DETERMINANTS OF MALARIA VECTOR HABITAT USE, SPATIAL DISTRIBUTION, AND COMMUNITY COMPOSITION, WITH A FOCUS ON ENVIRONMENTAL FACTORS AND INSECTICIDE-TREATED BED NETS

By

ROBERT SEAN MCCANN

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ABSTRACT

DETERMINANTS OF MALARIA VECTOR HABITAT USE, SPATIAL DISTRIBUTION, AND COMMUNITY COMPOSITION, WITH A FOCUS ON ENVIRONMENTAL FACTORS AND INSECTICIDE-TREATED BED NETS

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Malaria is one of the deadliest vector-borne diseases known. Controlling malaria requires an understanding of the ecology of local malaria vectors. The potential for microdams to provide larval habitats for malaria vectors is poorly understood, despite the importance of microdams for water management in rural areas of Africa. The perimeters of microdam reservoirs in western Kenya were sampled for *Anopheles* larvae in the dry and rainy seasons. In the dry season, both malaria vector and non-vector species were found in microdam-associated habitats, suggesting that microdams may contribute to population persistence through the dry season. In the rainy season, microdams may provide important habitat for *Anopheles gambiae* s.l., as this species dominated *Anopheles* communities in microdams. Thus, microdams may represent a conflict between public health concerns about malaria and people's need for stable and reliable water sources.

Anopheles gambiae s.l. larvae also use a range of smaller, temporary bodies of stagnant water as habitat. Predictive models of the locations of these habitats may provide a basis for understanding the spatial determinants of malaria transmission. Four landscape variables and accumulated precipitation were used to model larval habitat locations through two methods: logistic regression and random forest. The random forest models were more accurate than the logistic regression models, especially when accumulated

precipitation was included to account for seasonal differences in precipitation. Larval habitats were more likely to be present in locations with a lower slope to contributing area ratio, closer to streams, and in agricultural land use. Differences among soil types were also found, and the probability of larval habitat presence increased with increasing accumulated precipitation. This model was used to assess the contribution of larval habitat proximity to houses to the number of adult malaria vectors in those houses. Houses were sampled for adult *Anopheles* females, and variation in household-level insecticide-treated net (ITN) use was assessed. The number of adult female *An. gambiae* s.l. per house increased with increasing mean probability of larval habitat presence within 500 m, which was a better predictor than distance to the nearest larval habitat. Thus, the configuration of larval habitats within a given landscape may affect the relationship between larval habitat location and adult malaria vector spatial distribution. Also, houses in which all residents slept under ITNs the previous night had fewer adult female *An. gambiae* s.l. than other houses. There was no difference in the number of *An. gambiae* s.l. females collected in houses without ITNs and houses where only some of the residents slept under ITNs, highlighting an important difference between the effects of ITN ownership at the household-level and individual ITN use.

Finally, the reemergence of *Anopheles funestus* in an area of long-term ITN use is described here. ITN use in Asembo, a community in western Kenya, has been high since 1998. The abundance of *An. funestus* in Asembo was relatively low from 1998-2008. However, the majority of the *Anopheles* collected here in 2010 and 2011 were *An. funestus*. This has important implications for malaria transmission in the region, given the current reliance on ITNs as a long-term, stand-alone method of vector control.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Malaria as a public health concern

Malaria continues to be one of the most significant infectious diseases in the world. Although significant reductions in malaria-related morbidity and mortality have occurred globally since 2001, there were still an estimated 219 million cases of malaria worldwide in 2010, resulting in 660,000 deaths (World Health Organization 2012). Furthermore, the burden of malaria is heaviest for children living in poverty (World Health Organization 2012). Poor families are less likely to be able to prevent malaria through measures like window screens and insecticide-treated bed nets (ITNs), which reduce malaria vector to human contact (Noor et al. 2009). Families living in poverty are also less likely to be able to treat a child with malaria medication (Mbachu et al. 2012). At the same time, malaria contributes to keeping people in poverty, producing a 'malaria trap' (Berthélemy et al. 2013). Individuals with malaria are more likely to be absent from school or work, and families may deplete their savings for treatment (Sachs and Malaney 2002). However, when poverty is reduced through community-wide economic development, malaria prevalence decreases as people have more economic flexibility (Pronyk et al. 2012).

Spatial distribution of malaria

The fact that malaria displays marked variation across space has long been recognized, such that Italian law in 1809 prevented housing in the vicinity of irrigated meadows (Watson 1949). As the etiology and transmission of malaria became clear following the work of Laveran, Ross, and Grassi, the connection between malaria and water was made explicit (Boyd 1949). Given that transmission of malaria requires the

bite of an infected *Anopheles* mosquito, the disease is limited to locations where malaria vector populations can persist. Additional factors have been shown to contribute to the spatial heterogeneity of malaria at the local scale. Even relatively small socioeconomic differences among households in a community can lead to differential risk of infection with malaria (Greenwood 1989, Gamage-Mendis et al. 1991). Immunological differences among people due to genetics and previous exposure to malaria may also contribute significantly to variation in malaria risk within a community (Greenwood 1989, Mackinnon et al. 2000). Nevertheless, the locations of people's homes relative to that of the malaria vectors' larval habitats has been shown to be an important determinant of malaria risk in a wide range of ecological and epidemiological settings (Trape et al. 1992, Ribeiro et al. 1996, Thompson et al. 1997, Ghebreyesus et al. 1999). Clearly, the spatial distribution of malaria vectors contributes to the spatial distribution of malaria. Thus, identifying determinants of the spatial distribution of malaria vectors facilitates a better understanding of variation in malaria risk.

The association between water and malaria is driven by the requirements of the aquatic life stages of the malaria vector mosquitoes. The specific aquatic habitats used by local malaria vector species are unique to the ecology of each species (Silver 2008), but the immature life stages of every species are restricted spatially to the locations of the suitable aquatic habitat. In this sense, the aquatic habitat of malaria vector immature stages may be thought of as a focus of malaria transmission (Carter et al. 2000). Importantly, the spatial extent, or dimensions, of the these foci should be largely determined by: 1) the relative densities of the aquatic habitats and people's homes; and 2) the dispersal behavior of the adult female malaria vectors (Carter et al. 2000). Therefore,

it is vital to identify the specific aquatic habitat types used locally by malaria vector immature stages, and to understand where these habitats are located, to better understand a primary driver of the spatial distribution of malaria vectors (Killeen et al. 2004).

Agriculture, water management, and malaria

Agriculture has been linked with malaria transmission, especially malaria caused by *Plasmodium falciparum* (Welsh), probably since the very emergence of the disease in humans (Webb 2009). This relationship is driven by the effects of agriculture on water management, as irrigation and poor drainage can result in the creation of suitable aquatic habitat for the immature stages of malaria vectors (Packard 2007). Of course, agriculture alone does not cause malaria. In cases where modest capital investments in agriculture have resulted in community-wide economic improvements, malaria prevalence actually decrease because of residents increased ability to prevent and treat malaria (Ijumba and Lindsay 2001, Ijumba et al. 2002). However, larval *Anopheles* habitats have been associated with agricultural land use in a wide range of settings (Ramasamy et al. 1992, Afrane et al. 2004, Pope et al. 2005, Mutuku et al. 2009). The construction of dams for agricultural water management or hydroelectric power has also been long recognized as potentially leading to increased larval *Anopheles* habitat (Keiser et al. 2005). In this case, thoughtful design and management of dams to make the reservoir less suitable for local malaria vector species can limit this risk (Kitron and Spielman 1989, Yohannes et al. 2005).

The physical landscape, precipitation variation, and malaria

In addition to the influence of human land use in determining the locations of malaria vector larval habitats, the physical landscape also has considerable influence on

these locations (Smith et al. 2013). For instance, in landscapes with high topographic relief, larval *Anopheles* habitats are generally restricted to valley bottoms (Balls et al. 2004, Zhou et al. 2007, Himeidan et al. 2009). Even in landscapes with low topographic relief, the larval habitats of malaria vectors may be concentrated along a river or stream (van der Hoek et al. 2003, Bogh et al. 2003). One method for quantifying the hydrologic potential of a landscape is to use a topographic wetness index (Beven and Kirkby 1979), which has been used to show that larval *Anopheles* habitats may be found in locations having a combination of greater upslope area contributing to drainage and less slope (Clennon et al. 2010, Li et al. 2011, Nmor et al. 2013).

The spatial distribution of larval *Anopheles* habitats is not necessarily static over time. Indeed, seasonal differences in rainfall may cause variation in larval habitat abundance and distribution across the landscape (Gimnig et al. 2001, Mutuku et al. 2009, Munga et al. 2009, Clennon et al. 2010, Li et al. 2011). These changes have important consequences for malaria transmission. An increased availability of larval habitats contributes to higher malaria vector population growth (Killeen et al. 2004), while increased spatial range of larval habitats across the landscape results in an increased range of malaria transmission (Carter et al. 2000). At longer temporal scales and broader spatial scales, the size and distribution of malaria vector populations may expand or contract regionally due to climate change (Thomas et al. 2004, Ermert et al. 2013).

Vector control interventions

Public health interventions may also have a significant impact on the distribution of malaria vectors. One of the primary malaria vector control measures used since the 1990s is mass distribution of insecticide-treated bed nets (ITNs), which must be retreated

every six to nine months. More recently, ITNs have been largely replaced by long-lasting impregnated nets (LLINs), with effective insecticidal activity lasting up to three years (World Health Organization 2012). On a broad scale, ITNs impact malaria vectors by reducing their population sizes (Gimnig et al. 2003a, 2003b), which reduces community-wide morbidity and mortality due to malaria (Hawley et al. 2003b). At the household scale, variation in ITN ownership and use may lead to variation in the number of adult *Anopheles* found indoors. The presence of ITNs in houses may reduce the rate of entry (Mathenge et al. 2001), or increase the rate of exiting (Mathenge et al. 2001, Malima et al. 2008), by malaria vectors.

While the effectiveness of ITNs is well documented, many potential limitations exist, especially when ITNs are used as the only means of vector control (Hawley et al. 2003a, Beier et al. 2008). First, ITNs target only indoor-biting malaria vectors, leaving exophagic populations unaffected (Bayoh et al. 2010). Second, despite the substantial increase in the number of households owning ITNs in many malarious countries over the last decade (World Health Organization 2012), variation in ITN use by individuals within households leaves some people unprotected (Korenromp et al. 2003, Eisele et al. 2009). This is true even in countries implementing universal coverage with LLINs (Macintyre et al. 2011). Finally, there is considerable potential for the development of insecticide resistance in malaria vector populations (Ranson et al. 2011, Trape et al. 2011). Only one class of insecticides, the pyrethroids, is approved for use with ITNs (Zaim et al. 2000), and pyrethroid resistance has been reported in malaria vectors across Africa (Ranson et al. 2011).

Biology of African malaria vectors

The vast majority of malaria cases (80%) and deaths from malaria (91%) in 2010 occurred in Africa (World Health Organization 2012). The most widely distributed malaria vectors in Africa are *Anopheles funestus* Giles, *Anopheles gambiae* s.s. Giles and *Anopheles arabiensis* Patton (Sinka et al. 2010). The latter two are members of a species complex of eight closely related, morphologically indistinguishable species known collectively as *Anopheles gambiae* s.l. (Coetzee et al. 2013). *Anopheles funestus* and *An. gambiae* s.s. are two of the most important malaria vectors in the world due to their preference for feeding almost exclusively on human blood (Garrett-Jones 1964). *Anopheles arabiensis* demonstrates a broader variation in host preference for blood feeding, opportunistically taking blood meals from cattle in addition to humans (Takken and Verhulst 2013). However, *An. arabiensis* is the primary malaria vector in many regions (Massebo et al. 2013) and can become a more important vector when control measures reduce the size of other malaria vector populations (Bayoh et al. 2010).

These three species utilize a range of aquatic habitat types across their distributions, but they are generally limited to stagnant fresh water bodies (Gillies and De Meillon 1968). In many regions the larval habitats of *An. gambiae* s.s. and *An. arabiensis* are smaller, temporary bodies of stagnant water (Gimnig et al. 2001, Mutuku et al. 2006), and the two species are often sympatric within the same larval habitats (Charlwood and Edoh 1996, Minakawa et al. 1999, Gimnig et al. 2001). *Anopheles arabiensis* will additionally exploit larger, permanent habitats such as rice fields when they are available (Githeko et al. 1996). *Anopheles funestus* larvae are found more often in larger, more permanent aquatic habitats with emergent vegetation (Gimnig et al. 2001).

Objectives

In 2007, Bill and Melinda Gates called for the eradication of malaria from the globe within their lifetime (Roberts and Enserink 2007). If we are to succeed at this goal without experiencing the widespread resurgences of malaria that followed the Global Malaria Eradication Program of the 1950-60s (Feachem et al. 2010, Nájera et al. 2011), we must take into account the ecology of the local malaria vector species (Ferguson et al. 2010). Along these lines, this dissertation addresses four aspects of malaria vector biology in order to strengthen our understanding of the contribution of *Anopheles* mosquito malaria vectors to transmission dynamics in rural landscapes heavily altered by human activity. The objectives of this dissertation are to: 1) investigate the contribution of microdam-associated habitat to the production of malaria vectors in the dry and rainy seasons; 2) create a model for predicting larval *An. gambiae* s.l. habitat locations using landscape variables that predict the likelihood of stagnant water bodies and account for seasonal changes in habitat probability based on accumulated precipitation; 3) quantify the relative contributions of larval habitat proximity and household characteristics to the spatial distribution of adult *An. gambiae* s.l.; and 4) describe the reemergence of *Anopheles funestus* in a region with long-term ITN coverage.

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CHAPTER I: Anopheline larvae in microdam reservoirs.

Abstract

Microdams are important for water management in rural areas of Africa. Microdams retain water used by people for domestic and agricultural use, but the potential for malaria vector larvae to use aquatic habitats associated with microdams is poorly understood. The perimeters of microdam reservoirs in western Kenya were sampled for *Anopheles* larvae in the dry and rainy seasons. In the dry season, malaria vector species and non-vector species were found in microdam-associated habitats. The primary malaria vector *Anopheles funestus* was more common in vegetated habitats relative to open water and hoof print aggregations. In the rainy season, *Anopheles gambiae* s.l., another primary malaria vector, dominated *Anopheles* communities in microdam reservoirs. *Anopheles gambiae* s.l. larvae were more common in hoof print aggregations relative to other habitat types along the microdam reservoir perimeters. Microdams may provide a dry season refuge habitat for malaria vectors, contributing to population persistence through the dry season. Microdams also appear to provide important habitat for *An. gambiae* s.l. larvae in the rainy season. This suggests a potential conflict between public health concerns about malaria and people's need for stable and reliable sources of water. To alleviate this conflict, variation in habitat suitability within and among microdams could potentially be exploited to reduce conditions favorable to malaria vector larvae.

Introduction

Malaria transmission is intricately linked with water due to the obligate aquatic life stage of all malaria vector mosquito species, though the specific ecology of local

malaria vectors determines which types of water bodies contribute to malaria transmission in a region (Smith et al. 2013, McKeon et al. 2013). While factors such as adult stage dispersal (Carter et al. 2000), host preference (Garrett-Jones 1964) and survival (Macdonald 1957, Smith et al. 2007), determine a malaria vector population's capacity to transmit malaria, identification of the specific aquatic habitat types utilized by malaria vector immature stages is critical for understanding malaria vector population dynamics across space and time (Killeen et al. 2004). The three most widely distributed primary malaria vector species in Africa, *Anopheles arabiensis*, *Anopheles gambiae* s.s. and *Anopheles funestus*, utilize a range of aquatic habitat types across their distributions, but they are generally limited to stagnant fresh water bodies (Gillies and De Meillon 1968). *Anopheles gambiae* s.s. and *An. arabiensis* are two members of the *An. gambiae* s.l. species complex, a group of at least eight closely related species (Coetzee et al. 2013), which are morphologically indistinguishable but exhibit important ecological and behavioral differences in the larval and adult stages. Larvae of *An. gambiae* s.s. and *An. arabiensis* both utilize smaller, temporary aquatic habitats without vegetation and are often found in the same habitats (Charlwood and Edoh 1996, Minakawa et al. 1999, Gimnig et al. 2001), but *An. arabiensis* will additionally exploit larger, permanent habitats such as rice fields when they are available (Githeko et al. 1996). *Anopheles funestus* larvae are found more often in larger, more permanent aquatic habitats with emergent vegetation (Gimnig et al. 2001).

Infrastructure designed to manipulate the flow and retention of water, such as an irrigation scheme or dam, is often designed to meet societal needs for reliable sources of water for domestic or agricultural use. Effects on the production of malaria vectors and

thus effects on malaria transmission are not generally considered in the design of such infrastructure, and therefore the potential for increased malaria transmission exists (Keiser et al. 2005). Notably, water-related infrastructure projects can potentially reduce malaria transmission when the projects improve the socioeconomic status of local residents (Ijumba and Lindsay 2001, Ijumba et al. 2002) or the ecology of the local malaria vector can be exploited by management techniques (Kitron and Spielman 1989). An estimated 800,000 small dams, defined by Keiser and colleagues (2005) as impoundments less than 15 m high or storing less than 3 million m^3 of water, had been built worldwide as of 2001, yet the effects of these dams on malaria in Africa have not been widely studied. In Ethiopia, children living near microdams had an increased risk of malaria incidence compared to children living 8-10 km from microdams (Ghebreyesus et al. 1999), but environmental management of larval habitats has shown potential for reducing this risk (Yohannes et al. 2005).

The use of aquatic habitats along the perimeters of microdam-created reservoirs by malaria vectors in western Kenya has not been previously investigated. Therefore, the objective of this study was to quantify the abundance of malaria vector species along microdam reservoir perimeters. Additionally, we quantified the abundance of non-vectors and characterized the overall anopheline community. Furthermore, the contributions of environmental conditions and seasonal differences in rainfall to variation in the *Anopheles* communities within microdam reservoirs were assessed.

Study site

This study took place in Asembo, a rural community of 79 villages in western Kenya (Nyanza Province). The region sits in the lowlands along the shores of Lake

Victoria, with elevations ranging from 1,100 m to 1,400 m above sea level and low topographic relief. Networks of streams run across the region and drain into Lake Victoria. Microdams in Asembo are constructed along these drainage basins. The region is relatively densely populated, with about 60,000 residents living in an area of 200 km^2. The majority of residents are subsistence farmers, primarily growing maize, sorghum, cassava, millet, or vegetables, and raising cattle, goats, or chickens (Phillips-Howard et al. 2003). Cattle are an important resource in the community, being used primarily for plowing fields and as an economic investment in addition to sources of dairy and meat products. Thus, a source of water for cattle in Asembo is vital. Reservoirs created by microdams are a primary source of water for livestock and for domestic use, including drinking water (Crump et al. 2005).

Rainfall in Asembo is seasonally bimodal with peaks usually occurring in October-November and March-May. However, rainfall may occur year round, with monthly precipitation totals ranging from 7 to 49 mm and yearly totals ranging from 1,100 to 1,800 mm from 2003 through 2012. Malaria is holoendemic in the region, with parasitemia rates in children under 5 being around 50% in 2009 (Hamel et al. 2011). The predominant species of malaria is *Plasmodium falciparum* (Welch). The primary malaria vectors in the region are *An. funestus*, *An. gambiae* s.s. and *An. arabiensis*. Other species of *Anopheles* known to occur in the region include *Anopheles coustani*, *Anopheles rufipes*, *Anopheles pharoensis* and *Anopheles squamosus*. None of these species are known to known to transmit malaria in western Kenya, although some have been reported as locally important malaria vectors in other regions of Africa (Carrara et al. 1990, Mwangangi et al. 2013).

According to Jewsbury and Imevbore (1988), around 50,000 microdams were constructed in Nyanza Province over three years in the 1950s. Seventy-two functional microdams were identified within Asembo by local residents from each village for the current study, twenty-four of which had been built or renovated since 2000. Three of these were excluded from our study because they were located in a village where larvicide was being applied to larval *Anopheles* habitats, including microdam reservoirs. Eighteen microdams were excluded because they were within 1 km of villages where insecticide-treated wall lining was applied to every house as part of a separate, controlled study. Of the remaining 51 microdams in Asembo, 31 still held water in February 2012 and were included in the current study (Figure 1.1).

Methods

Sampling larvae. Microdams were sampled for *Anopheles* larvae in the first dry season and the first rainy season of 2012. Each microdam was sampled once from 3-22 February 2012 (dry season) and once from 30 April - 23 May (rainy season). Standardized samples were taken along the perimeter of each microdam reservoir at 20 m intervals. Each sample was taken from a quadrat measuring 2 m along the perimeter and 1 m into the reservoir, and consisted of twenty 300 ml dips taken at 20 s intervals. If sampling locations were primarily aggregations of hoof prints, dipping was considered impractical and all larvae within the 2 by 1 m quadrat were collected using plastic pipettes. All collected larvae were kept in separate containers for each instar and sampling location. The larvae were reared in the lab to the forth instar and identified to species according to Gillies and Coetzee (1987). In some cases the larvae were reared to adults, which were also identified to species according to Gillies and Coetzee (1987).

