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Characterization of potential larval habitats for *Anopheles* mosquitoes in relation to urban land-use in Malindi, Kenya

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Published: 04 May 2004

Received: 11 March 2004

International Journal of Health Geographics 2004, **3**:9

Accepted: 04 May 2004

This article is available from: <http://www.ij-healthgeographics.com/content/3/1/9>

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Abstract

Background: This study characterized *Anopheles* mosquito larval habitats in relation to ecological attributes about the habitat and community-level drainage potential, and investigated whether agricultural activities within or around urban households increased the probability of water body occurrence. Malindi, a city on the coast of Kenya, was mapped using global positioning system (GPS) technology, and a geographic information system (GIS) was used to overlay a measured grid, which served as a sampling frame. Grid cells were stratified according to the level of drainage in the area, and 50 cells were randomly selected for the study. Cross-sectional household and entomological surveys were conducted during November and December 2002 within the 50 grid cells. Chi-square analysis was used to test whether water bodies differed fundamentally between well and poorly drained areas, and multi-level logistic regression was used to test whether household-level agricultural activity increased the probability of water body occurrence in the grid cell.

Results: Interviews were conducted with one adult in 629 households. A total of 29 water bodies were identified within the sampled areas. This study found that characteristics of water bodies were fundamentally the same in well and poorly drained areas. This study also demonstrated that household-level urban agriculture was not associated with the occurrence of water bodies in the grid cell, after controlling for potential confounders associated with distance to the city center, drainage, access to resources, and population density.

Conclusions: Household-level urban agricultural activity may be less important than the other types of human perturbation in terms of mosquito larval habitat creation. The fact that many larvae were coming from few sites, and few sites in general were found under relatively dry conditions suggests that mosquito habitat reduction is a reasonable and attainable goal in Malindi.

Background

Malaria parasite transmission remains a serious international public health problem. In sub-Saharan Africa

(SSA), *Anopheles gambiae* s.s., *An. arabiensis*, and *An. funestus* are the primary vectors of malaria parasites and show highly anthropophilic tendencies [1-3]. Approximately

70% of the total SSA population lives in areas infested with malaria vectors, although urban areas typically have lower anopheline mosquito populations and less malaria as compared to rural areas [4]. It is generally thought that the abundance of clean, sun-lit, and shallow bodies of water makes rural populations especially vulnerable to increased contact with anopheline mosquitoes. Likewise, the absence of suitable habitat and increased water pollution generally inhibits the development of anopheline larvae in urban centers, resulting in fewer *Anopheles* mosquitoes [5,6]. Other studies on the epidemiology of urban malaria have demonstrated that the density of anophelines, and corresponding malaria parasite transmission, increases as the distance to the city center increases, with rural areas being more likely to harbor *Anopheles* mosquitoes as compared to urban areas [7-13]. These studies further concluded that city centers were significantly less likely to harbor mosquitoes as a result of decreased open space, increased pollution, different economic activities, and improved socioeconomic conditions. To what extent this is unequivocally true, or changing, is unclear, as many urban SSA populations live in overcrowded and denuded areas without access to safe water and sanitation services, paved roads, adequate housing, or engineered drainage systems [14], and continue to engage in rural-type activities to sustain an urban existence.

Commercial and household-level urban farming represents one rural-type activity that is both abundant in many SSA cities, and has been associated with anopheline mosquitoes [15-17]. On the coast of Kenya, urban residents often supplement their diet and income with produce grown on small patches of cultivated land around the household, and shallow garden wells are a common source of water for urban residents engaged in farming, or who are without access to piped water. Given that agricultural areas represent moist, disturbed environments that can sustain both larval-stage and adult mosquitoes [18], and water storage or irrigation is often necessary to sustain agricultural activity within the urban context, urban farming may be increasing the availability of potential habitat for *Anopheles* mosquitoes. Further, given that *An. gambiae* s.s. retract to relatively few water bodies in the absence of sustained precipitation, bite near the larval site of emergence [19,20], survive long dry periods with limited feeding activity and slower reproductive cycles [21], and disperse only so far as needed to acquire a blood meal [20], it stands to reason that populations in close proximity to mosquito habitat may be at an increased risk of receiving an infectious bite.

Other features of the SSA urban environment may also act to affect the overall propensity of an area to harbor *Anopheles* mosquitoes. High human population density for

example, provides additional blood meal opportunities, as well as additional habitat in the form of artificial water storage containers, debris, or human-made depressions that retain water. Conversely, in the presence of *Anopheles* mosquitoes, high human population density may act to reduce the overall chance of receiving an infectious bite, as an increase in the number of potential hosts may result in fewer overall bites per person. The built environment is likely to reduce the abundance of mosquitoes by reducing or eliminating standing water and vegetation, thus reducing potential habitat. Soil and vegetation are often replaced with asphalt, concrete, brick, or stone for housing, or commercial and industrial centers; and drainage systems are installed and enclosed to drain water. In general, the built environment decreases as the distance from the city center increases, and is accompanied with corresponding changes in community level land-use. Thus areas further away from the city center are less likely to have access to utilities, drainage systems and paved roads, and may be more likely to be supplement household incomes with rural-type activities. Distance to the city center may also be associated with community level socioeconomic status, as cities tend to expand outward with settlement occurring along the periphery where space permits. These newly formed communities may not have access to the resources needed to modify the environment in such a way as to reduce the overall threat of disease, or improve the respective infrastructures. Communities on the fringes of urban centers, often called peri-urban areas, represent the interface between rural and urban areas, where livelihoods are dependent upon on both natural resources and farming activities, and the economic and social opportunities found with urban areas.

The purpose of this research was to characterize larval habitats in Malindi, identify associations between the presence of anopheline larvae and human-ecological attributes, and describe the relationship between household-level urban agricultural activity and the occurrence of water bodies in the grid cell, while controlling for potential confounding effects associated with features of the urban environment. This paper builds upon previous ecological field studies conducted in Kisumu and Malindi, Kenya during 2001 [22,23]. A stratified geographic sampling strategy and cross-sectional study design provided the framework for this study.

