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Sampling of Adult Mosquito Using Human Bait Method, Spray-Sheet Method and the CDC Light Trap

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Abstract

Mosquito sampling in the Alulu-Nike community in Enugu East Local Goverment Area of Enugu state was performed using the human bait method, the CDC-light trap method and the spray sheet collections. The CDC-Light trap placed outdoor and the Spray sheet collection spread with a residual deltamethrin formulation were compared with the Human landing catch in the five locations. A total of 275 mosquitoes were collected and identified and they comprised of the three mosquito genera: *Aedes*, *Anopheles*, and *Culex*, and seven species *(Aedes aegypti*, *Aedes albopictus, Aedes africanus*, *Aedes taylori, Aedes luteocephalus*, *Anopheles gambiae*, and *Culex quinquefasciatus)*. Specifically, the occurrence of the species encountered included *Aedes aegypti* 63, *Aedes albopictus* 29, *Aedes taylori* 2, *Aedes africanus* 33, *Aedes luteocephalus*19, *Anopheles gambiae* 27, and *Culex quinquefasciatus* 102 with means of 156.0 mosquitoes for human bait catch, 103.0 mosquitoes for Spray sheet method and 16.0 mosquitoes for the CDC-light trap method. Among the studied locations, Umuogbe showed the highest mosquito abundance (80.00) compared to other areas, Umuchuba also have more mosquito abundance (65.00) which was significantly different from that of Enugwu (54.00 mosquitoes) and Negbune (45.00 mosquitoes), the least mosquito populated area according to this research was Amabo which collected only (34.00) mosquitoes. The Human landing catch showed a more efficient and reliable collection for mosquitoes than others but for ethical reasons spray sheet method should be used as in carrying out surveillance in this community because it demonstrated a good correlation with the human landing catch. A standardized evaluation of more promising methods for capturing host-seeking mosquitoes should also be considered in this community.

Keyword: Sampling, mosquitoes, landing catch, Light trap, Alulu – Nike, Spray sheet

Introduction

Mosquitoes are important vectors transmitting both human and animal diseases resulting to a variety of human efforts to eradicate or reduce their population. Among mosquitoes that do carry important diseases, not all species transmit the same kind of diseases and under the same circumstance, their habits differ. For example, some species attack people in houses, and others prefer to attack people walking in forests. Some mosquitoes that bite humans routinely act, as vectors for a number of infectious diseases affecting millions of people per year. Others that do not routinely bite humans, but are the vectors for animal diseases may become disastrous agent for zoonosis of new disease when their habitats are disturbed, for instance, sudden deforestation (WHO, 2007). Mosquito sampling manages the populations of mosquitoes to reduce their damage to human health and economics. Thus mosquito sampling is a vital public health practices throughout the world especially the tropics. In measuring the malaria transmission and other mosquito borne infection, adult mosquito sampling methods are essential. Preference for any sampling method however depends on both its field efficiency and the characteristics of local vector populations.

However, the use of an accurate mosquito trapping method is crucial, because several studies have reported significant differences in capture efficiencies between methods (Kline, 2006). The human bait trap (Human landing catch) which is the gold standard method has been a traditional method of estimating mosquito abundance. It provides the most accurate measure of man-vector contact, mainly sampling host seeking females which represent the mosquitoes responsible for disease transmission (Service, 1993). The collection of mosquitoes landing on human bait is considered the most direct and reliable method for determining human-biting activity since female mosquitoes are collected as they attempt to feed on human collectors (Jiang *et al,* 2007). However, the use of HLC has declined in recent years due to ethical concerns about potential exposure of collectors to mosquito-borne pathogens (WHO, 2003).

Many sampling methods have been evaluated as an alternative to human biting catches with varying degrees of success. One of the most commonly employed tools for catching host-seeking malaria vectors in particular is the centre for diseases and prevention miniature light trap (CDC-LT) which is typically positioned indoors near an occupied bed net (Chadwick *et al,* (2013); Sikaala *et a*l,(2013) and Wong *et al,* 2013). Other sampling methods such as resting boxes (RB), claypots, pitshelter, bednets traps have been evaluated under different epidemiological settings in African with varying degrees of success(Okumu *et al,* 2008). The window exit traps (WET) have been used for monitoring vector density trends in part of southern African and Bioko island in central African (Govella *et al,* 2011). Their efficiency is undoubtedly affected by variations in house design and behavioural pattern of both mosquitoes and humans. In recent years, BG-sentinels traps (BGS) designed by the biogents corporation have been used for collecting *Aedes* (*stegomyia*) species such as *Aedes aegypti*, *Aedes albopictus* and *Aedes polinesiensis* (Schmaedick *et al,* 2008). This type of trap can be used with a variety of mosquito attractants (e.g., CO_2, BG- lure, or Octenol), Nevertheless, only a few studies have investigated their efficiency regarding the capture of mosquito species other than those species of the genus *Aedes.* (Obenauer *et al,* 2009). Chaki *et al,* (2012) evaluated a newly developed ifakara tents trap design (ITT-C) which is a genuinely exposure free tool that could be used for routine epidemiological surveillance purposes. In this study the

sampling method considered are the Human bait trap; as Gold standard method, the Centre for disease control light trap, and the spray sheet collection. The study is generally aimed at sampling for adult mosquito in the given communities using different sampling techniques in order to compare the sampling efficiency of each technique and specifically determine the mosquito infestation status of the community.