Specimens identified morphologically as part of the *An. gambiae* species complex were further differentiated to the species level by PCR (Scott et al. 1993). It was not possible to differentiate *An. pharoensis* larvae from *An. squamosus* larvae. Mosquito voucher specimens were deposited in the Laboratory of Entomology at the Center for Global Health Research, Kenya Medical Research Institute/Centers for Disease Control and Prevention in Kisian, Kenya (Table 1.1).

The habitat type for each sample was classified as either open water, vegetated, or aggregations of hoof prints. The percent vegetated habitat for each microdam was determined by calculating the proportion of samples classified as vegetated. The soil type for each microdam was determined using the 1:1,000,000 exploratory soil map of Kenya, compiled by the Kenya Soil Survey in 1980 (Sombroek et al. 1982). The three soil types were 1) friable clay/sandy clay loam, 2) friable clay, and 3) firm, silty clay/clay. Daily precipitation totals for December 2011 through May 2012, as measured by the weather station at the Kisumu Airport (about 40 km east of Asembo), were downloaded from the National Climatic Data Center's Global Summary of Day (GSoD) database.

Statistical analyses. All analyses were performed separately for each season. To assess differences in anopheline abundance among habitats, the number of larvae of a given species per sample was related to habitat type using general linear mixed models. We used a compound symmetrical correlation structure to account for repeated measures within microdams. Separate analyses were performed for each malaria vector species and for the most abundant non-vector species.

We were also interested in determining how microdam characteristics influenced overall anopheline abundance within microdams. To account for variation in reservoir

size, the number of larvae collected per meter of reservoir perimeter was calculated for each microdam, separately for each species. This metric of larval density was related to percent vegetated habitat and soil type using general linear models. Finally, *Anopheles* species richness in each microdam was also related to percent vegetated habitat and soil type using general linear models.

Results

As expected, there was a dramatic difference in rainfall between the dry and rainy season sampling periods, both in the weeks preceding the sampling periods and during the sampling periods (Figure 1.2). Corresponding differences were found in the *Anopheles* larvae collected from microdams (Table 1.2). In the dry season, a total of 419 *Anopheles* larvae were collected, 85% of which were identified to species. The primary reason for not being able to identify a specimen was the larva dying as an early instar or pupa. A mixture of malaria vector species and non-vector species occurred in the dry season. The most common species was *Anopheles pharoensis/squamosus*. Species richness per microdam ranged from 0 (n = 10) to 6 (n = 1). In the rainy season, a total of 2,750 *Anopheles* larvae were collected (75% identified to species), most of which were *An. gambiae* s.l. (Table 1.2). A total of 980 *An. gambiae* s.l. were identified to species by PCR, 96% of which were *An. arabiensis*. Species richness in the rainy season ranged from 0 (n = 2) to 4 (n = 2) species per microdam.

The perimeters of the microdam reservoirs also changed between seasons. While the water level in many of the reservoirs remained relatively unchanged, the water level in some of the reservoirs changed dramatically. The mean perimeter length increased from 168 m (range: 60-540 m) to 183 m (60-580 m). Changes in reservoir water level

were associated with changes in the types of habitat occurring along the reservoir perimeter. Open water was the most common habitat type in the dry season, but vegetated habitat was more common in the rainy season (Figure 1.3).

Larval abundances of the most common *Anopheles* species differed among habitat types in both seasons. In the dry season, *Anopheles pharoensis/squamosus* larvae were more common in samples taken from hoof print aggregations and vegetated locations relative to open water (Figure 1.4). *Anopheles funestus* larvae were most common in vegetated locations. *Anopheles gambiae* s.l. larvae were found in all three habitat types at similar abundances in the dry season. In the rainy season, *An. gambiae* s.l. larvae were most common in hoof print aggregations (Figure 1.5).

Anopheles communities in the microdam reservoirs were associated with the percent vegetation of the reservoir perimeter. Species richness of *Anopheles* larvae increased with increasing vegetation in both seasons, though the effect was stronger in the dry season (Figure 1.6). Soil type did not have a significant effect on species richness. Table 1.3 shows the effects of percent vegetated perimeter and soil types on the relative density of larvae in the microdams. Higher densities of *An. funestus* larvae were found with higher percent vegetation in the dry season, and they were lowest in the firm, silty clay/clay soil type. In both seasons, the relative density of *An. pharoensis/squamosus* larvae was associated with the percent vegetation and soil type of the microdams. Finally, densities of *An. gambiae* s.l. larvae in the rainy season were higher in microdams with lower percent vegetation.

Discussion

Aquatic habitats along the edges of microdam reservoirs in western Kenya were utilized in this study by *Anopheles* larvae, including malaria vectors, indicating a potential conflict between public health concerns about malaria and people's need for stable and reliable sources of water. However, *Anopheles* communities in the microdam reservoirs varied considerably between seasons. This variation between seasons was likely driven by a combination of factors, including the population dynamics of *Anopheles* species, the availability of other aquatic habitats, and catchment-scale hydrology. The seasonal variation of *An. funestus* and *An. gambiae* s.l. population sizes are well characterized in this region, generally correlating positively with lagged precipitation (Beier et al. 1990, Taylor et al. 1990, Odiere et al. 2007). If microdam habitats were used equally, relative to other larval habitats on the landscape, higher collections of these two species would have been expected in the rainy season compared to the dry season.

The lower relative abundance of *An. funestus* larvae in the rainy season sampling suggests that other larval habitats are more important than microdam habitats for *An. funestus* during its yearly peak in population abundance. However, microdam habitats are potentially important for the reproduction of *An. funestus* and other anophelines in the dry season. Microdam reservoirs are some of the few water bodies available to anopheline larvae in the dry season in Asembo. Dry season precipitation accumulation in this region varies from year to year, but January-February 2012 was a particularly dry period for Asembo. Water bodies in these drier seasons are limited to a few small springs fed by ground water, Lake Victoria, and reservoirs at microdams. Thus, habitats along the

perimeters of microdam reservoirs potentially represent dry season refuge, which *An. funestus* and other anopheline populations use to persist when other aquatic habitats dry up.

Anopheles gambiae s.l. larvae were found in microdam habitats in both seasons. Contrary to the findings of Webb (1961) in Tanganyika (Tanzania), *An. gambiae* s.l. larvae were found along the perimeters of reservoirs that persisted throughout the dry season. The relative abundance of *An. gambiae* s.l. larvae was considerably higher in the rainy season, agreeing with previous findings regarding the population dynamics of *An. gambiae* s.l. However, the exact mechanisms by which *An. gambiae* s.l. larvae enter microdam habitats and the importance of these habitats for *An. gambiae* s.l. population dynamics remain unclear. Potentially, female anophelines may oviposit in microdam reservoirs. Additionally, larvae may be aggregated in microdams during the rainy season when they are flushed from more typical aquatic habitats (Gimnig et al. 2001, Paaijmans et al. 2007). Heavy rainfall during the rainy season in this region flows across the landscape, and microdams are designed to collect water flowing along these catchments. Therefore, even if *An. gambiae* s.l. females do not oviposit in microdam habitats, these habitats may contribute to *An. gambiae* s.l. population dynamics through larvae completing at least part of their lifetime in them. Of course, additional work must be done to determine whether *An. gambiae* s.l. adults emerge from microdam habitats, given the low correlation between larval abundance and adult emergence within larval habitats (Mutuku et al. 2006).

Variations in the use of microdam habitats within species and within a season were associated with the types of available habitat along microdam reservoir perimeters.

These associations corresponded with previous findings about habitat preferences of anopheline larvae. Many *Anopheles* species, including *An. funestus*, *An. squamosus*, and *An. pharoensis*, are associated with emergent vegetation in larval habitats (Gillies and De Meillon 1968) because of the protection it provides from predators. Accordingly, these species were found at higher abundances in vegetated habitats. Additionally, species richness was higher in microdam reservoir perimeters with higher percent vegetation, suggesting that vegetated perimeters provide more ecological niches for *Anopheles* larvae.

In contrast, *An. gambiae* s.l. larvae are often found in habitats without vegetation (Gimnig et al. 2001). The absence of predators in the small, temporary aquatic habitats generally associated with *An. gambiae* s.l. larvae suggests a strategy of avoiding predators altogether rather than being adapted to find protection in emergent vegetation. While all of the reservoirs sampled in this study were permanent, and therefore likely harbored predators, biological communities varied among habitats within the reservoirs. Thus, *An. gambiae* s.l. larvae may have been able to utilize specific habitats within the reservoir perimeters, such as hoof print aggregations, that did not contain predators.

In addition to the effects of vegetation, differences in soil type were associated with differences among microdams in the number of larvae found for some species. Soil type potentially influences the abundance of larvae in a microdam reservoir indirectly through its influence on microbial communities (Bossio et al. 1998). *Anopheles* larvae feed primarily on bacteria (Merritt et al. 1992) or algae (Kaufman et al. 2006), and variation in nutrient availability among soil types may lead to differences in microbial biomass, diversity, and community composition. Additionally, the microbial community

may produce semiochemicals that provide oviposition cues for *Anopheles* females (Lindh et al. 2008). However, the roles of soil type and microbial community ecology in the habitat use of *Anopheles* mosquitoes are not yet fully understood.

APPENDIX

Chapter I Tables and Figures

Table 1.1: Record of deposition of voucher specimens for Chapter I. The specimens listed below have been deposited in the Laboratory of Entomology at the Center for Global Health Research, Kenya Medical Research Institute/Centers for Disease Control and Prevention in Kisian, Kenya, as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number 2013-14 have been attached or included in fluid-preserved specimens.

Species	Life Stage	Quantity	Preservation
Anopheles gambiae s.l.	Larvae	5	70% EtOH
Anopheles pharoensis/squamosus	Larvae	5	70% EtOH
Anopheles rufipes	Larvae	5	70% EtOH
Anopheles coustani	Larvae	5	70% EtOH
Anopheles funestus	Larvae	5	70% EtOH
Anopheles ardensis	Larvae	5	70% EtOH

Table 1.2: Number of each species of *Anopheles* collected from the perimeters of microdam reservoirs in Asembo in the dry (February) and rainy (May) seasons of 2012. Percent of total *Anopheles* identified in each season shown in parentheses.

	No. Collected	
Species	Dry (%)	Rainy (%)
Anopheles gambiae s.l.	45 (12.6%)	1,963 (95.9%)
Anopheles pharoensis/squamosus	185 (52.0%)	55 (2.7%)
Anopheles rufipes	14 (3.9%)	17 (0.8%)
Anopheles coustani	53 (14.9%)	7 (0.3%)
Anopheles funestus	33 (9.3%)	3 (0.1%)
Anopheles ardensis	26 (7.3%)	2 (0.1%)

Table 1.3: Estimated changes in the density of larvae collected per microdam according to soil types and percent vegetation along the perimeter. Soil type 1, friable clay/sandy clay loam; soil type 2, friable clay; soil type 3, firm, silty clay/clay. Bold indicates $p < 0.10$. Effect on *Anopheles funestus* was not analyzed for rainy season sampling because only three *An. funestus* larvae were collected.

		Dry season		Rainy season	
Species	Habitat type	Estimated change ± SE	p	Estimated change ± SE	p
Anopheles funestus	Percent vegetation	**0.26 ± 0.11**	**0.027**	NA	NA
	Soil type 1-2	-0.10 ± 0.09	0.310	NA	NA
	Soil type 1-3	**-0.26 ± 0.11**	**0.035**	NA	NA
Anopheles gambiae	Percent vegetation	-0.06 ± 0.10	0.526	**-4.08 ± 2.09**	**0.061**
	Soil type 1-2	-0.09 ± 0.08	0.289	1.24 ± 1.79	0.497
	Soil type 1-3	-0.08 ± 0.10	0.457	1.77 ± 2.21	0.431
Anopheles pharoensis/squamosus	Percent vegetation	**0.45 ± 0.25**	**0.083**	**0.19 ± 0.08**	**0.027**
	Soil type 1-2	-0.40 ± 0.22	0.073	-0.03 ± 0.07	0.643
	Soil type 1-3	**-0.57 ± 0.27**	**0.040**	**-0.15 ± 0.08**	**0.081**

Figure 1.1: Pictures of six of the sampled microdams in Asembo. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this dissertation.

Figure 1.2: Daily precipitation totals for 1 December 2011 (12/01/11) through 31 May 2012. Horizontal black bar indicates dry season period for *Anopheles* sampling from microdams, while the red bar indicates the rainy season sampling period.

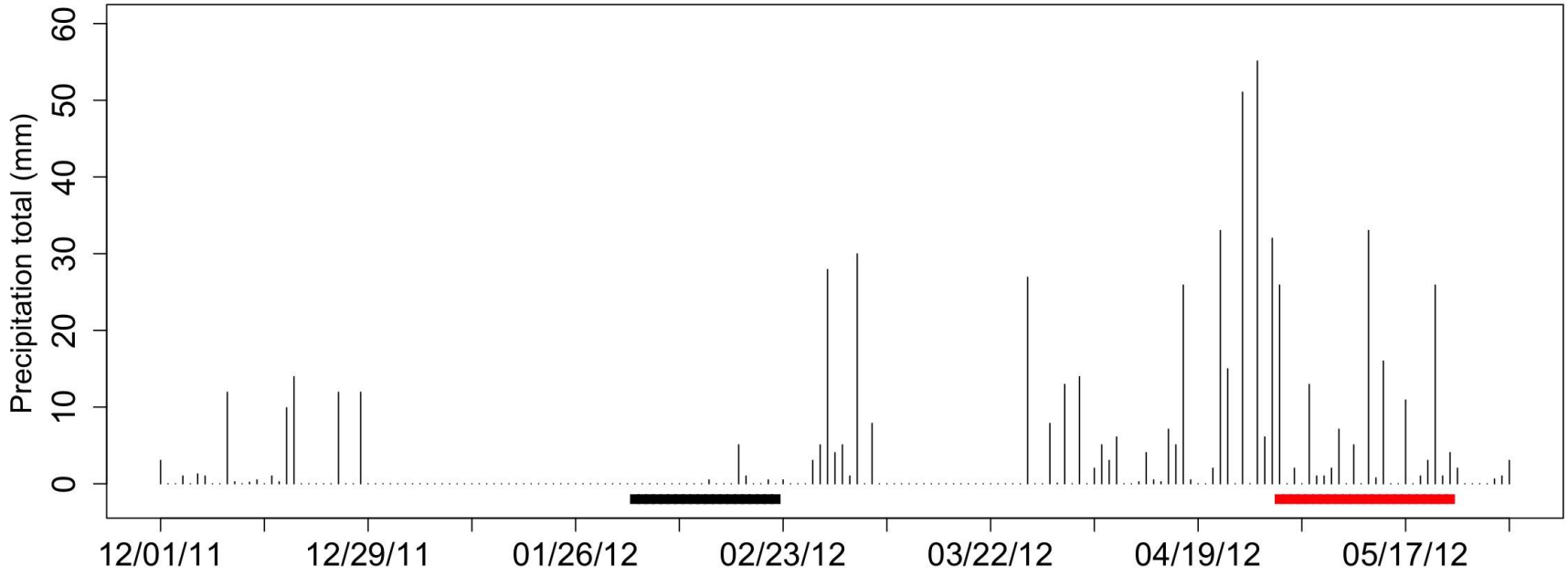


Figure 1.3: Total number of samples taken for *Anopheles* larvae by habitat type in the dry and rainy seasons of 2012. Hoof, hoof print aggregations; open, open water; veg, vegetated habitats.

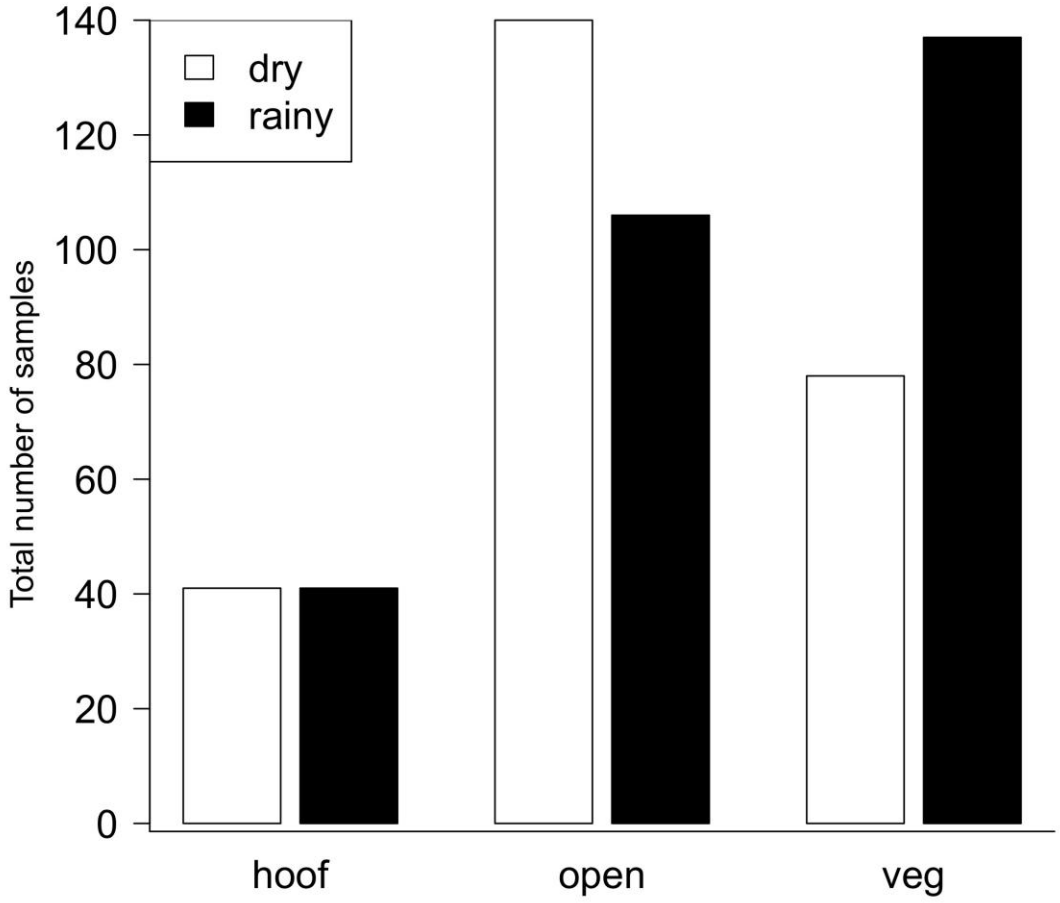


Figure 1.4: Mean number of *Anopheles* larvae per sample, collected from three habitat types on the perimeters of microdams in the dry season. Error bars are 95% confidence intervals. Results shown for *An. pharoensis* are for larvae identified as *An. pharoensis/squamosus.*

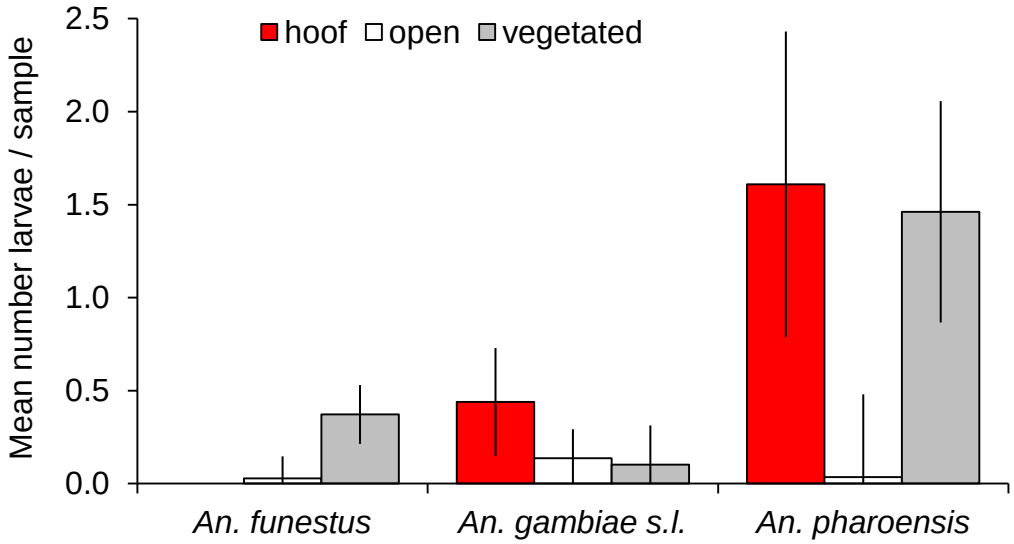


Figure 1.5: Mean number of *Anopheles gambiae* s.l. larvae per sample, collected from three habitat types on the perimeters of microdams in the rainy season. Error bars are 95% confidence intervals.

