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The association between distance to water pipes and water bodies positive for anopheline mosquitoes (Diptera: Culicidae) in the urban community of Malindi, Kenya

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ABSTRACT: The increasing risk of mosquito-borne diseases in African urban environments has been partly attributed to failed planning and resource underdevelopment. Though engineered systems may reduce mosquito proliferation, there are few studies describing this relationship. This study investigates how engineered systems such as roads and piped water systems affect the odds of anopheline immatures (i.e., larvae and pupae) occurring in water bodies located in Malindi, Kenya. *Anopheles gambiae* s.s. (Giles), *An. arabiensis* (Patton), and *An. merus* (Dointz) were identified in urban Malindi, with *Anopheles gambiae* s.s. being the predominant species identified. The Breslow-Day test was used to explore interactions among independent variables. Logistic regression was used to test whether water bodies positive for anopheline immatures are associated with engineered systems, while controlling for potential confounding and interaction effects associated with urban water body characteristics. Water bodies more than 100 m from water pipes were 13 times more likely to have anopheline immatures present, compared to water bodies that were less than 100 m from water pipes (OR = 13.54, 95% CI: 3.15 – 58.23). Roads were not significantly associated with water bodies positive for anopheline immatures. Statistical interaction was detected between water body substrate type and distance to water pipes. This study provides insight into how water pipes influence the distribution of water bodies positive with immature anophelines in urban environments. ***Journal of Vector Ecology*** **32 (2): 319-327. 2007.**

Keyword Index: Anopheles gambiae s.l. immatures, piped water systems, roads, urban environment, Malindi, Kenya.

INTRODUCTION

Nearly half of the world's population lives in urban areas, and the urban annual population growth rate is expected to remain around 2% through 2030 (United Nations 2003). As cities become larger, there is a need for more efficient planning and management of urban population growth and basic infrastructure development to maintain and improve environmental and public health systems. Given that processes such as deforestation, subsistence farming and ranching, human migration, water delivery and sanitation projects, and road development projects can contribute to the propagation of vectors and pathogen transmission through the creation of favorable habitats (Patz et al. 2000), it is important that we continue to explore the human ecological and environmental dynamics of malaria vector propagation in urban areas.

Though the heaviest burden of malaria falls disproportionately on poor residents in rural environments of sub-Saharan Africa (SSA) (Hay et al. 2005), malaria is also a significant problem for urban residents in SSA (Keiser et al. 2004, Robert et al. 2003). The three major malaria vectors in SSA are *Anopheles gambiae* sensu stricto and *An. arabiensis*, belonging to the *An. gambiae* complex (sensu lato), and *An. funestus*. Though the main habitats of these mosquitoes are found in rural environments, an increasing number of studies suggest that these mosquitoes are adapting to urban environments (Coene 1993, Khaemba et al. 1994, Knudsen and Slooff 1992, Robert et al. 1998, Sattler et al. 2005, Trape and Zoulani 1987). Alternatively, the breakdown of urban infrastructure and poor planning of urban environments may be providing adequate conditions for these malaria vectors to propagate (Knudsen and Slooff 1992).

Increasing emphasis has been put on the utilization of Integrated Vector Management (IVM) on a global scale (WHO 2004) to control mosquito-borne diseases. IVM involves not only the utilization of all appropriate entomological interventions such as chemical and biological insecticides or insecticide treated nets (ITN), but also the implementation of sound land planning and development strategies that contribute to improved public health (Loch and Howard 1994, WHO 1983). The importance of studying the effects of engineered systems in urban environments is to try to understand their capacity to interrupt mosquitoes proliferation by limiting water storage and generally making urban areas less conducive for mosquitoes to proliferate (WHO 1980). However, the lack of access, planning, and/or maintenance of engineered systems may provide opportunities for mosquitoes to proliferate (Loch and Howard 1994). For example, in Kisumu, Kenya, it was reported that residents who did not have access to a reliable water source broke into the local piped water system to acquire water, which caused temporary pools to form that were subsequently colonized by *Anopheles gambiae* s.l. mosquitoes (Keating et al. 2003).

