Investigating the dynamics of mosquito vector populations within Lake Ecosystems: The case of East African Rift Valley Lakes Nakuru, Bogoria and Lesser Flamingo (*Phoeniconaias minor)* mass-die offs.

By

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Abstract

Recent changes in the occurrence and spectrum of infectious diseases affecting wildlife have contributed to the growing importance of zoonotic diseases. Emerging infectious diseases in wildlife stem from turbulence in the complex interrelationships between host, pathogen and environment. Much remains unknown about the true prevalence of arboviruses in East Africa and the mosquito vectors responsible for maintenance and virus transmission. Such a deep and thorough representation is needed and will help make better sense of the effect, patterns and prevalence of mosquito vectors and arbovirus networks. This study involved the collection of mosquitoes around the Rift Valley Flamingo lakes Nakuru and Bogoria over a period of one month from December 2012 to January 2013. Using EVS CO_2 baited traps we collected a total of 2212 mosquitoes from four genera *Culex, Aedes, Mansonia and Anopheles*. From this collection 89.9% were from Lake Nakuru and 10% from Lake Bogoria with *Culex* species being the highest number overall and in Lake Nakuru whereas *Aedes* species dominated the Lake Bogoria collection. Lake Nakuru had a higher species diversity of the two lakes with a Shannon-Weiner Index value of 1.39 whereas Lake Bogoria had Shannon-Weiner Index value of 0.98. The study also assessed the potential species of medical importance and many of the species collected in this study have been previously associated with the transmission of arboviruses in Africa.

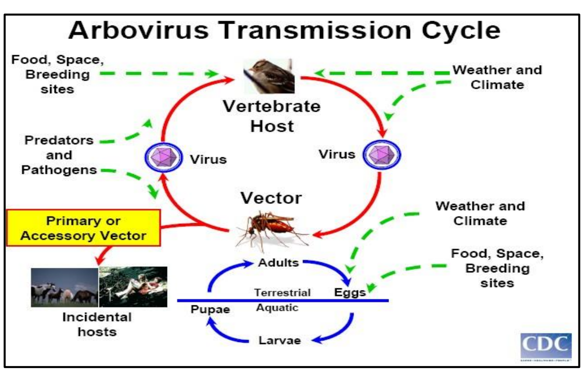
Introduction

In the last two decades East Africa has experienced episodic mass die-offs of Lesser Flamingo (*Phoeniconaias minor*) at the alkaline-saline Rift Valley lakes Nakuru, Elementaita, Bogoria,in Kenya and lakes Manyara, and Natron in Tanzania (Matagi, 2004). The recorded episodes include approximately 40,000 in 1993, 20,000 in 1995 and 50,000 in 2000 (Matagi, 2004; Owino, 2008). In 2004 over 50,000 Lesser Flamingo died in Lakes Manyara and the Big Mommela in Tanzania (Owino, 2008). Currently, the Lesser Flamingo are categorized globally as 'Near Threatened' in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species and listed as undergoing a 'moderately rapid reduction'. Numerous factors have been linked to these die-offs including heavy metal poisoning, cyanobacterial toxins, pesticide residues, septicemia and avian tuberculosis (Kairu, 1996; Kock, Kock, Wambua, Kamau, & Mohan, 1999; Ndetei & Muhandiki, 2005; Nelson et al., 1998).

One of the major issues surrounding these regional lake ecosystems and Lesser Flamingo research is there has not been a continuous ecological monitoring and thus there is a paucity of data on the biodiversity of species in and around these lakes (Matagi, 2004). It is difficult to point out the causes or contributors without previous data to compare where the problem may have occurred.

Recent changes in the occurrence and spectrum of infectious diseases affecting wildlife have contributed to the growing importance of zoonotic diseases. Disease emergence has occurred on a worldwide scale in a broad spectrum of wildlife species and habitats (Burroughs, Knobler, Lederberg, & Ebrary, 2002). Arboviral encephalitides are zoonotic and are maintained in nature through transmission between vertebrate hosts and hematophagus arthropods.