Materials and methods

Description of study area

The city of Malindi, which is a major tourist destination as well as a significant local market town and small industrial center, is located on the Indian Ocean in Coast Province, Kenya and has a population of 81,000 people [24]. The climate is considered tropical (3°14'S latitude and 40°04'E longitude). April through June and October

through November are considered the long and short rains, respectively, with precipitation varying from 750 to over 1,200 mm per year.

Malindi comprises commercial, undeveloped, farmed, and residential areas. Although most roads in Malindi are a mixture of sand and dirt, the city center is paved and has both covered and uncovered engineered drainage systems along main streets. The main roads used for travel and transport in and out of the city, as well as some roads along the beach are also paved. Tourism, fishing, commercial trade and retail, and service professions are the major economic activities. Many residents also engage in small-scale farming for personal consumption and sale. Skilled labor accounts for less than 5% of the workforce in Malindi [24]. The informal economic sector comprises street vendors, sex workers, and tour guide services. According to the latest census, approximately 60% of the urban population has access to piped water, either in the home or at community taps.

Malaria parasite transmission occurs year-round on the coast of Kenya, although the incidence usually increases shortly after the onset of the rains [25,26]. No malaria prevalence studies have been carried out within Malindi.

Sample frame development

Keating et al. (2003) described the sampling strategy as it was originally conceived and implemented [22]. Although the approach used in this study was fundamentally the same, changes to the design were made and are described herein. An existing base-map for Malindi was updated in September of 2002 using ArcView 3.3[®] and Garmin E-Trex[®] GPS receivers, to include additional landmarks and points of reference throughout the study area. Latitude, longitude, and elevation data were collected for roads and reference points, and information relative to land-use type and the level of drainage were noted. The information contained in the 1999 census, existing town maps, and environmental descriptions from the field formed the basis for urban boundary demarcation in this study. ArcView 3.2[®] was used in 2001 to generate and overlay a series of 270 meter × 270 meter grid cells on the base-map [22]. The grid created in 2001 was also used in this study because data collection teams were already familiar with the boundaries of the existing grid cell locations relative to important landmarks. Two hundred and forty eight grid cells fell within the urban study area, which constituted the sampling frame for this study.

Stratifying Malindi into two categories involved assessing the level of drainage in each grid cell and assigning a value of 1 if the grid cell was well drained and 0 if poorly drained. A grid cell was classified as well-drained if functional (e.g. clear of debris or vegetation at the time of

observation) engineered drainage systems covered the majority of the grid cell and no standing water was visible, or if the grid cell was located on a slope and no standing water was visible. A grid cell was classified as poorly drained if it was located in a depression or valley and had either no drainage systems, or the drainage systems were blocked with debris or vegetation. Topographical and town maps were used to assist with this process. Although the same person characterized all grid cells in this study, no algorithm or coding system was used to standardize the process. Seventy-three grid cells were classified as well drained and 175 grid cells were classified as poorly drained. Twenty-five grid cells were selected from each stratum ($n = 50$). A systematic random sample with a random start was used to select grid cells. This insured that the probability of selection was equal for each grid cell within the respective strata. The probability of selection was equal to 0.1429 (25/175) for the poorly drained stratum and 0.3424 (25/73) for the well-drained stratum. These numbers were used in the calculation of sampling weights. Figure 1 illustrates the randomly selected grid cells used in this study. The number of grid cells selected was a function of time and logistic feasibility. The boundaries of selected grid cells were located in the field using hand-held navigational units (GPS), a compass, and base-maps with landmarks, paths, and roads indicated. Latitude and longitude readings were taken at the corners and center of each selected grid cell to confirm the location and extent of grid cell boundaries.

Water body identification

Each selected grid cell was visually inspected for the presence of water bodies during November and December 2002. All accessible water bodies within the selected grid cells were identified to avoid the bias associated with sampling in areas most likely to contain anopheline larvae. In this analysis, multiple water-filled containers in close proximity (e.g. bucket or tire piles) were considered to be one aquatic habitat. Likewise, artificial water storage containers existing in isolation of other containers were considered to be one aquatic habitat.

Standard dipping methods were used to collect mosquito larvae at each water body [27]. Larvae were preserved and transported to the laboratory for further identification. Water bodies containing no larvae were revisited two weeks later to confirm the absence of mosquito larvae. Environmental and human-ecological information were recorded for each water body.

Household sampling

A two-stage cluster format was used to select households from within the 50 grid cells. The grid cells served as primary sampling units (PSU), or clusters, from which the ultimate sampling units (USU), or households, were

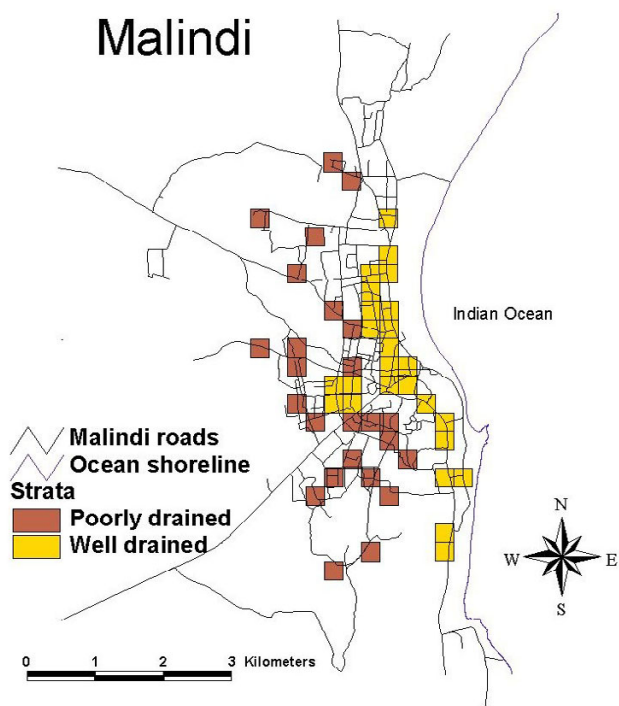


Figure 1
Randomly selected grid cells within Malindi by strata. All data collection occurred within the selected grid cells.

selected. Equal allocation, and a design effect of 3 [23], was used to calculate the target sample size. The most conservative estimate of p (0.50) was used in the sample size calculation. The alpha level was set at 95% ($\alpha = 1.96$). The maximum tolerable error was equal to 10%. The sample size formula was equal to: $n \geq 3(1.96)^2(0.50)(1 - 0.50) / (0.10)^2$. An additional 10% was added to account for non-response, yielding a target sample size of 318 households in the well-drained stratum and 318 households in the poorly drained stratum ($n = 636$). Because household level enumeration lists were not available for grid cells, a random direction method was used to approach approximately 13 houses ($318/25$) from within each selected grid cell. The middle of the grid cell was located and a random direction was selected for each interviewer. Interviewers traveled along their respective axis until a household respondent was identified. Additional houses were sampled along the same axis until the boundary of the grid cell was reached. At which time, a new direction from the center was selected and the process repeated until approximately 13 houses had been sampled. In grid cells containing fewer than 13 households, all responsive households were approached. Households selected, but

with no resident adult available were revisited once and then replaced with the closest house.

