academicJournals

Vol. 6(9), pp. 142-150, September 2014
DOI: 10.5897/JPVB12.010
Article No: F4708A847025
ISSN 2141-2510

http://www.academicjournals.org/JPVB

Journal of Parasitology and Vector Biology

Short Communication

Biodiversity and ecology of Culicidae and Simuliidae probable vectors of infectious diseases in villages of the Sanaga mid valley, Cameroon: Influence of the Sanaga River

Kuete Thomas[1]*, Nkoa Therese[2], Tassen Fabien[3], Baleguel Nkot Pierre[4] and Same-Ekobo Albert[1,5]

[1]Faculty of Medicine and Pharmaceutical Sciences, University of Douala, Cameroon.
[2]Faculty of Medicine and Biomedical Sciences, University of Yaoundé 1, Cameroon.
[3]Faculty of Sciences, University of Yaounde 1, Cameroon.
[4]Yaounde Initiative Foundation, Yaoundé, Cameroon.
[5]Laboratory of Parasitology, Yaoundé University Hospital Centre, Cameroon.

Received 6 April, 2012; Accepted 25 August, 2014

Most riverine villages of the Sanaga river are known endemic for vector-borne diseases. Two cross sectional surveys were set during two seasons in villages of the Sanaga mid valley to identify main Simuliids and mosquitoes genus and species, their specific biotopes and fluctuations of their abundance with respect to distance from the river banks and seasons. The study villages are located close to 5 and 35 km from the Sanaga river edges. Both larva and adult stages were assessed using known methods. All adults Simuliids, larvae and nymphs were identified as *Simulium damnosum*. Larvae and nymphs were collected only in the river stream and adults near the banks, farms and near households. Adults and larvae abundance was greater in the rainy season whereas nymphs were more abundant in the dry season. Endophilic mosquitoes harvested were *Anopheles* and *Culex*. Their abundance was greater in villages close to the river. Species and resting densities varied with distance from the river edges. Culicidae larvae collected belonged to *Aedes*, *Anopheles* and *Culex*. Fourteen species were identified, 3 of *Anopheles*, 5 of *Aedes* and 6 of *Culex*. Some species showed broad specificity to biotopes. This study indicates that mosquito fauna is more diversified at larval stage in the Sanaga mid valley; some having broad specificity to breading sites. The Sanaga river harbours most of the species found in the water bodies. Indoor adult mosquitoes are less diversified indicating that most of the mosquito found at larval stage may breed mostly outdoor. *Simulium* larvae and pupae breed specifically in the falls and rapids of the Sanaga stream. Both sexes of adult *Simulium* are found near the river, whereas only females are found near households and in farms. Adults *Simulium* density decreases with the distance from the river with two picks of abundance in the day.

Key words: Biodiversity, Culicidae, Simuliidae, Sanaga River.

INTRODUCTION

Insect borne diseases are most harmful in social economic importance among endemic diseases in most tropical areas. Transmission of these diseases is mostly related to insect's adult flies among which mosquitoes

(Culicidae) and black flies (Simuliidae) families are believed to be most important. Culicidae or mosquitoes are known to transmit either virus, bacterial or parasitic diseases, whereas Simuliidae or black flies are known as specific vectors of onchocerciasis in tropical areas (Rodhain and Perez, 1985; Rodhain, 1999; Ostfeld and Keesing, 2000).

The Sanaga mid valley is an area where despite mass distribution of ivermectin, the onchocerciasis-specific treatment, onchocerciasis is still highly endemic. Foremost, onchocerciasis endemicity level in this area is likely to favour the occurrence of epilepsy, a neurologic disease. Previous studies demonstrated that neighbouring villages of the Sanaga river are mesoendemic to hyperendemic for malaria (Gazin et al., 1989).

The Sanaga river is of most importance in Cameroon. The streams are mostly fast flowing with many rapids and falls on rocky substratum suitable as breeding sites for *Simulium* (Mouchet, 1962; Philippon, 1977; Same-Ekobo, 1997). Apart from favouring development of *Simulium* larva, the river can be expected to favour development of other flies like mosquitoes, thus influencing epidemiology of other vector borned transmitting diseases.

A descriptive study was done in the diversity and spatial distribution of potent endemic diseases transmitting Culicidae and Simuliidae flies in the Sanaga river and in three villages situated at different distances from the river edges in the Sanaga river mid valley. Studied villages were Mbebe, Ndomnjengue and Bot Makak.

The main objective of the study was to investigate species that can be found in the study villages with respect to distance from the Sanaga river edges and specify ecological peculiarities of species found in prospected areas.

MATERIALS AND METHODS

Study area

The Sanaga mid valley is located in a forest-savannah transition area covering almost 150 km distance between the Monatele town in the Lekie division upstream and Edea town in the Sanaga Maritime division downstream. This valley covers three divisions, namely, Lekie, Nyong-Ekelle and Sanaga-Maritime divisions. The Sanaga river is marked at this level by existence of rapids and one of it most important falls; the Mbebe-Kikot falls with an almost rocky substratum.

The studied villages were Ndomdjengue located in the Sanaga-Maritime division 5 km from the main Mbebe-Kikot falls of Sanaga, village Mbebe located close to the edges of the Sanaga river and close to the later falls of the area in the Nyong-Ekelle division and the village Bot-Makak located 35 km from the river edge in the Nyong-Ekelle division. Ndomdjengue and Mbebe villages are located in forest-like areas, whereas Bot-Makak is a small town surrounded by a forest area. Moreover, Ndomdjengue village is separated from the river by an evergreen forestry screen. Farming, bovine rearing and commerce are the main occupations in the three villages. Fishing is also made by residents in Mbebe. This study consisted in collecting and identifying adults as well as larvae stages of *Simulium* and Culicidae. Sampling techniques and conservation varied according to insect groups and stages.