Figure 1: Diagram showing the relationships between the various factors in the transmission of arboviruses in nature. (CDC, 2008)



Emerging infectious diseases in wildlife crop up from turbulence in the complex interrelationships between host, pathogen and environment. Wild animals serve as reservoir species for zoonotic pathogens and represent a critical target for emerging infectious disease surveillance. Birds are involved in transmission of a number of serious zoonoses, especially vector-borne diseases, this is of concern due to the geographic movement of many birds (Burroughs et al., 2002). Presently the international catalogue of arboviruses has listed 535 viruses including other viruses of vertebrates as having being isolated from birds or ornithophilic arthropods (Pattison, 2008) .Since wild birds are capable of carrying emerging zoonotic pathogens either as reservoir hosts or by dispersing infected arthropods they are of public health importance (Reed., Meece., Henkel., & Shukla., 2003). For several arborviruses, wild birds are considered amplifiers of infection. Much remains unknown about the true prevalence of arboviruses in East Africa and mosquito vectors responsible for maintenance and virus transmission.

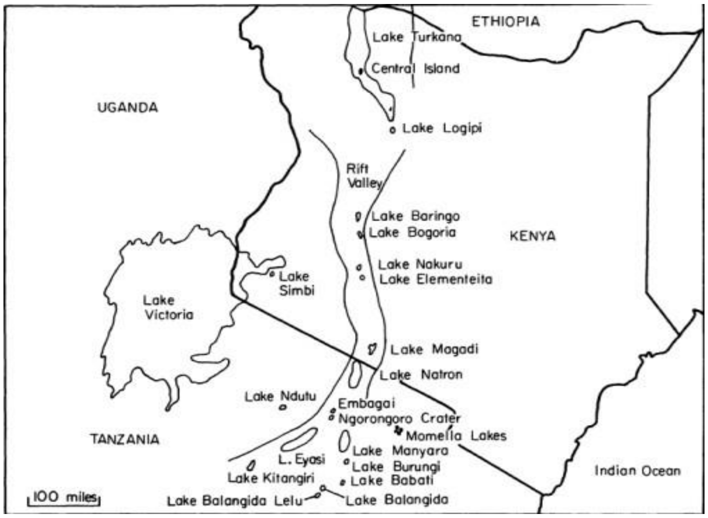
One of the neglected factors in the investigation of the mass die offs is vector borne zoonotic diseases particularly mosquito-borne arboviruses. Arboviral infections tend to have a seasonal pattern (Booss & Esiri, 2003), this should be a major focus in the investigation of the mass die-offs. Mosquitoes are vectors to bird, animal and human diseases; in addition they are the most abundant and widely dispersed vectors (Cox, 2001) and are responsible for the transmission of many medically important pathogens. Based on morbidity and mortality data they are the most dangerous vector threatening more than two billion people in tropical and subtropical regions (Becker, 2010).

The study involved continuous monitoring of endemic mosquito species, densities, and age structures and arboviral infection rates in adult mosquitoes within the flamingo lakes in the East African Rift Valley region for one month.

Objectives

- To reveal the distribution and density of endemic mosquito species of the region, as a necessary prelude to determining which vector-borne diseases may be circulating in that ecosystem.
- To gather preliminary data for establishing a long-term arboviral surveillance program that simultaneously monitors infectious disease and climate changes; and hence provide a more comprehensive understanding of the intersection of climate with major factors in disease emergence and re-surgence.
- To examine the preliminary data in order to guide the development of future works

Figure 2: Map showing location of Lakes Nakuru, Elementaita and Bogoria (WildlifeDivision, 2010)



Methodology

The disease dynamics study was conducted at two sites in the Kenyan Rift Valley Lakes Region: Lakes Nakuru, and Bogoria. These sites were chosen due to their utilization by Lesser Flamingos of East Africa and their recorded mass mortalities in recent years. The field study period was conducted from December 2012 to January 2013 which is the dry season in Kenya, a favorable climate for data collection.