A questionnaire was developed and pre-tested in Malindi during October 2002. Households were defined as residential units with one or more individuals in occupation. Multiple families residing in the same house were considered one household. Multiple structures within a compound occupied by dependents of household head were considered one household. The total number of households per selected grid cell was obtained by counting the total number of occupied households contained within each selected grid cell.

Interviews were conducted with any resident adult (>15 years) willing to be interviewed. A brief explanation of the study was provided and informed consent obtained. Variables used in this analysis were created based on responses to specific questions related to agricultural practices, land-use, home ownership, and access to electricity. The Tulane University Institutional Review Board (IRB) and the Kenya National Ethical Review Board approved the study.

Data analysis

The first objective was to describe the water bodies identified and determine if the characteristics were fundamentally the same in well and poorly drained areas, and to identify human-ecological factors associated with the probability of anopheline larvae occurring in a water body. The variables used were based on field observations and information obtained at the time the water body was identified. Land-use was equal to 1 if the water body was located in a residential, or residential and commercial area, and equal to 0 if located in an agricultural or undeveloped area. Water body type was recorded as a description (e.g. ditch, pond). Water body size was equal to 1 if less than or equal to 3 meters squared, and 0 if not. Water body nature was dichotomized as natural (0) or human-made (1). The level of permanency was equal to 1 if the water body was permanent or semi-permanent (> 3 mo), and 0 if temporary (< 3 mo). Because this study used a cross-sectional approach, and water bodies could not be observed over time, permanency was determined based on the source of water, water body size, previous experience with specific habitat types, and expert opinion. Substrate type was classified as cement (1), mud or soil (2), or rubber or plastic (3). The substrate variable was equal to 1 if substrate was cement or plastic and 0 if mud or soil. No rubber substrates were observed. The distance to the nearest house variable was recorded as 1 if a water body was located < 20 meters from the nearest house, and 0 if > 20 meters. A 20-meter dichotomization criterion was used based on the distribution of the data, as many of houses were less than 20 meters from a water body, with very

little variation in values. Pollution and floating debris was recorded as 1 if debris were present or the water body was discolored or foul smelling, and 0 if absent. This distinction was also based on expert opinion, as no physiochemical analysis of water samples were conducted. The shade variable was equal to 1 if some canopy coverage was present, or if a structure provided shade, and 0 if no coverage was present.

Chi-square analysis was conducted to determine if the proportions of the respective independent variable categories for water bodies differed by strata ($n = 29$). Chi-square analysis was also conducted to determine if the proportions of water bodies positive for anopheline larvae differed by strata, and by the respective categories of the data described above ($n = 29$). Although the original intention was to test the direction and magnitude of the controlled effect of the respective covariates using logistic regression, the low number of water bodies identified, coupled with the low number of anopheline larvae collected and lack of variability in some habitat characteristics precluded further analysis on the distribution or abundance of anopheline larvae.

The second research objective was to quantify the effect of household-level farming on the probability of water body occurrence within the grid cell, while controlling for potential confounders. Chi-square analysis was first conducted to investigate whether grid cells differed by strata in terms of household level farming, the existence of water bodies, and access to resources ($n = 50$). The research hypothesis was that the abundance of farming within or around the household, as recorded on the household questionnaire, increases the probability that at least 1 potential anopheline larval habitat (water body) exists within the community. The density of houses, distance from the city center, the level of access to resources, and the level of drainage per grid cell were treated as potential confounders. The dependent variable was binary and equal to 1 if at least one water body was found within the grid cell, and 0 if no water bodies were found within the grid cell (PSU = 50, USU = 629).

In this analysis, the grid cell (cluster) served as a surrogate for a community. Although communities are rarely uniform in space or character, and spatial units may fail to capture physical or human-ecological heterogeneity at a smaller scale, this analysis assumes a specific level of homogeneity within the grid cell based on previous studies conducted within Malindi [22,23]. The proportion of households sampled per grid cell reporting house ownership plus access to electricity was used as a surrogate for the community's overall ability to access resources and thus control their own environment. The proportion of households reporting home ownership plus electricity for

each selected grid cell was calculated from the household survey. The variable was dichotomized to equal 1 if the grid cell value was above the overall mean and 0 if below the overall mean. The proportion of households engaged in farming in or around the household per grid cell was calculated in the same way, and further dichotomized to equal 1 if the grid cell value was above the overall mean and 0 if below. In all cases where household level survey data were used to create a grid cell level variable, the number of households satisfying each respective condition was divided by the total number of households sampled within each grid cell to obtain the respective proportion.

The number of households per grid cell was a continuous variable and served as a surrogate measure of population density per grid cell. The distance from the city center variable was created using ArcView 3.3[®]. The spatial join function was used to calculate and assign a distance from the centroid of each selected grid cell to a point designated as the city center. This study used a roundabout just south of the old commercial district as the center of town based on its centrality, urban features, and access. The variable was also continuous and served as a proximate determinant of a range of community level variables, including access to infrastructure and services, levels of pollution, community level land-use and the relative socioeconomic status of the area.

Multi-level logistic regression was used to quantify the controlled effect of household-level agricultural activity on the probability of water body occurrence. Sampling weights, equal to the inverse of the probability that a grid cell was selected, were applied to the regression. The grid cell, and corresponding data from households interviewed therein, was treated as a cluster using the "cluster" option in STATA for the regression. Robust standard errors were used because data were collected at both the grid cell and household level. An alpha level of 0.05 was used to indicate significance. Data management and analysis were done using STATA version 7. ArcView 3.3[®] was used to generate the maps in this study.