The relationship between mosquito larval habitats and engineered networks such as roads (Dutta et al. 1998, Ebsworth et al. 2001, Mazine et al. 1996), piped water systems (Barrera et al. 1993), underground sewer systems (Batra et al. 1995, Byrne and Nichols 1999, Chernin 1987, Strickman and Lang 1986), and above-ground drainage systems (Dua et al. 2000) have been studied in various urban sites with mosquito species known to prefer urban environments. A few studies have investigated the relationships between urban infrastructure and anopheline larval habitats in SSA. In Cameroon, for example, it was shown that while a *Pistia*-based waste stabilization pond supported high *Mansonia* and *Culex* mosquito populations, low densities of *An. gambiae* larvae were found in the ponds (Kengne Noumsi et al. 2005). The study concluded that *Pistia*-based treatment plants are probably not favorable to *An. gambiae*. In the urban highlands of Kenya, it was shown that there was more *An. gambiae* breeding near dams than in swamps (Khaemba et al. 1994). It has been suggested that dams may attract both humans and mosquitoes as a result of the year-round supply of water (Ripert and Raccurt 1987). These two examples highlight the potential impact of engineered infrastructure on the occurrence of anopheline immatures (i.e., larvae and pupae) in water bodies.

In urban SSA, environmental characteristics, such as land use, shade, substrate, and topography, may also be interacting with engineered systems to affect the occurrence and abundance of anopheline larvae in water bodies. Land use has been demonstrated to influence *Anopheles gambiae* populations in both rural and urban communities in SSA. In the western rural highlands of Kenya, it has been shown that agricultural land tended to be more conducive for mosquito growth than uncultivated land (Munga et al. 2006). Urban agriculture has been studied in Kumasi, Ghana and Malindi, Kenya with contradictory results. The study in Ghana showed urban agriculture was associated with increased numbers of *Anopheles* mosquitoes (Afrane et al. 2004), while the study in Kenya was not able to detect relationships between water bodies and agricultural land use at the household level (Keating et al. 2004). Shade has been shown to be associated with vegetation and houses (Foley et al. 2002, Schultz 1989). Negative association between the presence of shade and *Anopheles gambiae* has also been demonstrated: habitats that are shaded are less likely to have mosquitoes than habitats that are not shaded (Jacob et al. 2005, Munga et al. 2006). Differences in anopheline larval habitat substrate type have also been found to influence mosquito abundance in both rural and urban environments (Jacob et al. 2005, Minakawa et al. 1999), and drainage has been suggested to affect the abundance of *Anopheles gambiae* in rural areas (Klinkenberg et al. 2003, Shililu et al. 2003). In urban Malindi, Kenya, no difference was found between well-drained and poorly-drained areas (Keating et al. 2004). While each variable mentioned above impacts mosquito ecology in different ways, it is not clear how these environmental variables interact with engineered systems to influence the occurrence of anopheline immatures in urban water bodies.

In this study, we use ecological and geographic data collected in 2001 and 2002 (Keating et al. 2003, Keating et al. 2004), to determine whether the distance to water pipes and the distance to roads are significantly associated with the proportion of water bodies positive for anopheline immatures in urban Malindi, Kenya, while controlling for survey year and season, land use, drainage, shade, substrate, and interaction effects associated with urban water body characteristics. The hypothesis we tested was that as distance to engineered systems such as roads and water pipes increases, the likelihood that a water body contains anopheline immatures increases.

MATERIALS AND METHODS

Description of study area

This study was conducted in the coastal town of Malindi, located 108 km north of Mombasa, Kenya. Malindi has been described in previously published literature (Keating et al. 2003, Keating et al. 2004, Macintyre et al. 2002). Coastal Kenya has two distinct wet seasons, April to June (long rains) and October to November (short rains), with the climatic condition being classified as semi-arid with hot and humid weather most of the year.

