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THE DISTRIBUTION OF MOSQUITO (DIPTERA: CULICIDAE) ACTIVITY IN ZIKA FOREST, UGANDA: PUBLIC HEALTH IMPLICATIONS

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Abstract

The distribution of mosquito activity can be highly variable even in the same geographical area. This suggests that mosquito distribution is influenced by a number of factors which in turn affect emergence/re-emergence of disease. To test whether such differences affect the vertical and horizontal distributions of mosquitoes, we used human-landing, carbon dioxide-baited (CO_2) CDC light and oviposition traps from Zika Forest, Uganda. Significant differences were observed between mosquito species collected from the tower, within and along the edge of the forest (Kruskal Wallis test, $\chi^2 = 17.58$, df = 2, $p < 0.05$). Majority of *Aedes* species were collected from the tower, *Anopheles* at the forest edge and *Coquillettidia* and *Culex* were present in all points. Thirty species were constant, three frequent and 13 had sporadic appearances. Fifteen species presented densities above one and 46 less than one. Our results suggest a high proportion of mosquito vectors are present within the forest which might influence the emergence/re-emergence of infections.

Keywords: Mosquito activity, distribution, horizontal-vertical, surveillance, ecology, Zika

Introduction

There has been a developing awareness of the importance of mosquitoes linking man and animals through disease (Haddow, 1945; Simpson et al., 1965; Haddow et al., 1968; McCrae & Kirya, 1982). For a number of years, investigations focused on the circadian pattern of biting behavior by 24-hour man-baited catches. In the earlier series mosquito catches were carried out at a tower of different levels above ground. In addition to man or animal baits, lighted or unlighted suction traps (often with animal bait) were used (Haddow et al., 1964). Previous studies demonstrated that mosquitoes are efficient vectors of various pathogens (Haddow et al., 1947; Henderson et al., 1970; Haddow & Ssenkubuge 1973). However, differences in mosquito activity distribution were found in the same species (Haddow et al., 1947; Derraik et al., 2005) including populations that resided in the same geographic area. Differences in mosquito activity have been reported to affect group size and abundance for example; Adebote et al., 2008 and Abel-Hamid et al., (2009) noted that the mosquito distribution was a result of suitable ecological conditions between reservoir and host. Haddow & Ssenkubuge, (1973) reported mosquito distribution was affected by abundance of blood meal host vectors. de Souza et al., (2010) showed that the distribution of *Anopheles gambiae* s.s. varied with environmental factors. Mushizimana et al., (2006) reported that landscape factors affected anopheline mosquito distribution. When competing species enter a niche, natural selection drives them into different parts allowing resource sharing. Groups of the same species partition their confined habitat without outcompeting other species (Bradshaw & Holzapfel, 2007).

While previous studies analyzed the distribution of mosquito activity with various methods, this was done mainly along the vertical dimension (Haddow et al., 1964; Haddow et al., 1968; Kirya, 1977; McCrae & Kirya, 1982). The activity of mosquitoes in Zika forest continued till the late 1970's as a result of the instabilities in the country and had not been updated for over 40 years. Very little infor-

mation is presented about mosquito activity distribution over a horizontal dimension in the forest. We address this gap by using carbon dioxide baited CDC light and oviposition traps in addition to the Human Landing Collections over both the horizontal and vertical dimensions. The methods have been shown to reliably generate meaningful patterns of mosquito behavior and density in different ecological conditions (Mboera, 2005; Okumu et al., 2010; Roiz et al. 2012). We hypothesized that variation in distribution of mosquito activity would follow similar patterns across species.

Materials and Methods

Study area

Zika Forest is described in Kaddumukasa et al. 2014

Sampling

Mosquitoes sampling was conducted from July 2009 through June 2010 following three different sampling protocols. The first collections were done along a vertical gradient on the steel tower at six different heights separated by a 6-metre distance (20, 40, 60, 80 and 100 and 120 ft (6.1 to 36.6 m) above the ground on a steel tower. In the second and third protocol collections were conducted along a horizontal gradient within and at the forest edge separated by 50-metre distances from the tower. Collections along the horizontal were conducted one-metre above the ground. Mosquito collections were conducted using Carbon dioxide (CO2) baited CDC light traps (light/Co2), Human Landing Catches (HLCs) and oviposition (ovitraps) traps as described in Kaddumukasa et al., (2013). All collected mosquitoes were quickly sorted on dry ice and transferred to the laboratory for identifications. Using the nomenclature of Knight and Stone (1977) supplemented with notes and updates from the Walter Reed Biosystematics Unit website *(http://www.wrbu.org/docs/mq* ClassificationTraditional201307.pdf), mosquito identifications were conducted with help of the keys of Edwards (1941), de Meillon, (1947), Gillett, (1972), Gilles & Coetzee, (1987) and Jupp, (1996). Voucher specimens for each species are kept at the Uganda Virus Research Institute, Entebbe, Uganda.