Results

The timing and near failure of the short rains were such that data were collected under unusually dry conditions. In 2002, 126 mm and 86.5 mm of precipitation was recorded for the months of November and December, respectively. Only 34% of the 50 grid cells contained water bodies during this study. Twenty-nine water bodies were identified from within 17 of the grid cells. Twenty-seven percent of the 29 water bodies identified were positive for anopheline larvae. Twenty-one percent of the water bodies had anophelines only, and 28% had culicines only. Seven percent had both anopheline and

Table 1: Distribution of mosquito larvae in water bodies identified within Malindi during November and December 2002.

	Habitats							
	# Habitats(%)	Swimming pool	Swamp	Container	Bathtub	Pond	Fountain	Ditch
Anopheline vs. culicine								
Presence of anopheline larvae only	6 (20.7)	4	1	1	0	0	0	0
Presence of culicine larvae only	8 (27.6)	2	0	3	1	0	0	2
Presence of both	2 (6.9)	1	0	0	0	0	1	0
Absence of both	13 (44.8)	12	0	0	0	1	0	0
Total	29 (100)	19	1	4	1	1	1	2

culicine larvae. Table 1 lists the distribution of anopheline larvae in relation to culicine larvae and habitat type. Fifty-five percent ($n = 110$) of all *Anopheles* larvae were collected from the well-drained grid cells. The number of anophelines per water body ranged from 0 to 30 in both strata, with 27% of the total number of larvae coming from one habitat in the poorly drained stratum. Of the 110-anopheline larvae collected, 101 were morphologically identified as *Anopheles gambiae* s.l.. The remaining nine larvae were unidentifiable due to rearing difficulty in the laboratory. Subsequent analysis of the 101 larvae by polymerase chain reaction (PCR) yielded all *An. gambiae* s.s.. A total of 826 culicine larvae were also collected from within the 29 water bodies, although not identified to species nor considered further in this analysis.

Ninety percent of the 29 water bodies identified were classified as human-made. Types of human-made water bodies in Malindi included drained or abandoned swimming pools, drainage channels, abandon bathtubs, roadside ditches, water tanks, and broken pipes. Natural habitats consisted of swamp and a pond. No natural water bodies were found in the well-drained stratum. Eighty percent of the habitats identified were classified as temporary habitats, while the remaining 20% were classified as permanent bodies of water. Fifty percent of all water bodies contained debris such as plastics, paper, aluminum, polypropylene gardening bags, and organic refuse. Two water bodies positive for anopheline larvae contained both raw sewage and decaying wood. Another contained decaying animal corpses. Ninety percent of the water bodies were located within 20 meters of a house. Animals were present at 40% of the water bodies. Most water bodies had cement or rock substrates (83%), although 3 (10%) had mud substrate and 2 (7%) were comprised of plastic. All 29 water bodies were found in either residential or commercial areas. Figure 2 illustrates the types of water bodies identified

during this study. Figure 2A,2C &2E illustrate the types of water bodies found in the well drained stratum, while figure 2B,2D &2F represent the types of water bodies identified in the poorly drained stratum. Although many of the selected grid cells contained various levels of both commercial and household farming activities, no water bodies were identified from within these areas.

Table 2 presents results from the first stage bivariate analysis. Although the proportion of water bodies classified as human-made versus natural, the proportion of water bodies with some shade versus no shade, and the proportion of water bodies located within 20 meters of a house were different by strata, insufficient variability in the distribution of data about the 2×2 table rendered the results difficult to interpret; thus, no test statistic was calculated. The proportion of water bodies with animals present versus without animals present was the only variable significantly different by strata, with water bodies in well-drained areas significantly less likely to have animals present, as compared to water bodies in the poorly drained stratum (O.R. = 0.13; 95% C.I. = 0.02, 0.86). This result is most likely due to the fact that animals typically eat grass, and grass tends to grow best in wet areas, which may be more abundant in poorly drained areas.

Table 3 presents results from a second first-stage bivariate analysis. The proportion of water bodies with anopheline larvae present was significantly different for water bodies with pollution versus without pollution only (O.R. = 14.0; 95% C.I. = 1.43,137.32). In this analysis, polluted water bodies were 14 times more likely to have anopheline larvae present as compared to water bodies with no pollution or floating debris, although the confidence interval is quite large. This finding was opposite what one would expect and was most likely due to the low number of water bodies identified. The proportion of water bodies