I. Mosquito collection (Rift Valley, Kenya)

Material & Supplies Included:

- ✓ 2801A EVS-CO2-Mosquito-Trap (4)
- ✓ Dry ice (6lbs/week)
- ✓ Batteries (AA size)
- ✓ Anemometer (Kestrel)
- ✓ Falcon tubes
- ✓ Kimwipes
- ✓ Camera
- ✓ Garmin GPS unit
- ✓ Data entry sheets
- ✓ Packing tape
- ✓ Labels

II. Data Collection Methods:

Trap set-out:

Mosquito CO_2 baited EVS traps were set out one hour prior to sunset which was normally around 6.30 pm. Due to the nature of the terrain, speed limit and the 6pm curfew at the National Parks the field team with their equipment were ready and started by 5pm. For our safety we were accompanied by a park reserve guard each time we went out. Traps were hang on accessible trees at the two possible sites of interest within each lake and climate data collected and recorded. On average we recorded temperatures of 25.5± 0.4 °C at L. Nakuru Park and 29.7 ± 1.2° C at L.Bogoria Park during trap set up. In addition, our 3rd week of fieldwork was interrupted by the heavy rainfall which made the terrain impassable and thus no data was collected on that week.

Trap pick-up:

EVS traps (Figure 1) were picked up one hour after sunrise around 7.30 am. On average we recorded temperatures of 18.8 ± 1.8 °C at L. Nakuru Park and 25.2 ± 1.6°C at L.Bogoria Park during trap pick up. The catch nets were immediately placed in a deep cooler containing dry ice in an upright position and transported back to the Kenya Wildlife Service Headquarters where they were transferred to a deep freezer for one hour before sorting. After which the collected mosquitoes were sorted by site and carefully transferred into falcon tubes. Upon completion of the four week sampling period, mosquito samples were morphologically identified at the Kenya Medical Research Institute with the immense help of the Entomology team who were very familiar with the African mosquito species. The identified mosquitoes were then pooled into tubes according to their species, site and collection date.

Data and Descriptive Statistics

Figure 3: EVS Trap

Data was collected from six (6) randomly chosen sites around each lake, Lake Nakuru and Lake Bogoria. These sites all used the same instruments to trap and collect mosquitoes. Each collection site was set in place at around the same times of the day and allowed to remain active for an equal duration of time. Each week, the site locations were changed, according to sound field survey methodology practices, in order to seek to capture the distribution of mosquito populations and species around the lake; these data would form a crucial component of scientific investigations, which involve mosquito populations as vectors, within the lake regions.

Results and Discussion

In general, the lakes differed in terms of mosquito population, species richness and species diversity. The statistical differences generally pointed toward the following: the need for further investigation as to why the different lakes bear population difference; as well as to the fact that expectations related to disease outcomes or vector mitigation strategies that are geared toward mosquito populations may differ between lakes. The data suggest, for example, that a disease model, given mosquitoes as a disease vector, may have to incorporate locale differences between lakes; as an extended example, an investigation say into the impact of mosquitoes on flamingo population reductions may differ by lake. All in all, these differences offer merit to explore the reasons for the differences between the two lakes given that both lakes have similar characteristics and both regions surrounding the lakes are seemingly homogenous in terms of their ecological characteristics.