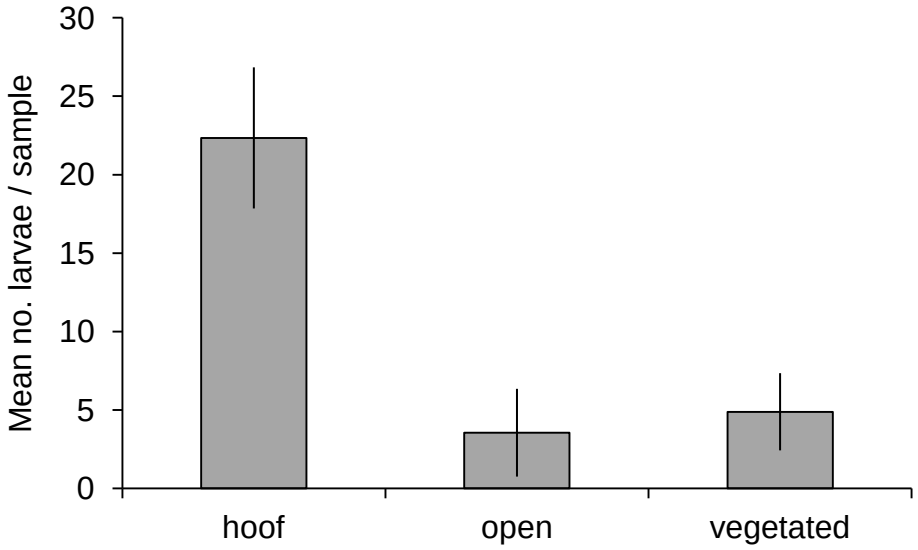
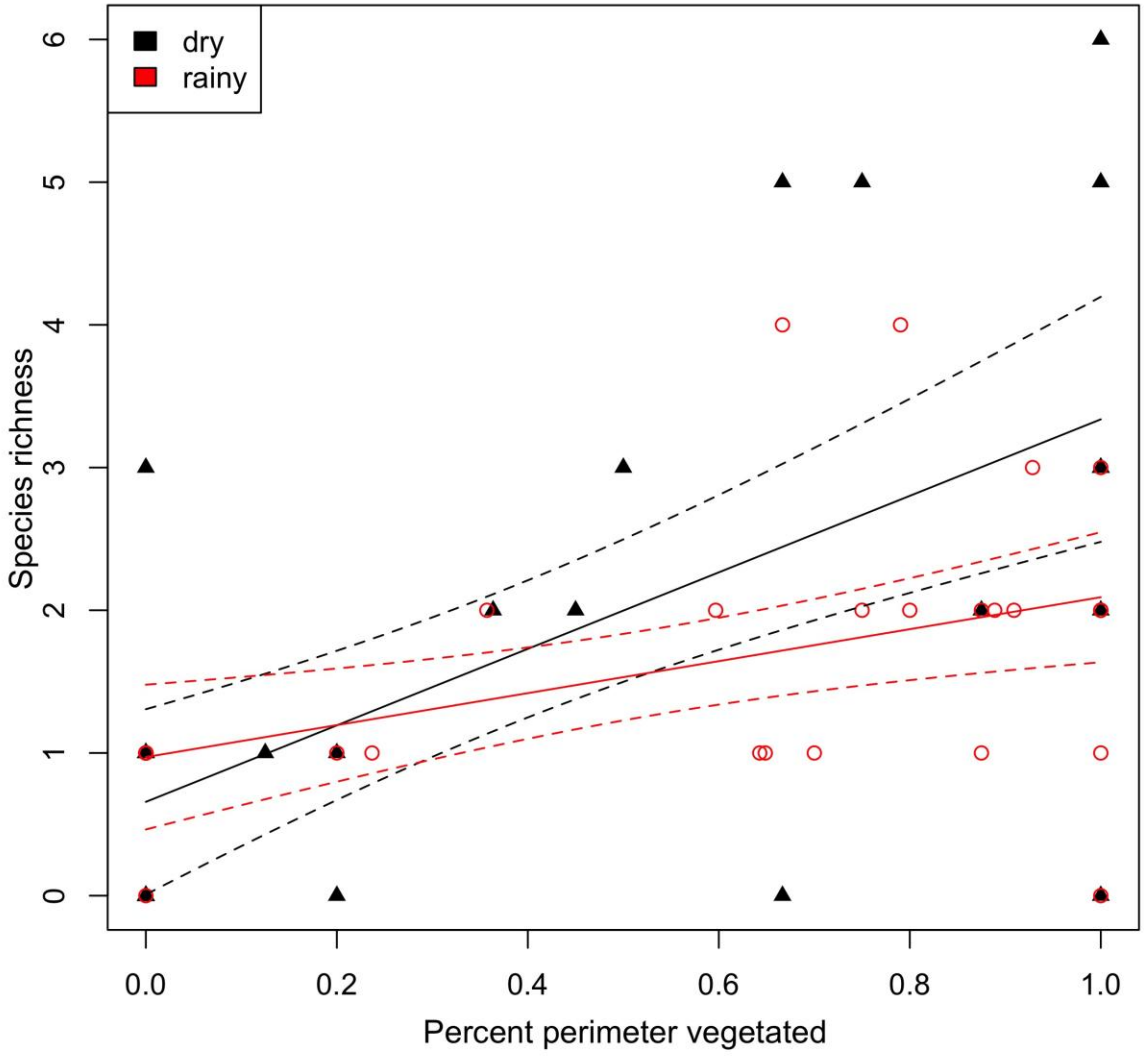


Figure 1.6: Dry and rainy season species richness of *Anopheles* larvae collected from microdam reservoir perimeters, by the percent of the perimeter that was vegetated. Points show observed data (n = 31 microdams per season). Solid lines show estimates from separate linear regressions for each season, with broken lines showing 95% confidence intervals.



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CHAPTER II: Precipitation data improve predictions of larval malaria vector habitat locations because of seasonal differences in precipitation.

Abstract

Predictive models of malaria vector larval habitat locations may provide a basis for understanding the spatial determinants of malaria transmission. Four landscape variables (topographic wetness index, soil type, land use-land cover, and distance to stream) and accumulated precipitation were used to model larval habitat locations through two methods: logistic regression and random forest. The random forest models were more accurate than the logistic regression models, especially when accumulated precipitation was included to account for seasonal differences in precipitation. Larval habitats were more likely to be present in locations with a lower slope to contributing area ratio, closer to streams, and in agricultural land use. Differences among soil types were also found, and the probability of larval habitat presence increased with increasing accumulated precipitation. Estimated larval habitat probabilities from the random forest model produced here can be used to study the influence of larval habitat proximity to houses on the number of malaria vectors resting indoors in this landscape. Finally, the sampling strategy employed here for model parameterization could serve as a framework for creating predictive larval habitat models to assist in larval control efforts.

Introduction

Malaria remains as one of the most significant infectious diseases affecting people in poverty, with an estimated 219 million cases of malaria worldwide in 2010 killing 660,000 people (World Health Organization 2012). In many settings, the spatial distribution of malaria is heterogeneous, with malaria prevalence differing among households within a community (Greenwood 1989, Gamage-Mendis et al. 1991, Trape et

al. 1992, Clark et al. 2008). Of course, socioeconomic and immunological differences contribute to heterogeneity of malaria prevalence in people (Greenwood 1989, Gamage-Mendis et al. 1991). Additionally, the spatial distribution of malaria vectors may coincide with the spatial distribution of parasitemia in people, suggesting a causative relationship (Trape et al. 1992). Furthermore, the spatial distribution of the adult malaria vectors is determined, in part, by the spatial distribution of the aquatic habitats of the vector mosquito larvae. In many landscapes, houses closer to larval habitats have more adult malaria vectors resting indoors relative to houses farther from larval habitats (Trape et al. 1992, Ribeiro et al. 1996, Zhou et al. 2004, Bogh et al. 2007). Therefore, understanding the factors that determine the distribution of the larval habitats facilitates our understanding of the spatial determinants of malaria transmission.

The vast majority of deaths from malaria (91%) now occur in Africa (World Health Organization 2012), where the primary vector mosquitoes are perhaps the most efficient vectors of malaria in the world. Two of the most widely distributed vectors in Africa are *Anopheles gambiae* s.s. Giles and *Anopheles arabiensis* Patton, which are both members of a species complex of eight closely related, morphologically indistinguishable species known collectively as *Anopheles gambiae* s.l. (Coetzee et al. 2013). In many regions the larval habitats of *An. gambiae* s.s. and *An. arabiensis* are similar, and in fact, the two species are often found within the same larval habitats (Charlwood and Edoh 1996, Minakawa et al. 1999, Gimnig et al. 2001). These larval habitats are generally smaller, temporary bodies of stagnant water (Gimnig et al. 2001, Mutuku et al. 2006) with rain being the main source of the water.

The locations of larval *An. gambiae* s.l. habitats are associated with environmental features in many landscapes. Previous studies have found more larval habitats closer to streams (Mutuku et al. 2009) and in locations with agricultural land uses (Mushinzimana et al. 2006, Mutuku et al. 2009). Others have used a topographic wetness index (TWI; Beven and Kirkby 1979) to predict the locations of larval habitats, finding more larval habitats in locations having a combination of greater upslope area contributing to drainage and less slope (Clennon et al. 2010, Li et al. 2011, Nmor et al. 2013). The influence of soil types on the presence of larval habitats has largely been ignored, although Bogh and colleagues (2003) found larval habitats exclusively in alluvial soils in The Gambia. Finally, seasonal differences in rainfall likely influence the number of larval habitats on the landscape (Gimnig et al. 2001, Mutuku et al. 2009, Munga et al. 2009, Clennon et al. 2010, Li et al. 2011).

The objectives of this study were to create a model for predicting larval *An. gambiae* s.l. habitat locations using landscape variables that predict the likelihood of stagnant water bodies, and to account for seasonal changes in habitat probability based on accumulated precipitation. A model predicting accurately the locations of malaria vector larval habitats would allow researchers to investigate the links between larval habitat distribution and adult malaria vector distribution across large landscapes where manually mapping the larval habitats is infeasible (Bogh et al. 2007). Additionally, such a model could be useful for malaria control programs, allowing program managers to focus their efforts to areas where larval habitats are most likely to occur.

Study site

The Asembo region of Rarieda District in western Kenya (Figure 2.1A) is a rural community of about 60,000 people covering about 200 km^2. Most of the residents are subsistence farmers, and the landscape is largely dominated by small-scale agriculture. Small plots of land generally surround family-based groups of houses, or compounds, further arranged into villages. While the compounds are highly dispersed within villages, the boundaries between villages are often discernable only by residents (Phillips-Howard et al. 2003) (Figure 2.1B). Asembo sits in the lowlands along the shores of Lake Victoria, with elevations ranging from 1,100 m to 1,400 m above sea level and low topographic relief. Networks of streams run across the region and drain into Lake Victoria. Farmland is common in these low-lying drainage basins, as well as throughout the region. Houses are mostly absent within 100 m of the streams. Rainfall is seasonally bimodal but may occur year round, with monthly precipitation totals ranging from 7 to 49 mm and yearly totals ranging from 1,100 to 1,800 mm from 2003 through 2012.

Malaria is holoendemic in Asembo, with parasitemia rates in children under 5 being around 50% in 2009 (Hamel et al. 2011). Similar to rainfall patterns, malaria transmission occurs year round, with seasonal peaks in May-July and October-November. The predominant species of malaria is *Plasmodium falciparum* (Welch). Two of the primary malaria vectors in the region are *An. gambiae* s.s. and *An. arabiensis*, the only two members of the *An. gambiae* s.l. species complex found here. Larval *An. gambiae* s.l. habitats are numerous and widespread in Asembo yet heterogeneously distributed. This makes it difficult to establish a relationship between larval habitats and the spatial distribution of the adult vectors or malaria prevalence in people. A 10 by 10 km study site

was defined within Asembo to capture variation in the determinants of larval habitat location across a relatively large area. Because we wanted to include the lakeshore in the study site, the southern border fell largely within the lake, leaving 96.43 km^2 of actual landmass in the 10 by 10 km site.

Methods

Larval habitat ground surveys. The study site was divided into 500 by 500 m quadrats for larval habitat ground surveys. After excluding the quadrats that fell completely in the lake, from the remaining 393 quadrats, we selected 31 for larval habitat ground surveys using spatially stratified random sampling (Figure 2.1C). Thirty-one quadrats were the maximum we could sample during the targeted time frame, which was the end of the long rainy season to coincide with the peak *An. gambiae* s.l. population level (Beier et al. 1990, Taylor et al. 1990, Odiere et al. 2007). For spatial stratification the study area was divided into 25, 2 by 2 km quadrats, containing 16 of the 500 by 500 m quadrats each. The smaller quadrats were randomly selected from groups defined by the larger quadrats. Spatial stratification was implemented to avoid the problem of sampling a cluster of quadrats in a certain area of the grid and missing variation in the predictor variables (Hurlbert 1984).

The quadrats were each surveyed exhaustively on 1 of 22 days between 17 May 2011 and 4 July 2011, during the end of the long rainy season. All potential *An. gambiae* s.l. habitats found in the quadrats were georeferenced with GPS units. Six field workers spaced 20 m from each other walked from one end of a quadrat to the other, using ArcPad on a GPS unit for navigating the borders of the quadrat. This was repeated until the entire quadrat was covered, usually in four to five passes. This approach allowed us to

say, with certainty, where habitats were absent during the survey. Where habitats were present, we recorded the location and presence or absence of *Anopheles* larvae. Larval *An. gambiae* s.l. habitats were defined as any stagnant body of water, regardless of whether *Anopheles* were present on the day of the ground survey, and falling under the following categories: drainage channel, burrow pit, rain pool, runoff, cluster of hoof prints, stream bed pool, pond/reservoir, wet meadow, well and tire track (Mutuku et al. 2006). For a subset of habitats (the first five *Anopheles*-positive habitats for each of the four to five passes across each quadrat), *Anopheles* larvae and pupae were collected and transported to the lab for species identification to confirm that the habitats were being used by *An. gambiae* s.l. Larvae were raised to fourth-stage instars for identification, while pupae were allowed to eclose as adults before identification. All identifications were done according to Gillies and Coetzee (1987). Mosquito voucher specimens were deposited in the Laboratory of Entomology at the Center for Global Health Research, Kenya Medical Research Institute/Centers for Disease Control and Prevention in Kisian, Kenya (Table 2.1).

To capture variation in habitat location across time due to seasonal rainfall patterns, monthly ground surveys were done in two neighboring villages, Aduoyo-Miyare and Nguka, covering an area of 6.22 km^2 within the 10 by 10 km study site (Figure 2.1D). Two local field workers with extensive knowledge of the villages walked throughout the whole of each village over the course of one to three days, depending on the number of habitats encountered, each month from April 2011 through June 2012. Potential *An. gambiae* s.l. habitats were defined and recorded as above, and we had the ability to say where habitats were absent within the two villages each month.

Environmental data. Spatial data for soils, land use-land cover (LULC), distance to the nearest stream, and TWI were created at a 20 m resolution across the study site. All data were assembled in ArcGIS (ESRI, Redlands, CA) in raster data structures. Soil data were taken from the 1:1,000,000 exploratory soil map of Kenya, compiled by the Kenya Soil Survey in 1980 (Sombroek et al. 1982). The three soil types in Asembo were 1) friable clay, 2) friable clay/sandy clay loam, and 3) firm, silty clay/clay. A satellite image from the IKONOS-2 sensor was used to create the LULC classification. Briefly, unsupervised classification was done using the K-means method. Classes were combined into a binary data layer of agricultural or non-agricultural land use. All streams in Asembo were mapped using GPS units, and the Euclidean distance to the nearest stream was calculated.

The TWI data were derived from a digital elevation model (DEM) of the study site. The DEM was created using local universal kriging to interpolate 11,130 GPS elevation records previously taken within Asembo (Hightower et al. 1998, Ombok et al. 2010). The ArcGIS extension TauDEM 5.0 (Tarboton, Utah State University) was used to calculate a TWI. First, flow direction and slope were calculated. Contributing area catchments were calculated using the flow direction and slope data. Finally, TWI was calculated as the ratio of the slope to the contributing area, and the values rescaled to the range, from 0-100, by taking:

$$(TWI_i - \min(TWI)) \times 100 / (\max(TWI) - \min(TWI)) \text{ for each } i \text{ value of TWI.}$$

Because the TWI was calculated as the ratio of slope to contributing area, the lowest values (near 0) represent the wettest locations, while the highest TWI values represent the driest areas.

Daily precipitation totals for March 2011 to July 2012, as measured by the weather station at the Kisumu Airport (about 40 km east of Asembo), were downloaded from the National Climatic Data Center's Global Summary of Day (GSoD) database (Figures 2 and 3). For missing daily data at the Kisumu weather station (n = 19), the inverse distance weighted mean of surrounding GSoD weather stations (within 250 km) was used. We summed cumulative precipitation totals from 0 to 30 days prior to each day of larval habitat ground surveys, and selected the best cumulative n-day total based on the criteria outlined below.

Statistical methods. We used two approaches for modeling the distribution of larval habitats across the landscape, logistic regression and random forest, and each approach was used separately on the two datasets (one from the 10 by 10 km area and the other from the 15 monthly ground surveys in Aduoyo-Miyare and Nguka). While logistic regression has been used in species distribution modeling more frequently, ecologists have recently started using the random forest method as well, because it does not require any assumptions about the distribution of the data (Bisrat et al. 2011, Hernández et al. 2013).

We built a series of candidate logistic regression models to select the most useful predictor variables. Univariate models were built for each of the cumulative precipitation totals from 0 to 30 days, and the model with the lowest BIC was selected as the best precipitation measure to use in further logistic regression models. All possible combinations of our five predictor variables (TWI, soil, LULC, distance to stream, and precipitation) were used to build 31 candidate logistic regression models, with the top models again being selected according to the lowest BIC. These analyses were

implemented in the statistical software R 2.14.2 (R Development Core Team, Vienna, Austria).

Random forest is a machine learning classification method extending on the classification and regression tree approach (CART), which works by recursive binary partitioning of the data space into increasingly homogenous regions (Breiman et al. 1984, Bisrat et al. 2011). Random forest works by fitting and combining many CARTs to create a more accurate prediction (Breiman 2001, Bisrat et al. 2011). We implemented the random forest approach using the R package 'randomForest' (Liaw and Wiener 2002). The best cumulative precipitation measure for use in the random forest models was determined by the mean decrease in the Gini impurity criterion when removing the variable from the full model (TWI, soil, LULC, distance to stream, and precipitation). The cumulative precipitation measure with the highest mean decrease in the Gini impurity criterion was used in the final random forest models.

The top models from both approaches within each dataset were evaluated by determining their accuracy at predicting larval habitat presence and absence for holdout data. Fifty percent of each dataset was randomly selected as a holdout dataset before model building. Evaluation of model accuracy required the selection of a threshold at which to convert predicted probabilities into larval habitat presence or absence. Because threshold specific accuracy statistics can be sensitive to the threshold used for conversion, we generated an optimal threshold value by minimizing the absolute value of the difference between sensitivity and specificity (Liu et al. 2005). This approach was chosen because both sensitivity and specificity were equally important for the intended application of the predictive models. To assess the performance of each model, we

calculated the sensitivity, specificity, percent correctly classified (PCC), and kappa of each approach at each of the thresholds from the methods above. We also calculated the threshold-independent area under the ROC curve (AUC) statistic from ROC plots using the R package 'SDMTools'.

Finally, we assessed correlation among the cumulative precipitation measures using Pearson's correlation coefficient. The contribution of cumulative 30-day precipitation to variation in the number of habitats found each month in Aduoyo-Miyare and Nguka was quantified using simple linear regression.

Results

In the 31 sampling quadrats selected from the 10 by 10 km study site, we recorded the locations of 1,673 larval *An. gambiae* s.l. habitats. Six of the quadrats did not have any larval habitats, while the mean number of habitats per 500 by 500 m quadrat was 54. *Anopheles* larvae were present in 921 of the 1,673 habitats on the day each habitat was recorded. Of the *Anopheles* larvae and pupae collected from 141 of the habitats, 79% were identified as *An. gambiae* s.l. In 15 monthly ground surveys in Aduoyo-Miyare and Nguka, a total of 6,770 larval *An. gambiae* s.l. habitats were recorded. The number of larval habitats in this area varied by month, ranging from 104 to 953 with a mean of 451. The number of larval habitats recorded in the two villages each month increased with increasing cumulative 30-day precipitation on the final day of ground surveys each month ($R^2 = 0.1931$, $p = 0.1012$; Figure 2.2).

The best cumulative precipitation total to use in the models differed between the datasets and between the modeling approaches, although all cumulative precipitation totals were highly correlated with each other (Table 2.2). For the 15 monthly ground

surveys in Aduoyo-Miyare and Nguka, the univariate logistic regression model with the lowest BIC within the precipitation candidate models used the cumulative 30-day precipitation (Figure 2.3), whereas the random forest model using the cumulative 21-day precipitation (Figure 2.3) had the highest mean decrease in the Gini impurity criterion. For the 10 by 10 km data, the univariate logistic regression model with the lowest BIC used the cumulative 6-day precipitation (Figure 2.4), while the random forest model using the cumulative 14-day precipitation (Figure 2.4) had the highest mean decrease in the Gini impurity criterion. Each of these precipitation measures was used within its respective dataset and modeling approach moving forward.

