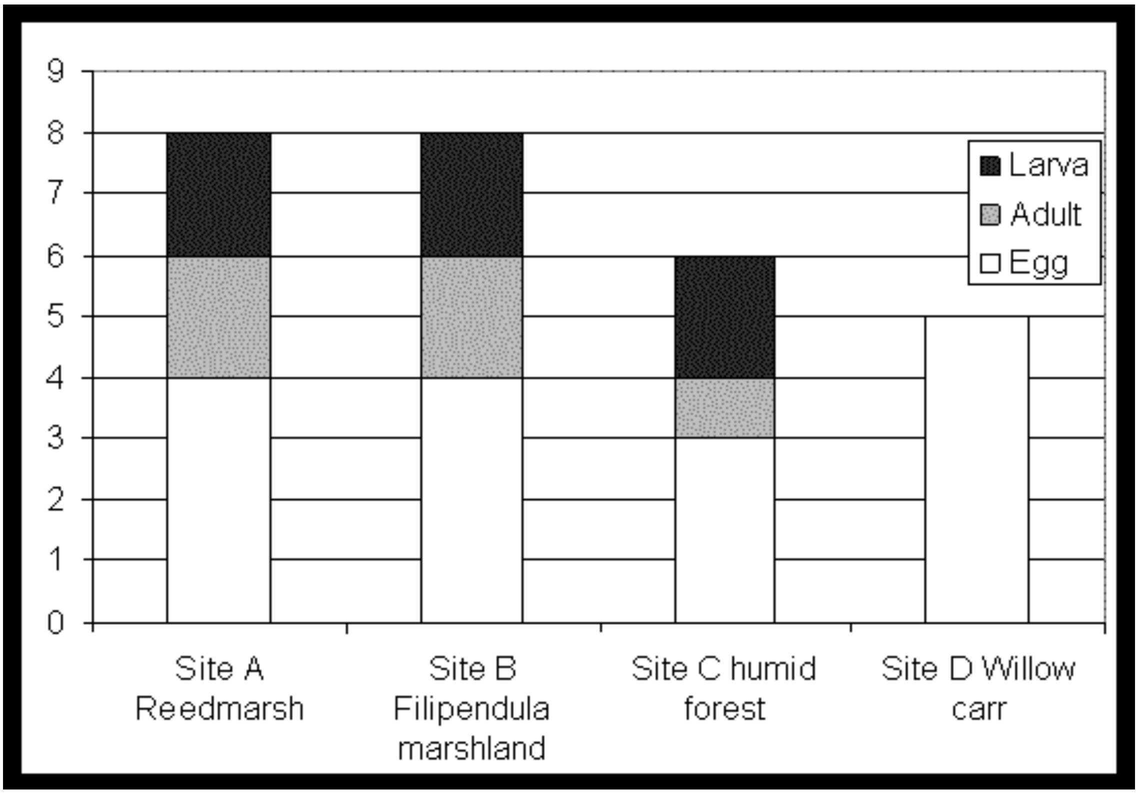
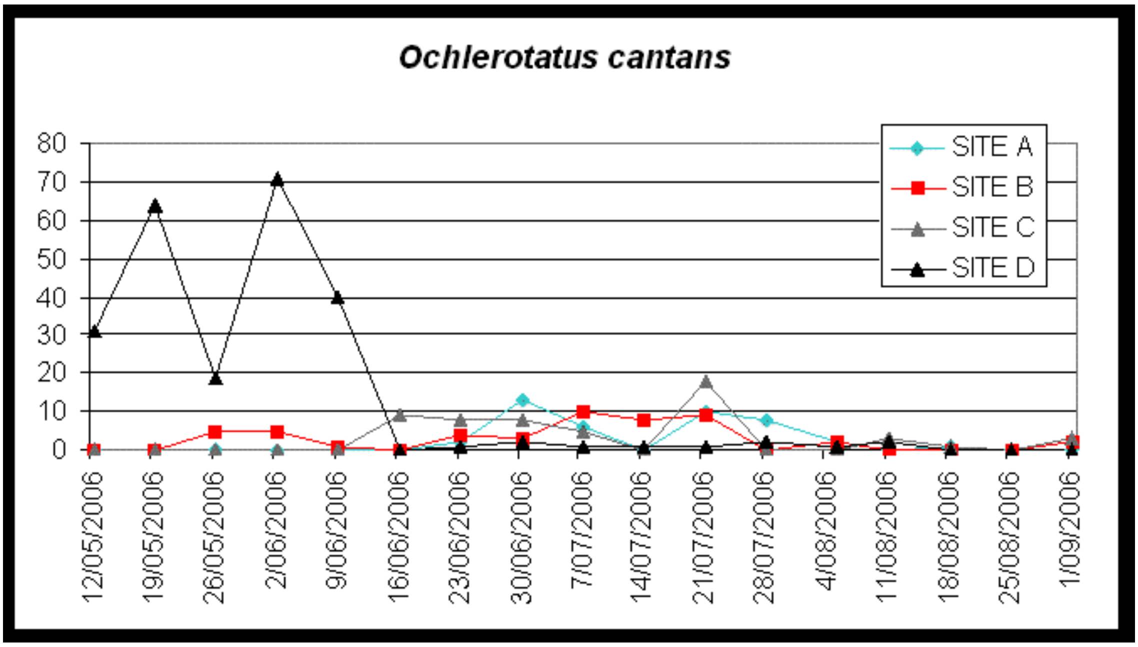