Population Distribution Dynamics

In general, Lake Nakuru recorded significantly more mosquitos in terms of absolute numbers compared to Lake Bogoria. In total, Lake Nakuru recorded 1989 mosquitos while Lake Bogoria recorded 223 mosquitos, making the total number of mosquitos collected at 2212 (Table 1). Most of the mosquitoes in Lake Nakuru were of the *Culex* genus, while Lake Bogoria was overwhelmingly dominated by the *Aedes* genus; while it is interesting that Lake Bogoria was dominated by only one particular genus it is unclear how this fact translates into any biological consequences or disease outcomes. Additionally, the *Mansonia* genus of mosquitos had a moderate presence in Lake Bogoria but recorded no presence in Lake Nakuru; moreover, while the Anopheles genus has a leading presence in Lake Nakuru, this particular genus barely showed a presence in Lake Bogoria. The distribution of mosquito genera clearly differed by lake. From all the sites in L. Nakuru Park we collected 89.9% and 10% from all sites at the Lake Bogoria Park. It is important to mention that we noted most of the Kenyan flamingo population was at Lake Bogoria during our study as evidenced in the pictures below. Previous studies conducted have shown that different mosquito genus have breeding preference influenced by the environment and habitats (Diallo et al., 2012; Igbinosa, 1989; Okogun, Nwoke, Okere, Anosike, & Esekhegbe, 2003). This reinforces the need to have more data on the lake surroundings.

There are many mosquito species that are considered to be of potential medical importance due to the viruses previously isolated from them consequently they are considered to be a threat to potential hosts bird or human. Below we have a list of potential arboviruses associated with the mosquitoes captured in this study.

- *Ae. mcintoshi* : Rift Valley Fever Virus (RVFV), Wesselsbron virus (WSLV), Ngari virus (NRIV) (McIntosh, 1986; Mutebi et al., 2012).
- *A. christyi* : RVFV (Linthicum, Davies, Kairo, & Bailey, 1985)
- *Cx. pipiens* : Japanese Encephalitis virus (JBEV), LaCrosse Encephalitis virus (LACV), Semiliki Forest virus (SFV), St. Louis Encephalitis virus (SLEV), Tahyna virus (TAHV),Western Equine Encephalitis (WEEV), West Nile virus (WNV), Banzi virus (BANV), Bunyamwera virus (BUNV) (Berge, 1975; Mutebi et al., 2012).
- *Cx univitattus* : Sindbis virus (SINV), Spondweni virus (SPOV) , Usutu virus (USUV), WSLV and WNV ((Berge, 1975; Hubalek & Halouzka, 1999; Jupp, 2001; Mutebi et al., 2012).
- *Cx. zombaensis* : BUNV, Pongola virus (PGAV) and RVFV (Linthicum et al., 1985; Meegan & Bailey, 1988; Mutebi et al., 2012).
- *Cx. vansomereni* being ornithophilic and the most abundant specie collected has been studied by other groups and found to be a competent vector of WNV under laboratory conditions (Lutomiah et al., 2011) and a previous study has also isolated RVFV (Linthicum et al., 1985) .

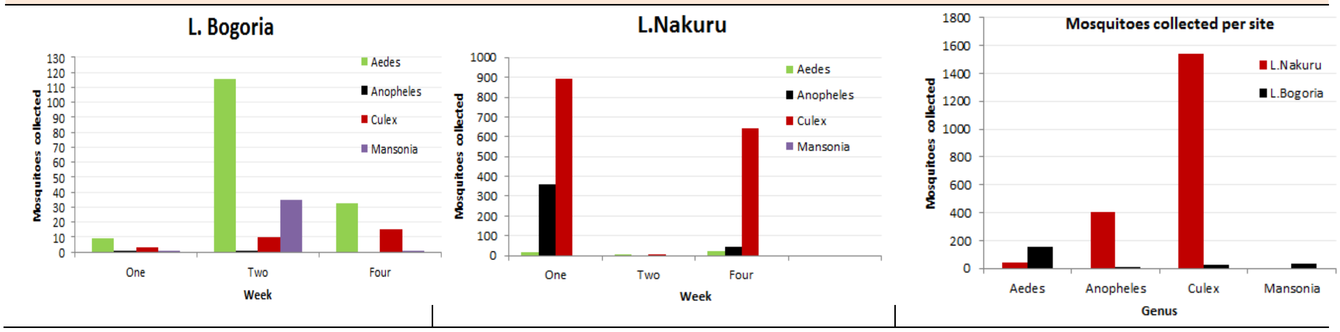
With these possibilities of arboviruses circulating in the lake ecosystems it is imperative to earnestly consider what the role of such a high presence of ornithophilic mosquitoes species actually means for the Lake Nakuru bird populations. *Culex vansomereni* particularly were the most abundant, it is clear that the surroundings at Lake Nakuru favor the breeding and survival of this specie it would be interesting to explore deeper how this has influenced this particular ecosystem.