Materials and Methods

This study was conducted in Alulu-Nike community in Enugu East Local Government Area of Enugu State located at the tail end of Abakpa Nike. The study locations comprises of Negbune, Enugwu, Umuogbe, Umuchuba, and Amabo which are the five villages of Alulu- Nike. There is presence of private Nursery and Primary schools in the community but no Secondary or Tertiary Institution. The community is surrounded by bushes, forest and rivers. There are presence of goats, dog chickens and other few livestock in the areas. Houses mainly built with a low thatched roof, and the eaves of most houses were open which facilitates mosquito ingress and egress. Cooking occurs typically inside the home or under the eaves of a porch. The areas are littered all over with domestic water holding containers including gutters. This dirty environment gives rise to breeding site for mosquitoes.

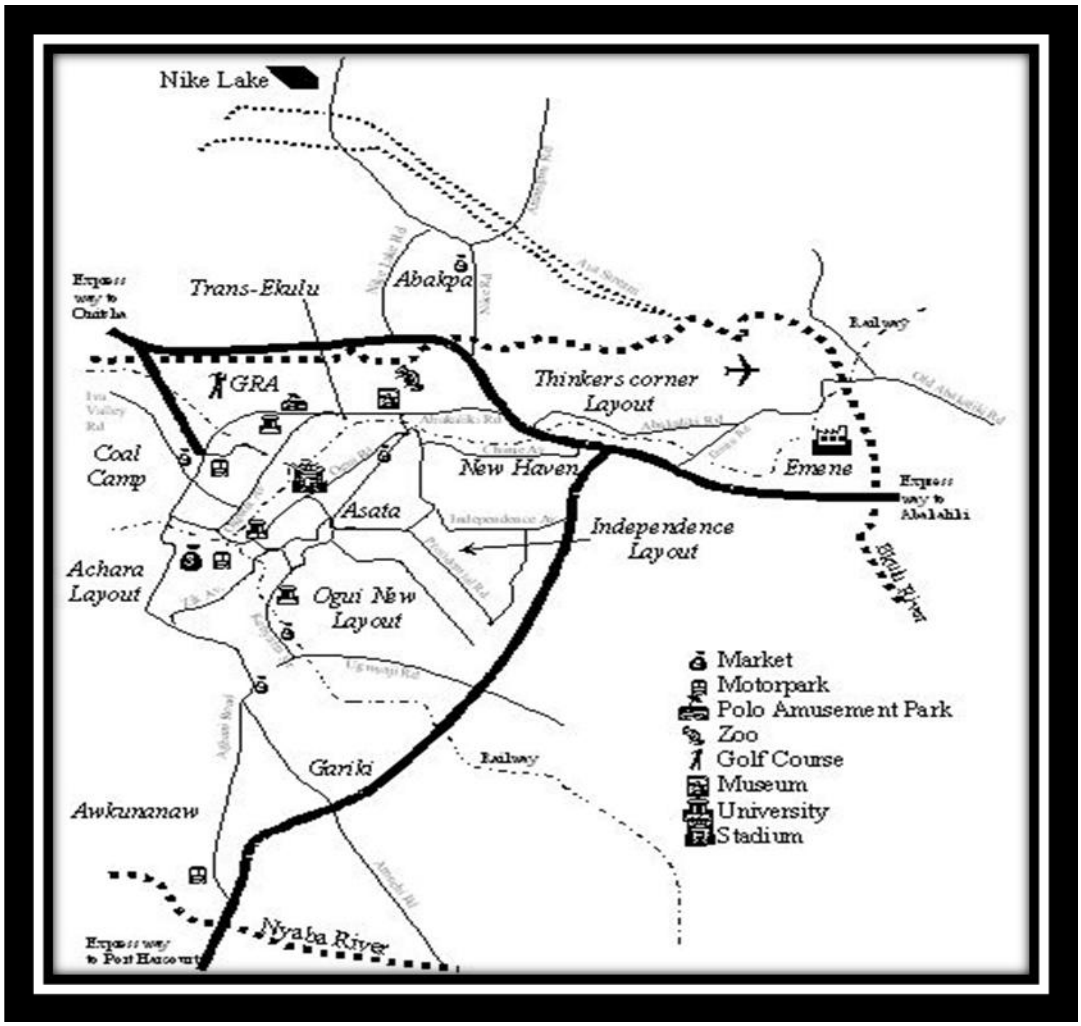

Figure 1: Map of Enugu east Local Government Area; showing the study area (Nike-Lake) located at the tail end of Abakpa Nike.

GPS Reading in the Communities

Community	Elevation	Longitude	Latitude
Negbune	168m	06.20356	006.22533
Enugwu	193m	06.28542	006.24141
Umuogbe	179m	06.27651	006.21218
Umuchuba	250m	06.20056	006.36693
Amabo	238m	06.20034	006.37020

Mosquito Collctions

Sampling was conducted in the month of October 2013 in the five villages of Alulu Nike community. Three sampling methods: Human landing catch (HLC), Spray sheet collection (SSC) and Centre for disease control and prevention light trap (CDC-LT) were used for this study.