Simuliidae collection and preservation

Adult *Simulium* were captured using aluminium panel trap (100×100×0.55 cm) coiled with glue made of Tween 20 and 95° alcohol. The trap was set from 6 am to 6 pm at 2 m from the ground and flies caught at each hour were collected, counted and transferred into tubes. These tubes were then closed with a dry cotton wool and brought to laboratory for identification. The traps were set in farms, near habitations, at the river edges and in the forest undergrowth.

Larvae and pupae of *Simulium* were collected in the stream of the river on rocky substratum, and in the falls from leaves and stems of submerged and floating vegetation. Soft forceps and plastic pipettes were used to collect larvae on rocky substratum. Larvae hanged on submerged and floating vegetations were collected with soft pipettes after tearing the vegetation using a hook. Larvae and pupae collection lasted 30 min at each site. Larva stages were then transferred in test tubes containing 70° ethanol, and then carried to laboratory for identification.

Culicidae collection and preservation

Indoor resting adult mosquitoes were harvested in each village through pyrethrum insecticide spray in a room of known dimensions and where a 2×2 m white sheet was previously placed. The spraying took place between 10 pm and midnight. Dead and alive mosquitoes collected on the white sheet were transferred into test tubes, and then brought to the laboratory for identification. Indoor-resting density for adults Culicidae was calculated with the following formula: Total number of adults collected/Number of rooms screened.

Larva and pupa of mosquitoes were searched in different water bodies across the villages, namely, Sanaga river falls, rapids, marshes, pools, ponds, springs, holes in trees, abandoned containers like flasks, tyres, and dishes. Culicidae larvae and pupae were collected in large water bodies using the classical “dipping” technique as described by Service (1976). In small water bodies like abandoned containers or tyres and tree holes, larva stages were collected using a plastic pipette. All larva collected were brought to the laboratory in flasks containing water from the collecting site for identification.

Some of the mosquito larvae were bred in laboratory for adult emergency for accurate identification. The larvae were fed in plastic containers until adult harvest with organic detritus from water collected in their natural breeding sites. These adults were then identified using morphological keys.

Identification of harvested insects

Both Culicidae and Simuliidae adult and larval stages were

*Corresponding author. E-mail: thomaskuete@hotmail.com. Tel: (237) 77 92 12 30.

examined under a stereomicroscope and identified using the specific morphological characteristics identification keys for *Anophelinae* species (Gillies and De Meillon, 1968; Gillies and Coetzee, 1987), Culicinae (Jupp, 1996) and Simuliidae (Freeman and De Meillon, 1953). Data were analysed using Chi^2 test at confidence interval of 0.05.

RESULTS

Biodiversity and abundance of Simuliidae

A total of 996 adults *Simulium* (black flies) were collected in the villages with 90.7% in May (rainy season) and 9.3% in December (dry season). All adult black flies were identified as *Simulium damnosum* Theobalt. Abundance of adult stages decreased significantly with distance from the Sanaga river edges with adult *Simulium* collected, 819 (53.2%) were harvested at Mbebe, 166 (43.9%) at Ndomnjengue and 11 (2.9%) at Bot Makak.

Adult black flies abundance varies also within the same village with distance from the breeding site. At Mbebe for example, of 766 adult black flies caught in May (rainy season), 618 (80.7%) were caught at the vicinity of rapids in the Sanaga river and the remaining far from the breeding site including 97 (12.7%) near households and 51 (6.7%) in farms.

Abundance of adult black flies also showed variation with seasons. In fact, 378 adult *Simulium* were harvested close to households and farms. 93 (24.6%) of them were collected in December and 285 (75.4%) caught in the rainy season (May). The difference recorded is statistically significant between the two seasons ($p<0.001$) indicating that the rainy season may favour the spread of adult black flies throughout the farms and near human habitats.

Daily cycle of adult *Simulium* flies

Hour to hour harvesting of adult black flies from 6 am to 6 pm allow identification of two peaks of maximal activities of adult flies, with one in the morning between 8 and 9 am (20% of adults caught) and the second in the late afternoon between 5 and 6 pm. Flies were less abundant between 1 and 3 am with an aggregate 2% of adult *Simulium* caught during the 2 h.

Abundance variation between households and farms

In all villages screened, adult black flies were found mostly near households than in farms with differences being statistically significant. In fact, adult *Simulium* caught outside from the Sanaga river edges, 65.5, 100 and 50.7% were caught near households at Mbebe, Bot Makak and Ndomdjengue, respectively. The remaining was captured in farms from the other village.

Distribution of adult *Simulium* flies according to sex in the screened areas

Of the adult *Simulium* caught on traps, males were caught only along the Sanaga river edges and Mbebe, whereas only female black flies were caught in the other villages near households far from the river edges and farms. Of the adult *Simulium* harvested at Mbebe, 44 (7%) were males and 574 (93%) females.

Larvae and pupae

Simulium larvae and pupae were found only in the Sanaga River mostly hanged on submerged aquatic rocky substratum and vegetables. This submerged and floating vegetation was identified to family Podostemaceae, namely, *Dicraeananthus africanus*.