Statistical analysis

Species distribution and density were determined as described by (Rydzanick & Lonc, 2003). Distribution classes described as; C1 - sporadic appearance (constancy 0 - 20 %), C2 - infrequent (20.1 - 40%), C3 - moderate (40.1 - 60%), C4 - frequent (60.1 - 80%), C5- constant (80.1-100%) were used. These were determined as the percent of sampling sites in which a species was noted as: C = n/N X 100% where: C = distribution, n = number of sites of the species, N = number of all sites. Density classes were grouped according to; satellite species (D < 1%), subdominant species (1< D <5%), and dominant species (D > 5%) and determined as D- I/L *100% where: D = density, I = number of specimens of each mosquito species, L = number of all specimens.

Collection points were of three categories; within, on the edge and tower platforms. Mosquito population distribution count data from the three categories were compared using Kruskal Wallis test in the STATA ver 12. A Mann-Whitney U test was conducted to evaluate the hypothesis that there were no differences between mosquito collections over sites. Associations amongst individual mosquito genera and collection points were examined using Chi-square test. Values were considered significantly different at $p < 0.05$.

Results

Sixty one species belonging to 12 genera were collected between July 2009 and July 2010. Collection points 3 & 4, within the forest had highest abundance of mosquitoes 9.4% (15360) and 9% (14675) respectively and the tower platforms 100, 1.1% (1837) and 120, 1.8% (3012) had least. Coquillettidia, Mansonia and Uranotaenia species were present in majority of the collection points (Figure 1). Mosquitoes from the *genera Anopheles, Eretmapodites, Mansonia, Aedes* were most frequent within and at the forest edge rather than on the tower. The primary vectors of yellow fever *Aedes aegypti* formosus (Walker), *Aedes africanus (Theobold), Aedes apicoargenteus* (Theobold) were collected from within the forest and the tower platforms, none at the forest edge. High numbers of Ae. africanus at the higher levels (60-100ft) were collected. The two species of *Anopheles (An. coustani and An. implexus)* were frequent at the forest edge. *Toxorynchites brevipalpis* (Theobold) was most recorded at one-meter levels within the forest (Table 1).

Table 1
Distribution of Satellite and least common mosquito species from Zika forest,Uganda

Spp/Collection points	WF1	WF2	WF3	WF4	WF5	WF6	WF7	Tw20	Tw40	Tw60	Tw80	Tw100	Tw120	FE1	FE2	FE3	Total	Distribution	Density
Tx. Brevipalpis (Theo)		3	4	1													**8**	C1	S
Mi. plumosa (Theo)	3	1	1													2	**7**	C2	S
Ae. longipalpis (Zavor)										2	2						**4**	C1	S
Cx. horridus (Edw.)		1		1						2							**4**	C1	S
Mi. mediolineata (Theo)		1	2													1	**4**	C4	S
Ae. albocephalus (Theo)		1									1						**2**	C1	S
Ae. luridus (Mcintosh)				1	1												**2**	C1	S
Ae. albomarginatus (Newstead)	1						1										**2**	C1	S
Ae. marshalii (Theo)			1														**1**	C1	S
Cx. bitaeniorynchus (Edw.)									1								**1**	C1	S
Cx. kingianus (Edw.)	1																**1**	C1	S
Mi. splendens (Theo)				1													**1**	C1	S

Key
WF – Within Forest

Tw – Tower platforms

FE- Forest Edge

C1- Sporadic appearance (0-20%)

S – Satellite species

Six mosquitoes in the *genus Coquillettidia* were collected. *Coquillettidia aurites* (Theobold) and *Coquillettidia fraseri* (Theobold) were most distributed above 60 ft (24 metres), with very low numbers within and along the forest edge. *Coquillettidia fuscopennata* (Theobold) was most common in points along the edge of the forest and *Coquillettidia pseudoconopas* (Theobold) most prevalent within the forest and lower tower platforms especially 20-40 ft (6-12m). *Coquillettidia metallica* (Theobold) and *Coquillettidia maculipennis* (Theobold) were most collected within and at the edge of the forest.