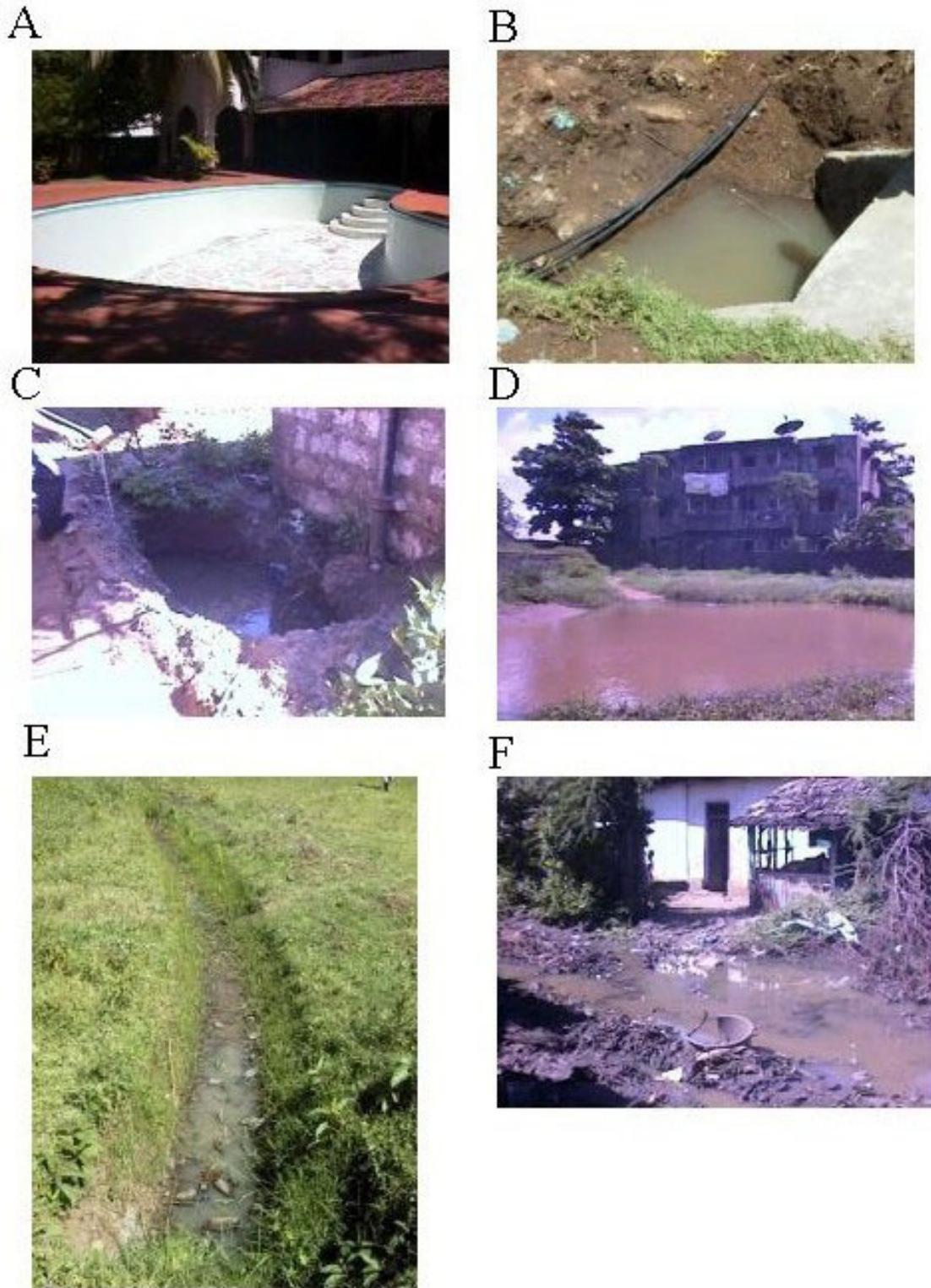


Figure 2

Pictures illustrating the types of habitat identified by strata during this study: (A) Swimming pool in well drained tourist area; (B) Broken water pipe in well drained residential area; (C) Open water tank in poorly drained area; (D) Pond in poorly drained area; (E) Drainage channel in well drained area; and (F) Ditch and tire tracks in poorly drained area.

Table 2: Bivariate results for proportions of water bodies located in well drained versus poorly drained grid cells by environmental variables collected about the water body (n = 29).

Variables	Well drained (n = 21)	Poorly drained (n = 8)	χ^2	P-value	O.R.	C.I.
Land-use (% residential or commercial)	20 (95.2)	6 (75.0)	0.055	0.814	6.7	0.51, 86.9
Size (% small habitats)	6 (28.6)	4 (50.0)	1.177	0.278	0.4	0.75, 2.14
Nature (% human-made)	20 (95.2)	6 (75.0)	**			
Shade (% some shade)	12 (57.1)	6 (75.0)	**			
Pollution (% polluted)	9 (42.9)	5 (62.5)	0.895	0.344	0.45	0.09, 2.4
Substrate (% cement or plastic)	16 (76.2)	6 (75.0)	0.004	0.947	1.07	0.16, 7.06
Permanency (% perm. or semi-perm.)	3 (14.3)	2 (25.0)	0.466	0.495	0.5	0.07, 3.75
Animals present (%)	6 (28.6)	6 (75.0)	5.148	0.023	0.13	0.02, 0.86
Nearest house (% < 20 meters)	21 (100)	5 (62.5)	**			

*d.f. = 1, **Insufficient variability in the data

Table 3: Bivariate results for proportions of water bodies with anopheline larvae versus no larvae by environmental variables collected about the water body (n = 29).

Variables	Anophelines present (n = 8)	Anophelines absent (n = 21)	χ^2	P-value	O.R.	C.I.
Land-use (% residential or commercial)	5 (62.5)	21 (100)	**			
Size (% small habitats)	3 (37.5)	7 (33.3)	0.045	0.83	1.2	0.22, 6.53
Nature (% human-made)	20 (95.2)	6 (75.0)	**			
Shade (% some shade)	5 (62.5)	13 (61.9)	0.01	0.98	1.03	0.19, 5.51
Pollution (% polluted)	7 (87.5)	7 (33.3)	6.8	0.01	14	1.43, 137.32
Substrate (% cement or plastic)	7 (87.5)	15 (71.4)	0.82	0.36	2.8	0.28, 27.91
Permanency (% perm. or semi-perm.)	0 (0.0)	5 (23.8)	**			
Animals present (%)	4 (50.0)	8 (38.1)	0.34	0.56	1.63	0.32, 8.4
Nearest house (% < 20 meters)	6 (75.0)	20 (95.2)	**			
Drainage (% well drained)	5 (62.5)	16 (76.2)	0.54	0.46	0.52	0.09, 3.0

*d.f. = 1 **Insufficient variability in the data

with anopheline larvae present versus absent was not significantly different for other variables measured. Further, calculating a meaningful chi-square statistic was not possible for 4 of the variables due to the lack of variability in the data about the 2 × 2 table.

Thirty-three grid cells contained no water bodies during the study period. The well-drained stratum contained 67% of the 29 water bodies identified, distributed across 11 grid cells, while the poorly drained stratum contained 33% of the total, distributed across 6 grid cells. All selected grid cells were within 3 km of the city center, while the average number of households per grid cell was equal to 110 (min. = 2; max. = 542). The average proportion of households harboring animals and the average proportion of households engaged in agricultural activity within or around the property were both equal to 41%. Only 15% of households per grid cell reported doing some form of larval source reduction on a regular basis,

including removing standing water and clearing debris or vegetation from blocked drains. Forty-seven percent of the 629 households reported having access to electricity and 52% reported owning their own home. Twenty-one percent reported having both home ownership and access to electricity.