Larva and pupa were mostly abundant in the rainy season. In fact, of *Simulium* larvae and pupae harvested in the two seasons, 69.4% of the larvae and 52% of the pupae were caught in the rainy season. All the larvae and pupae were identified as *S. damnosum* complex. The difference found between abundances of larvae collected is statistically significant between the two seasons with the rainy season being more suitable for development of these stages ($p<0.001$), whereas this difference is not statistically significant among pupa harvested in either season ($p>0.30$).

Culicidae species diversity in the study area

Forty species were identified in this sample. Three species were found among mosquitoes of the genus *Anopheles*: *Anopheles gambiae*, *Anopheles nili*, and *Anopheles funestus*. Those of the genus *Aedes* belonged to five species, namely, *Aedes aegypti*, *Aedes vittatus*, *Aedes chaussieri*, *Aedes ledgeri*, and *Aedes albopictus*. Six species were found in the genus *Culex*, *Culex quinquefasciatus*, *Culex perfuscus*, *Culex rubinotus*, *Culex chorleyi*, *Culex simpsoni*, and *Culex insignis*.

Species diversity in each village

The number of Culicidae spp. and development stages varied among villages. Thus, *A. gambiae*, *A. aegypti* and *C. quinquefasciatus* were harvested in the three villages visited, while *A. ledgeri* was harvested only at Ndomdjengue and Bot Makak. Species *A. nili* and *A. funestus* were found at Mbebe and Ndomdjengue. Species *A. Albopictus* and *C. simpsoni* were found only at Bot Makak. Species *A. vittatus*, *A. chaussieri*, and *C. Insignis* were harvested only at Mbebe whereas *C. perfuscus*, *C. rubinotus*, and *C. chorleyi* were collected only at Ndomnjengue.

Stage-related diversity

Of Culicidae spp. harvested, *A. gambiae* and *C. quinquefasciatus* were found either as adult or larval stages, whereas *A. nili* and *A. funestus* were found only as adult stage. The other ten mosquito species were found only as larval stage.

Mosquitoes of the genus *Aedes* were found only at larval stages in our sampling. Five species were identified among specimen harvested including: *A. aegypti* (88.9%), *A. vittatus* (2.6%), *A. chaussieri* (1.1%), *A. ledgeri* (2.3%) and *A. albopictus* (5.1%).

Six species were identified among mosquitoes of the genus *Culex*, namely, *Culex quinquefasciatus* (16.2%), *C. perfuscus* (1.6%), *C. rubinotus* (52.7%), *C. chorleyi* (15.1%), *C. simpsoni* (4%) and *C. insignis* (10.4%). *C. quinquefasciatus* was the only species found at adult stage, the others were found at larval stage.

Culicidae larva stages in breeding sites

Thirty four (34) breeding sites were screened: 9 (26.47%) at Ndomnjengue, 6 (17.65%) at Bot-Makak, 19 (55.9%) at Mbebe. Of those screened at Mbebe, 78.95% were located at the edge of the Sanaga river and the other away from the edges, so the rocky banks of the Sanaga river at Mbebe seem propitious for development of mosquito larva.

Of the larvae collected, 857 (63%) were collected at Mbebe, 371 (27.25%) collected at Ndomnjengue, and 133 (9.75%) collected at Bot Makak. Culicidae larvae were harvested in a variety of breeding sites: permanent, temporary and natural water bodies.

Mosquito larvae from Ndomnjengue belonged to the genera *Aedes* and *Culex*. Of the total number collected in this village, those of the genus *Aedes* belonged to two species, namely, *A. aegypti* (16.2%) and *A. ledgeri* (2.9%). Four species were identified among those of the genus *Culex*, namely, *C. quinquefasciatus* (5.65%), *C. perfuscus* (1.9%), *C. rubinotus* (61.2%) and *C. chorleyi* (12.1%). No *Anopheles* larva was found in this village.

At Mbebe, *A. gambiae* was the only Anopheline larva collected. This species represented 8.75% of the sample collected in this village. Mosquitoes of the genus *Aedes* belonged to three species including *A. aegypti*, *A. vittatus*, and *A. chaussieri* accounting for 75.95, 2.6 and 1%, respectively of the sample collected in this village. Culex larvae belonged to three species: *C. quinquefasciatus*, *C. insignis*, and *C. Chorleyi.* These Culicidae spp. represented 4.1, 5.2 and 2.3% of the total Culicidae larvae collected as in Table 1.

At Bot Makak, no *Anopheles* larva was found. Mosquitoe larvae collected were *Aedes* and *Culex*. *Aedes* larvae were of three species: *A. aegypti*, *A. ledgeri* and *A. albopictus* representing 38.3, 6.8 and 33.1% of the specimen collected, respectively. Culex larvae were of two species: *C. quinquefasciatus* and *C. simpsoni* comprising for 9 and 12.8% of Culicidae larvae collected in this area.

Distribution of mosquito larvae in breeding sites

A. aegypti was found in natural temporary as well as permanent breeding sites notably in abandoned water-storage containers near households and holes in trees. This species was usually found together with one or more of the following species: *C. rubinotus*, *C. perfuscus*, *A. albopictus*, *A. ledgeri* and *C. simpsoni*.

Species *A. aegypti* and *C. quinquefasciatus* were found in the three villages, while *A. gambiae*, *A. vittatus*, and *A. chaussieri* were present only at Mbebe, and species *A. albopictus* and *C. simpsoni* collected only at Ndomnjengue. Species *A. gambiae*, *A. vittatus*, *A. chaussieri*, and *C. insignis* were found mostly in clean water contained in holes on the rocky substratum of the Sanaga river edges.