Fig. 1. Overwintering stage of the species collected at sampled sites with Egg = species overwinters as egg, Larva = species overwinters as larva and Adult = species overwinters as adult, according to Schaffner *et al*. (2001).



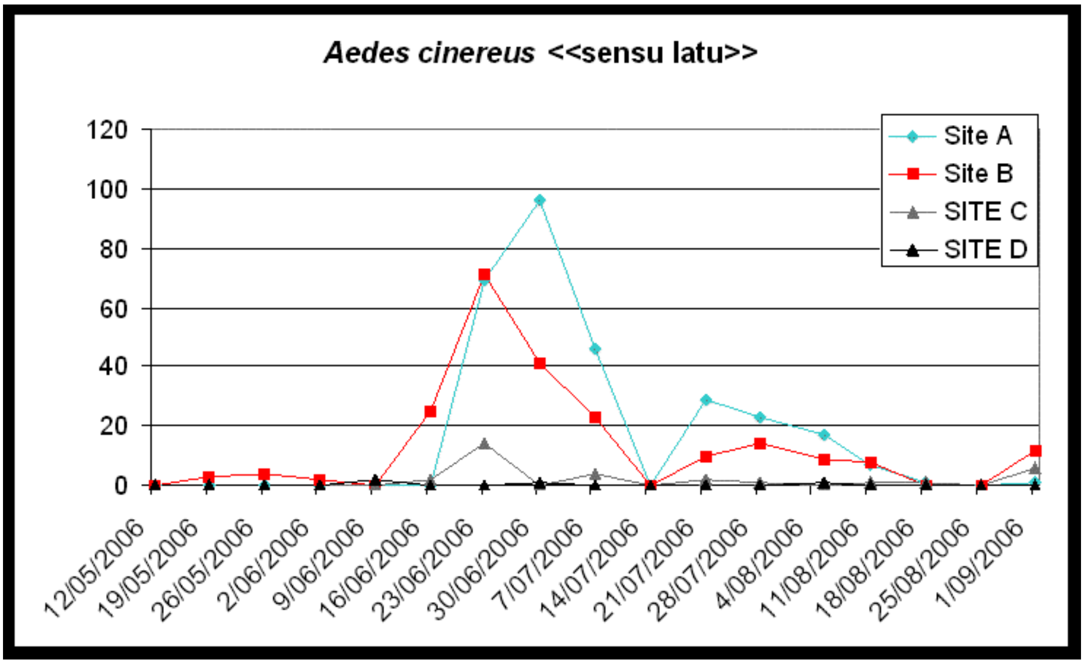


Fig. 2. Weekly catches with one Malaise trap of *Ochlerotatus cantans* and *Aedes cinereus/geminus* at four sites along the Lake Virelles from 12/5/2006 until 1/9/2006.