The Human Landing Catch Method

In each location, two adult volunteers were recruited to carry out the mosquito collections. The volunteers were trained on sampling using the Human bait and given instructions on how to collect the mosquitoes. The collectors stayed with the both legs and arms exposed for the duration of the experiment (Usip *et al*, 2003). Any mosquito perching on the exposed part was caught before it fed by inverting a small glass tube over it. The caps of the tubes were immediately

replaced. All tubes containing mosquitoes were labelled to indicate date and time of capture(Opara *et al*, 2005) The catch was performed at dusk between 17.00 to 19.00 hours as most specie has biting peak after sunset.

The catch was performed by two immunized adult volunteers. These immunized human baits catch the mosquitoes as they alight to feed on them. Mosquitoes coming to feed were detected and collected with glass tubes and placed in a screened pint-size container with labelled location according to Duo-qua *et al* (2012). The bait sat on a stool with legs exposed and caught the mosquitoes as they attempted to bite. After the collections, the mosquitoes were taken to the laboratory (National Institute for Arbovirus and Research, Enugu) for identifications.

Plate 1: Volunteers in human landing catch

The Center for Disease Control and Prevention Light Trap (CDC-LT) Method

Five light traps (one in each of the village) were operated outside of every selected house. Each light trap was hung about 1.5-2m above the ground operated by a 6volts battery. The light trap used in this surveillance was the New Dersey mosquito light trap model 512.

Plate 2: CDC-Light trap set outdoor

Mosquitoes were attracted to the brightness of the light at night and they entered the hood of the trap where they were exposed to a strong downward air current produced by an electric motor fan. The mosquitoes were trapped into a funnel mesh screen, which were later taken to the laboratory for identification as described by Adeleke *et al* (2010).

The Spray Sheet Collection Method

In spray–sheet collection, a two-man spray team was employed in order to get a complete catch as possible; one man stood in the room and sprayed the inside, while the other sprayed outside the house, around the eaves and the inner walls that divide the room from the rest of the house, putting up a barrier of spray round all possible exits, the spraying was synchronised so that the eaves of air space above each wall were sprayed simultaneously from inside and outside. In each location five rooms were randomly chosen and the collection was performed between the hours of 7.00AM.

The floor surfaces of the room, as well as beds, furniture were completely covered with white sheets. The windows and doors were closed and the eaves (openings) were blocked to prevent mosquitoes from escaping. Then the aerosol (New Mortein powerGard® containing- Imiprothrin, d-Phenothrin, and D-trans allethrin) was used to flit the room and around the eaves outside the room, then the room was closed for 10 minutes. After 10 minutes, the sheets were carefully retrieved from the floor, starting from the door by lifting them at the four corners and moving them gently so that the mosquitoes were gathered at the middle of the sheet. The mosquitoes were collected into a labelled Petri dish using a forceps and then taken to the laboratory for identification.

Mosquito Processing

The mosquitoes in Human landing catch (HLC), spray sheet collection (SSC) and the centre for disease control and prevention light trap (CDC-LT) were collected and counted each morning, into a well labelled container and taken to

field laboratory (National Institute for Arbovirus and Research, Enugu) for identifications. Mosquitoes were identified to specie using a microscope and appropriate taxonomic keys according to Becker *et al* (2010).

Data Analysis

Data were entered using the 2007 Microsoft Excel version. The mean number of mosquitoes collected per night was analyzed using ANOVA with the following factors (village, method, and species) using the LSD test.

Result and Analysis

This study was carried out in five different locations in Alulu-Nike community (Enugu east) of Enugu state for 25 trap nights in the month of October, 2013.In all, 275 mosquitoes were caught belonging to three different genera: *Aedes*, *Culex*, and *Anopheles* which were identified to specie level. A total of seven (7) different species were identified: *Aedes aegypti*(63), *Aedes albopictus*(29), *Aedes taylori(2)*, *Aedes africanus*(33), *Aedes luteocephalus*(19), *Culex quinquefaciatus(102) and Anopheles gambiae*(27).

The human bait caught 156(56.73%) mosquitoes; the spray sheets 103(37.45%); while the CDC Light trap collected 16(5.82%). Tables below show the results performed by each sampling methods at different locations.

Table 1a: Sampling of Adult Mosquitoes in the five Locations Using the Human Bait Method

LOCATION (NAME OF CLAN)	DURATION	NUMBER OF BAIT	TOTAL MOSQUITOES COLLECTED	MOSQUITO SPECIES IDENTIFIED
Negbune	2 hours	2 persons	16	*Ae.aegypti(9) Ae.albopictus(7)*
Enugwu	2 hours	2 persons	35	*Ae.luteocephalus(8) Cx.quinquefasciatus(1) Ae.aegypti(11) ,Ae.albopictus(2) Ae.africanus (13)*
Umuogbe	2 hours	2 persons	48	*Ae.aegypti(24) Ae.taylori(2) Ae.fricanus(15) Ae.luteocephalus (7)*
Umuchuba	2 hours	2 persons	41	*Ae.aegypt(12) Ae.albopictus(19) Cx.quinquefasciatus (10)*
Amabo	2 hours	2 persons	16	*Ae.aegypti(7) Ae.africanus(5) Ae.luteocephalus (4)*
Total	10 hours	10 persons	156	*Ae.aegypti(63) Ae.albopictus(28) Ae.taylori(2) Ae.africanus(33) Ae.luteocephalus(19) Cx.quinquefasciatus(11)*

A total of 156 (one hundred and fifty six) mosquitoes were collected and identified. Mosquito species identified were *Aedes aegypti*, *Aedes albopictus*, *Aedes taylori*, *Aedes africanus*, *Aedes luteocephalus*, and *Culex quinquefaciatus* with distributions of 63, 28, 2, 33, 19, and 11 respectively. 48 mosquitoes were caught in Umuogbe, 41in Umuchuba, 35 in Enugwu, while 16 were caught at Negbune and Amabo respectively.