Engineered systems that can be found in Malindi are paved roads, located in the city center and along the beach (other roads consist of a mixture of sand and dirt), and a piped water system, which runs through much of the city. Other engineered systems are covered and uncovered engineered storm water drainage systems that run along the main streets of Malindi town.

Anopheline immatures identified in water bodies in Malindi consisted of: *Anopheles gambiae* s.s. (Giles), *An. arabiensis* (Patton), and *An. merus* (Dointz) (Mbogo, unpublished data). Culicine immatures identified in water bodies in Malindi consisted of: *Culex quinquefasciatus* (Say), *Cx. simpsoni* (Theobald), *Cx. decens* (Theobald), *Cx. tigripes* (DeGrandpré and DeCharmony), and *Aedes aegypti* (Linnaeus) (Mbogo, unpublished); this study focused on anophelines only.

Sample frame development

The development of the geographic sample frame used in this study has been described elsewhere (Keating et al. 2003, Keating et al. 2004, Macintyre et al. 2002). Briefly, base-maps for Malindi were prepared using ArcView 3.2® (Environmental Systems Research Institute, Redlands, CA). ArcView 3.2® was then used to overlay a series of 270 × 270 m grid cells, corresponding to a 9 × 9 pixel LANDSAT Thematic Mapper remote-sensing satellite image, on a map of Malindi. The grid cells served as the sample frame, where random sampling was used to select individual grid cells (clusters) for data collection. The total number of grid cells (n = 42 grid cells) selected in this study was predetermined from studies conducted in 2001 and 2002.

Entomological sampling and environmental attributes

All accessible water bodies with and without mosquitoes were sampled within each selected grid cell during April and May of 2001 (a characteristically wet period) and during November and December 2002 (an uncharacteristically dry period) (Keating et al. 2003, Keating et al. 2004). This was done to avoid bias from sampling only water bodies where mosquitoes would likely be found. Standard methods were used to sample and identify the various species of mosquito immatures present in each water body found within each selected grid cell (Keating et al. 2003, Service 1976). The mean and standard deviation of sampling dips taken per water body was 15 dips ± 15 (range = 2 – 85 dips). The number of dips taken was dependent on the water body size. Data on water body type and environmental attributes of the surrounding area such as drainage and land use were recorded at each water body. Data from water bodies identified in 2001 and 2002 were pooled. All water bodies sampled from the selected grid cells were included in the data analysis described below. In 2001, all paved and unpaved roads in the area were mapped and entered into a GIS. In 2005, a map of Malindi's piped water system in place during 2001 and 2002 (1:2500) was acquired, digitized, georeferenced, and entered into the GIS. Using ArcView 3.3®, we calculated the shortest distance of all sampled water bodies to the nearest water pipe, nearest paved road, and nearest unpaved road. These three variables were dichotomized to equal 0 if the distance value was below the median and 1 if the distance value was above the median. We did not map out the drainage systems in Malindi nor were we able to obtain a map of the covered and uncovered storm water drainage system; however, we did determine the level of drainage within each grid cell based on the presence of functional drainage system and topographic features.

Statistical analysis

Our hypothesis was that increasing distance away from engineered water and road systems increases the odds of a water body having anopheline immatures. A total of eight variables were selected for analysis: three engineered systems and five water body characteristics (Table 1). Predominant land use in the grid cell was defined as cultivated/undeveloped land (i.e., farmed or open space) or built-up land (i.e., industrial, commercial, or residential space). Water body substrate type was defined as water bodies contained within sand, mud, or rock/gravel (i.e., natural substrates) or water bodies contained within rubber, concrete, or plastic (i.e., artificial substrates). For drainage, well-drained areas vs poorly-drained areas were determined by the presence or absence of functional engineered drainage systems and topographic features within each grid cell.