Single or double individual *Culex species* were collected. *Culex annulioris* (Edwards) among the dominant species from within and along the edge of the forest, *Culex insignis* (Carter) and *Culex univittatus* (Theobold) had highest abundances on the tower platforms. Mansonia species were collected within and along the forest edge. *Uranotaenia species* were most abundant within the forest and least on the tower platforms and forest edge. Among abundant *Uranotaenia species* were; *Ur. alboabdominalis* (Theobold) and *Ur. mashonaensis* (Theobold). Other *Uranotaenia species* had very sporadic appearances.

Thirty mosquito species namely; *Aedes africanus* (Theobold), *Anopheles coustani* (Laveran), An. *implexus* (Theobold), *Coquillettidia aurites, Cq. fraseri, Cq. fuscopennata, Cq. metallica, Cq. pseudoconopas, Culex annulioris, Cx. antennatus (Becker), Cx. cinereus (Theobold), Cx. cinerellus (Theobold), Cx. insignis (Carter), Cx. moucheti (Evans), Cx. nebulosus (Theobold), Cx. niveai (Edwards), Cx. poicilipes (Theobold), Cx. quinquefasciatus (Say), Cx. univittatus (Theobold), Cx. vansomereni (Edwards), Ma. africana africana (Theobold), Ma afr. nigerrima (Theobold), Ma. uniformis (Theobold), Mimomyia hispida (Theobold), Uranotaenia alboabdominalis, Ur. mashonaensis, Ho. cyptopus (Theobold)* and groups of unidentified Uranotaenia and *Hodgesia species* were constant in the collections (C5 = 80.1-100%). In this class majority were from the *genera Anopheles, Coquillettidia, Culex, Hodgesia* and *Mansonia*. Five species namely; *Mimomyia medilineata* (Theobold), *Mi. mimomyiaformis* (Newstead), *Uranotaenia connali* (Edwards), *Ur. nigromaculat*a (Theobold) and *unidentified Culex species* were frequent (C4=60.1-80%). Four species namely; *Aedes mcintonshi* (Theobold), *Ae apicoargenteus* (Theobold), *Ae. tarsalis* (Theobold) and *Eretmopodites chryosogaster* (Graham) were in the moderate class C3 (40.1 - 60%). Eleven species including; *Aedeomyia furfurea* (Enderlein), *Aedes argenteopunctatus* (Theobold), *Ae aegypti formosus* (Walker), *Ae. circumluteolus* (Theobold), *Ae. cumminsii* Theobold), Ae. ingrami (Edwards), *Ae metallicus* (Edwards), *Ae. tarsalis* (Newstead), *Lutzia tigripes* (de Grandpre and de Charmoy), *Mimomyia plumosa* (Theobold) and *Uranotaenia nivipous* (Theobold) had infrequent C2 (20.1 - 40%) occurrences. Eleven species namely; *Aedes albocephalu*s (Theobold), *Ae. marshal*ii (Theobold), Ae. luridus (Mcintosh), Ae. albomarginatus (Newstead), *Ae. longipalpis* (Gruenberg), *Culex bitaeniorynchus* (Edwards), *Cx. horridus* (Edwards), *Cx. perfuscus* (Edwards), *Cx. kingianus* (Edwards), *Mimomyia splendens* (Theobold) and *Toxorynchites brevipalpis* (Theobold) had *sporadic* occurrences (C1 = 0 – 20%) constituting majority of *Aedes* and *Toxorynchites species.*

Six species (*Cq. fuscopennata, Cq. metallica, Cx. annulioris, Cq. aurites & Cq. pseudoconopas* and *Cq. maculipennis*) formed the dominant class. Nine species (*An. implexus, Cq. fraseri, Cx. insignis, Cx. univittatus*. Six species (*Cq. fuscopennata, Cq. metallica, Cx. annulioris, Cq. aurites & Cq. pseudoconopas and Cq. maculipennis*) formed the dominant class. Nine species (*An. implexus, Cq. fraseri, Cx. insignis, Cx. univittatus, Ma. africana, Ma. nigerrima, Ma. uniformis, Ur. alboabdominalis* and *Uranotaenia spp*) subdominant, the rest (46) were satellite species. Overall 15 mosquito species had densities above one and 46 species much less than 1. Mosquitoes were active over all collection points however; mosquito numbers decreased from tower platforms 100 and 120 (Figure 1). Mosquitoes from the genera *Aedes, Aedeomyia, Coquillettidia, Culex, Hodgesia, Mansonia* and *Uranotaeni*a showed a representation of mosquito species in sites within the forest, tower platforms and least proportions along the forest edge (Figure 1).