TABLE 1b: Adult Mosquito Sampling in the Different Locations Using the Spray Sheet Method.

LOCATION (NAME OF CLAN)	NUMBER OF HOUSES SAMPLED	TOTAL NUMBER OF MOSQUITO	MOSQUITO SPECIES IDENTIFIED
Negbune	5	27	*An.gambiae(11) Cx.quinquefasciatus (16)*
Enugwu	5	14	*Cx.quinquefasciatus (14)*
Umuogbe	5	32	*An.gambiae (7) Cx. quinquefasciatus (25)*
Umuchuba	5	18	*An.gambiae (6) Cx.quinquefasciatus (12)*
Amabo	5	12	*An.gambiae (3) Cx.quinquefasciatus (9)*
Total	25	103	*An.gambiae(27) Cx. quinquefasciatus(76)*

The result of spray sheet method in which 25 houses were sampled, 5 from each clan and a total of 103 mosquitoes were collected.

TABLE 1c: Adult mosquito sampling in the different location using the CDC-Light Trap

LOCATION (NAME OF CLAN)	NUMBER OF TRAP SET	NUMBER OF MOSQUITOES COLLECTED	MOSQUITO SPECIE IDENTIFIED
Negbune	1	2	*Cx.quinquefasciatus(2)*
Enugwu	1	5	*Cx.quinquefasciatus(4) Ae.albopictus(1)*
Umuogbe	1	0	*Nil*
Umuchuba	1	6	*Cx.quinquefasciatus(6)*
Amabo	1	3	*Cx.*quinquefasciatus*(3)*
Total	5	16	*Cx.quinquefasciatus(15) Ae.albopictus(1)*

A total of 16 mosquitoes were caught belonging to the species *Culex quinquefasciatus* (15), and *Aedes albopictus (*1). 6 mosquitoes were collected in Umuchuba, 5 at Enugwu, 3 at Amabo and 2 at Negbune. There was no catch in Umuogbe using this method.

TABLE 2a: Mean mosquitoes collected using different collection methods in the five locations.

LOCATION	HUMAN BAIT	SPRAY SHEET	CDC-LIGHT TRAP
Negbune	16.00^{d}	27^{b}	2^{d}
Enugwu	35.00^{c}	14^{d}	5^{b}
Umuogbe	48.00^{a}	32^{a}	0
Umuchuba	41.00^{b}	18^{c}	6^{a}
Amabo	16.00^{d}	12^{e}	3^{c}

Mean in the same column having the same letter are not significantly different at $p \leq 0.05$, according to LSD test.

Result show that, there was significant difference in the number of mosquitoes collected by using the different collection methods in the five locations in Alulu-Nike community. In Human bait method, Umuogbe location recorded the highest mosquitoes collected. This location has a mean mosquito collection of 48 which is significantly different from the number of mosquitoes collected in the other locations (Table 2(a)). The lowest mosquito collected was recorded in Negbune and Amabo with a mean mosquito collection of 16 each which was significantly different from that of Umuogbe, Umuchuba, and Enugwu that recorded 48, 41, and 35 respectively. Similarly, Umuogbe recorded the highest mosquito collection by the spray sheet method. The mosquito collected in this location (32) is significantly different from the mosquitoes collected in other locations using spray sheet method (Table 2a). However, Negbune recorded the second highest mosquito collection with a mean of 27 which is also significantly different from the mean mosquito collection of Umuchuba (18), Enugwu (14) and Amabo (12) which was the lowest.

Mean number of mosquitoes collected by using CDC-light trap in the five locations of Alulu-Nike show that Umuchuba recorded the highest mean number(6) of mosquitoes collected which was significantly different from the mean number of mosquitoes collected in other locations (Table 2a). The least mosquito collection with the CDC-light trap was recorded in Negbune with a mean of (2) which was significantly different from the other locations. On the contrary, there was no mosquito collection using CDC-light trap method in Umuogbe.

Table 2b: Mean mosquitoes collected by the three different methods of collection.

METHOD OF MOSQUITO COLLECTION	NUMBER OF MOSQUITOES COLLECTED
Human Bait	156.0^{a}
Spray sheet	103.0^{b}
CDC-Light trap	16.0^{c}

Means having the same letter are not significantly different at $p \leq 0.05$, according to the LSD test.

Table 2b shows number of mosquitoes collected by using different collection method of human bait, spray sheet and CDC-light trap. Human bait method recorded the highest mean number of mosquito collection (156) which was significantly different from mean number of mosquitoes collected by the spray sheet (103) and the CDC-light trap (16) methods.