The Mann-Whitney U test (Glantz 2002) was used to identify significant differences in the distance to engineered systems between water bodies positive for anopheline immatures and water bodies negative for anopheline immatures.

Chi-square statistics were used to identify significant differences in the proportion of water bodies positive for anopheline immatures and water bodies negative for anopheline immatures, by categories of the independent variables. The Breslow-Day test was conducted to test the homogeneity of odds ratios for different strata and to identify statistical interaction between water bodies positive for anopheline immature and all two-way combinations of distance to water pipes and water body characteristics (Lachin 2000). This was done to determine if the association between presence of anopheline immatures and a second variable would be modified in the presence of a third variable, thus guiding the development of the subsequent logistic regression model (Hennekens and Mayrent 1987, Jaccard and Turrisi 2003).

A logistic regression was performed with the presence or absence of anopheline immatures in water bodies as the outcome variable and the engineered and water body characteristics as the covariates. The Breslow-Day test mentioned above was used to select the interaction terms to be used in the logistic regression. All analyses were done using Microsoft Excel ® and SPSS 11.5 (Chicago, IL).

RESULTS

Within the 42 grid cells sampled, a total of 115 water bodies was identified and sampled for mosquito immatures in 2001 (n=86) and 2002 (n=29) survey years combined. Anopheline immatures were found in a variety of water body types: car-track pools, drainage channels, a flower garden, a fountain, ponds, stream pools, swamps, swimming pools, tires, water tanks, a water trough, and a well (Jacob et al.

Table 1. Summary of entomological, environmental, and engineered systems variables used in multivariable logistic regression.

Variables	Classification (n) [a]			
Entomological Variable				
Water bodies positive for anopheline immatures [b]	Anopheline (-)	(77)	Anopheline (+)	(38)
Engineered Systems Variables [c]				
Distance to piped water system	<100 m	(58)	>100 m	(57)
Distance to paved roads	<200 m	(58)	>200 m	(57)
Distance to unpaved roads	<30 m	(58)	>30 m	(57)
Environmental Variables				
Survey year and season	2001-wet period	(86)	2002-dry period	(29)
Drainage [d]	Well-drained areas	(39)	Poorly-drained areas	(76)
Land-use [e]	Cultivated/ Undeveloped land	(28)	Built-up land	(87)
Shaded water bodies [b]	Shade (-)	(52)	Shade (+)	(63)
Water body substrate type [f]	Natural	(63)	Artificial	(52)

[a] A total of 115 water bodies were sampled between 2001 (a wet period) and 2002 (a dry period); n = the frequency of water bodies with classification.

[b] (-) = absence of characteristic, (+) = presence of characteristic.

[c] The three engineered systems variables were classified into the distance value below and above the median value.

[d] Well-drained areas = grid cells with functional engineered drainage systems and/or topography that allows drainage; Poorly-drained areas = grid cells without functional engineered drainage systems and topography that allow drainage.

[e] Cultivated/Undeveloped land = farmed or open space; Built-up land = Industrial/Commercial/Residential space.

[f] Natural substrate type = water bodies contained within sand, mud, or rock/gravel; Artificial substrate type = water bodies contained within rubber, concrete, or plastic.

Table 2. Summary of 2 × 2 table testing the association between water bodies positive for immature anophelines and distance to water pipes while controlling for water body substrate type.[a]

Substrate type [b]	Distance to water pipes	Anopheline		Odds Ratio (OR)	95% CI for OR
		Present (cases)	Absent (references)		
Natural	>100 m	17	13	13.08	3.26 – 52.46
	<100 m*	3	30		
Artificial	>100 m	10	17	1.25	0.40 – 3.94
	<100 m*	8	17		
Total	>100 m	27	30	3.84	1.66 – 8.88
	<100 m*	11	47		

[a] The Breslow-Day test was done to determine homogeneity between odds ratios in different strata (i.e. substrate type). Odds ratio between natural substrate type and artificial substrate type are significantly different: χ^2=6.90, df=1, *p*=0.009.