The environmental variables used in the best logistic regression models for predicting the locations of larval *An. gambiae* s.l. habitats differed slightly between the datasets. For the 15-month dataset from Aduoyo-Miyare and Nguka, the logistic regression model with the lowest BIC included all five of the variables (Table 2.3). No other model had a ΔBIC less than 20. Larval habitats were more likely to be found in locations with a lower TWI (i.e. wetter because of a lower slope to contributing area ratio), closer to streams, in agricultural land use, and in the friable clay/sandy clay loam soil type (Table 2.4). The probability of a larval habitat increased with increasing cumulative 30-day precipitation (Table 2.4).

For the 10 by 10 km dataset, the logistic regression model with the lowest BIC included four of the variables (TWI, distance to stream, soil type, and cumulative 6-day precipitation). No other model had a ΔBIC less than 9 (Table 2.5). Larval habitats were again more likely to be found in locations with a lower TWI, closer to streams, and in the friable clay/sandy clay loam soil type (Table 2.6). Counterintuitively, the probability of a

larval habitat according to this model decreased with increasing cumulative 6-day precipitation.

The most accurate model for predicting larval *An. gambiae* s.l. habitat locations in the 10 by 10 km area was built using the random forest method and all five variables (AUC = 0.864). Removing the cumulative 14-day precipitation from this model reduced the accuracy of the model (Table 2.7). The best logistic regression model for the 10 by 10 km area was less accurate than the random forest model when evaluated against the holdout data (Table 2.7). When the probabilities of larval habitat location across the entire 10 by 10 km study site were estimated using the most accurate models from each method, the random forest model clearly produced a more heterogeneous landscape at a fine scale than that produced by the logistic regression method (Figure 2.5).

Similarly, the most accurate model for predicting larval *An. gambiae* s.l. habitat locations over the 15 monthly surveys in Aduoyo-Miyare and Nguka was built using the random forest method and all five variables (Table 2.8). Again, removing the cumulative 21-day precipitation from this model reduced its accuracy, and the best logistic regression model for the 15 monthly surveys in Aduoyo-Miyare and Nguka was less accurate than the random forest model when evaluated against the holdout data (Table 2.8).

Discussion

The use of models to predict the distribution of species is common in ecology (Guisan and Zimmermann 2000), and novel approaches to building these models such as random forest have become more widely available in recent years. We used two methods to predict the probability of larval *An. gambiae* s.l. habitat across the landscape and over time, and the random forest method created more accurate models than the logistic

regression method. This may be due to the differences between the two methods in heterogeneity of larval habitat probability on a fine scale, which can be seen in the estimates across the entire 10 by 10 km study site (Figure 2.5). The estimates from the most accurate random forest model are more fragmented, in that high-probability locations are nearer to low-probability locations relative to the estimates from the logistic regression model. At a broad scale, a general pattern for higher probability locations is still apparent with the random forest estimates. However, the fine scale heterogeneity in the random forest estimates more closely reflects the nature of actual larval habitat distribution on the ground, where larval *An. gambiae* s.l. habitats are distributed as many small patches rather than one continuous, large patch.

The creation of larval *An. gambiae* s.l. habitats (which are temporary, small bodies of stagnant water) depends on rainfall, which varies seasonally across the range of the species complex. Therefore, an important question in the application of predictive larval habitat models is whether models parameterized with data for habitat locations in one season are applicable to another season (Li et al. 2011). One strategy to deal with differences between seasons is to account for variation in precipitation. We found more larval habitats in months with more precipitation compared to the same area in months with less precipitation. Thus, including accumulated precipitation in our models improved the accuracy of larval habitat location predictions.

While this confirms that variation in precipitation influences larval *An. gambiae* s.l. habitats, the relationship may be more complex than it first appears. For example, it may not be linear. Rather, the number of larval habitats may increase monotonically with accumulated precipitation up to a threshold, after which more of the water on the

landscape flows as surface sheet or channeled water, which is unsuitable aquatic habitat for *An. gambiae* s.l. larvae. Additionally, different habitat types may respond differently to increasing accumulated precipitation. Stagnant water forming in drainage channels and stream bed pools may be described better by a threshold relationship than the water filling burrow pits, hoof prints and tire tracks, because the former develops from channel and sheet water made stationary by diminished water flows, whereas the latter forms from water accumulating into various catchments not associated with channels. These additional factors may explain some of the uneven residual error seen in Figure 2.2, where the 4 months in the red box falling above the fitted regression line have more larval habitats with a lower accumulated precipitation relative to the 3 months in the blue box falling below the fitted regression line.

An unexpected result was found for the best logistic regression model of the 10 by 10 km data, as the odds of larval habitat presence decreased with increasing cumulative 6-day precipitation. Most likely this reflects a limitation of the 10 by 10 km data collection rather than the true influence of precipitation on larval habitat presence, given the range of cumulative 6-day precipitation over the 49-day period (1.5 mm – 51.1 mm; Figure 2.4). The sampling strategy for those data was designed to capture variation in landscape variables over space. While precipitation varied among the days of the ground surveys, we were not able to capture that variation over the full range of values for the landscape variables. Instead, the effect of accumulated precipitation in this particular model may be an indication of some other property differing between the quadrats sampled on days of higher and lower accumulated precipitation. Furthermore, the temporal scale over which larval habitats respond to variation in accumulated

precipitation is probably closer to monthly than daily. That is, ground surveys conducted at monthly intervals in the same area are more likely to be different than daily samples within a month in the same area.

The sampling designs of these two datasets allowed us to address two complementary goals. The monthly surveys in Aduoyo-Miyare and Nguka captured variation in precipitation across both dry and rainy seasons in the same landscape. This provided a stronger logical basis for inferences about the relationship between seasonal variation in precipitation and variation in the location and number of larval habitats. However, the small spatial extent of that landscape limited the applicability of the model results across a larger area. Conversely, limiting the ground surveys of the 31 quadrats from the 10 by 10 km study site to be within one season likely impeded our ability to infer much about the affect of precipitation on these data. On the other hand, replicating our sampling effort across space in the 31 quadrats captured more variation in landscape variables, allowing us to apply the results of models based on these data to a larger area. Thus, we are currently using estimated larval habitat probabilities from the random forest model in the 10 by 10 km study site to study the influence of larval habitat proximity to houses on the number of malaria vectors resting indoors in this landscape where mapping all of the larval habitats across 100 km^2 is infeasible.

Additionally, the sampling strategy used in the 10 by 10 km site could serve as a framework for creating predictive larval habitat models for larval control. Targeted larval control is often cited as a useful application of predictive larval habitat models (Li et al. 2011, Nmor et al. 2013), and we agree that there is potential for this application. For example, malaria control programs could identify areas suited to environmental

management such as filling in burrow pits and engineering drainage channels to drain more completely. Additionally, allowing application crews to focus on areas with a higher probability of larval habitat presence would reduce the time, and therefore the cost, of larviciding. However, models fitted to data from a single geographic location may have limited generalizability (Strauss and Biedermann 2007). Spatially stratified random samples, repeated across a variable landscape, may allow malaria control programs to build models that are useful over larger areas.

While the models developed here exclusively used physical and environmental factors as predictor variables, the formation of larval *An. gambiae* s.l. habitats also depends on human behavior. For example, landowners in Asembo create small drainage channels around fields. Stagnant water left behind in the channels creates habitats for *An. gambiae* s.l. larvae (Mutuku et al. 2006). The locations of these drainage channels are often in low-lying agricultural areas, and therefore our models were able to predict the locations of most of the drainage channels. However, drainage channels are not found in 100% of low-lying agricultural areas, probably in part because of individual variation in landowner decision-making. Larval habitats formed from burrow pits and aggregations of hoof prints are also subject to variation in human behavior. While our models were able to correctly predict the locations of most of these habitats, interactions between the physical landscape and human behavior likely account for some of the locations identified incorrectly by the models.

APPENDIX

Chapter II Tables and Figures

Table 2.1: Record of deposition of voucher specimens for Chapter II. The specimens listed below have been deposited in the Laboratory of Entomology at the Center for Global Health Research, Kenya Medical Research Institute/Centers for Disease Control and Prevention in Kisian, Kenya, as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number 2013-14 have been attached or included in fluid-preserved specimens.

Species	Life Stage	Quantity	Preservation
Anopheles gambiae s.l.	Larvae	5	70% EtOH

Table 2.2: Pearson's correlation coefficient (r) matrix of four cumulative precipitation measures for (A) 22 days of larval habitat ground surveys in the 10 by 10 km area from 17 May to 4 July 2011, and (B) the last day of larval habitat ground surveys each month in Aduoyo-Miyare and Nguka from April 2011 to June 2012. 0-day refers to precipitation total for the day of ground surveys, while 6-day, 14-day, 21-day, and 30-day refer to the cumulative precipitation total for the day of the ground surveys plus the previous 6 days, 14 days, 21 days, or 30 days, respectively. Note that similar correlations were found among other cumulative n-day precipitation totals at 1-day increments from 1-day to 30 day (data not shown).

	0-Day	6-Day	14-Day	21-Day	30-Day
A) 17 May - 4 July 2011					
0-Day	1.000				
6-Day	0.411	1.000			
14-Day	0.332	0.721	1.000		
21-Day	0.405	0.477	0.794	1.000	
30-Day	0.392	0.611	0.578	0.517	1.000
B) April 2011 - June 2012					
0-Day	1.000				
6-Day	0.812	1.000			
14-Day	0.665	0.826	1.000		
21-Day	0.408	0.555	0.841	1.000	
30-Day	0.336	0.537	0.819	0.979	1.000

Table 2.3: The top four logistic regression candidate models for the 15 monthly ground surveys in Aduoyo-Miyare and Nguka based on Bayesian information criterion (BIC), in order of increasing difference in BIC from the top model (ΔBIC) and decreasing BIC weight, *w*. TWI, topographic wetness index; LULC, land use-land cover; DS, distance to the nearest stream; Precip., cumulative 30-day precipitation total; NA, not applicable.

Model	BIC	ΔBIC	*w*
TWI + LULC + DS + Soil + Precip.	45933.1	NA	0.9999
TWI + DS + Soil + Precip.	45955.6	22.5	<0.001
TWI + LULC + DS + Precip.	46045.5	112.3	<0.001
TWI + DS + Precip.	46073.7	140.5	<0.001

Table 2.4: Parameter estimates as odds ratios with 95% CI for the top logistic regression model for the 15 monthly ground surveys in Aduoyo-Miyare and Nguka. TWI, topographic wetness index; LULC, land use-land cover; DS, distance to the nearest stream; Precip., cumulative 30-day precipitation total; Ag:NonAg, odds ratio of agricultural to nonagricultural LULC; Type2:Type3, odds ratio of the friable clay/sandy clay loam soil type to the firm, silty clay/clay soil type.

	Odds ratio	95% lower	95% upper	p-value
(Intercept)	0.0378	0.0334	0.0426	< 0.001
TWI	0.9365	0.9324	0.9405	< 0.001
LCLU, Ag:NonAg	1.3371	1.2096	1.4780	< 0.001
DS	0.9980	0.9978	0.9981	< 0.001
Soil, Type2:Type3	0.7127	0.6715	0.7564	< 0.001
Precip.	1.0033	1.0029	1.0036	< 0.001

Table 2.5: The top five logistic regression candidate models for the 10 by 10 km area based on Bayesian information criterion (BIC), in order of increasing difference in BIC from the top model (ΔBIC) and decreasing BIC weight, *w*. TWI, topographic wetness index; LULC, land use-land cover; DS, distance to the nearest stream; Precip., cumulative 30-day precipitation total.

Model	BIC	ΔBIC	*w*
TWI + DS + Soil + Precip.	6482.6	0	0.9822
TWI + LULC + DS + Soil + Precip.	6491.8	9.3	0.0096
TWI + DS + Precip.	6492.2	9.6	0.0082
TWI + LULC + DS + Precip.	6502.0	19.4	< 0.001
TWI + DS + Soil	6658.0	175.4	< 0.001

Table 2.6: Parameter estimates as odds ratios with 95% CI for the top logistic regression model for the 10 by 10 km area. TWI, topographic wetness index; DS, distance to the nearest stream; Precip., cumulative 6-day precipitation total; Type1:Type3, odds ratio of the friable clay soil type to the friable clay/sandy clay loam soil type; Type2:Type3, odds ratio of the friable clay soil type to the firm, silty clay/clay soil type.

	Odds ratio	95% lower	95% upper	p-value
(Intercept)	0.3156	0.2472	0.4030	< 0.001
TWI	0.9117	0.8988	0.9248	< 0.001
DS	0.9973	0.9969	0.9977	< 0.001
Soil, Type1:Type2	1.9105	1.4994	2.4343	< 0.001
Soil, Type1:Type3	1.4970	1.2024	1.8639	< 0.001
Precip.	0.9700	0.9656	0.9745	< 0.001

Table 2.7: Comparison of models predicting the presence of larval *Anopheles gambiae* s.l. habitats in the 10 by 10 km area. Two random forest (RF) models are shown with and without Precip. (the cumulative 14-day precipitation total). The best logistic regression (LR) model is also show. TWI, topographic wetness index; LULC, land use-land cover; DS, distance to the nearest stream; AUC, area under the receiver operating curve; PCC, percent correctly classified.

Model	AUC	Sensitivity	Specificity	PCC	Kappa
RF: TWI + DS + Soil + LULC + Precip.	0.864	0.806	0.789	0.790	0.216
RF: TWI + DS + Soil + LULC	0.808	0.750	0.725	0.726	0.145
LR: TWI + DS + Soil + Precip.	0.799	0.748	0.709	0.711	0.133

Table 2.8: Comparison of models predicting the presence of larval *Anopheles gambiae* s.l. habitats in Aduoyo-Miyare and Nguka. Two random forest (RF) models are shown with and without Precip. (the cumulative 14-day precipitation total). The best logistic regression (LR) model is also shown. TWI, topographic wetness index; LULC, land use-land cover; DS, distance to the nearest stream; AUC, area under the receiver operating curve; PCC, percent correctly classified.

Method of optimizing	AUC	Sensitivity	Specificity	PCC	Kappa
RF: TWI + DS + Soil + LULC + Precip.	0.871	0.820	0.773	0.774	0.102
RF: TWI + DS + Soil + LULC	0.827	0.659	0.936	0.930	0.268
LR: TWI + DS + Soil + Precip.	0.733	0.621	0.704	0.703	0.045

Figure 2.1: (A) Map of Kenya with red square indicating location of the study region in western Kenya. (B) Map showing the boundaries of Asembo and the streams within the community, with black dots representing all households. (C) 10 by 10 km study site shown as dashed black line with thirty-one 500 by 500 m quadrats surveyed for larval habitats shown as red boxes. (D) Location of the neighboring villages of Aduoyo-Miyare and Nguka, sites of the 15 monthly ground surveys for larval habitats, shown in red.

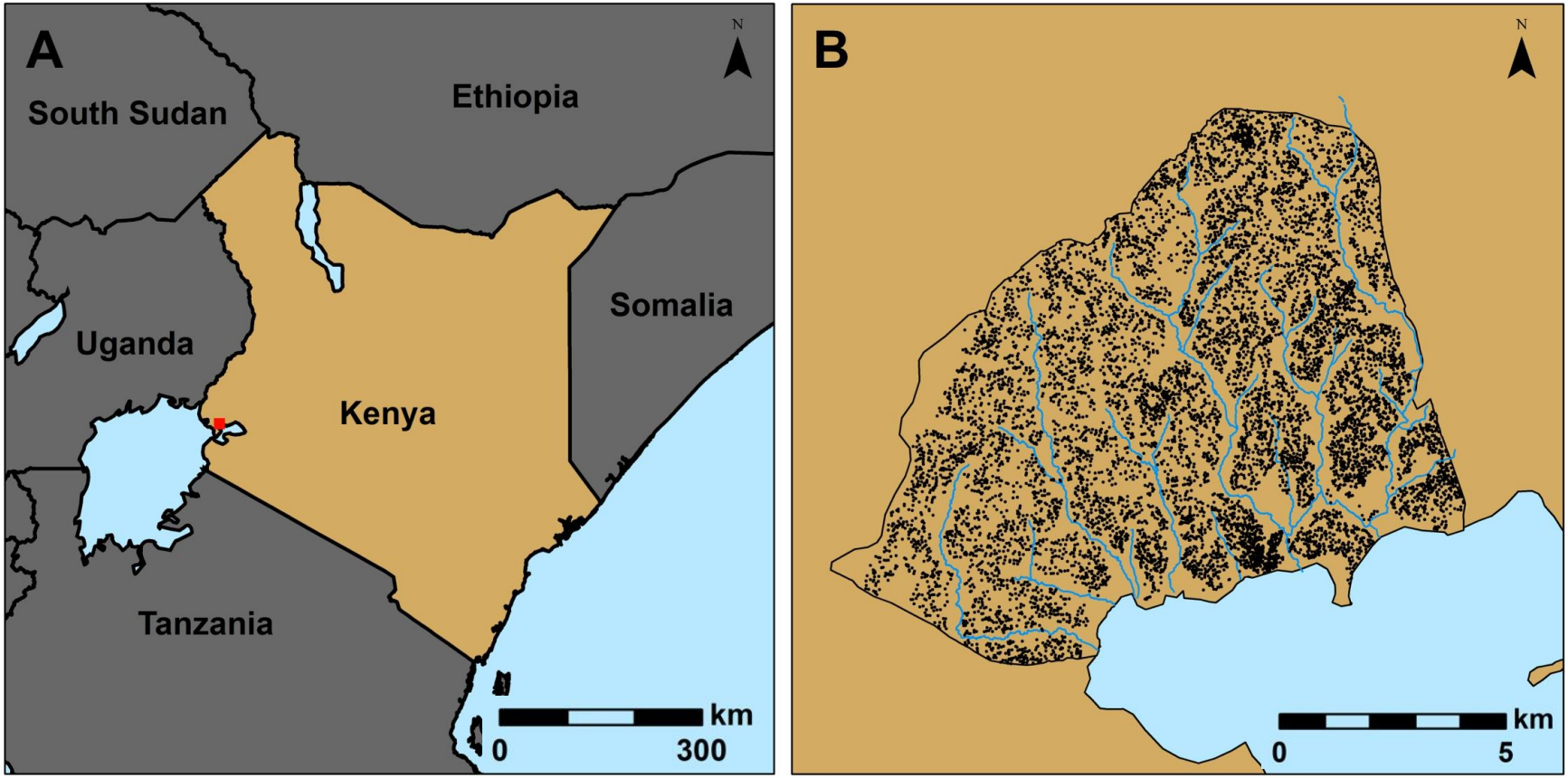


Figure 2.1 (cont'd)

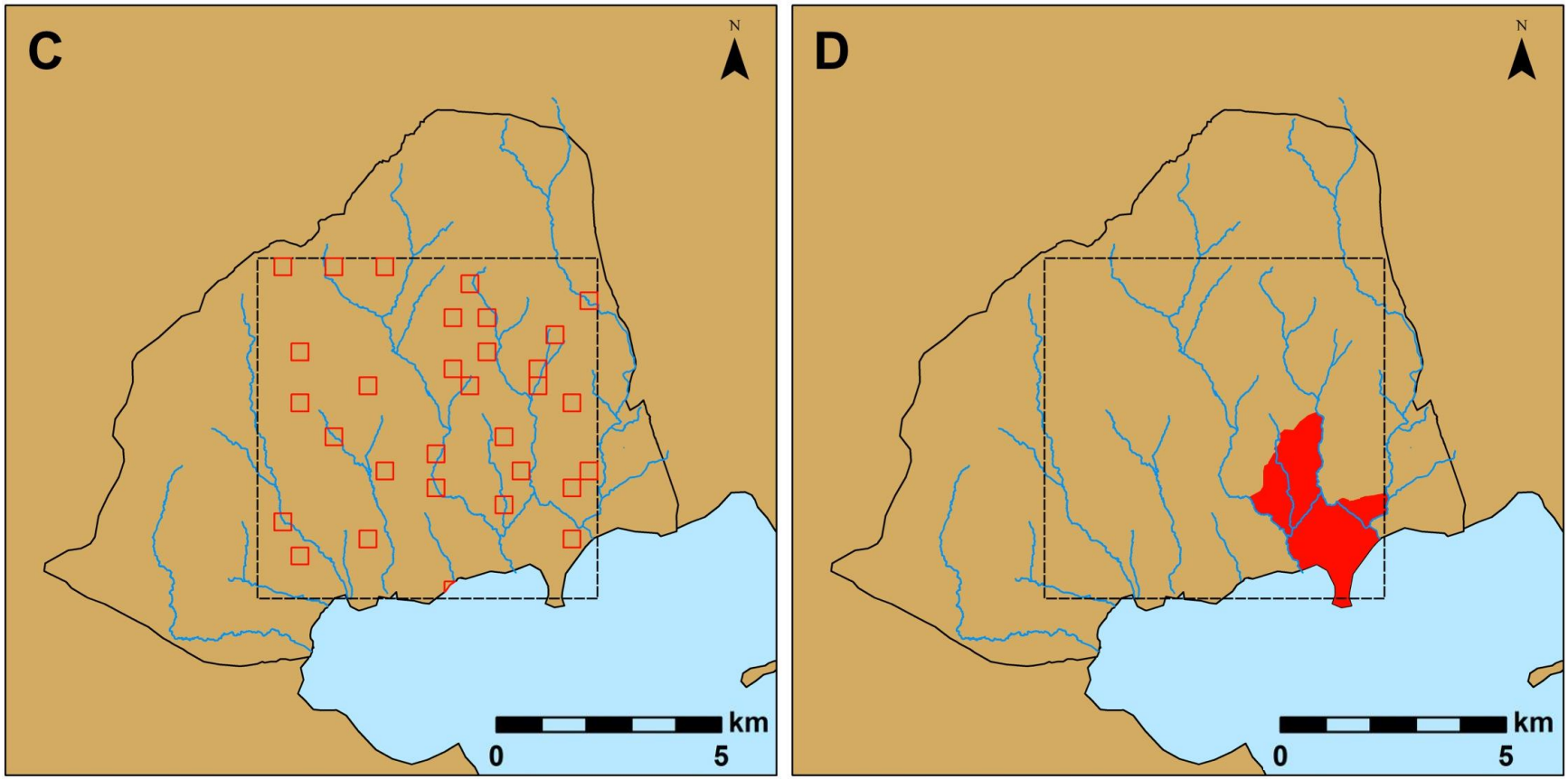


Figure 2.2: Scatter plot of the number of habitats recorded in Aduoyo-Miyare and Nguka each month (n = 15) by the cumulative 30-day precipitation for the last day of ground surveys for that month. Line shows prediction of linear regression, $R^2 = 0.1931$, $p = 0.1012$. The red and blue boxes highlight variation in the residual error discussed further in the text.