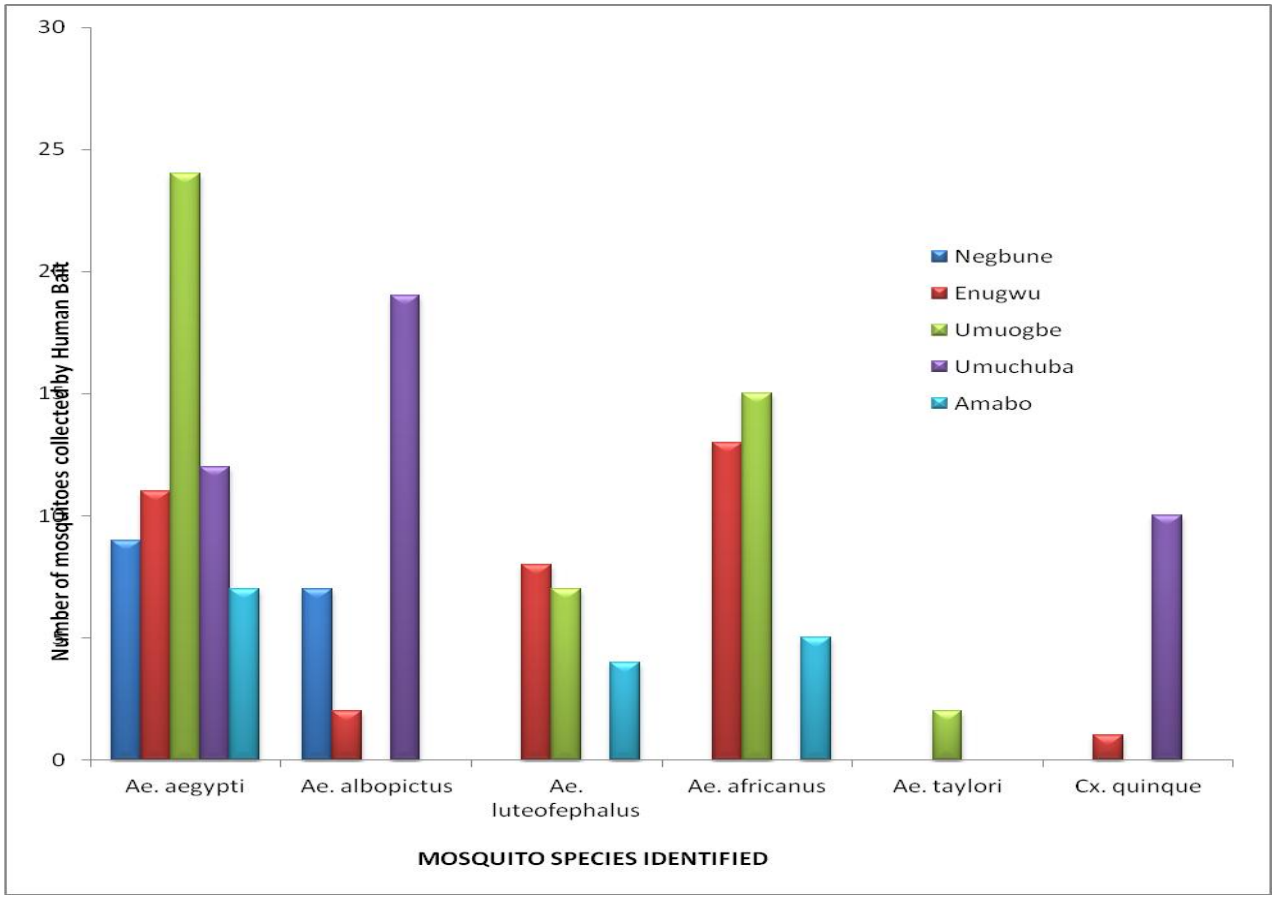

Figure 3(a): Distribution of mosquito species collected by Human Bait in the sampled communities.

Results show that *Aedes aegypti* (63) was more predominant than other species of mosquito collected in the five locations. The second highest specie collected from the HLC was the *Aedes africanus (33)* which was significantly different from *Aedes. Albopictus* (28), *Aedes luteocephalus*(19), *Culex quinquefasciatus* (11) and *Aedes taylori* (2) The lowest specie recorded was the *Aedes.taylori*.

As can seen from the above, *Aedes aegypti* was more predominant in Umuogbe, *Aedes albopictus* in Umuchuba, *Aedes luteocephalus* in Enugwu, *Aedes africanus* was also more in Umuogbe, *Culex quinquefasciatus* in Umuchuba, whereas *Aedes taylori* was only caught in Umuogbe and not at other locations.