[b] Natural substrate type: Mud, Sand, Gravel/Rock; Artificial substrate type: Concrete, Rubber, Plastic.

* Reference category.

Table 3. Multivariable logistic regression testing the association between water bodies positive for anopheline immatures and engineered systems while controlling for urban factors.

Variables	Odds ratio (OR)	95% CI for OR	
		Lower	Upper
Distance to water pipes (DWP)			
>100 m	13.54	3.15	58.23
<100 m*			
Distance to paved roads			
>200 m	1.14	0.41	3.18
<200 m*			
Distance to unpaved roads			
>30 m	1.06	0.31	3.54
<30 m*			
Survey year and season			
2002-dry period	0.47	0.12	1.92
2001-wet period *			
Drainage [a]			
Well-drained areas	0.78	0.20	2.97
Poorly-drained areas*			
Land-use [b]			
Built-up land	0.59	0.20	1.985
Cultivated/Undeveloped *			
Shade			
Shade	0.50	0.19	1.82
No Shade *			
Water body substrate type (SUB) [c]			
Artificial	8.94	1.63	49.01
Natural *			
DWP by SUB	0.11	0.01	0.73

* Reference group.

[a] Well-drained areas = grid cells with functional engineered drainage systems and/or topography that allows drainage; Poorly-drained areas = grid cells without functional engineered drainage systems and topography that allow drainage.

[b] Cultivated/Undeveloped land = farmed or open space; Built-up land = Industrial/Commercial/Residential space .

[c] Natural substrate type = Mud, Sand, Gravel/Rock; Artificial substrate type = Concrete, Rubber, Plastic.

2003, Keating et al. 2004). Other water body types found in Malindi included septic tanks and pit latrines; culicines were found in these water bodies but not anophelines. In the two survey years, 889 anopheline immatures were collected. More than 95.0% were identified by polymerase chain reaction (PCR) (Scott et al. 1993) as *An. gambiae* s.s., while 1.1% were identified as *An. arabiensis*, 0.3% were identified as *An. merus,* and 3.6% were unidentifiable (Mbogo, unpublished data).

Water bodies positive for anopheline immatures had a significantly greater mean and median distance to the nearest water pipe than water bodies negative for anopheline immatures (Mann-Whitney U test: Z = -3.119, p = 0.002). The mean and median distance from water bodies positive and negative for anopheline immatures to paved roads were similar (Mann-Whitney U test: Z = -1.442, p = 0.149), as were the distances to unpaved roads (Mann-Whitney U test: Z = -0.312, p = 0.755).

Chi-square analyses showed that the proportion of water bodies positive for anopheline immatures was significantly different by distance to water pipes ($\chi^2 = 10.48$, p = 0.001) but not the other variables. Results of the 2 × 2 table and Breslow-Day test are reported in Table 2. Results revealed one significant statistical interaction: distance to water pipes by water body substrate type ($\chi^2 = 6.90$, df = 1, p = 0.009). In water bodies that were of a natural substrate type, the proportion of water bodies positive for anopheline immatures was significantly different by distance to water pipes. Conversely, in water bodies that were artificial substrate type, the proportion of water bodies positive for anopheline immatures was not statistically different by distance to water pipes.

Only distance to water pipes, water body substrate type, and the interaction between water body substrate type and distance to water pipes were significant predictors of the presence of anopheline immatures in urban water bodies. Paved and unpaved road variables were not significant. Results of logistic regression are reported in Table 3. We also conducted a sensitivity analysis to determine whether changes in median distance to engineered systems would change our results. The median distance to engineered systems was increased and decreased by 50%. In each case, we found consistent results as found in the use of the median distance to engineered systems (data not shown).