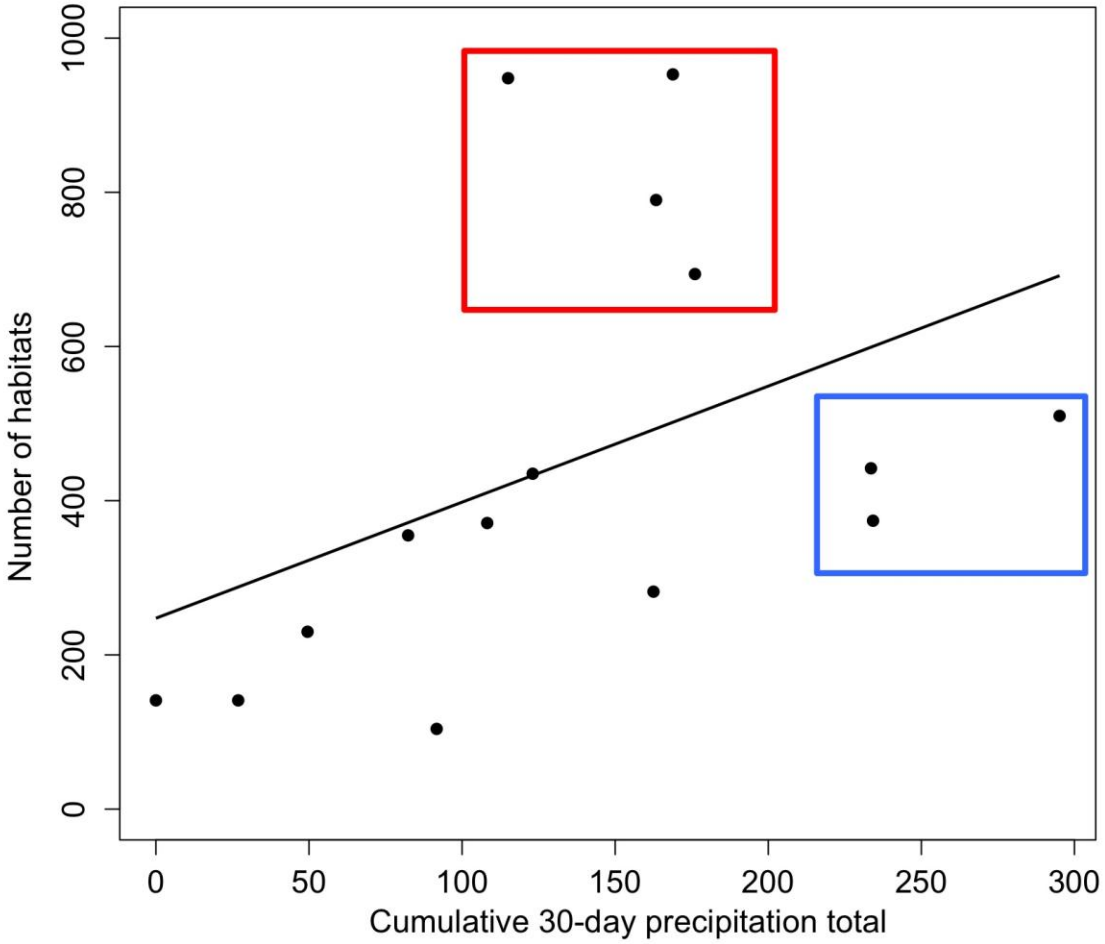


Figure 2.3: Precipitation data from the Kisumu airport (about 40 km east of Asembo) for March 2011 through July 2012. Bars show daily precipitation total. Solid line shows the cumulative 30-day precipitation total used in the logistic regression model for the 15-month dataset, while the dashed line shows the cumulative 21-day total used in the random forest model of the same data. Circles and squares show the last day of ground surveys for each month in Aduoyo-Miyare and Nguka.

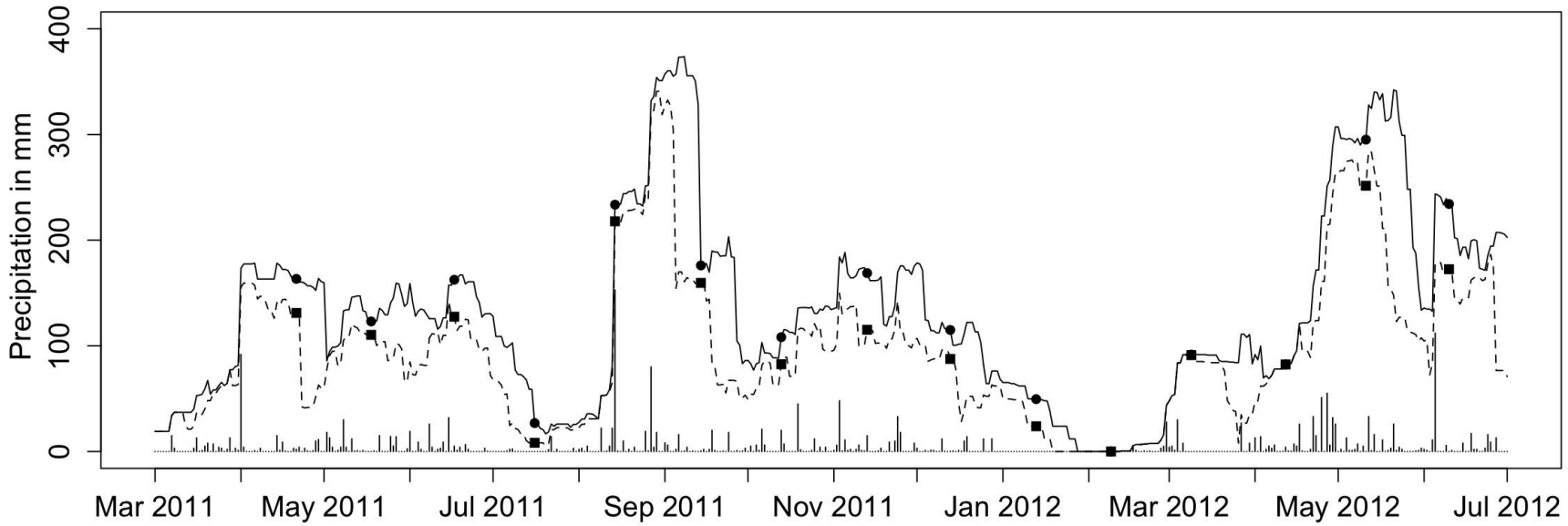


Figure 2.4: Precipitation data from the Kisumu airport (about 40 km east of Asembo) for 2 May 2011 to 4 July 2012. Bars show daily precipitation total. Solid line shows the cumulative 6-day total used in the logistic regression model of the 10 by 10 km dataset, while the dashed line shows the cumulative 14-day precipitation total used in the random forest model of the same data. Squares and circles show the days of the ground surveys in the 31 quadrats.

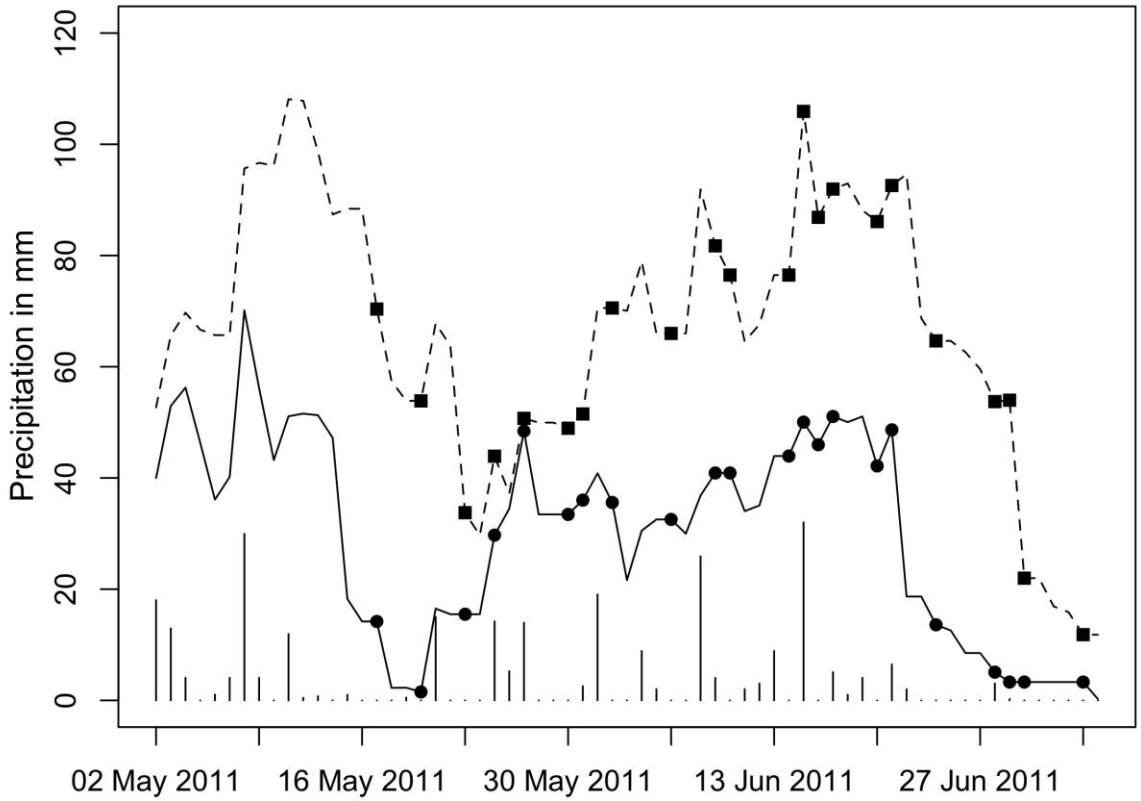
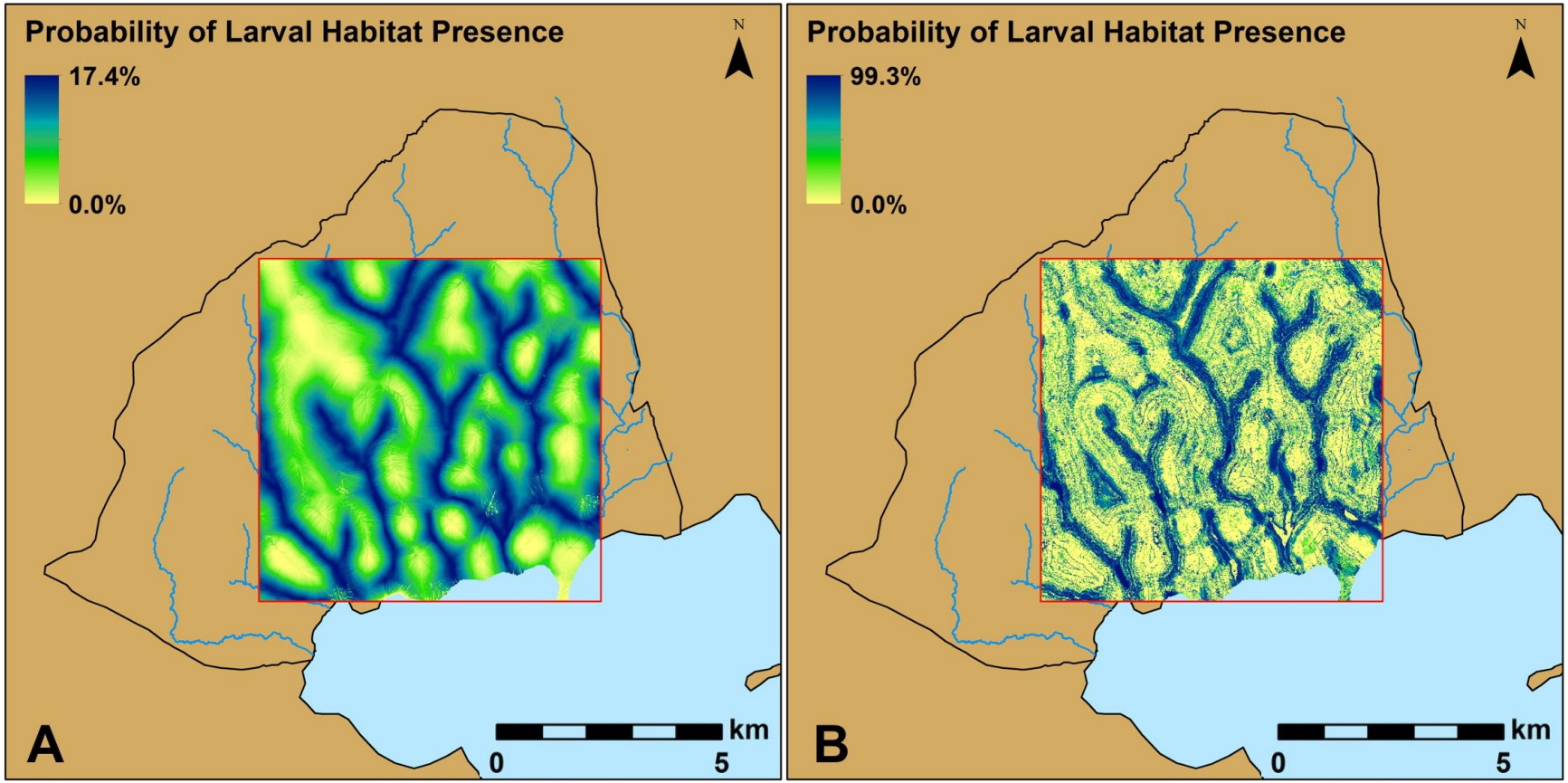


Figure 2.5: Probability of larval habitat presence across the 10 by 10 km study site as predicted by (A) the logistic regression model using TWI, distance to stream and soil type; and (B) the random forest model using TWI, distance to stream, soil type and LULC.



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CHAPTER III: Proximity of larval *Anopheles gambiae* habitats to houses and insecticide-treated bed net use both explain variation in adult *An. gambiae* indoor resting densities.

Abstract

The spatial heterogeneity of malaria has long been recognized, but the specific determinants of spatial variation in malaria risk may vary according to the landscape. Additionally, public health interventions may influence the spatial distribution of malaria. Houses in western Kenya were sampled for adult *Anopheles* females at the end of the rainy season in 2011. The proximity of the sampled houses to larval habitats was assessed using the predictive larval habitat model described in Chapter III. Variation in household-level factors, including use of long-lasting impregnated nets (LLINs), was also assessed. The number of adult female *An. gambiae* s.l. per house increased with increasing mean probability of larval habitat presence within 500 m. This index of habitat proximity was a better predictor of *An. gambiae* s.l. female abundance than simply measuring the distance to the nearest larval habitat. Also, houses in which all residents slept under LLINs the previous night had fewer adult female *An. gambiae* s.l. than houses in which only some, or none, of the residents used LLINs the previous night. There was no difference in the number of *An. gambiae* s.l. females collected in houses without LLINs and houses where only some of the residents slept under LLINs. Our results describing the relationship between larval habitat locations and the number of adult malaria vectors in houses differ from previous findings, suggesting that this relationship may vary depending on the configuration of larval habitats within a given landscape. The difference between individual LLIN use and LLIN ownership at the household-level highlights an important consideration for malaria control program managers, as well as those modeling the potential effects of LLINs as an intervention.

Introduction

The spatial distribution of malaria prevalence within communities is heterogeneous in a wide range of ecological and epidemiological settings, especially when community-wide transmission intensity is low to moderate (Carter et al. 2000). While the recognition of spatial variation in malaria risk predates even the etiology of the disease (Boyd 1949), the specific determinants of malaria's spatial distribution vary locally. Even within communities, socioeconomic and immunological differences affect people's risk of malaria prevalence (Greenwood 1989, Gamage-Mendis et al. 1991, Mackinnon et al. 2000). Additionally, the ecology of the local malaria vectors determines the spatial distribution of malaria, often through the relative locations of the vectors' larval habitats and people's homes (Trape et al. 1992, Ribeiro et al. 1996, Thompson et al. 1997, Ghebreyesus et al. 1999). While closer proximity to the larval habitats of malaria vectors may lead to increased (Clark et al. 2008) or decreased (Clarke et al. 2002) incidence of clinical malaria, depending on the epidemiological context, it is nevertheless clear that the spatial distribution of malaria vectors has important implications for malaria transmission.

The spatial distribution of adult malaria vectors is determined, in part, by the landscape through its effects on hydrology and the locations of aquatic habitat for *Anopheles* larvae (Smith et al. 2013). In landscapes where larval habitats are restricted to a linear feature such as along a river or swamp, adult *Anopheles* densities per house increase with decreasing distance to the nearest larval habitat (Trape et al. 1992, van der Hoek et al. 2003, Bogh et al. 2007, Zhou et al. 2007). A similar relationship exists for some *Anopheles* species in landscapes where relatively few larval habitats are dispersed

among people's homes (Minakawa et al. 2002, Walker et al. 2013). If larval *Anopheles* habitats are considered as foci of vector production or malaria transmission (Carter et al. 2000), this relationship is intuitive. However, in regions where numerous larval habitats are spread across the landscape, the relationship between larval habitat locations and the spatial distribution of adult *Anopheles* is less clear, possibly because the foci of vector production overlap.

Public health interventions aimed at reducing malaria transmission also influence the spatial distribution of malaria. As one of the primary vector control measures used since the 1990s, insecticide-treated bed nets (ITNs) have a significant impact on malaria vectors, reducing their population sizes on a broad scale (Gimnig et al. 2003a, 2003b). At the household scale, variation in ITN ownership and use may lead to variation in the number of adult *Anopheles* found indoors. The presence of ITNs in houses may reduce the rate of entry (Mathenge et al. 2001), or increase the rate of exiting (Mathenge et al. 2001, Malima et al. 2008), by malaria vectors. Furthermore, despite the substantial increase in the number of households owning ITNs in many malarious countries over the last decade (World Health Organization 2012), variation in ITN use by individuals within households leaves some people unprotected (Korenromp et al. 2003, Eisele et al. 2009), even in countries implementing universal coverage with long-lasting impregnated nets (LLINs) (Macintyre et al. 2011). Quantifying the contribution of these interventions to the heterogeneity of malaria, relative to that of landscape factors such as the distribution of larval habitats, is critical for the effective implementation and evaluation of interventions.

The primary objective of this study was to quantify the contribution of larval habitat proximity to variation in the number of *Anopheles gambiae* s.l. resting in houses, within a landscape where larval habitats are numerous yet heterogeneously distributed. As an observational study, we also accounted for household-level variables that potentially influenced the number of *An. gambiae* s.l. in houses. In particular, LLIN use was of interest because of the relatively high proportion of households owning LLINs in the study site. We included additional variables in our analysis based on previous studies' findings of variation in the number of *Anopheles* found indoors associated with the following factors (Table 3.1): presence of cattle in the compound (Minakawa et al. 2002); whether the inhabitants had cooked in the house (Bockarie et al. 1994, Hiscox et al. 2013); different wall types (Zhou et al. 2007, Kirby et al. 2008); different roof types (Zhou et al. 2007); and the number of residents in the house (Kirby et al. 2008, Walker et al. 2013).