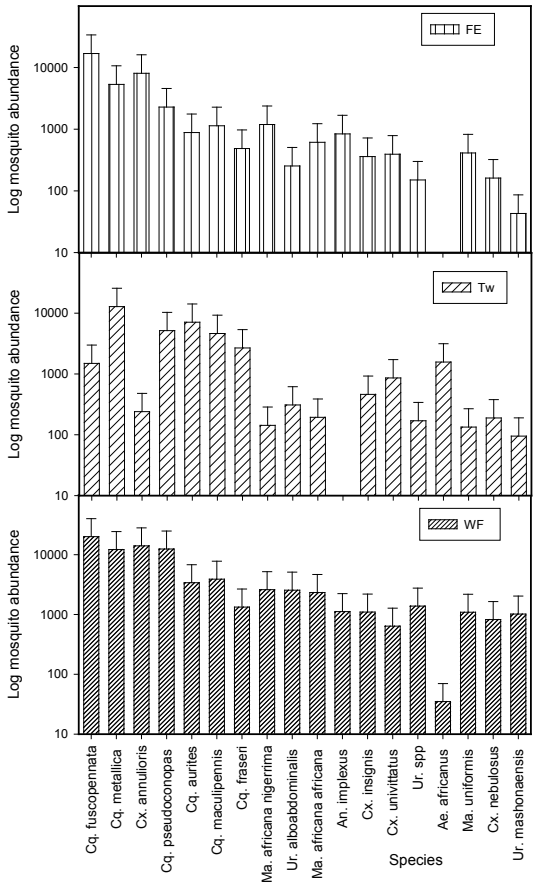

Figure 1

Distribution of the most dominant and constant medically important mosquito species from Zika forest

Aedes subgenus Stegomyia species were most associated with the tower in the forest. *Anopheles, Eretmopodites, Hodgesia, Mansonia, Mimomyia* and *Uranotaenia* species had the highest occurrences from collection points within the forest. *Coquillettidia* and *Culex* species had uniform occurrences over all collection points. *Toxorynchites brevipalpis* only occurred from points within the forest.

A significant difference was observed between mosquitoes collected from the horizontal and vertical sites (*Kruskal Wallis* test, $\chi^2 = 17.58$, df = 2, $p < 0.05$). Mean mosquito abundance was highest at forest edge (13415 ± 406), followed by points within the forest (12220 ± 2555) and least at the tower (6533 ± 3132). No significant difference was observed between mosquitoes collected within and along the forest edge (*Mann-Whitney U* test $z = -0.703$, $p > 0.05$). Mosquitoes collected within the forest differed significantly from the tower species (*Mann Whitney U* test, $z = 0.000$, $p < 0.05$) and collections from the tower also differed significantly from those along the edge of the forest (*Mann Whitney u* test $z = 0.001$, $p < 0.05$).

Discussion

Our results show that mosquito vectors of various animal pathogens are distributed throughout the forest. Apart from *Ae. africanus*, few varieties of *Aedes* vectors occurred in the forest. The high abundance of *Ae. africanus,* the principal *sylvatic YF* vector suggests the forest provides a hot spot suitable for its breeding and survival, the well defined tree holes that species takes advantage of (*Haddow et al.,* 1964). In addition, Ae. africanus is capable of transmitting Chikungunya, another significant human disease (Weinbren et al., 1958, Diallo et al., 1999).

Aedes aegypti the principal vector of *dengue virus, chikungunya* and urban yellow fever virus, was one of the satellite species. The East African *Ae. aegypti* has been noted as most competent yellow fever species in the world (*Tabachnick et al*., 1985). Presence of *Ae. aegypti* and *Ae. africanus* could easily result in *sylvatic* yellow fever and *dengue* transmission spilling to the surrounding urban areas. However, our study did not distinguish between *Ae. aegypti aegypti* and *Ae. aegypti* formosus. The small numbers of the *Aedes subgenus Neomelaniconion* including; c*ircumluteolus, mcintonshi, luridus suggest that these* species may have limited epidemiological significance in the above arbovirusess but may play a big role in their transmission. Trapping of most mosquitoes from the genus *Aedes*, along the tower shows that they are arboreal species which may easily acquire unique pathogens through blood-meal interaction with arboreal animals.