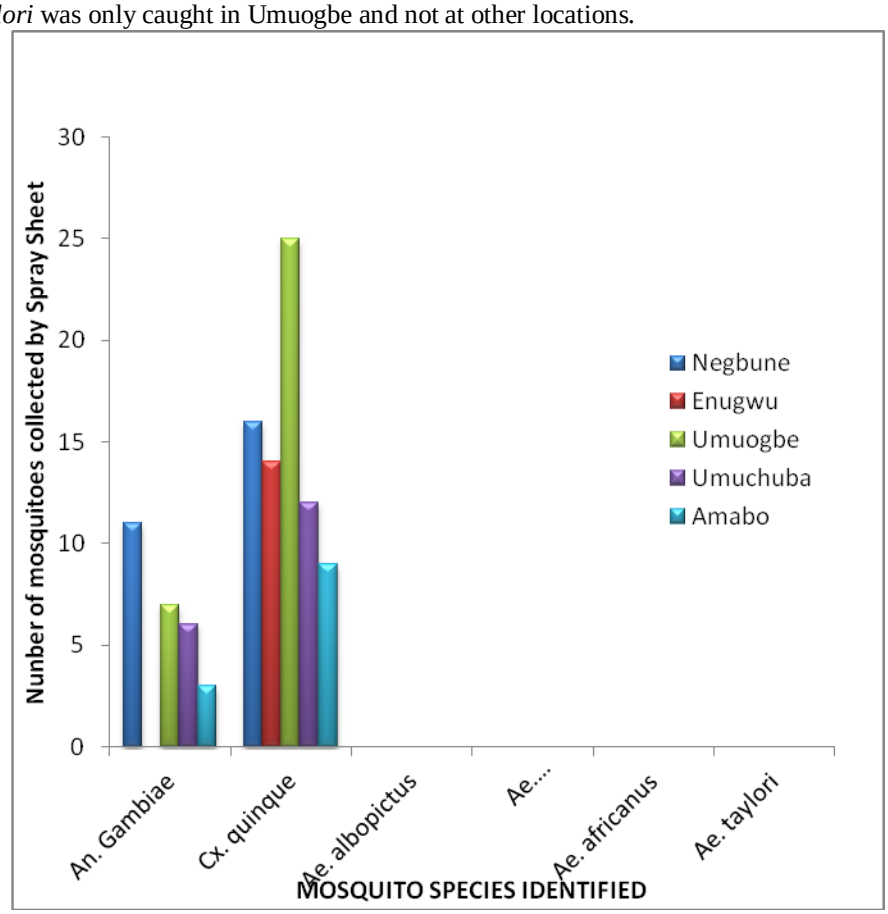

Figure 3(b): Distribution of mosquito species collected by Spray Sheet in the sampled communities.

The species recorded using the spray sheet method was the *Culex quinquefasciatus* and the *Anopheles gambiae*. Other species were not collected. The *Culex quinquefasciatus* recorded the highest collections (recorded in the five locations) which was significantly different from that of *Anopheles gambiae* which only collected in four locations (Negbune, Umuogbe, Umuchuba, and Amabo).Umuogbe recorded the highest collection of *Culex quinquefasciatus*. And Negbune recorded the highest collection of *Anopheles gambiae*.

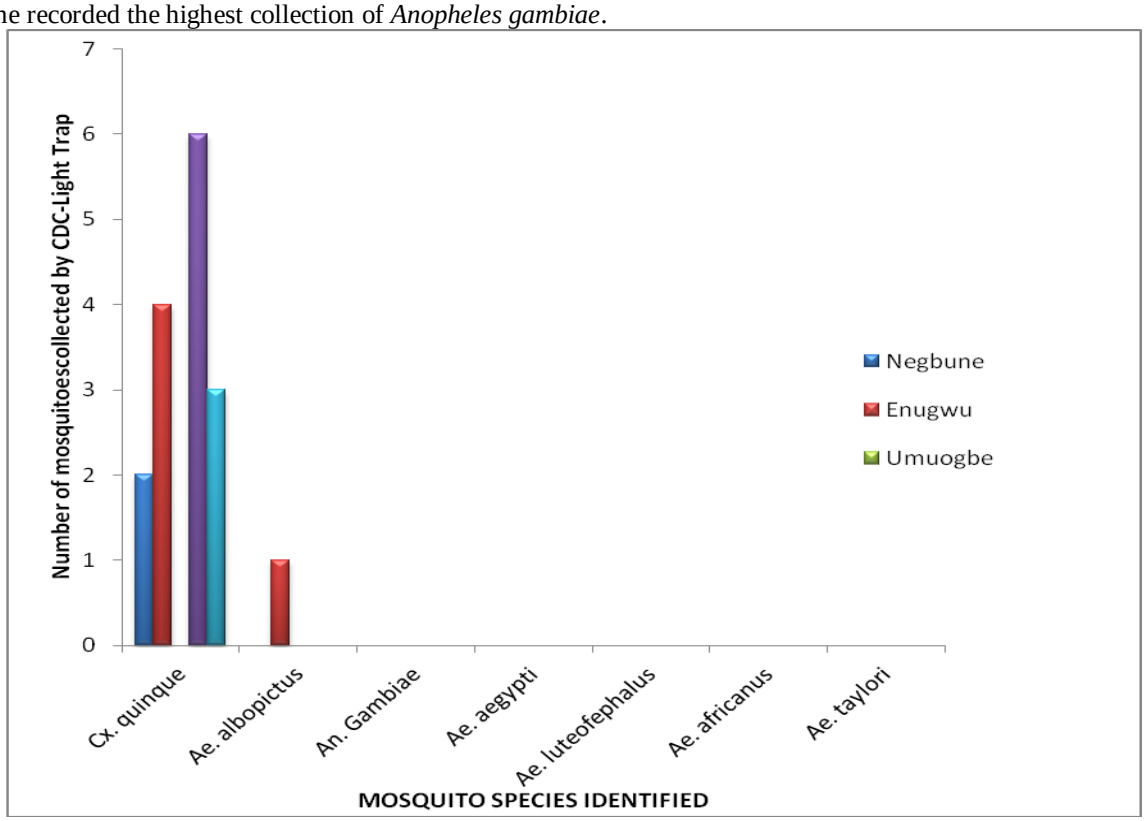

Figure 3(c): Distribution of mosquito species collected by CDC-Light Trap in the sampled communities.