C. quinquefasciatus specimen were found in water bodies bearing organic materials in permanent ponds of the undergrowth forest at Ndomdjengue, and in clean water bodies with holes of rocky substratum of the Sanaga banks.

Of the 8 larvae of mosquito species found at Mbebe, 6 (75%) were harvested in the Sanaga river and the 2 (25%) outside.

Spatial distribution of adult *Culicidae spp.*

At Ndomdjengue, 67.7% of the Anophelines collected were identified as *A. gambiae*, 25.8% as *A. nili* and 6.5% as *A. funestus*. *Culex* and *Aedes* mosquitoes were not harvested at adult stage in this village. At Mbebe, *A. nili* specimen accounted for 55.6% of the Anohelines collected, *A. gambiae* for 40.7 and 3.7% were *A. funestus*. *Culex* and *Aedes* mosquitoes were not harvested at adult stage in this village. At Bot-Makak, 86.7% of the adult mosquito specimens were *A. gambiae* and 13.3% were *C. quinquefasciatus.* Adult *Aedes* was not found in this area.

Therefore, three *Anopheles* species, namely, *A. gambiae*, *A. nili* and *A. funestus* were found in the two villages close to the Sanaga River (Mbebe and Ndomnjengue), whereas *A. gambiae* is the species found at Bot Makak.

Of the mosquitoes harvested in the three villages, *A. gambiae* specimen account for 46.7, 24.4 and 28.9% at Ndomnjengue, Mbebe and Bot Makak, respectively. The species *A. nili* and *A. funestus* found only at Ndomnjengue and Mbebe representing 34.8 and 66.7%, respectively in the first village, 65.2 and 33.3% at Mbebe.

Adult mosquitoes resting densities

Average resting densities of mosquitoes recorded in the villages were 1.5, 1.48 and 0.65 at Mbebe, Ndomnjengue and Bot-Makak, respectively.

Considering mosquito species, resting densities for *A. gambiae*, *A. nili*, *A. funestus* and *C. quinquefasciatus* are 0.73, 0.37, 0.05 and 0.03 in the three villages. The highest resting densities per species are 1.00 for *A. gambiae* at Ndomdjengue, 0.83 for *A. nili* at Mbebe, 0.1 for *A. funestus* at Ndomdjengue and 0.09 *C. quinquefasciatus* at Bot Makak.

DISCUSSION

Culicidae and Simuliidae are leading transmitters of vector borne diseases in the tropics. Their abundance is mainly influenced by the existence of water bodies for larvae breeding. However, occurrence and spread of chemoresistance to pathogens as well as the vectors is the main difficulty to effective control of these diseases. Vector control is becoming more difficult due to the complexity of vectorial system in addition to chemo-resistance, since some strains or species are becoming more susceptible to insecticides than others. The complexity of the vectorial systems become harmful with the zooanthropophilic capacity of some insects vectors which feed both on human and livestock, thus facilitating transportation of the pathogens to human neighbourhood. The launching and implementation by the National Malaria Control Program of the prevention of disease transmission through fighting vectors intend to eliminate or lower abundance of potent anthrophilic insects that feed on human being.

This study was a first step on the estimation of the risk of vector borne diseases outbreaks or persistence in villages situated at different distances from the Sanaga river in a forest/savannah area and to assess the influence of this river on the biodiversity, the spatial distribution of major Culicidae and Simuliidae species among investigated habitats in the Sanaga mid valley.

Fourteen species were identified among Culicidae larvae from the 34 potent larval breeding sites investiga-ted from the Sanaga river banks to Bot Makak area situated at 35 km from river edges. Most of these species have been mentioned in previous studies on mosquito fauna in Cameroon (Rageau and Adam, 1952; Rickenbach et al., 1976a, b; Fontenille and Toto, 2001; Awono-Ambene et al., 2004). The Sanaga river banks seemed more suitable for the development of mosquito larvae, since these banks harboured six of the eight larvae Culicidae spp. found in village Mbebe the closest village. The river banks may thus offer better physical and chemical conditions for larval stages development. This finding corroborates reports from the Comoe River in Ivory Coast which bears more larval mosquito species than other water bodies in the village (Adja et al., 2006).

Mosquito species were found more diversified at larval stage and also showed broad specificity for breeding sites. *A. aegypti* and C*. quinquefasciatus* larvae were found in all areas screened indicating its ubiquity as previously reported in Cameroon (Rageau and Adam, 1952, 1953; Rickenbach et al., 1976a). However, the presence of C*. quinquefasciatus* larvae in all water bodies types, both sunny and shady sites, clear and organic materials bearing water bodies, though similar to reports from Cap Vert (Larivière and Abonnenc, 1958), contrast with earlier data reporting *C.* species to prefer organic materials bearing water bodies in urban settings of Africa (Subra, 1973; Robert et al., 1986; Hougard et al., 1993).

C. perfuscus and *A. ledgeri* larvae showed a broad specificity to tree holes bearing water bodies, while *A. vittatus* larvae specific habitats were holes on stones along the Sanaga river banks. This study is a first demonstration of *A. vittatus* larvae in the Sanaga mid valley. Demonstration of *A. ledgeri* in Cameroon is a new observation. *A. albopictus* larvae showed microhabitat specificity to small size natural and artificial water bodies mostly abandoned tyres and containers corroborating previous observations in Cameroon (Fontenille and Toto, 2001), though this species seemed not largely distributed as previously thought. Further investigations throughout the four seasons in this area will enable conclusion of this findings.