Methods

Study site. The Asembo region of Rarieda District in western Kenya (Figure 3.1A) is a rural community of about 60,000 people covering about 200 km^2. Most of the residents are subsistence farmers, and the landscape is largely dominated by small-scale agriculture. Small plots of land generally surround family-based groups of houses, or compounds, further arranged into villages. While the compounds are highly dispersed within villages, the boundaries between the 79 villages in Asembo are often discernable only by residents (Phillips-Howard et al. 2003). This is apparent in Figure 3.1B, which shows the roughly 10,500 compounds georeferenced within Asembo as of 2009 (Hightower et al. 1998, Ombok et al. 2010). Asembo sits in the lowlands along the shores

of Lake Victoria, with elevations ranging from 1,100 m to 1,400 m above sea level and low topographic relief. Rainfall is seasonally bimodal but may occur year round.

Similar to rainfall patterns, malaria transmission occurs year round, with seasonal peaks in May-July and October-November. Parasitemia rates in children under 5 were around 50% in 2009 (Hamel et al. 2011), although entomological inoculation rates have been estimated at less than 15 infectious bites per person per year since 2003 (MN Bayoh, unpublished data). The predominant species of malaria is *Plasmodium falciparum*. Two of the primary malaria vectors in the region are *An. gambiae* s.s. and *Anopheles arabiensis* (Beier et al. 1990, Taylor et al. 1990, Bayoh et al. 2010), the only two members of the *An. gambiae* s.l. species complex found here.

Larval habitats. Larval *An. gambiae* s.l. habitats are numerous and widespread in Asembo, yet heterogeneously distributed. The locations of 1,673 larval *An. gambiae* s.l. habitats were recorded in ground surveys conducted in thirty-one 500 by 500 m quadrats from 17 May to 4 July 2011 (Chapter III). The surveyed quadrats were randomly selected, after spatial stratification, from a 10 by 10 km area within Asembo. Using a topographic wetness index, land use-land cover, soil type, and distance to stream, we applied the random forest method (Breiman 2001) to produce a landscape model of larval habitats, predicting the probability of larval habitat presence at a 20 m resolution across the 10 by 10 km area (Figure 3.1C).

Adult mosquito sampling. Houses were sampled for indoor-resting adult *Anopheles* between 16 May and 24 June 2011 using the pyrethrum spray catch method (PSC) (Gimnig et al. 2003a, Silver 2008). This period was chosen to coincide with seasonal peaks of *Anopheles* populations, which occur after the March-May rainy season

(Beier et al. 1990, Taylor et al. 1990, Odiere et al. 2007). All collected *Anopheles* were morphologically identified to species according to Gillies and Coetzee (1987). Mosquito voucher specimens were deposited in the Albert J. Cook Arthropod Research Collection at Michigan State University (Table 3.2). To avoid edge effects in the assessment of larval habitat proximity on the number of adult *Anopheles* collected in houses, no houses were sampled within 1 km of the larval habitat model border (Figure 3.1D). Houses within the 8 by 8 km area were selected for sampling using two-stage cluster sampling. First, the 8 by 8 km area was divided into 1 by 1 km quadrats. Forty of these quadrats were randomly selected, and one house in each quadrat was randomly selected as the starting point for cluster sampling. The order in which the clusters were sampled was also randomly selected. Nine to nineteen nearest neighbor houses were sampled in each cluster, as time permitted, resulting in 40 clusters of 10 to 20 houses being sampled over the six-week period.

Seven household-level variables that potentially influenced the number of *Anopheles* in the sampled houses (Table 3.1) were assessed at the time of mosquito sampling through a visual inspection of the house and via a standardized survey. While specific numbers of LLIN users were counted in the surveys, houses were later categorized into three groups of LLIN use. The first group was houses where everyone who slept in the house the previous night used an LLIN. The second group of houses included those where some residents had slept under an LLIN the previous night while other residents had not. The third group of houses was those where no one had slept under an LLIN the previous night.

The locations of the sampled houses were recorded using GPS units to quantify the proximity of the houses to larval *An. gambiae* s.l. habitats based on the model described above. The best method for measuring the proximity of the houses to larval habitats was not clear *a priori*. In contrast to previous studies using only the distance to the nearest larval habitat, larval habitats in Asembo are abundant and may surround a house from multiple directions. Thus, the number of adult mosquitoes found in the house may depend on the density of larval habitats surrounding the house. Accordingly, several indices of larval habitat proximity were calculated for each house sampled during PSC (Table 3.3), and each index was evaluated as a univariate candidate model during model selection (below).

To calculate the distance to the nearest larval habitat and the number of larval habitats surrounding a house, the probability output from the predictive habitat model was converted to a binary surface of presence and absence using a threshold value (Chapter III). Alternatively, variation in the probability of habitat presence within locations above the threshold could contribute additional information about how the number of adult *An. gambiae* s.l. in a house is influenced by the surrounding landscape. Therefore, we assessed the probability values surrounding each house by calculating their maximum, minimum, and mean. Finally, the scale at which to measure these statistics was not obvious, though the dispersal range of *An. gambiae* s.l. is thought to be less than 1 km, generally (Carter et al. 2000). Thus, the statistics were calculated across a range of distances from 50 to 1,000 m (Table 3.3).

Statistical analysis. We used multiple regression with generalized linear models to quantify the relative contribution of the measured variables to the number of *An.*

gambiae s.l. in houses. These generalized linear models used a log link function and negative binomial error distribution to account for the over-dispersion and high number of zeros in the observed count data. To control for potential dependence among houses within the clusters of sampled houses described above, we used generalized estimating equations (GEE) within the generalized linear models. Additionally, we compared models with and without GEE to determine the potential effect of sampling the houses in clusters.

We evaluated a series of candidate models with the measured variables, using an information-theoretical approach for selecting models that best explained the observed variation in the number of *An. gambiae* s.l. in houses (Burnham and Anderson 2002). We considered eight variables (seven listed in Table 3.1 plus habitat proximity) to be potentially useful as predictors of adult female *An. gambiae* s.l. in houses, and we fitted models with all possible subsets of these parameters, calculating Bayesian Information Criterion (BIC) and model weights (w_i) for the candidate models. BIC quantifies the fit of the model to the observed data based on maximum likelihood while accounting for a bias of increased likelihood with an increasing number of parameters in the model. While models the lower BIC values were considered the best-fitting models to the data, model weights were used to reflect the degree of uncertainty about differences among competing models (Burnham and Anderson 2004). Habitat proximity indices were not combined into the same candidate model. Rather, univariate models with each of the habitat proximity indices were compared first. For the purposes of comparing the habitat proximity indices to each other, we compared the model weights of those univariate models. To quantify the contribution of larval habitat proximity to adult *An. gambiae* s.l.

abundances relative to the seven other household-level variables, we used the habitat proximity index from the univariate model with the lowest BIC.

Results

A total of 227 female *An. gambiae* s.l. were collected in the 525 houses sampled (mean = 0.432, range: 0-8). Houses varied considerably in their proximity to larval *An. gambiae* s.l. habitats. Distance from the sampled houses to the nearest predicted larval habitat ranged from 1 to 539 m (mean = 48 m). The number of predicted larval habitats within 500 m of the sampled houses ranged from 0 to 977 (mean = 462), while the mean probability of larval habitat presence within the same area ranged from 0.0 to 11.3% (mean = 3.5%). The larval habitat proximity index that best fit the observed variation in female *An. gambiae* s.l. collected per house was the mean probability of larval habitat presence within 500 m of a house (Table 3.4). However, there was support for considering three additional indices of larval habitat proximity to houses, though all three were highly and positively correlated with the mean probability of larval habitat presence within 500 m of a house. The number of habitats within 500 m and 300 m, as well as the mean probability of larval habitat presence within 300 m of a house, combined to make up the top 90% of model weights for the univariate larval habitat proximity candidate models (Table 3.4).

Overall, the model that best fit the observed variation in female *An. gambiae* s.l. collected per house included four variables: larval habitat proximity (mean probability within 500 m), LLIN use, whether residents cooked in the house, and whether cattle were present in the compound. Three additional models could not be ruled out based on the top

90% of model weights, but all three models included the four variables from the leading model (Table 3.5). Furthermore, in each of those models, the 95% confidence intervals for the regression estimates of the fifth variable overlapped with an estimate of no effect. In the leading model, after accounting for LLIN use, cooking in the house, and cattle in the compound, the number of adult female *An. gambiae* s.l. per house increased with increasing mean probability of larval habitat presence within 500 m (Figure 3.2). Houses in which all residents slept under an LLIN the previous night had fewer adult female *An. gambiae* s.l. than the other two groups of houses, in which only some, or none, of the residents used an LLIN the previous night (Table 3.6). We also collected fewer *An. gambiae* s.l. adult females from houses in which residents had cooked the previous night, and from houses without cattle in the compound (Table 3.6). Controlling for the effect of sampling in clusters resulted in a change of the standard errors for model coefficients of less than one percent.

Discussion

We have shown that the proximity of houses to larval *An. gambiae* s.l. habitats contributes significantly to the spatial distribution of adult *An. gambiae* s.l. in a region where larval habitats are numerous and spread widely across the landscape. This agrees broadly with findings in other landscapes (Trape et al. 1992, Bogh et al. 2007, Zhou et al. 2007), suggesting that *An. gambiae* s.l. females preferentially disperse relatively short distances between aquatic larval habitats, which are also oviposition sites, and adult-stage blood feeding sites. Presumably, this relates to the energetic costs of flight (Nayar and Van Handel 1971, Foster 1995).

We found variation in the predictive ability of different indices of larval habitat proximity. Indices related to the density of larval habitats within 300 to 500 m were better predictor variables for our data than simply measuring the distance to the nearest larval habitat (Table 3.4). This is likely due to a combination of the relatively large number of habitats present during the rainy season in this region and the distribution of the habitats around people's houses in this landscape. For houses less than about 100 m from the nearest larval habitat, we found considerable variation in the mean probability of larval habitat presence within 500 m (Figure 3.3). In contrast, in landscapes where the distance to the nearest larval habitat and the density of larval habitats surrounding a house are more closely related, their usefulness for explaining the number of adult *Anopheles* in houses will be similar. Thus, although larval habitats are related to the distribution of adult *Anopheles* in general, the specific predictive relationship between the two may vary depending on the configuration of larval habitats within a given landscape.

Variation in LLIN use also contributed to variation among houses in the number of *An. gambiae* s.l. in this study. In the houses we sampled, only households that did not own LLINs made up the group of houses in which none of the residents used LLINs. As expected, these houses had more *An. gambiae* s.l. than houses in which all of the residents used LLINs the previous night. Notably, houses in which only some of the residents used LLINs the previous night also had more *An. gambiae* s.l. than houses in which all of the residents used LLINs the previous night. In fact, there was no difference between the number of *An. gambiae* s.l. females collected in houses without LLINs and houses where only some of the residents slept under LLINs. A likely explanation is that people not sleeping under LLINs, even in houses with LLINs, represent potential hosts

for *An. gambiae* s.l. females. This is an important consideration for malaria control program managers, as well as those modeling the potential effects of LLINs as an intervention.

In addition to larval habitat proximity and LLIN use, the presence of cattle in the compound also contributed to variation in the number of *An. gambiae* s.l. among sampled houses. *Anopheles arabiensis* females are relatively opportunistic blood feeders, readily utilizing cattle as hosts in addition to humans (Takken and Verhulst 2013). *Anopheles gambiae* s.s. females are highly anthropophilic and respond preferentially to human odors at short distances (Pates et al. 2001). However, *An. gambiae* s.s. females respond to carbon dioxide (CO_2) alone (i.e. in the absence of other host cues) at distances up to 50 m (Lorenz et al. 2013), potentially using the CO_2 emitted from cattle to orient toward their eventual human host when cattle are kept near people's homes. Together, these responses to cattle potentially account for the higher number of *An. gambiae* s.l. in houses with cattle present in the compound.

Finally, cooking in the house the previous night was associated with collecting fewer *An. gambiae* s.l. females. Wood and charcoal are the predominant fuels for cooking in Asembo, and the smoke from firewood may reduce the number of *An. gambiae* s.l. indoors (Bockarie et al. 1994). However, Biran and colleagues (2007) found little evidence for a protective effect of smoke from domestic fires against mosquitoes in a recent systematic review. They note that three observational studies from the early 20th century in South and East Africa (De Meillon 1930, Symes 1930, Gibbins 1933) found no difference in the numbers of *Anopheles* between homes with and without smoke from

domestic fires. Furthermore, the increased risk of respiratory diseases linked to smoke from biofuel sources (Po et al. 2011) likely outweighs any potential decrease in the number of *Anopheles* indoors.

This study was aimed at quantifying the determinants of variation in *An. gambiae* s.l. females in houses during the yearly peak in population size. Clearly, *Anopheles* populations in the region vary seasonally (Beier et al. 1990, Taylor et al. 1990, Gimnig et al. 2003a, Odiere et al. 2007), and the relationships found here may change in magnitude or even direction with the seasons. Additionally, variation among years in precipitation patterns could influence the relationships found here. The advantage to this cross-sectional approach was our ability to cover a relatively large area, capturing greater variation in the landscape and potentially making our results more generalizable.

APPENDIX

Chapter III Tables and Figures

Table 3.1: Household-level variables recorded for houses where sampling was done for female *Anopheles*. For factors, all levels of the factor are listed, with the number of houses observed at each level shown in parentheses. LLIN, long-lasting impregnated net.

Variable	Summary of observed values		
Number of total residents	mean = 3	range: 1 - 9	
LLIN use	all (314)	some (81)	none (130)
Wall type	mud (280)	plastered (109)	other (136)
Roof type	thatch (161)	iron (364)	
Cattle present	no (166)	yes (359)	
Goats present	no (209)	yes (316)	
Cooked in house	no (342)	yes (183)	

Table 3.2: Record of deposition of voucher specimens for Chapter III. The specimens listed below have been deposited in the Albert J. Cook Arthropod Research Collection, Michigan State University (MSU) museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number 2013-14 have been attached.

Species	Life Stage	Quantity	Preservation
Anopheles gambiae s.l.	Adult female	5	Pinned
Anopheles gambiae s.l.	Adult male	5	Pinned

Table 3.3: Indices used to quantify the proximity of larval habitats to houses where sampling was done for female *Anopheles.* NA, not applicable.
*larval habitats predicted using a random forest model, converting probabilities to presence and absence with a threshold
†probability of larval habitat presence predicted using a random forest model

Larval habitat proximity index	Measured at x =
Distance to nearest habitat*	NA
Number of habitats* within x meters	50, 100, 300, 500, 1000 m
Minimum probability† of a habitat within x meters	50, 100, 300, 500, 1000 m
Maximum probability† of a habitat within x meters	50, 100, 300, 500, 1000 m
Mean probability† of a habitat within x meters	50, 100, 300, 500, 1000 m

Table 3.4: Leading candidate models (sum of $w_i > 0.90$) for comparison of larval habitat indices contribution to the number of *An. gambiae* s.l. in houses. Results for the distance to the nearest habitat are also shown for comparison. BIC, Bayesian Information Criteria; ΔBIC, difference in BIC from the lowest BIC; w_i, model weight; r, correlation of habitat index with mean probability of larval habitat within 500 m; P(habitat), probability of habitat presence. Remaining candidate models had ΔBIC > 7.0 and $w_i < 0.020$.

Habitat index	BIC	ΔBIC	w_i	r
Mean P(habitat) within 500 m	897.0	0.0	0.616	1.00
Number of habitats within 500 m	899.8	2.8	0.152	0.86
Number of habitats within 300 m	900.6	3.7	0.098	0.69
Mean P(habitat) within 300 m	900.9	4.0	0.084	0.77
Distance to nearest habitat	910.6	13.6	< 0.001	-0.22

Table 3.5: Leading candidate models (sum of $w_i > 0.90$) for quantifying the variation in number of *An. gambiae* s.l. in houses using household-level variables. BIC, Bayesian Information Criteria; ΔBIC, difference in BIC from the lowest BIC; w_i, model weight; df, degrees of freedom; LLIN, long-lasting impregnated nets. Remaining candidate models had ΔBIC > 6.5 and $w_i < 0.025$.

Model	df	BIC	ΔBIC	w_i
Cattle + Cooked + LLIN use + Larval habitat prox.	7	862.0	0.0	0.745
Cattle + Cooked + LLIN use + Larval habitat prox. + Goats	8	865.7	3.7	0.116
Cattle + Cooked + LLIN use + Larval habitat prox. + Number of people	8	868.2	6.2	0.034
Cattle + Cooked + LLIN use + Larval habitat prox. + Roof type	8	868.2	6.2	0.033

Table 3.6: Relative rate ratios of the number of *An. gambiae* s.l. per house, according to the variables in the leading negative binomial regression model. Note that all models were analyzed with larval habitat indices as continuous variables; mean probability of habitat, P(habitat), within 500 m is presented here as a factor for a more intuitive interpretation. LLIN use, long-lasting impregnated nets.

Variable	Relative Rate Ratio (95% Confidence Interval)
Mean P(habitat) within 500 m	
0.000 - 0.020	1.00
0.021 - 0.040	1.68 (1.01, 2.79)
0.041 - 0.070	2.01 (1.24, 3.26)
0.071 - 0.113	3.99 (2.10, 7.59)
LLIN use	
all	1.00
some	2.13 (1.29, 3.51)
none	2.46 (1.63, 3.72)
Cooked in house	
no	1.00
yes	0.32 (0.20, 0.52)
Cattle in compound	
no	1.00
yes	2.44 (1.52, 3.92)

Figure 3.1: (A) Map of Kenya with red square indicating location of the study region in western Kenya. (B) Map showing the boundaries of Asembo and the streams within the community, with black dots representing all compounds in the community. (C) Probability of larval habitat presence as predicted by a random forest model across a 10 by 10 km area in Asembo. (D) 8 by 8 km border for pyrethrum spray catch sampling shown as dashed black line, with red dots showing the 525 houses sampled for adult *Anopheles* and solid red line representing the extent of the larval habitat model.