Results from a grid cell level bivariate analysis ($n = 50$) indicated that the proportion of grid cells with at least one water body did not differ significantly between the poorly drained and well-drained strata (O.R. = 2.4; C.I. = 0.74, 8.4). The number of grid cells with higher than the average proportion of households engaged in some form of agriculture within or around the property was not different by strata, although grid cells in the well drained stratum were less likely to be above the mean, as compared to grid cells within the poorly drained stratum (O.R. = 0.375, C.I. = 0.12, 1.1). Results from a second grid cell level chi-square analysis ($n = 50$) indicated that the presence of at

least one water body per grid cell was not significantly different between high and low household farming groups (O.R. = 1.9; C.I. = 0.59, 6.4), although grid cells with higher than the mean number of households engaged in some form of farming within or around the property were almost twice as likely to have at least one water body present. Figure 3 illustrates the type of urban farming activity present in Malindi. Figures 3A and 3B illustrate a large commercial plot of maize behind a house and a household farm plot on the edge of town, respectively. Figure 3C illustrates ornamental agricultural activities around a residence. Although large commercial farms also exist in Malindi, few were included within the selected grid cells, and even fewer were adjacent to, and managed by, households selected for an interview.

Results from the logistic regression suggest that the distance to the city center, drainage, and the house ownership plus electricity variables were significantly associated with the occurrence of water bodies. The abundance of households per grid cell was marginally significant, and the household-level farming variable tested insignificant. Although not significant, the odds of the outcome occurring was almost 6 times more likely for grid cells with higher than average levels of household farming as compared to those with less than the average, while controlling for other covariates in the model (OR = 5.77; $p = 0.199$). The unadjusted odds of the outcome occurring was 1.5 more likely for grid cells with higher than average levels of household-level agricultural activity, as compared to those with less than the mean (O.R. = 1.49; C.I. 0.32, 6.34), while controlling only for the clustering of houses in the regression.

The signs of the beta coefficients were in the expected direction for the house density variable, the distance to the city center variable, and the household farming variable. That is to say, the probability of the outcome occurring increased as house density decreased, increased as distance to the city center increased, and increased for grid cells with higher than average levels of households engaged in farming activities within or around the property. Both the drainage and house ownership plus electricity variable signs of the betas were opposite what one might expect, suggesting that an increased level of house ownership plus electricity was associated with an increased probability of the outcome occurring. The well-drained stratum was also at increased odds. The household-level farming variable did not test significant in the model after accounting for potential confounders, suggesting that other factors may be important determinants of water body occurrence. However, the sign of the beta coefficient was in the expected direction, suggesting that farming may have a positive effect on the probability of water body occurrence at some level. As well, irrigated

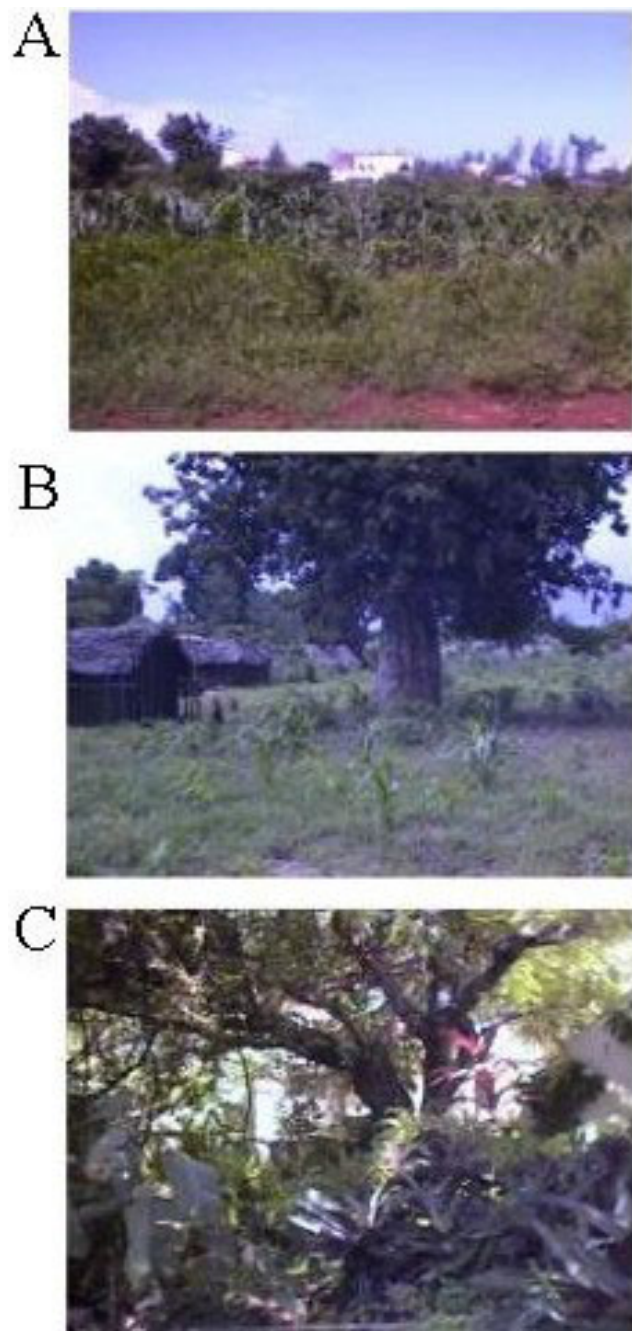


Figure 3
Pictures illustrating the types of urban agricultural activity in Malindi. Fig. 3A illustrates a cultivated field, 3B illustrates a household farm, and 3C represents an ornamental garden.

commercial farm plots also exist in Malindi, but may not have been adequately represented within selected grid cells. Figure 4 illustrates the estimated risk of water body