From the above figure 3(c), only two species (*Culex quinquefasciatus* and *Aedes albopitus*) were recorded using the CDC-light trap method, with *Culex quinquefasciatus* showing the highest collection which was significantly different from that of *Aedes albopictus*. However, *Culex quinquefasciatus* was the highest collection in Umuchuba which was significantly different from *Culex* collected in the other locations. *Culex quinquefasciatus* was not recorded in Umuogbe. *Aedes albopictus* was only recorded in Enugwu but not in other locations of the community using CDC-light trap.

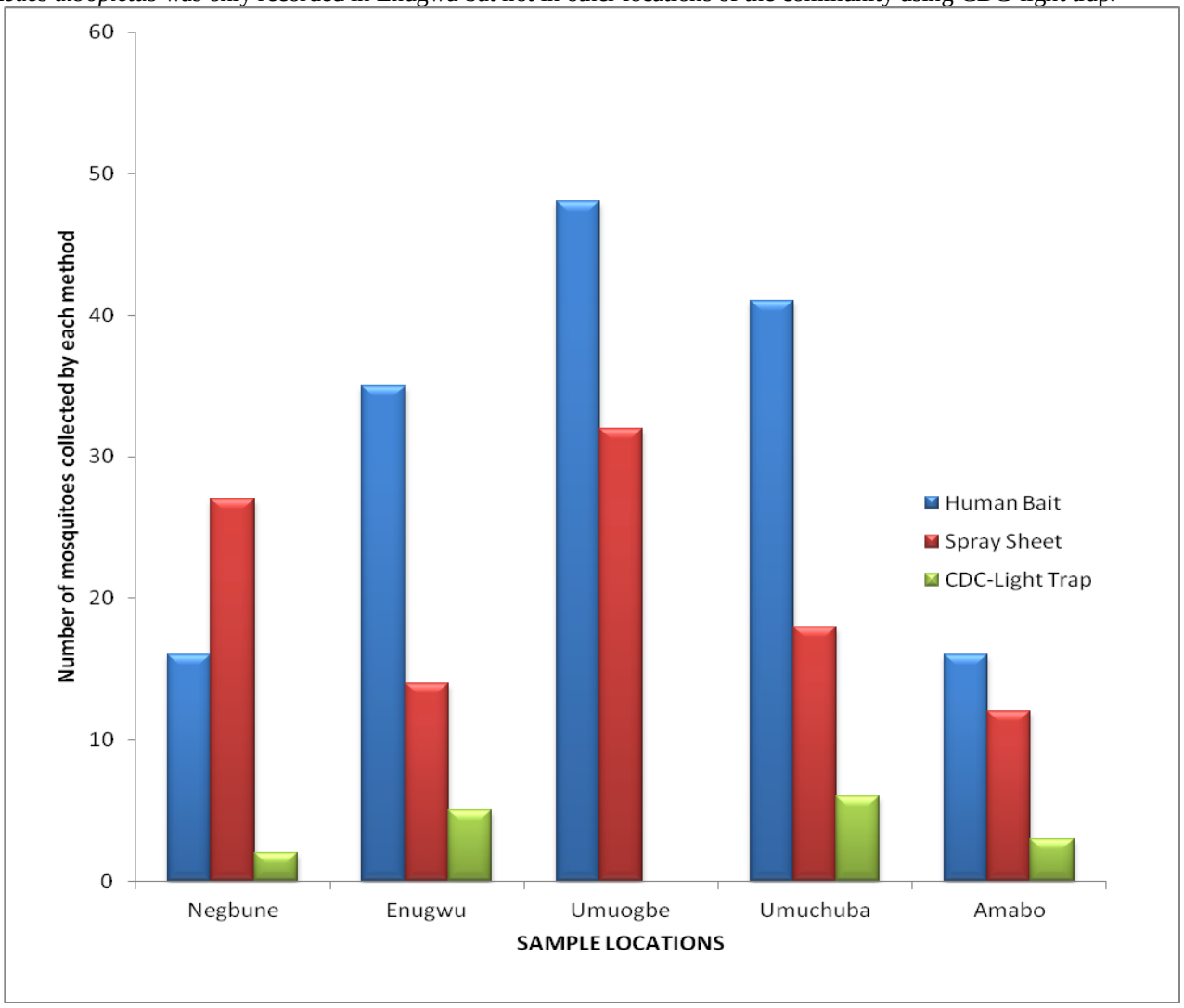

Figure 3d: Number of mosquitoes collected with each method in the sample locations.

The chart represents the mosquitoes collected in the different locations of Alulu-Nike community using the different sampling techniques. Human bait catch recorded the highest collections in Umugbe, Umuchuba, Enugwu, and Amabo.Spray sheet method was the highest recorded in Umuogbe and are significantly efficient in other locations. Whereas, the CDC-light trap method got the least catch in all the locations.