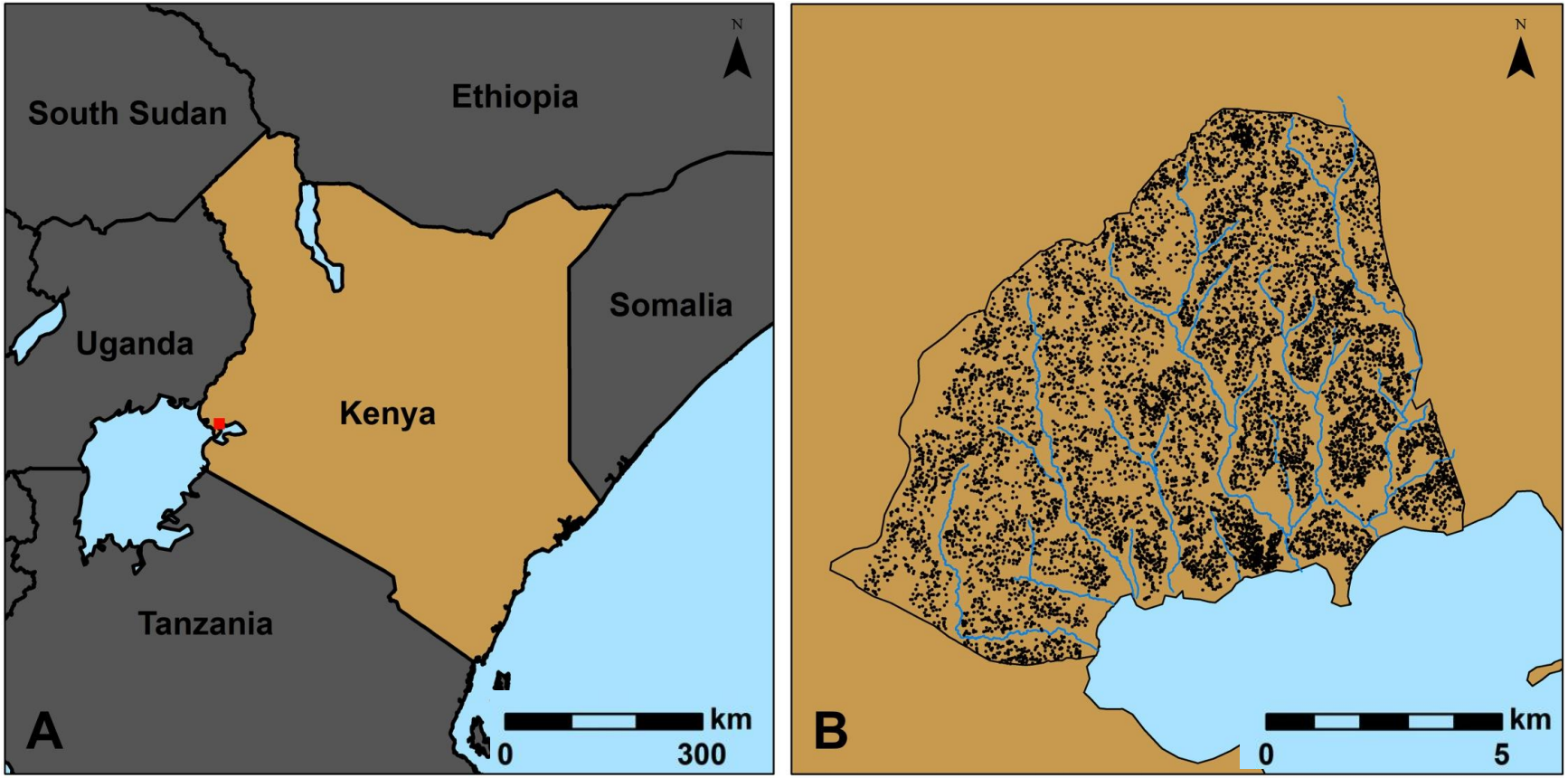


Figure 3.1 (cont'd)

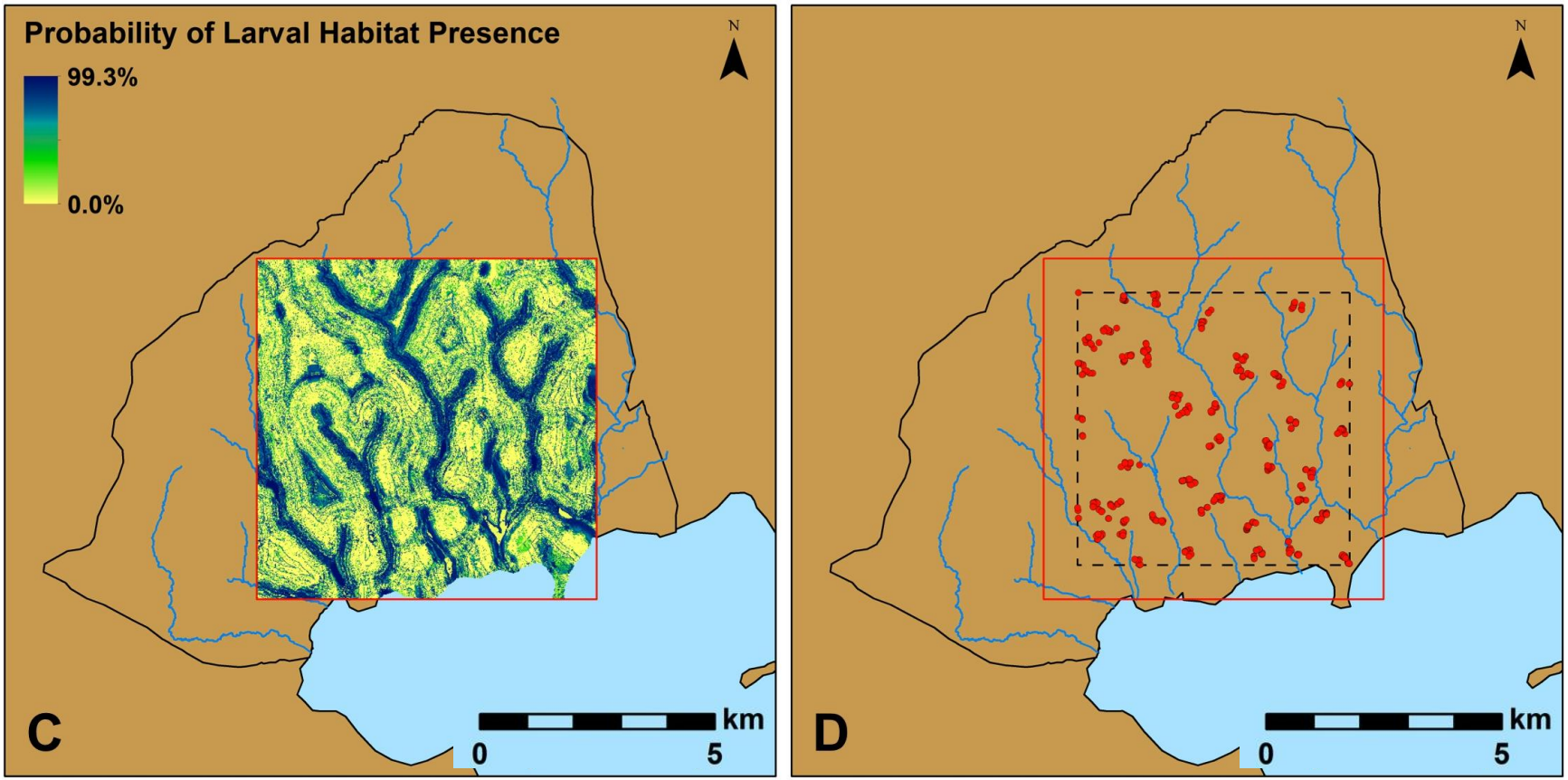


Figure 3.2: Estimated number of adult female *An. gambiae* s.l. per house according to the leading model. Shading bounded by dashed lines indicates 95% confidence intervals. Levels of LLIN use refer to whether all, some, or none of the residents used LLINs in the house the previous night. Estimates are shown for houses where cattle are present in the compound (i.e. cattle = yes), but residents did not cook in the house (cooked = no).

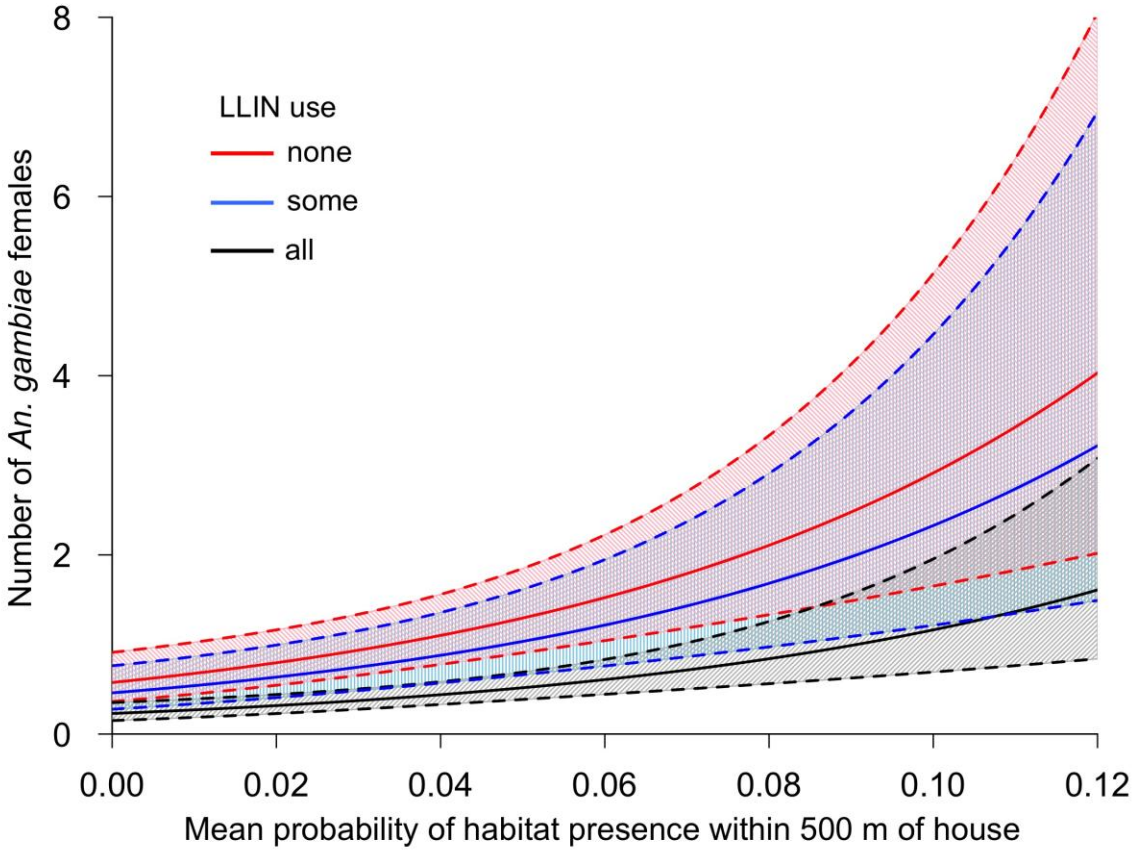
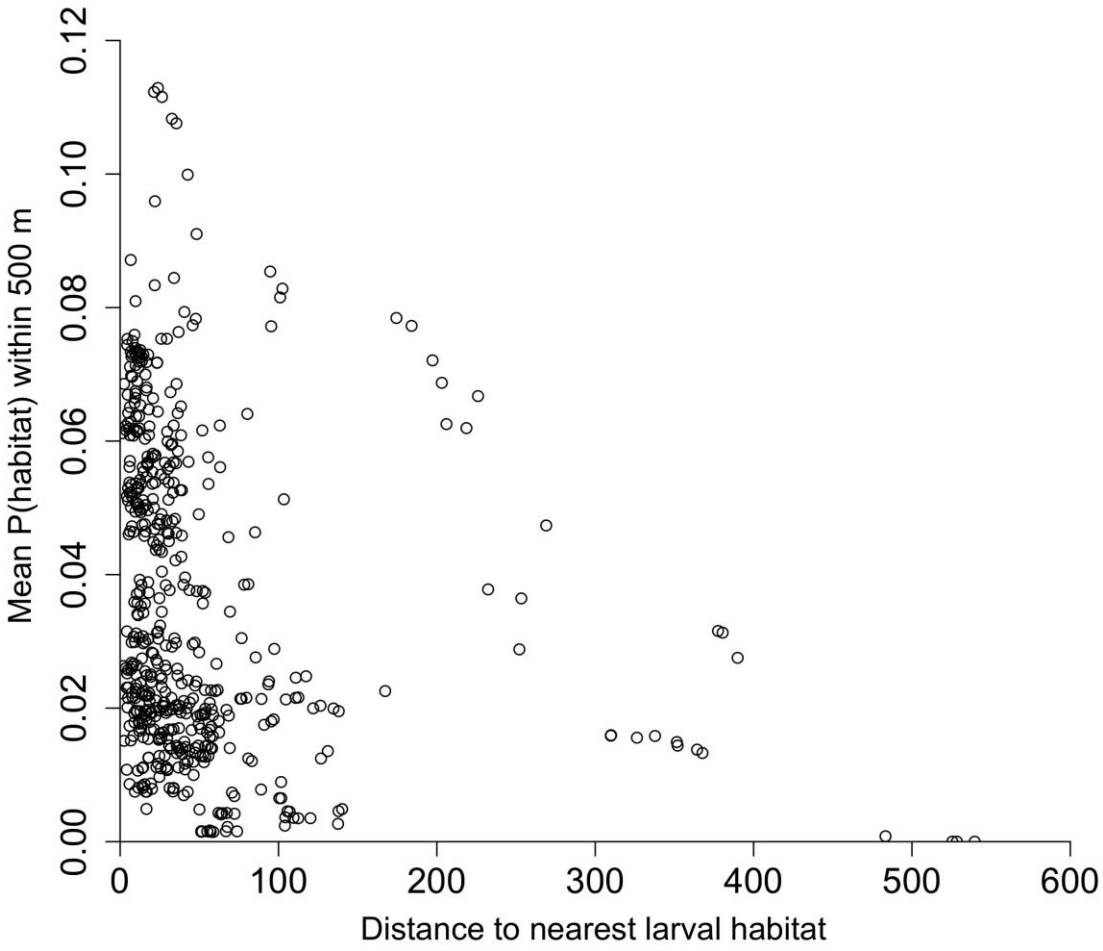


Figure 3.3: Scatterplot of the mean probability of larval habitat presence within 500 m of a house and the distance to the nearest larval habitat to the house (r = 0.22) for the 525 houses sampled in this study.



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CHAPTER IV. Reemergence of *Anopheles funestus* as a vector of *Plasmodium falciparum* in western Kenya after long-term implementation of insecticide-treated bed nets

Abstract

Historically, the malaria vectors in western Kenya have been *Anopheles funestus*, *Anopheles gambiae* s.s., and *Anopheles arabiensis*. Of these species, *An. funestus* populations declined the most after the introduction of ITNs in the 1990s in Asembo, and collections of *An. funestus* in the region remained low until at least 2008. Contrary to findings during the early years of ITN use in Asembo, the majority of the *Anopheles* collected here in 2010 and 2011 were *An. funestus*. Female *An. funestus* had characteristically high *Plasmodium falciparum* sporozoite rates and showed nearly 100% anthropophily. Female *An. funestus* were found more often indoors than outdoors and had relatively low mortality rates during insecticide bioassays. Together, these results are of serious concern for public health in the region, indicating that *An. funestus* may once again be contributing significantly to the transmission of malaria in this region despite the widespread use of ITNs/LLINs.

Introduction

Significant regional reductions in malaria-related morbidity and mortality have occurred globally since 2001 (World Health Organization 2012), but these outcomes may reverse if lessons from the past are not heeded. The Global Malaria Eradication Program (GMEP) of the 1950-60s succeeded in eliminating malaria from many countries and reducing malaria vector populations and malaria transmission substantially in others where elimination was not achieved (Feachem et al. 2010, Nájera et al. 2011). Unfortunately, resurgences in vector populations and transmission occurred in many of

these countries, likely due to a combination of reductions in program funding, economic and political changes, reintroductions of the malaria parasite by movement of people, development of drug resistance in malaria parasites, and evolution of insecticide resistance in vector populations (Feachem et al. 2010, Nájera et al. 2011). As the global health community strives once again toward the goal of malaria elimination and eradication, it is therefore vital to quantify and understand the causes of any resurgence in malaria transmission or malaria vector population size.

In western Kenya, malaria continues to cause significant morbidity and mortality despite public health efforts to reduce malaria prevalence (Zhou et al. 2011, Hamel et al. 2011). These efforts include the widespread distribution of long-lasting impregnated nets (LLINs), which target indoor biting malaria vectors (Bayoh et al. 2010). While high community-level coverage with LLINs reduces morbidity and mortality caused by malaria in the short term through impacts on malaria vector populations (Hawley et al. 2003), the long-term effectiveness of this intervention depends, in part, on whether insecticide resistance evolves in vector populations (Ranson et al. 2011, Trape et al. 2011).

Historically, the primary malaria vector mosquito species in western Kenya were *Anopheles gambiae* sensu stricto (s.s.) and *Anopheles funestus* (Beier et al. 1990, Taylor et al. 1990). *Anopheles arabiensis* was historically considered a secondary malaria vector in the region (Beier et al. 1990, Taylor et al. 1990), though the role of *An. arabiensis* in malaria transmission has become increasingly important in the last decade as conventional insecticide treated bed nets (ITNs) and LLINs have reduced the abundance of the other vector species (Bayoh et al. 2010). Following the introduction of ITNs in the

Asembo region of western Kenya in 1997 during a large scale, randomized trial, the population of *An. funestus* declined the most of these three species (Gimnig et al. 2003). The population of *An. funestus* remained low for several years after the trial ended and as ITNs were distributed nationally (Lindblade et al. 2004, Bayoh et al. 2010).

In Kenya resistance to the pyrethroid insecticides used in ITNs has been reported largely in *An. gambiae* sensu lato (s.l.) (Vulule et al. 1994, Mathias et al. 2011, Ochomo et al. 2013), a species complex of at least eight closely related species including *An. gambiae* s.s. and *An. arabiensis* (Coetzee et al. 2013). There are few published studies on insecticide resistance in *An. funestus* from Kenya, although reports of resistance to pyrethroids, carbamates, and DDT in *An. funestus* from other regions of Africa have increased over the last decade (Coetzee and Koekemoer 2013). Notably, pyrethroid resistance in *An. funestus* was associated with an increase in malaria cases in South Africa in the 1990s (Hargreaves et al. 2000).

The purpose of this study was several-fold. First, we investigated relative abundances, human biting rates, and malaria infection rates of *An. funestus* and other malaria vectors in the same western Kenyan region (Asembo) where vector abundances had decreased previously due to ITN distribution programs (Gimnig et al. 2003, Lindblade et al. 2004), to determine if vector abundances and malaria transmission remained similarly low or had increased despite continued high coverage of ITNs and LLINs. Additionally, the sensitivity of the vector populations to pyrethroid insecticides in ITNs and LLINs was measured through standardized bioassays to investigate the possibility that pyrethroid resistance could explain changes in the malaria vector populations.

Methods

Study site. Adult *Anopheles* mosquitoes were sampled in the Asembo region of Rarieda District (Figure 4.1). Numerous studies of malaria vectors have been conducted here since the 1970s, and the region has been described in detail (Phillips-Howard et al. 2003, Gimnig et al. 2013). Malaria is holoendemic in Asembo and is caused chiefly by *Plasmodium falciparum*. Rainfall occurs year-round but is seasonally bimodal, with peaks occurring from March through May and in November and December. Mosquito sampling intervals for the indoor resting and human landing studies in 2010 and 2011 (below) were chosen to coincide with seasonal peaks of *Anopheles* populations, which occur after the March-May rainy season (Beier et al. 1990, Taylor et al. 1990, Odiere et al. 2007).

As mentioned previously, Asembo was the site of a randomized, controlled trial of ITNs from 1997 to 1999 (Phillips-Howard et al. 2003). Following the trial, ITNs were distributed to control villages, and the ITNs were retreated every 6 to 9 mo with permethrin until 2002, then with alphacypermethrin until 2007 (Lindblade et al. 2004, Bayoh et al. 2010). This program in Asembo covered a population of approximately 55,000 persons as of 1997 (Phillips-Howard et al. 2003), which grew to 66,727 by 2011 (F. Odhiambo, personal communication). Nationwide, ITNs became available at partially subsidized rates through the retail sector in 2002, and at heavily subsidized rates through health clinics in 2004 (Noor et al. 2007). Furthermore, the Kenya Division of Malaria Control provided LLINs in mass distribution campaigns in 2006 and in June 2011, resulting in high ownership with the goal of reaching universal coverage, or one LLIN for every two people (Division of Malaria Control 2009).

Sampling the indoor resting populations. Indoor-resting *Anopheles* were sampled from 14-18 June 2010 and 16 May to 24 June 2011 using the pyrethrum spray catch method (PSC) as described by Gimnig and colleagues (2003). Sampling was done in 416 houses across an area of 15 km^2 in 2010 and in 806 houses across an area of 100 km^2 in 2011, and each house was sampled once. Differences between years were examined to determine whether grouping the data across the two sampling periods was appropriate, with analyses performed using R version 2.14.2 (R Development Core Team, Vienna, Austria). Differences between years in the number collected per house within each *Anopheles* species within each sex were determined using non-parametric Mann-Whitney tests. Differences between years within each *Anopheles* species in the proportions of blood meals from humans and cattle, and in the ELISA positivity rate (see below), were determined using Fisher's exact test.

Sampling the human landing populations. Host-seeking *Anopheles* were sampled indoors and outdoors in 75 villages (150 houses) covering about 200 km^2 from 13 June to 22 July 2011 using the human landing collection method (HLC) as described by Gimnig and colleagues (2013). Local adult men were trained as collectors and organized into 38 teams of 4 persons from two neighboring villages with the exception of 1 team that consisted of only 2 collectors. Each team rotated among four collection sites, sampling for four nights every week. Collectors working outdoors were given discretion to stop collections during rainfall, indicating any hour for which collections were stopped because of rain. Collectors working indoors were instructed to continue regardless of

rainfall. All collectors were provided atovaquone proguanil as malaria prophylaxis during the sampling period and for 7 days after the last night of collection.

Historical comparison. *Anopheles* females were sampled weekly in 19 villages in southern Asembo from 1993 through September 1997 using the bed net trap sampling method (BNT). From December 1996 to September of 1997, collections were done in 10 of the villages as the remaining villages had received ITNs as part of the large-scale trial. BNTs consisted of untreated nets hung above the sleeping spaces of volunteers so that the bottom edge of the net was approximately 6 cm above the bed. Mosquitoes that fed on the sleeper and remained resting inside the net were collected the following morning using mouth aspirators. The sleepers were not at an additional risk for malaria during BNT sampling as ITNs were not the standard of care at the time.

From 2002 through 2008, *Anopheles* females were sampled monthly using CDC light traps (LT). Light traps were set inside houses next to persons sleeping under their own ITNs. Each person was instructed to turn on the trap just before they went to sleep and mosquitoes were collected from traps in the morning. The traps were set in 30-60 houses each month and run for two consecutive nights in each house.

Collection rates of LT relative to HLC from Wong and colleagues (2013) were used to compare the LT data from 2002 through 2008 to the HLC data from 2011. Collection rates of BNT relative to HLC, calculated as described below, were used to compare the BNT data from 1993 through 1997 to the HLC data from 2011. Only data from June and July of each year of BNT and LT sampling were used in these comparisons to avoid any potential bias caused by seasonal variation in the abundance of either *An. gambiae* s.l. or *An. funestus*.