Since statistical interaction was identified between substrates and distance to water pipes in our analysis, we also conducted an additional two separate logistic regressions for water bodies that were natural substrate type, and water bodies that were artificial substrate types to examine the direction and magnitude of each substrate type. In the natural water body substrate analysis, there was a significant positive association between water bodies positive for anopheline immatures and distance to the water pipes, where natural substrate type water bodies that were >100 m from the water pipes were 12 times (OR=12.34, 95% CI: 2.72 – 56.07) more likely to have anopheline immatures compared to water bodies that were <100 m from the water pipes. Neither paved roads or unpaved roads, nor the other water body characteristics were significant in this analysis. In the artificial water body substrate analysis, none of the variables were significant.

DISCUSSION

This study demonstrates that engineered systems may influence the occurrence of water bodies positive for anopheline immatures in urban environments. Water bodies that were greater than 100 m from the water pipes had higher odds of having anopheline larvae present, compared to water bodies that were less than 100 m from the water pipes. This finding demonstrates that water pipes could play a significant role in preventing anopheline mosquitoes from colonizing urban water bodies. Increased access to a reliable piped water system for urban residents has been suggested as a means of reducing the mosquito burden (WHO 1987). One mechanism by which a reliable piped water system may reduce anopheline propagation is through the modification of human behavior around water sources. In SSA for example, urban wells are a major source for propagation of mosquitoes (Awono-Ambene and Robert 1999, el Shazly et al. 1998, Fletcher et al. 1992 , Robert et al. 1998). Wells serve as good meeting places for humans and mosquitoes because of the abundance of water and human activity occurring near wells. In this example, increased access to piped water systems may reduce the need to use wells. However, this was irrelevant in Malindi because in our study, we only found one well during our sampling; however, the well was found to be colonized with an abundance of anopheline larvae and pupae. The presence of a piped water system may also relate to other factors that limit mosquito proliferation. For example, piped water systems are more likely to be concentrated in city centers. Anopheline density has been shown to increase as distance from the city center increases (Coene 1993, Keating et al. 2004, Robert et al. 1993, Trape and Zoulani 1987). This observation has been attributed to decreased open space, increased pollution, different economic activities, and improved socioeconomic conditions.

In this study, statistical interaction was found between water body substrate type and distance to piped water system. We found that natural water bodies located >100 m from water pipes were more likely to be positive for anopheline immatures compared to water bodies located <100 m from water pipes. In the artificial substrate water bodies, we found no difference in the proportion of water bodies positive for immatures by distance to water pipes. This distinction in the presence of different substrate types may be attributed to the stability of the water body for different substrate types. Water body stability, defined as the probability of water occurrence in a water body, is associated with the occurrence of both culicines and anophelines (Minakawa et al. 2005, Mutuku et al. 2006, Sota et al. 1994). Permanency is often used as a surrogate for water body stability. We found that natural water bodies were more likely to be temporary pools of water compared to permanent pools of water. Given that piped water systems are more likely to be concentrated in the city center, it may be that the modified differences between water bodies positive for anopheline immatures and distance to piped water systems by water body substrate type is a function of water body stability where the farther the distance to the piped water system (or to the city center), the higher the stability of the water body. This higher stability leads to a greater probability of occurrence of the anopheline immatures in the water body. Conversely, greater human activity around water pipes may negatively affect the stability of neighboring temporary water bodies, which subsequently leads to reduced probability of occurrence of anopheline immatures. We also found that artificial water bodies were more likely to be permanent pools than temporary pools (data not shown); hence, distance to the piped water systems (or to the city center) probably had less of an effect on the water stability.

Resource development projects that focus on increasing access to piped water may reduce the abundance of mosquitoes. This may provide an added benefit to chemical or biological control approaches used in IVM programs. This study lends insight into how engineered systems such as piped water systems may be influencing anopheline proliferation in urban environments. By refining our understanding of the mechanisms by which anopheline propagate in urban environments, additional tools for reducing mosquito populations can be developed and implemented.

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