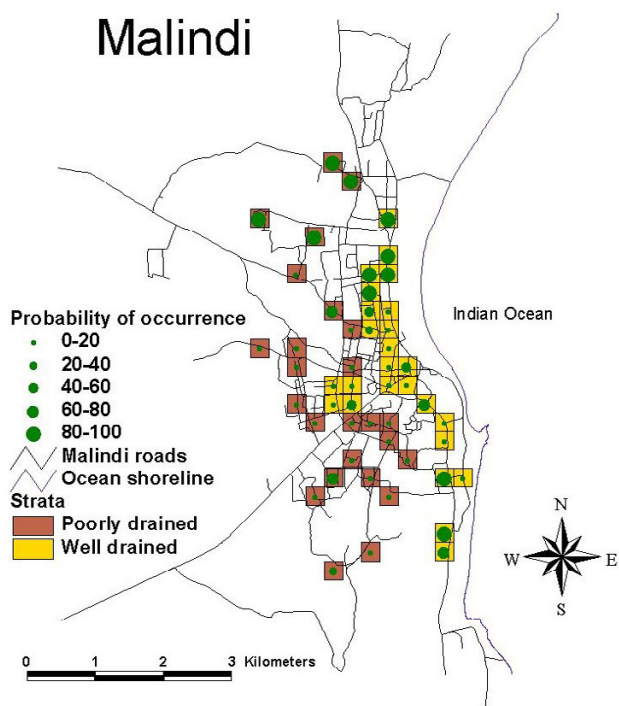


Figure 4
Map illustrating the estimated risk of water body occurrence throughout Malindi town. The probabilities were estimated at the grid cell level from the logistic regression.

occurrence within the selected grid cells. In this illustration, the distribution of risk suggests that areas in close proximity to the city center may be at a reduced risk, as compared to areas further away. As well, areas of high and low risk appear to be somewhat clustered, suggesting possible spatial correlation in the outcome variable, as well as the presence of additional factors operating to effect the overall risk of water body occurrence in Malindi. As such, a properly designed spatial analysis may yield more precise standard errors for model coefficients.

Discussion

This study characterized larval habitats within Malindi, examined relationships between the occurrence of anopheline larvae and human ecological characteristics of the water body, and quantified the effect of household-level farming on the occurrence of water bodies. Nearly all water bodies were human-made, with the well-drained stratum harboring 67% of the total larvae collected. *Anopheles gambiae* s.s. was the only anopheline species collected. Although this observation was interesting and may be important with respect to the epidemiology of malaria parasite transmission within Malindi, the limited

number of water bodies located, and the lack of simultaneous adult mosquito sampling in the area precludes our ability to unequivocally draw conclusions regarding associations with malaria parasite transmission. A key assumption in this analysis was that the abundance and distribution of water bodies was a proximate determinant of the number of adult mosquitoes in the area, although we recognize that a plethora of factors interact on multiple biological and physical scales to affect the actual abundance and distribution of anophelines. As such, the results from this study may hold true in dry periods only, and in areas where humans are modifying the urban landscape in similar ways.

Two main findings are evident from the first bivariate analysis. First, water bodies with pollution or floating debris were 14 times more likely to have anopheline larvae, as compared to water bodies identified with no pollution or floating debris. This is in sharp contrast with what one might expect, where the general rule is that pollution has an inhibitory affect on anopheline larval development [5,6]. It is possible that the types of pollution and debris found in Malindi have little biological affect on oviposition preference or mosquito larval development. As well, the types of debris and pollution found may have an inhibitory affect on natural predators of mosquito larvae, rendering aquatic habitats free of predation, which in theory could lead to increased nutrients and space for larval development within the habitat. No statistical difference was detected between water bodies with anopheline larvae present and categories of additional human-ecological variables measured in this study. This result is most likely affected by the small sample size used and the lack of variability in some of the data. To understand the mechanisms driving the observed relationships increased sample sizes and in depth case studies on the physiochemical properties of the habitat are needed.

Secondly, the results from the first bivariate analysis suggest that characteristics of water bodies do not fundamentally differ between well and poorly drained areas. More habitats were identified and larvae collected in the well-drained stratum, suggesting that water body occurrence and subsequent oviposition operates independent of precipitation in certain circumstances. Drained or abandoned swimming pools, which have subsequently filled with rainwater, were the most important type of habitat identified. These swimming pools within the well-drained, and high-income and tourist areas are situated for access to sunshine, rendering water temperatures sufficient for larval development, and typically have high levels of natural and ornamental vegetation nearby for adult mosquito resting habitat. This suggests that although the influence of drainage in terms of oviposition behavior may be similar across the strata, the strata are actually

measuring human-ecological variations in socioeconomic status and community level infrastructure development, which influence the behavior of the adult mosquito. This is directly related to the oviposition preference of gravid female mosquitoes. Further, the well-drained stratum is predominantly characterized by commercial and tourism related activities, while the poorly drained stratum consists mostly of residential and patches of undeveloped or cultivated land. This observation is important in terms of the types of human activities that may be influencing mosquito population dynamics. In the absence of sustained precipitation, such as in this study, no difference appears to exist between the two strata.

This study also quantified the effect of household-level farming on the probability of water body occurrence. Results from the logistic regression analysis suggest that farming within or around the home does not necessarily contribute to the occurrence of water bodies, after accounting for the effects of distance, house density, access to resources, and drainage typology of the area. Further, independent variables used within this analysis may actually be measuring the same thing. Although the grid cells were relatively homogeneous within the well-drained and poorly drained areas in terms of human activities, land-use, and drainage, there exist pockets of heterogeneity within the grid cell that this analysis failed to capture. This unobserved heterogeneity might have indirectly influenced the outcome, which suggests that the scale and assumptions used regarding the study area may not be the most appropriate. The sampling strategy employed may have also failed to capture important heterogeneity, as clusters tend to be more homogeneous with respect to human activity.

Two main findings are evident from this analysis. First, farming within or around the household was not significantly associated with the occurrence of water bodies, after controlling for community confounders, although the sign of the beta coefficient was in the expected direction. This is in contrast to the results from a study in Ghana, which found that irrigated urban farm plots were associated with increased numbers of *Anopheles* mosquitoes [18]. In this analysis, it is possible that the distance to the city center variable was acting as a surrogate for a vector of community level variables, including small-scale land-use and the likelihood of urban agriculture within an area. Because the built environment and the density of people generally decrease as the distance from the city center increases, and the types of habitats found were predominantly human-made, the abundance of people was more important in terms of predicting the occurrence of water bodies. The house density variable had a negative effect on the outcome and the distance to city center variable had a positive effect on the outcome, suggesting that

human perturbation, reductions in open space and the built environment influence mosquito ecology. Increasing human perturbation reduces open space and increases the level of pollution in the area, if adequate sewer and sanitation services are lacking. The built environment is assumed to reduce the abundance of mosquitoes by reducing or eliminating standing water. Soil and vegetation are replaced with asphalt, concrete, brick, or stone for housing, commercial, or industrial activities. Drainage systems and paved roads are built and enclosed water delivery systems introduced, thus further reducing potential anopheline habitat. However, as population settlements extend toward the rural areas, new construction activities, excavation sites, and irrigation schemes are introduced, which can provide important larval habitats in the presence of precipitation.