Table 1: Summary of mosquitoes collected during study period

		Collection Sites	
Genus	**Species**	**L. Nakuru (%)**	**L. Bogoria (%)**
Aedes (Ae.)	*mcintoshi*	10 (0.45)	
	dentatus	2 (0.09)	
	chaussieri	1 (0.045)	
	tricholabis	4 (0.18)	
	hirsutus	24 (1.08)	158 (7.143)
	unknown	1 (0.045)	
Anopheles(A.)	*funestus*		1 (0.045)
	christyi	405 (18.3)	
Culex (Cx.)	*bitaeniorhynchus*	7 (0.32)	
	pipiens	268 (12.12)	24 (1.08)
	univittatus	20 (0.90)	
	unknown	4 (0.18)	3 (0.136)
	vansomereni	980 (44.3)	1 (0.045)
	zombaensis	263 (11.88)	
Mansonia	*uniformis*		6 (0.27)
	africanus		30 (1.36)
Total		**1989 (89.92)**	**223 (10.08)**
Grand Total	**2212**		

Notably, in terms of weather conditions, during the collection period, the average temperatures around Lake Nakuru were relatively even; while the wind speed steadily declined from week 1 to week 4. Precipitation, however, showed the more variance peaking in-between week 2 and 4, before returning to similar precipitation levels that were recorded in week 1, in week 4. Between week 1 and week 4, the average trap-setting temperature conditions were within 2.95°C deviations from each other in Lake Bogoria; also at this lake, the precipitation levels at trap-collection times increased only modestly between week 1 and 4. Thus, overall, during the collection period the weather conditions did not show significant variations in condition and as such I did not suspect that weather conditions alone would have had a large (and potentially biasing) impact on the mosquito collections.

Figure 4: Mosquito Populations Per Week By Lake & Genus & Mosquitoes Collected Per Site



Species Diversity and Species Richness

Given a sample, species diversity is defined as the relative abundance of species in the sample data; thus the total number of observations in a data set is weighted by taxonomical breakdown of the number of species in that sample data set. This is important because a large sample does not necessarily imply diversity of specie groups within the total number of observations. In order to measure diversity, researchers have relied on nonparametric measures to incorporate species richness and abundance information into an index to serve as measurement of species diversity. One of the most commonly used measures of species diversity is the Shannon-Wiener Diversity Index (Apostolaki, Tsagaraki, Tsapakis, & Karakassis, 2007; Hopkins & Mudge, 2004; Khera, Mehta, & Sabata, 2009; Kuehl, Wood, Marsh, Schmidt, & Young, 2005; Samuelson, 2001; Shannon & Weiner, 1963; Wetherbee, Crow, & Lowe, 1996). The index is calculated by:

$$H' = \sum_{i=1}^{s} p_i \ln p_i$$

Where s is the number of species and p_i is the proportion of the total sample that is represented by each species. Species diversity indices imputer for both lakes and compared. Lake Bogoria had a Shannon-Weiner Index value of 0.98, while Lake Nakuru has a value of 1.39; indicating that Lake Nakuru had a higher species diversity of the two lakes. Nevertheless, the data suggests that the area around Lake Nakuru tends to attract a more diverse distribution of mosquito species.