Similar to studies by Goma, (1965) and Haddow and Ssenkubuge, (1965), An. *implexus* was confined to levels below 20 ft (6 metres). *Anopheles* coustani has been reported to transmit *Onyong nyong* and *Bwamba* viruses (Lutwama et al., 1999; 2002), CHIKV (Diallo et al., 1999) and *RVFV* virus (Ratovonjato et al., 2011). Other *Anopheles* species below one meter height in an open farmland have been reported (Gilles and Wilkes, 1976). *Anopheles* species may be strategically placed at lower heights where they can easily find a blood meal host. *YFV* (Kirya et al., 1977), *RVF* (Haddow, 1961) and *Sindbis* (SIND) (Haddow, 1964) viruses have been isolated from *Coquillettidia fuscopennata* which was among the most abundant. *Sindbis* virus was also isolated from a *juvenile hooded crow* (Corvus corone sardonius) (Taylor et al., 1955). This may suggest that it is a likely transmitter of infections from animals. This species has been reported as a catholic feeder compared to other *Coquillettidia specie*s (Mukwaya, 1972, Crabtree et al., 2013). It may play a big role in the transmission of *pathogens* among human and animal sources. *Coquillettidia metallica* has been implicated in the transmission of *WNV* from birds to humans (Hubalek and Halouzka, 1999). *Coquillettidia aurites* and *Cq. fraseri* are reported as principal vectors with a bird-mosquito natural cycle and would most likely circulate *pathogens* among birds as they were most collected above 60ft (18 metres) (Mukwaya, 1972; Crabtree et al, 2013). *Coquillettidia aurites, Cq. maculipennis* and *Cq. pseudoconopas* have been implicated in the transmission of *avian* malaria (Njabo et al., 2011).

Culex univittatus, a vector of *WNV* and Rift Valley Fever virus in East Africa *(*Huba lek and Halouzka, 1999, Miller et al., 2000) was abundant. RVFV a member of the genus *Phlebovirus* (Bunyaviridae), was isolated from a number of Culex mosquito species (Miller et al., 2000, Lutomiah et al., 2011). *Culex insignis, Cx. nebulosus* and *Cx. quinquefasciatus* are *considered* secondary vectors. RVFV was isolated from *Culex poicilipes* (*Diallo et al.*, 2000). *Culex annulioris*, has been implicated in the transmission of *Kamese v*irus (Arbocat ID 223, 1984). This species was previously reported as a ground haunting species (Haddow and Ssenkubuge, 1965). *Mansonia* species are also implicated in the transmission of RVFV (Lutomiah et al., 2011, 2013). *Mansonia* species were reported as mainly ground haunting thus their abundance within and along the forest edge

(Haddow and Ssenkubuge, 1965). The medical significance of *Hodgesia, Mimomyia, Uranotaenia* and *Toxorynchites sp*ecies has not yet been disclosed.

Other factors such as *niche segregation* may influence mosquito distribution with specific restriction to certain areas in the forest. Land-use change can lead to environmental effects such as the elimination or severe disruption of *ecological niches* of resident species whilst new *ecological niches* may be formed such as has happened in Zika forest. With altered *ecological niches* vector mosquito species exploit vacant niche space or extend their range (*Fagan et al.,* 1999). *Hubbell's* (2005) neutral theory of community structure predicts that ecologically equivalent species can co-exist for a long time.

Association of mosquito *genera* with collection points within, at the forest edge, and tower showed the presence of most species where they were most likely near breeding sites and sources of blood meals. However other factors may also influence distribution of *arboviral* diseases including extrinsic and intrinsic factors and the presence of the virus within the area (Turell et al., 1985). More than half the mosquito species from this study have been implicated as vectors of human and wildlife diseases. This suggests a high potential for maintenance and transmission of a wide variety of pathogens in Uganda. The presence of various mosquito vectors leaves the population surrounding the forest and the country at great risk of transmission of different pathogens. Knowing which collection point's mosquito vectors prefer would help in entomological surveillance and vector control efforts.

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