The species *C. rubinotus* found at Ndomdjengue has already been suspected in Cameroon, but the authors did not confirm this species in Cameroon since it was known as an Asian species (Rageau and Adam, 1952). *C. rubinotus* has already been identified in the Culicids fauna of Gabon (Service, 1976; Mouchet, 1971), a border country with Cameroon enables the confirmation of these findings which indicates that this *C. rubinotus* may be migrating upward from Gabon.

The presence of *A. nili* and *A. funestus* adult specimen and their absence at larval stages in our sampling may be due to the heavy rainfall before our arrival in the area which washed of larval breeding sites. Previous studies in Cameroon reported *A. nili* and *A. funestus* larval stages usually attached to aquatic plants (Le Goff at al., 1990; Huang, 2004).

The results of this study are of medical importance. Demonstration of *Aedes* species indicates a potent for arboviruses transmission in Cameroon (Huang, 2004; Cattand et al., 2006). *Anopheles* spp. found in this study are foremost of malaria vectors system in Cameroon (Fontenille and Lochouarn, 1999; Carnevale et al., 1992). The simultaneous existence of three species at adult as well as larval stages may favour permanent transmission of malaria and other parasitic disease like lymphatic filariasis in the area. A previous parasitological study in village demonstrated high prevalence rates for malaria and onchocerciasis (Gazin et al., 1989). These high Onchocerciasis high prevalence rates in the area indicate

Table 1. Larvae and adult Culicidae species distribution in the study area.

Species	Mbebe		Ndomdjengue		Bot-Makak	
	Larvae	Adult	Larvae	Adult	Larvae	Adult
A. Gambiae	2	Present	0	Present	0	Present
A. Nili	0	Present	0	Present	0	Absent
A. Funestus	0	Present	0	Present	0	Absent
A. aegypti	4	Absent	3, 4, 5	Absent	4, 5	Absent
A. vittatus	2	Absent	0	Absent	0	Absent
A. chaussieri	2	Absent	0	Absent	0	Absent
A. ledgeri	0	Absent	5	Absent	5	Absent
A. albopictus	0	Absent	0	Absent	4, 5	Absent
C. quinquefasciatus	2	Absent	3	Absent	1	Present
C. perfuscus	0	Absent	4	Absent	0	Absent
C. rubinotus	0	Absent	5	Absent	0	Absent
C. chorleyi	3	Absent	3, 4	Absent	0	Absent
C. simpsoni	2	Absent	0	Absent	5	Absent
C. insignis	2	Absent	0	Absent	0	Absent

Larvae microhabitats: 0, Absent; 1, ponds with clear water; 2, holes in rocky substratum in the Sanaga river; 3, ponds bearing organic materials in the forest undergrowth; 4, containers near households and farms; 5, holes in trees bearing organic materials.

a good efficiency of *S. damnosum* in the transmission.

Studies on the epidemiology of arboviruses in Cameroon are scarce and we can not comment on the efficiency of the potent vector in the transmission of these pathogens in the area

Indoor Pyrethrum spraying sampling technique collected only female Culicidae with *Anopheles* specimen being most abundant. This abundance of Anophelines upon *Culex* and *Aedes* has already been demonstrated in previous studies at Ndomnjengue and Mbebe using joint capture with indoor spraying and mosquito net (Le Goff et al., 1990, 1994; Huang, 2004; Carnevale et al., 1992). Adult *A. nili* were harvested at Ndomnjengue more than 1.5 km from the larvae breeding sites in the Sanaga river banks at Mbebe, indicating that adult *A .nili* dispersal capacity is greater than thae stated in previous studies (Le Goff et al., 1990). However, adult *A .nili* do not reach farther distances as indicated by their absence at Bot-Makak (35 km from the Sanaga river).

Rapid decrease in indoor resting densities of adult *A. nili* and *A. gambiae* with distance from the river edges is indicative of the influence of the Sanaga river stream. This influence of the Sanaga river is also indicated by the distribution of larvae breeding which shows *A. nili* larvae mostly located in water bodies on the left hand of the Sanaga river under vegetations, whereas *A. gambiae* larvae were mostly harvested on the right hand. The dense vegetation which separates the two arms may force adult *A. nili* to fly towards the Mbebe area and *A. gambiae* to go mostly towards Ndomdjengue.

The rapids and falls at Mbebe-Kikot are found to be the main *Simulium* larvae breeding sites in the study areas. At this level, stream turbulence favours optimal development of *Simulium* larvae and pupae. Absence of larvae breeding sites downstream may be due to smooth flow of the stream. Larvae and pupae were collected only on immerged vegetation. Their absence on rocky substratum may be due to the presence of algae overcastting on rocks. Such observations have already been made in Ghana where the presence of algae on rocky substratum was thought to limit development of *Simulium* larval stages (Opoku, 2006).