Notably, despite having a slightly higher score for genera richness, the population of mosquitoes around Lake Bogoria were concentrated within a single genus, furthermore the species evenness within Lake Bogoria was low as most of the mosquitos collected from the this lake area belonged to the *Ae. hirsutus* species. Thus not only was the species diversity lower at Lake Bogoria, but the relative abundance within each species was unevenly biased toward a particular specie, and also a particular genus. Conversely, Lake Nakuru was more diverse at the specie level and exhibited a more evenly spread abundance across each species.

Spatial Clustering Considerations

Recall, each collection site around each lake was geo-coded. Given the importance of common geo-coding, spatial statistics rise in relevance especially given that mosquito populations are distributed across space; the process that generates this distribution could involve spatial correlations. Spatial statistics are usually applied at lower resolution, such as the context of this study. Spatial statistics now differentiate between spatially related data which is only the by-product of some system behavior from cases where clustering is an intrinsic function - or to cluster is part of an identity or a survival strategy.

Within the purposes of this study, since spatial autocorrelation is a well-known potential property of among observations across geographic space, one of our investigative goals was to explore if there were any spatial dependencies within the data, to test if there was any spatial clustering within the mosquito genera and to detect if unobserved spatial relationships within the sample of mosquito populations existed.

Although there was no obvious reason for us to believe spatial dependencies existed, we believed it was a worthwhile statistical exercise. To our knowledge, there are no studies that have attempted to investigate if there are spatial dependencies with regard to habitat selection within mosquito populations at the genus level. However, we believe that should there spatial relationships exist across genera, this would informative implications on how mosquito populations distribute and also help inform investigations into the unobserved spatial relationships (if any) between mosquito populations and the observed features of ecosystems.

Here, then, our goals were to test for existence of spatial interactions within the sample of mosquitos within around the lakes Bogoria and Nakuru. The idea was to test, at each lake, if mosquitos of the genus were spatially correlated. Two most widely applied spatial autocorrelation coefficients are Moran I and Geary's C statistic(Geary, 1954; Moran, 1948). Here, we employed Moran's I statistic. Moran's I (like Geary's C) measures the degree of spatial association as reflected in the data set as a whole. A significant Moran I would imply that similar values for a genus, are more likely to occur around each other, providing evidence of spatial clusters by genus. Moran's I is calculated using the formula:

$$I = \frac{N}{\sum_i \sum_j w_{ij}} \cdot \frac{\sum_{ij}^{N} w_{ij}(x_i - \bar{x})(x_j - \bar{x})}{\sum_{i=1}^{N}(x_i - \bar{x})^2}$$

Moran's values can vary from 1 to -1, indicating strong positive spatial correlation (1) and strong negative spatial correlation (-1). Similar spatial test were conducted at the species level. However, at both the genus and species level there was little evidence to suggest spatial clustering within the data. This, however, does not negate the possibility of spatial relationships between as mosquitos interact with the ecosystems of the lake regions. To confirm this, however, would require data on the ecology of the lake regions.

Conclusions

In conclusion, how the composition, functional diversity, and species richness of mosquito populations affect the biological processes of ecosystems within the lakes Bogoria and Nakuru, particularly as with regard to effect of mosquitos as disease vectors. In order to estimate the degree to which the aforementioned influence flamingo populations, or other avian or non-avian animal populations requires data about the ecology and built environment around the lakes, as well as the disease data of the mosquitos around collected at the sites, in order to sow and integrated model that would offer more reliable inference about the impact of mosquitoes as disease vectors within the ecosystem around the lakes Bogoria and Nakuru.

Potential Further Works

Although the major drawback of this study was the technical and governmental barriers that limited our ability to transport samples across seas to the continental United States, the information gathered from this study was enough to spark further interest into mosquito populations around the lake regions.