Discussion

Surveys are essential for the planning operation and evaluation of any effective mosquito control whether for the prevention of mosquito borne diseases or the lowering of the population of these biting insects to a level permitting normal activities without undue discomfort. Mosquito borne diseases still remain the major public health problem in Africa and their transmission is becoming frequent on a daily basis due to wide spread of the insects. The presence of three genera of mosquito*: Aedes*, *Anopheles*, and *Culex*, and seven species *(Aedes aegypti*, *Aedes albopictus, Aedes africanus*, *Aedes taylori, Aedes luteocephalus, Anopheles gambiae*, and *Culex quinquefasciatus)* is an indication that the climatic and environmental conditions in Alulu-Nike community are conducive to support the survival and development of wide range of mosquitoes that could invariably contribute to vector disease risks in these study areas.

The human bait method which is referred to as the gold standard method collected more mosquitoes compared to other sampling methods used in this surveillance, this result compares well with reports from other researchers (Ndiath *et al*, 2011). Results from this study reviewed that the Human bait catch recorded 156 mosquitoes which comprised of the seven different species *(Aedes aegypti* (63), *Aedes albopictus* (28*), Aedes africanus* (33), *Aedes luteocephalus* (19), *Aedes taylori* (2), and *Culex quinquefasciatus* (11). From this result, it was observed that the human bait method collected more *Aedes* species, especially the "*Aedes aegypti"* more that other species identified in the study locations. This was due to the fact that, the *Aedes* species are outdoor feeders and mostly feeds late in the afternoon or even in full sunlight. The *Aedes* species can transmit Yellow fever, Dengue fever, Chikungunya, Heartworm, Encephalitis, Ross River virus, and West Nile virus.

The spray sheet method collected total of 103 mosquitoes which included the *Culex quinquefasciatus* (76) and the *Anopheles gambiae* (27). In this study, this sampling technique was the only method that collected *Anopheles gambiae* and more *Culex quinquefasciatus*, this was because the species are highly anthropophilic and can only be found indoors. They are very active at night and are allegedly attracted to smelly feet. The *Culex quinquefasciatus* is a primary vector of

several arboviral diseases particularly the West Nile Virus, Lymphatic filariasis and elephantiasis, while the *Anopheles gambiae* specie plays a very important role in the transmission of the most dangerous malaria parasite to humans. This species carry the parasite *Plasmodium falciparum* which causes malaria and has also been associated with the transmission of filariasis (Verma, 1989).

The CDC-Light trap collected the least mosquito species (caught *Culex quinquefasciatus* (15) and *Aedes albopictus*) (1). The parallel trap evaluation in Tanzania demonstrated similar result; it recorded low capture rates for CDC-light trap in Urban Dares Salaam (Govella *et al*, 2011). The low catch in CDC-light trap method may be because the trap was set-up outdoors, and predominant species in the community are highly anthropohilic and are less influenced by light and also, many large types of vegetation in the community might have covered the brightness of the light thereby preventing the attractions. It was observed from the result that more *Culex quinquefasciatus* were collected using the CDC light-trap which could be due to the fact that, other species were not really attracted to the light.

In this study, Umuogbe recorded the highest number of mosquito density; this is as a result of the high population in the location leading to dirty environments, presence of stagnant water, water holding containers, tires etc. But there was no catch using CDC in this location due to the presence of vegetations and the mosquitoes are indoor feeders.Umuchuba recorded the second highest collection of mosquitoes compared to other locations; collected a total of (65) mosquitoes using the three sampling techniques.The least mosquito density was recorded at Amabo (Table 2a), this community happens to be less populated and the presence of mosquito breeding sites was less compared to other locations. It has been established that occurrence of mosquitoes is associated with the presence and activities of humans which was higher in other places compared to Amabo. O'Meara *et al*, (1997) stated that human activities are responsible for the establishment of vast majority of aquatic habitats used by *Cx quinquefasciatus.*

The results also showed that *Culex quinquefasciatus* (102 mosquitoes) were present in four of the five sampled locations of Alulu-Nike community and was collected in two of the three sampling techniques used in this study, this shows that the specie was more predominant in the community compared to other species. The *Aedes* species, especially *Aedes aegypti* (63 mosquitoes) was second most abundant in the study locations compared to other Aedes species because this specie commonly bites at dusk and dawn, and are mostly in shady areas and they preferred breeding in stagnant water.The *Anopheles gambiae* (27) recorded in this study was collected only with the spray sheet method contradicts the report of Ndiath *et a*l, (2011) in Senegal where the mean *Anopheles* density was lower with pyrethrum spray catch for all species compared to other sampling methods used. This result reviewed that *Anopheles gambiae* mosquito is not predominant in the community. The low density of this mosquito could be attributed to the period of this study (October) which happens to be the onset of dry season in the country, for it is believed that the amount of rainfall is a principal factor that promotes breeding especially by providing the high humidity which prolongs the longevity of the adult mosquitoes. This corroborates the results of Gillet and Smith (1972) and Okorie (1978), in which *Culex* and *Aedes* predominate all year round differing from *Anopheles* which reach peak in abundance in wet months.

A large variety of traps have been developed for entomological studies and their relative efficiency has been highly variable. This study confirms that, the method used influences the quantity and the variety of mosquitoes' collected. In this study, the Human landing catch showed a more efficient and reliable collection than others but for ethical reasons spray sheet method should be used as in carrying out surveillance in this community because it demonstrated a good correlation with the human landing catch. A standardized evaluation of more promising methods for capturing host-seeking mosquitoes should also be considered in this community.

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