Only *S. damnosum* larvae and pupae were found in our sample. Further studies based on monthly collection during a year period need to be undertaken to identify the presence of other species that may compete with *S. damnosum*. In river Pra in Ghana, *S. damnosum* was shown to dominate over *S. adersi* and *S. unicornutum* (Opoku, 2006). Such competitive domination by *S. damnosum* has also been demonstrated in river Maraoue in Ivory Coast against *S. adersi* and *S. tridens* (Elouard and Gibon, 1985). However, the results of this study are consistent with previous studies in the mid Sanaga which demonstrated *Simulium squamosum* (s.s) to be the main species of the *S. damnosum* complex in this area (Traore-Lamizana and Lemasson, 1987; Traore-Lamizana et al., 2001). Our results have extrapolated to the mid Sanaga valley, but absence of respiratory filaments in pupae collected in our sample may be indicative of particular strain of *S. squamosum*. In previous

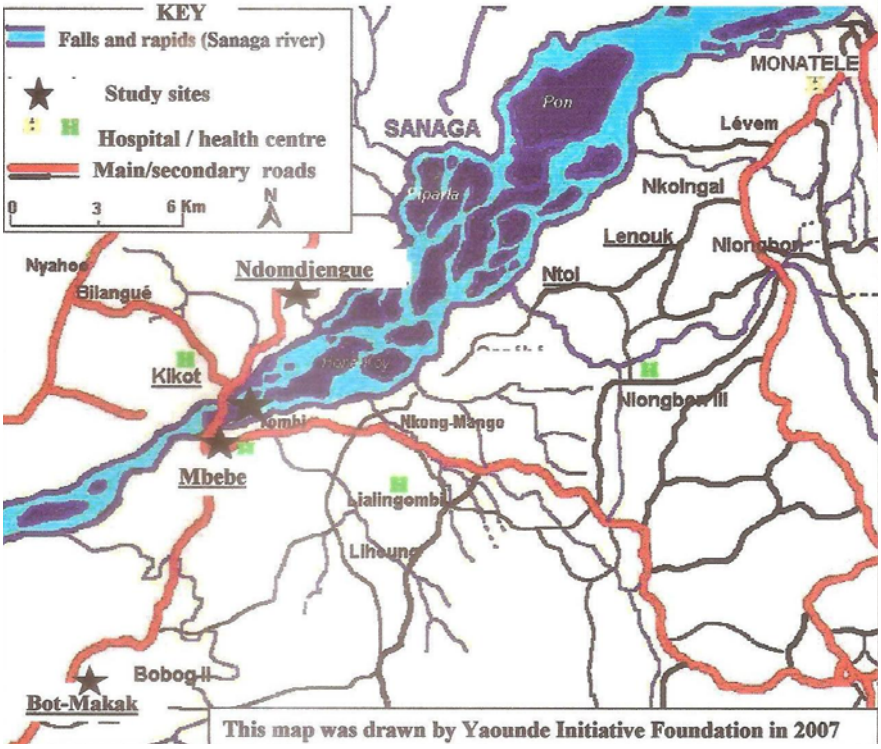

Figure 1. Map of the study area.

previous studies, the species found in this area was described as “form B” of *S. squamosum* (Traore-Lamizana et al., 2001).

Larvae and pupae were found more abundant in the rainy season (May) than the dry season (December). This seasonal increase can be explained by the increase of flow of the river which also leads to an important increase of water turbulence thus improving the nutritional status of larvae (Opoku, 2006). Pupae abundances were not significantly affected by season alternation, indicating that increase in larvae abundance does not systematically influence pupae stage formation. Therefore, larvae stage may extend in the rainy season to favour constant pupae densities as demonstrated in Ivory Coast where temperature fall is demonstrated to slow down the development of *Simulium* immature stages (Bellec and Hebrard, 1983). The constant abundance of *Simulium* pupae throughout seasons leads to permanent emergence of adult through the year in the rapids and falls of Mbebe-Kikot.

Adults *Simulium* specimen were found more abundant in the rainy season than the dry season. Females had a greater dispersal capacity than males. Adult males were caught only close to the breeding sites at Mbebe whereas female reached 35 km from the larval breeding sites. These females showed greater abundance all along the day in areas mostly visited by human like households vicinity; farms indicating man biting rates may be important and transmission of related filarial continuous in our study area as shown in Figure 1.

The circadian rhythm of adult black flies in the villages in the rainy season indicate two picks one early in the morning (8 to 9 am) and the second late in the evening (5 to 6 pm). Between the two peaks, adult flies abundances was weak, indicating that *Simulium* flies are less active during hot periods of the day and very active during cooler hours. This bimodal in the *Simulium* daily activity are closed to findings in the Soudanian savannah area in Mali (Western Africa) where a peak was recorded from 8 to 11 am and the second in the evening ((Bellec and Hebrard, 1983).

Abundance of adult *Simulium* was greater near households than farms in all villages, indicating high nuisance by black flies near households than in farms. This finding may be due to the existence of a chemical attraction by man vis-à-vis of flies which need to feed on human being. This attractive effect can be justified at Ndomdjengue which is a savannah area where habitants are mostly farmers and adult flies were found at similar abundance near households as well as in farms. In forest areas like Mbebe, human occupations take place mostly close to households whereas the working zones can exist farther from households in the savannah areas for their occupational activities.

Demonstration of the diversity and widespread insect fauna in the Sanaga mid valley has epidemiological

implications since most of the species collected are major known vectors of endemic diseases in Cameroon and other countries. Anopheline species *A. gambiae* harvested in all the study areas, *A. nili* and *A. funestus* collected in villages close to the river edges, namely, Mbebe and Ndomnjengue are well known efficient malaria vectors in Cameroon (Antonio-Nkondjo et al., 2006). Simultaneous existence of the three species in villages close to the Sanaga river edges is relevant for amplifying the exposition risk to malaria parasites and other anopheline transmitted diseases.