Secondly, the opposite direction of the beta coefficient for the drainage variable suggests that engineered drainage systems and topography may be less important than suspected in terms of water body development during periods of little precipitation. Although in periods of increased precipitation, as in our earlier studies in Kisumu and Malindi, Kenya [22], many water bodies were observed in the poorly drained strata, suggesting that drainage does effect water body development at some level. The informal economy and lack of access to piped water may also be acting to distort the effect of drainage in this model. In Malindi, water pipes are broken in an attempt to procure free water for sale or consumption in many of the well-drained areas (Figure 2B). Furthermore, pipes are often poorly installed and break as a result of passing vehicles. Stagnated water provides potential larval habitat. Community taps and urban garden wells, in areas of high population density, can also provide excellent aquatic habitat for anopheline mosquitoes. As people move into areas with little access to piped water, there is more community use at the taps, increases in the number of artificial water storage containers, and an increase in the amount of standing water around the tap. Car washing activities also contribute to the creation of potential larval habitat in Malindi. Open containers, excavation projects, and container type equipment also provide ample habitat if water-filled [22]. However, most aquatic habitats were found in the well-drained stratum and on properties that were considered either high-income or tourism related. Swimming pools that were drained or abandoned, and containing rainwater, were the most important larval sites found in this study. This observation suggests the need to reconsider the potential deleterious health effects of tourism development, as well as the environmental and health effects associated with the mismanagement of private property in Malindi.

Given the relatively low number of habitats identified, and the highly significant relationship between water body occurrence and distance to the city center, community level source reduction may be effective in reducing the number of mosquitoes in the area in the absence of sustained precipitation. Although urban agriculture may play a role in the creation of anopheline habitat at some level, individual or household-level human agricultural activity may be less important than community level land-use and the overall abundance of human perturbation in the area. During the data collection phase of this study, engineered drainage systems and buried water delivery and sewer systems were being installed throughout Malindi and peri-urban areas. Although the excavation and movement of earth, as well as the machinery tire tracks left in the area may have a positive effect on the development of potential larval habitat in the short-term, the long-term benefits of access to piped water, covered drainage systems, and improved sanitation service will reduce the propensity of the area to harbor anopheline mosquitoes.

Conclusions

In this analysis, household -level agricultural activity was not found to be a significant factor affecting the occurrence of water bodies within the community. However, because areas of standing water have been observed in many areas under cultivation in Malindi (Keating per observation 2001 & 2002), it is crucial that we continue to explore methods for mitigating the development of larval habitats. The importance of proper drainage should be stressed as one tool for reducing urban mosquito popula-

tions. Further, the fact that most aquatic habitats were human-made suggests that increased awareness at all levels, as to the benefits of reducing standing water and corresponding mosquito populations, should be enforced. The fact that many larvae were coming from few sites, and few sites in general were found under relatively dry conditions suggests that mosquito populations could be reduced by active targeting of specific areas. A door-to-door effort may then be necessary, as most water bodies in this study were found on private or commercial property. As well, because drained swimming pools, which have subsequently filled with rainwater, were found to be an important source of standing water, it is paramount that efforts be made to disseminate the appropriate information to both residents and businesses with swimming pools. Secondly, the importance of economic development at both the community and household level cannot be understated. In the absence of economic opportunities normally associated with urban livelihoods, individuals will continue to engage in rural-type activities, which may modify the urban landscape in unforeseen ways. In Malindi, the presence of active community groups, municipal councils, mosquito abatement programs, economic incentive in the form of tourism, suggests that reducing mosquito populations and the overall burden of malaria disease is a reasonable and attainable goal.

List of abbreviations

GPS – Global positioning system

GIS – Geographic information system

Table 4: Summary statistics for a multi-level logistic regression model estimating controlled effect of agricultural activity within or around households on the probability that a water body exists within the grid cell.

Variables	O.R.	95% C.I.
Drainage		
• Well drained	7.61	1.78, 32.49
• Poorly drained	1	Reference
Household farming		
• Above the mean	5.72	0.39, 81.95
• Below the mean	1	Reference
Home ownership plus electricity		
• Above the mean	60.58	1.24, 295.2
• Below the mean	1	Reference
Distance to city center	*	*
House density per grid cell	*	*
Pseudo R ²	0.55	
Wald's chi-square	24.06	
P-value	<0.001	
PSU = 50 USU = 629		

*Continuous variables, no odds ratio reported

SSA – Sub-Saharan Africa

IRB – Institutional review board

PCR – Polymerase chain reaction

PSU – Primary sampling unit

USU – Ultimate sampling unit

Authors' contributions

JK led the data collection, analysis, and write-up of the manuscript; KM co-developed the research protocol and sampling strategy, co-wrote sections of the manuscript and assisted with the editing; CM co-developed the study design, supervised data collection, and assisted with the editing; JG co-developed the study design and assisted with the editing; JB co-developed the research protocol and sampling design, co-developed the study design, and assisted with the write-up and editing.

Acknowledgements

We would like to thank two anonymous reviewers for their helpful comments. NSF Grant DEB-0083602 and NIH Grant U19 AI45511 supported this research. We are grateful for the assistance provided by the scientific and technical staff at the Center for Geographic Medicine Research-Coast, KEMRI. We would like to thank Nellie Njoki, Samuel Kahindi, Lydia Kibe, Bonface Nyaga, and Roselyn Kasati for data collection efforts. We would also like to thank Maurice Ombok and Richard Amimo at KEMRI-Center for Vector Biology and Control Research, Kisumu, Kenya, who assisted with the mapping in 2001. This paper is published with the permission of the Director of the Kenya Medical Research Institute.

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