The three of the major points of interest that we have gathered through this study are the need to analyze: mosquitos as disease vectors in general within these lake regions, mosquitoes as disease vectors to flamingo populations within these lake regions and how mosquito distributions – in terms of numbers and species – are affected by the ecological attributes of the lake regions; we believe there is significant scientific merit to examine these dimensions further. Moreover, the data collection needs that would be required to implement these studies are similar across these three study agendas. Furthermore, in this study we used CO_2 baited EVS traps which are broader and attract a wider variety of mosquito species, it would be advisable to next time cast a wider net and set more of different kinds of mosquito traps.

The first challenge toward gaining an understanding of mosquitoes as disease vectors would be to screen a sample of mosquitoes from these lakes regions for disease. Recall, that there is currently no scientific consensus on the reasons of mass flamingo deaths. Disease is a certain possibility, and has been hypothesized as a culprit, however most hypotheses in this regard have failed to identify and describe the nature (and thus source) of disease. Mosquitoes, thus, cannot be ruled out as a potential candidate of a disease vector affecting mosquito populations. Here, blood samples from flamingoes would be needed in order to study the samples for disease prevalence. With general baseline knowledge of the prevalence and nature of disease found in samples of mosquitoes as well as in blood samples from flamingoes, one can start to answer the question if mosquitoes are potentially acting vectors to diseases found in flamingo blood. Notably, whether those diseases lead to death is a different question, however the first step toward approximate such a conjecture would be to accomplish the aforementioned.

This study provides proof of the diversity in mosquito species and the baseline difference in mosquito population distributions across these lake regions. One of the reasonable, immediate and logical next steps would be to gather basic data about the features of the lake regions in order to examine how the surrounding ecological systems influence mosquito populations. Although one would not be able to collect all the information about the ecosystems surrounding the lakes, guided by theory and scientific knowledge, a researcher could select relevant ecological attributes in order to analyze marginal correlations between ecological features and mosquito populations; and further investigate of spatial relations therein. The methods can be extended to not only include ecological features but also marginal effects of the built environment as well animal and wildlife populations on mosquito distributions.

One can then merge the expectations of how the ecological features and/or built environment and/or animal and wildlife distribution affects potential mosquito distributions with the knowledge on the workings of mosquitoes as disease vectors. This would form a rich information base.

With a little more data collection on factors related to mosquito populations would result in valuable inferences; the possibilities are exciting and would be highly informative across various disciples.

Lake Nakuru

Lake Bogoria

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Appendix: Charts

Chart 1:Mosquito Genus By Sample Location, Lake Nakuru

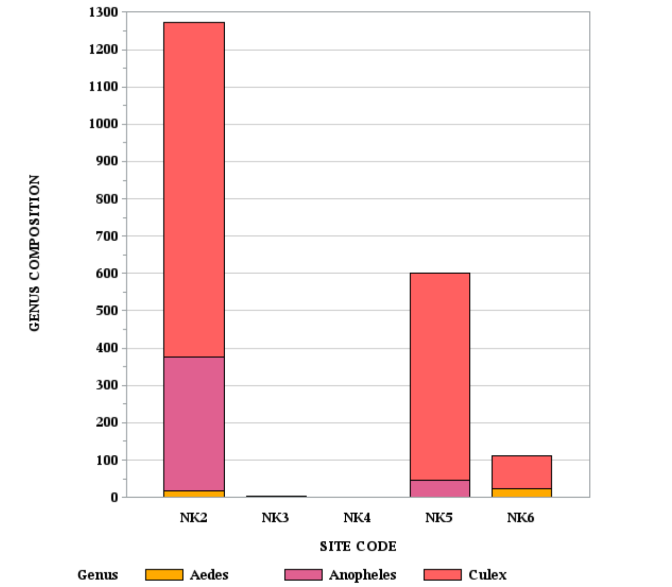

Chart 2:Mosquito Genus By Sample Location, Lake Bogoria