Onchocerciasis in the study area is of social and economical importance since many residents suffer from sight impairment, blindness. Furthermore, pest due to *Simulium* flies is of economical importance in hampering farming for example. Nuisance due to *C. quinquefasciatus* flies are of same effect though this fly is not known for transmitting infectious disease in Cameroon. Lymphatic filariasis infections are reported in health centers record books, but specific pathogens are not yet found. However, the presence of *A. gambiae* and *A. funestus* known to be vectors of this filariasis in Cameroon is evocative of existence of lymphatic filariasis in the study area. Parasitological investigations are ongoing to assess filariasis endemicity in the area.

Knowledge on human arbovirosis is scarce in Cameroon. However, the presence of known yellow fever and dengue virus mosquitoes vectors in Cameroon and elsewhere in the world notably *A. aegypti* and *A. albopictus* in our study sample indicate a possible transmission risk of these pathogens in the study areas. Other mosquito species like *A. vittatus* and *C. rubinotus* potent vectors of arboviruses in other parts of Africa were found in our collections, though only at larval stages. Occurrence of *A. ledgeri* is however a new observation in Cameroon.

Conclusion

The mid Sanaga valley harbours a great variety of potent vectors of parasites and arboviruses. *Anopheles*, *Culex*, *Aedes* and *Simulium* species recorded in the Sanaga mid valley are of the man or animal biting-flies mostly involved in diseases transmission in Africa.

Culicidae larvae are widely distributed in the study area, some having broad specificity for their micro-habitats, whereas *Simulium* larvae are found only in the Sanaga river. Distribution of Culicidae larvae varies among villages with distance from the Sanaga river, this river harbours almost all Culicidae spp. recorded at larval stage. The Sanaga River thus influences largely the biodiversity of the entomological fauna in the mid Sanaga valley. Adult Culicidae spp. are less diversified than larvae. Their distribution also decreases with distance from the Sanaga river edges. Occurrence of *A. ledgeri* as well as *C. rubinotus* is however a new observation in Cameroon.

S. damnosum was the species found. Adult *Simulium* had a high dispersal capability reaching up to 35 km from the main breading site. Their daily activity had a bimodal dispersion with a peak in the morning and the second late in the evening.

ACKNOWLEDGEMENTS

The authors sincerely thank the chiefs and guides of the villages for their collaboration. They are also indebted to technicians of the Faculties of Medicine, Douala and Yaounde1 Universities, namely, Mrs. Koagne Eveline and Mr. Essono Edmond.

Conflict Interests

The authors declare that there is no conflict of interests.

REFERENCES

Adja AM, N'Goran KE, Kengne P, Koudou GB, Toure M, Koffi AA, Tia E, Fontenille D, Chandre F (2006). Transmission vectorielle du paludisme en savane arboisée à Gansé en Côte d'Ivoire. Med. Trop. 66:449-455.

Antonio-Nkondjo C, Kerah CH, Simard F, Ambene AP, Chouaibou M, Tchuinkam T, Fontenille D (2006). Complexity of the malaria vectorial System in Cameroon: contribution of secondary vectors to malaria transmission. J. Med. Entomol. 43(6):1215-1221.

Awono-Ambene P, Kengne P, Simard F, Antonio-Nkondjio C, Fontenille D (2004). Description and bionomics of Anopheles (Cellia) ovengensis (Diptera: Culicidae), a new malaria vector species of the *Anopheles nili* group from south Cameroon. J. Med. Entomol. 41(4):561-568.

Bellec C, Hebrard G (1983). Les heures d'activité de vol des adultes du complexe *Simulium damnosum* en secteur pré-forestier de Côte d'Ivoire. Cah. O.R.S.T.O.M., Sér. Entomol. Méd. Parasitol. 21(4):261-278.

Carnevale P, Le Goff G, Toto JC, Robert V (1992). *Anopheles nili* as the main vector of human malaria in villages in southern Cameroon. Med. Vet. Entomol. 6:135-138.

Cattand P, Desjeux P, Guzmán MG, Jannin J, Kroeger A, Medici A, Musgrove P, Nathan MB, Shaw A, Schofield CJ (2006). Tropical Diseases Lacking Adequate Control Measures: Dengue, Leishmaniasis, and African Trypanosomiasis. In: Jamison DT, Breman JG, Measham AR, Alleyne G, Claeson M, Evans DB, Jha P, Mills A, Musgrove P (eds.), Disease Control Priorities in Developing Countries. Washington (DC): World Bank; 2006. P 23.

Elouard JM, Gibon FM (1985). Compétition interspécifique entre les stades préimaginaux de quelques espèces de simulies Ouest-Africaines. Bull. Ecol. 16(3):223-229.

Fontenille D, Lochouarn L (1999). The complexity of the malaria vectorial system in Africa. Parasitologia 41(1-3):267-271.

Fontenille D, Toto JC (2001). *Aedes* (*Stegomia*) *albopictus* (skuse), a potential new dengue vector in southern Cameroon. Emerg. Infect. Dis. 7(6):1066-1067.

Freeman P, De Meillon B (1953). Simuliidae of the Ethiopian region. British Museum, London, 224p.

Gazin P, Le Goff G, Ambassa P, Mulder L, Loue P, Carnevale P (1989). Etude du paludisme à Edéa et Mbebe : 1-Premiers résultats. Yaoundé: OCEAC 680/SEM.

Gillies MT, Coetzee M (1987). A supplement to the Anophelinae of Africa, South of the Sahara. S. African Institute for Medical Research, 55:1-143.

Gillies MT, De Meillon B (1968). The Anophelinae of Africa South of the

Sahara. Publication of the South African Institute for Medical Research, 13(54).
Hougard JM, Mbentengam R, Lochouarn L, Escaffre H, Darriet F, Barbazan P, Quillévéré D (1993). Lutte contre *Culex quinquefasciatus* par *Bacillus sphaericus* : résultats d'une campagne pilote dans une grande agglomération urbaine d'Afrique équatoriale. Bull. World Health Organ. 71(3-4):367-375.
Huang YM (2004). The subgenus Stegomyia of *Aedes* in the Afrotropical Region with keys to the species (Diptera: Culicidae). Magnolia press Aucklang, New Zealand. 82:78-82.
Jupp PG (1996). Mosquitoes of southern Africa: Culicinae and Toxorhynchitinae. Hartebeespoort (South Africa): Ekolgilde publishers. p. 155.
Larivière M, Abonnenc E (1958). Les gîtes larvaires de Culicidae dans la Presqu'île du Cap Vert. Waterhouse. pp. 334-356.
Le Goff G, Carnevale P, Vincent R (1997). Low dispersion of Anopheline malaria vectors in the African equatorial forest. Parasite 35:187-189.
Le Goff G, Robert V, Gazin P, Carnevale P (1990). Prospections entomologiques de la faune culicidienne dans le bassin de la Sanaga: 1. Premiers résultats. Yaoundé: OCEAC. P 4.
Le Goff G, Toto JC, Carnevale P (1994). Evaluation entomologique de l'effet insectifuge du DMP sur trois vecteurs du paludisme au Sud-Cameroun. Bull. Liais. Doc. OCEAC, 27(3):126-129.
Mouchet J (1962). Influence des fleuves sur la biologie d' *Anopheles gambiae* pendant la saison sèche dans le Sud-Cameroun. Bull. Soc. de Pathol. Exot. 55(6):1163-1171.
Mouchet J (1971). Surveys of potential yellow fever vectors in Gabon and Tchad. WHO/VBC/71.279.
Opoku AA (2006). The ecology and biting activity of black flies (Simuliidae) and the prevalence of onchocerciasis in an agricultural community in Ghana. W. Afr. J. Appl. Ecol. 9:51-59.
Ostfeld RS, Keesing F (2000). The function of biodiversity in the ecology of vector-borne zoonotic diseases. Can. J. Zool. 78:251-254.
Philippon B (1977). Rapport de mission à Song-Loulou (République Unie du Cameroun). N° 460/ 77/ O.R.S.T.O.M/ Bouaké.
Rageau J, Adam JP (1952). Culicinae du Cameroun. Ann. Parasit. 27(6):610-635.
Rageau J, Adam JP (1953). Note complémentaire sur les Culicinae du Cameroun. Ann. Parasitol. Hum. Comp. 28(5-6):412-424.
Rickenbach A, Eouzan JP, Ferrara L, Bailly-choumara H (1976a). Données nouvelles sur la présence et la répartition des Toxorhynchinae et Culicinae (Diptera, Culicidae) au Cameroun 1. Genres Toxorhynchites, Malaya, Hodgesia, Uranotaenia, Aedeomyia, Culiseta, Orthopodomyia, Ficalbia, Mansonia et Aedes. cah. O.R.S.T.O.M., sér. Entomol. Méd. Parasitol. 14(1):61-68.
Rickenbach A, Eouzan JP, Ferrara L, Bailly-choumara H (1976b). Données nouvelles sur la présence et la répartition des Toxorhynchinae et Culicinae (Diptera, Culicidae) au Cameroun 2-Genres Eretmapodites et Culex. Cah. ORSTOM, Sér. Entomol. Méd. Parasitol. 14(2):93-100.
Robert V, Gazin P, Ouédraogo V, Carnevale P (1986). Le paludisme urbain à Bobo-Dioulasso (Burkina Faso)- 1. Etude entomologique de la transmission. Cah. ORSTOM, Ser. Entomol. Med. Parasitol. 24(2):121-128.
Rodhain F (1999). *Les maladies à vecteurs*. Presses Universitaires de France, Paris. P 127.
Rodhain F, Perez C (1985). Précis d'entomologie médicale et vétérinaire. *Maloine s.a. éditeur,* Paris. p. 458.
Same-Ekobo A (1997). Santé Climat et environnement au Cameroun. Edition Jutely-Sciences. P 329.
Service MW (1976). Contribution to the knowledge of the mosquitoes (Diptera, Culicidae) of Gabon. Cah. ORSTOM, Sér. Entomol. Méd. Parasitol. 14(3):259-263.
Subra R (1973). Etudes écologiques sur *Culex pipiens fatigans* Wiedemann, 1828 (Diptera, Culicidae) dans une zone urbaine de savane soudanienne Ouest-Africaine: dynamique des populations imaginales. Cah. ORSTOM, Sér. Entomol. Méd. Parasitol. 11(2):79-100.
Traore-Lamizana M, Lemasson JJ (1987). Participation à une étude de faisabilité d'une campagne de lutte contre l'onchocercose dans une région du bassin du Logone. Répartition des espèces du complexe *Simulium damnosum* dans la zone Camerounaise du projet. Cah. ORSTOM, Sér. Entomol. Méd. Parasitol. 25(34):171-186.
Traore-Lamizana M, Somiari S, Mafuyai H, Vajime C, Post R (2001). Sex chromosome variation and cytotaxonomy of the onchocerciasis vector *Simulium squamosum* in Cameroon and Nigeria. Med. Vet. Entomol. 15(2):219-223.