A comparison of the relative collection rates of HLC and BNT was done using weekly samples of *Anopheles* from a pool of 91 houses in 15 villages covering about 70 km^2 in Asembo from 1 Nov 1992 to 1 May 1993. BNT and HLC methods were carried out as described above. HLC and BNT were done in the same houses, with HLC always done 1-3 nights after BNT for a given house. The collection rate of BNT relative to HLC was calculated using a generalized linear model with a log link function and negative binomial error distribution. To account for repeated measures within houses, an autoregressive correlation structure was used, allowing for correlation between collections to decrease with increasing time. Analyses were performed using PROC GENMOD in SAS version 9.2 (SAS Institute, Cary, NC, USA).

Mosquito identification, sporozoite rates and blood meal analysis. All *Anopheles* were morphologically identified to species according to Gillies and Coetzee (1987). Specimens from PSC and HLC sampling identified morphologically as part of the *An. gambiae* s.l. species complex were further differentiated to the species level by PCR using primers specific to *Anopheles gambiae* s.s. and *An. arabiensis* (Scott et al. 1993). All females collected during PSC and HLC were separated at the line of the thorax and abdomen to allow for separate diagnostic tests on each specimen. The heads and thoraces of those females were tested for *P. falciparum* sporozoite proteins by ELISA (Wirtz et al. 1987) using the *P. falciparum* sporozoite ELISA reagent kit (MRA-890, MR4, ATCC® Manassas, VA). The blood meal hosts of all fed and half-gravid females collected during PSC were identified by direct sequencing of the vertebrate mitochondrial cytochrome B gene (Hamer et al. 2008). Mosquito voucher specimens were deposited in the Laboratory

of Entomology at the Center for Global Health Research, Kenya Medical Research Institute/Centers for Disease Control and Prevention in Kisian, Kenya (Table 4.1).

Insecticide bioassays. Insecticide susceptibility assays were carried out following the World Health Organization protocol (World Health Organization 1998) using *An. gambiae* s.l. and *An. funestus* adults collected from houses in Asembo using backpack aspirators from May through October 2012. Only unfed females not injured during collections were retained for bioassays. These mosquitoes were held for 1 h with access to 10% sugar solution, then exposed in the field to insecticide-impregnated filter paper for 1 h. Following exposure the mosquitoes were transferred to a clean holding tube with 10% sugar solution, and mortality was determined 24 h later. The mosquitoes were exposed to the pyrethroids permethrin (at a concentration of 0.75%) and deltamethrin (0.05%), and the carbamate bendiocarb (0.01%).

Ethical approval. This work was approved by the Institutional Review Boards of the U.S. Centers for Disease Control and Prevention and Michigan State University and by the Ethical Review Committee of the Kenya Medical Research Institute.

Results

Indoor resting samples. A total of 1,897 *Anopheles* females and 1,033 *Anopheles* males were collected indoors during PSC sampling, and greater than 99% of the *Anopheles* collected were identified morphologically as either *An. funestus* or *An. gambiae* s.l. The only other anopheline species found indoors during PSC sampling was *Anopheles rufipes*. A total of 830 (85%) of the *An. gambiae* s.l. specimens were successfully differentiated into *An. gambiae* s.s. and *An. arabiensis*, while PCR amplification failed or was not done in 146 *An. gambiae* s.l. specimens. The number of

Anopheles collected per house differed between the two PSC sampling periods, though *An. funestus* made up a large proportion of the *Anopheles* collected in both years (Table 4.2). In 2010 three quarters (75.2%) of all female *Anopheles* collected were *An. funestus*, and in 2011 most of the female *Anopheles* collected were either *An. funestus* (37.9%) or *An. arabiensis* (37.0%).

Blood meal hosts were identified in 946 of the 1,328 fed or half-gravid *Anopheles* females collected during PSC (Figure 4.2). The proportions of blood meals from humans and cattle in *An. funestus* did not differ between years (Fisher's exact test, $p = 1.00$). Nearly all of the 715 *An. funestus* blood meal hosts identified were from humans, though 2.5% were from cattle. The proportions of blood meals from humans and cattle in *An. gambiae* and *An. arabiensis* differed between years (Fisher's exact test, $p = 0.017$ and $p = 0.037$, respectively). In 2010, 75.7% of the 33 *An. gambiae* s.s. blood meals and 51.3% of the 115 *An. arabiensis* blood meals identified were from humans. In 2011, 94.5% of the 55 *An. gambiae* s.s. blood meals and 35.6% of the 73 *An. arabiensis* blood meals identified were from humans. The remaining blood meals in both species from both years were from cattle, except for a total of five blood meals from goats in *An. arabiensis*.

With the exception of *An. rufipes*, a proportion of individuals of all *Anopheles* species collected during PSC sampling were positive by ELISA for *P. falciparum* sporozoite infection, but the percentage positive varied by mosquito species (Table 4.3). The ELISA-positivity rate varied between years only for *An. funestus* (3.4% in 2010 and 8.9% in 2011, $p < 0.001$). The highest rate across both years was in *An. gambiae* s.s., and the lowest rate was in *An. arabiensis*.

Human landing samples. HLC sampling was done indoors for a total of 888 collector-nights (12,432 collector-hours) and outdoors for 889.4 collector-nights (12,451 collector-hours). During HLC sampling, 1,936 female *Anopheles* were collected. Greater than 98% of these were morphologically identified as either *An. funestus* or *An. gambiae* s.l., while the remaining specimens were *Anopheles coustani* and *An. rufipes*. A total of 380 (71%) of the *An. gambiae* species complex specimens were differentiated into *An. gambiae* s.s. (43%) and *An. arabiensis* (28%), while PCR amplification failed in 159 (29%) of the specimens. Most of the *Anopheles* females collected during HLC sampling were *An. funestus*, and more females of each species were collected indoors than outdoors (Figure 4.3). Similar to specimens from PSC sampling, ELISA-positivity rates varied among the three malaria vector species during HLC sampling. Overall ELISA-positivity rates for these specimens were highest in *An. gambiae* s.s. and *An. funestus*, while the lowest rate was found in *An. arabiensis* (Figure 4.3).

Historical comparison. In our analysis of relative collection rates, BNT sampling produced similar numbers of *An. funestus* relative to HLC sampling, but fewer *An. gambiae* s.l. were collected from BNT relative to HLC (Table 4.4). According to Wong and colleagues (2013), collections of both *An. funestus* and *An. gambiae* s.l. were lower from LT relative to HLC in Rarieda District. The collection rate of *An. funestus* from LT relative to HLC was 0.69 (95% CI: 0.49-0.98), while the relative collection rate of *An. gambiae* s.l. was 0.76 (95% CI: 0.61-0.96) (Wong et al. 2013).

Prior to the introduction of ITNs in Asembo, the populations of both *An. funestus* and *An. gambiae* s.l. were large according to BNT sampling (Figure 4.4). Few *An. funestus* were collected in Asembo using LT from 2002 through 2008. For the months of

June and July, which historically were the months when *An. funestus* populations peaked, zero *An. funestus* were collected in two years from the period, and never were more than 0.17 *An. funestus* per trap collected. During the same sampling period, the monthly mean of *An. gambiae* s.l. collected per trap ranged from 0.13 to 1.53 with a mean of 0.75 (Figure 4.4).

Insecticide bioassays. Figure 4.5 depicts mortality to bendiocarb, deltamethrin, and permethrin in wild caught *An. gambiae* s.l. and *An. funestus* adults from Asembo. A total of 78 *An. funestus* were exposed to deltamethrin, 38 to permethrin, and 34 to bendiocarb. A total of 159 *An. gambiae* s.l. were exposed to deltamethrin, 6 to permethrin, and 48 to bendiocarb. While mortality rates following exposure to bendiocarb were high, much lower rates were seen for *An. funestus* samples exposed to deltamethrin and permethrin, indicating resistance in these specimens to the insecticides used in ITNs and LLINs.

Discussion

Anopheles funestus was the dominant species collected in all three of our sampling efforts in 2010 and 2011, comprising between one third and three quarters of the female *Anopheles* collected (Table 4.2; Figure 4.3). Historically, *An. funestus* was common in our study region (Evans and Symes 1937, Beier et al. 1990, Taylor et al. 1990), and it remained abundant until the introduction of ITNs in the late 1990s (Figure 4.4). Following ITN distribution, *An. funestus* became rare and likely played a minor role in malaria transmission in the period thereafter as ITN coverage increased (Gimnig et al. 2003, Lindblade et al. 2006). The population of *An. funestus* in Asembo remained low through 2008 according to the LT sampling reported here (Figure 4.4), but our sampling

in 2010 and 2011 indicates a marked reversal of this trend. The reemergence of this species has important implications for malaria transmission, which has increased recently despite widespread distribution of ITNs and LLINs (Bayoh et al. 2010, Zhou et al. 2011, Hamel et al. 2011).

The low mortality rate of *An. funestus* collected from Asembo when exposed to the pyrethroids used in ITNs and LLINs (Figure 4.5) supports one possible mechanism by which the *An. funestus* population in the region has been able to increase, though a more extensive analysis is necessary to determine the scope of insecticide resistance in *An. funestus* populations within western Kenya. The potential for insecticide resistance to reduce the effectiveness of ITNs is widely recognized (Ranson et al. 2011), and the appearance of pyrethroid resistance in populations of *An. funestus* after indoor residual spraying in South Africa (Hargreaves et al. 2000) underscores that potential for this malaria vector. A recent review by Coetzee and Koekemoer (2013) covers the extent to which insecticide resistance in *An. funestus* is currently understood. Varying degrees of resistance have been reported across many regions of Africa, notably including Tororo District in eastern Uganda near the Kenyan border (Morgan et al. 2010). In addition to the reemergence of *An. funestus* in the 1990s after evolving pyrethroid resistance in South Africa (Hargreaves et al. 2000), the development of insecticide resistance in other malaria vectors during the Global Malaria Eradication Program provides numerous examples of the detrimental effects of insecticide resistance on malaria control (Busvine and Pal 1969). Thus, the reemergence of *An. funestus* in this region of high ITN and LLIN coverage indicates the need to further investigate the resistance characteristics of this population.

Anopheles funestus and *An. gambiae* s.s. were found here to have taken their blood meals almost exclusively from humans, highlighting one factor contributing to their high vectorial capacity, a measure of the rate at which a vector population transmits malaria (Garrett-Jones 1964). Similarly to *An. gambiae* s.s. (Bayoh et al. 2010), the presence of ITNs or LLINs appears not to shift *An. funestus* host selection away from humans. *Anopheles arabiensis* took a larger proportion of blood meals from cattle than did either *An. gambiae* s.s. or *An. funestus*, but *An. arabiensis* also took blood meals from humans, demonstrating broader variation in feeding behaviors typical of *An. arabiensis* (Takken and Verhulst 2013). Overall, the results from this blood meal analysis emphasize the need for additional vector control methods beyond the indoor, insecticide-based strategies that target only highly anthropophilic vectors preferring late-night, indoor feeding.

The high rate of sporozoite ELISA-positive females collected during both PSC and HLC sampling implies a substantial infective human reservoir of malaria in Asembo, corresponding with recent measures of *P. falciparum* prevalence in the region (Hamel et al. 2011). We found the highest ELISA-positivity rates in *An. gambiae* s.s. females, implying the continued importance of this vector to malaria transmission in the region. Similarly high ELISA-positivity rates were found in *An. funestus*, further indicating its reemergence as one of the primary malaria vectors in Asembo. The relatively lower rates of ELISA-positive *An. arabiensis* females during this study agree with findings from before the introduction of ITNs suggesting it has a lower vectorial capacity in the region than *An. gambiae* s.s. and *An. funestus* (Taylor et al. 1990).

The high proportion of *An. funestus* relative to *An. arabiensis* and *An. gambiae* in our indoor resting and human landing samples is an important indication of changes to malaria transmission in this region, and demonstrates how our most efficient and widely implemented malaria control interventions may have important limitations. *Anopheles funestus* is well known as an efficient malaria vector due to near 100% anthropophily, which was confirmed in this study along with high sporozoite rates. The distribution of ITNs in Asembo in the 1990s significantly impacted *An. funestus*, and collections of *An. funestus* in the region had remained low until at least 2008. The increased abundance of *An. funestus* reported in this study suggests a reemergence of this species as a primary malaria vector in western Kenya, and might be explained by the development of insecticide resistance. Public health officials here and throughout regions where ITNs or IRS are deployed should be aware of changes in malaria vector populations, especially as our findings indicate reduced effectiveness of ITNs as a long-term, stand-alone method for malaria control. The GMEP of the 1950-60s revealed the potential dangers of relying on a single method of vector control, as malaria transmission resurged in many areas where significant gains had been made previously. Strategies to attenuate insecticide resistance development and complementary control methods for integrated vector management (World Health Organization 2004) deserve a renewed focus as the global health community continues to fight malaria.

APPENDIX

Chapter IV Tables and Figures

Table 4.1: Record of deposition of voucher specimens for Chapter IV. The specimens listed below have been deposited in the Laboratory of Entomology at the Center for Global Health Research, Kenya Medical Research Institute/Centers for Disease Control and Prevention in Kisian, Kenya, as samples of those species, which were used in this research. Voucher recognition labels bearing the voucher number 2013-14 have been attached.

Species	Life Stage	Quantity	Preservation
Anopheles gambiae	Adult female	5	Pinned
Anopheles gambiae	Adult male	5	Pinned
Anopheles arabiensis	Adult female	5	Pinned
Anopheles arabiensis	Adult male	5	Pinned
Anopheles rufipes	Adult female	5	Pinned
Anopheles rufipes	Adult male	4	Pinned
Anopheles coustani	Adult female	5	Pinned
Anopheles funestus	Adult female	5	Pinned
Anopheles funestus	Adult male	5	Pinned

Table 4.2: Number of *Anopheles* collected per house during pyrethrum spray catch sampling shown by species, sex, and year. Percentages of the total for each column are shown in parentheses. P-values are from non-parametric Mann-Whitney tests to determine differences between years for each species within each sex. NA, not applicable.

*These specimens were identified as members of the *Anopheles gambiae* species complex according to morphological methods but could not be further differentiated by polymerase chain reaction.

	Females			Males		
Species	2010	2011	p-value	2010	2011	p-value
An. funestus	2.305 (75.2%)	0.293 (37.9%)	< 0.001	1.327 (80.0%)	0.231 (54.2%)	< 0.001
An. arabiensis	0.498 (16.2%)	0.285 (37.0%)	0.007	0.127 (7.7%)	0.104 (24.5%)	0.374
An. gambiae s.s.	0.099 (3.2%)	0.122 (15.8%)	0.230	0.149 (9.0%)	0.083 (19.5%)	0.052
An. gambiae s.l.*	0.163 (5.3%)	0.066 (8.5%)	< 0.001	0.055 (3.3%)	0.002 (0.6%)	< 0.001
An. rufipes	0.000 (0.0%)	0.006 (0.8%)	NA	0.000 (0.0%)	0.005 (1.2%)	NA

Table 4.3: *Plasmodium falciparum* sporozoite-negative and positive female mosquitoes, according to enzyme-linked immunosorbent assay (ELISA), from pyrethrum spray catch (PSC) sampling, shown by species with percent positive in parentheses.

[*] ELISA positivity rates for *Anopheles funestus* were significantly different between PSC sampling periods in 2010 and 2011 (3.4 and 8.9% respectively; Fisher's exact test, $p < 0.001$).

[†] These specimens were identified as members of the *Anopheles gambiae* species complex according to morphological methods but could not be further differentiated by polymerase chain reaction.

Species	Negative	Positive (%)
An. funestus	1140	54 (4.5)[*]
An. arabiensis	433	4 (0.9)
An. gambiae s.s.	127	12 (8.6)
An. gambiae s.l.[†]	119	2 (1.7)

Table 4.4: Comparison of human landing catch (HLC) and bed net trap (BNT) sampling methods for *Anopheles funestus* and *Anopheles gambiae* s.l., Nov 1992 to May 1993. Relative rates of collection and associated 95% CI and p-value were calculated using negative binomial regression. P-values indicate the probability that the relative collection rate of BNT sampling was different from that of HLC. NA, not applicable.

Method	Total	Mean per night (95% CI)	Relative rate (95% CI)	p-value
An. funestus				
HLC	730	1.25 (1.05, 1.45)	1.00	NA
BNT	917	1.57 (1.34, 1.79)	1.27 (0.92, 1.76)	0.145
An. gambiae				
HLC	2408	4.12 (3.47, 4.78)	1.00	NA
BNT	1389	2.38 (2.01, 2.74)	0.57 (0.44, 0.74)	< 0.001

Figure 4.1: (A) Map of Kenya with box indicating location of the study region in Nyanza Province. (B) Map showing the location of Asembo on the shores of Lake Victoria, about 50 km west of the city of Kisumu.

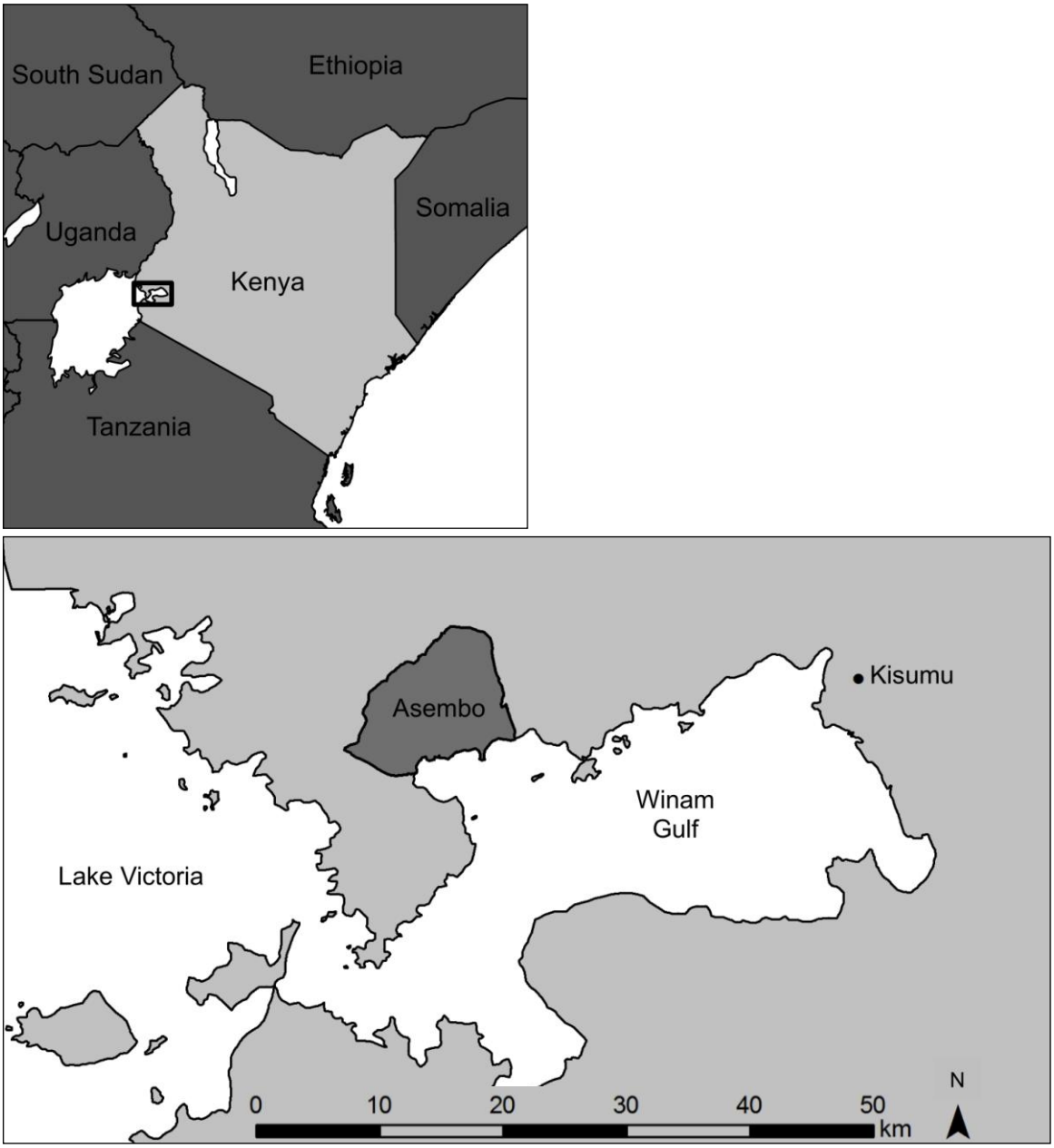


Figure 4.2: Proportion of blood meals taken from humans and cattle by *Anopheles* species collected during pyrethrum spray catch in Asembo. Non-amplifiers to polymerase chain reaction for blood meal identification are not shown (29%).
* Data pooled across years for *Anopheles funestus* because the proportions of blood meals from humans and cattle did not differ between years (Fisher's exact test, p = 1.00).

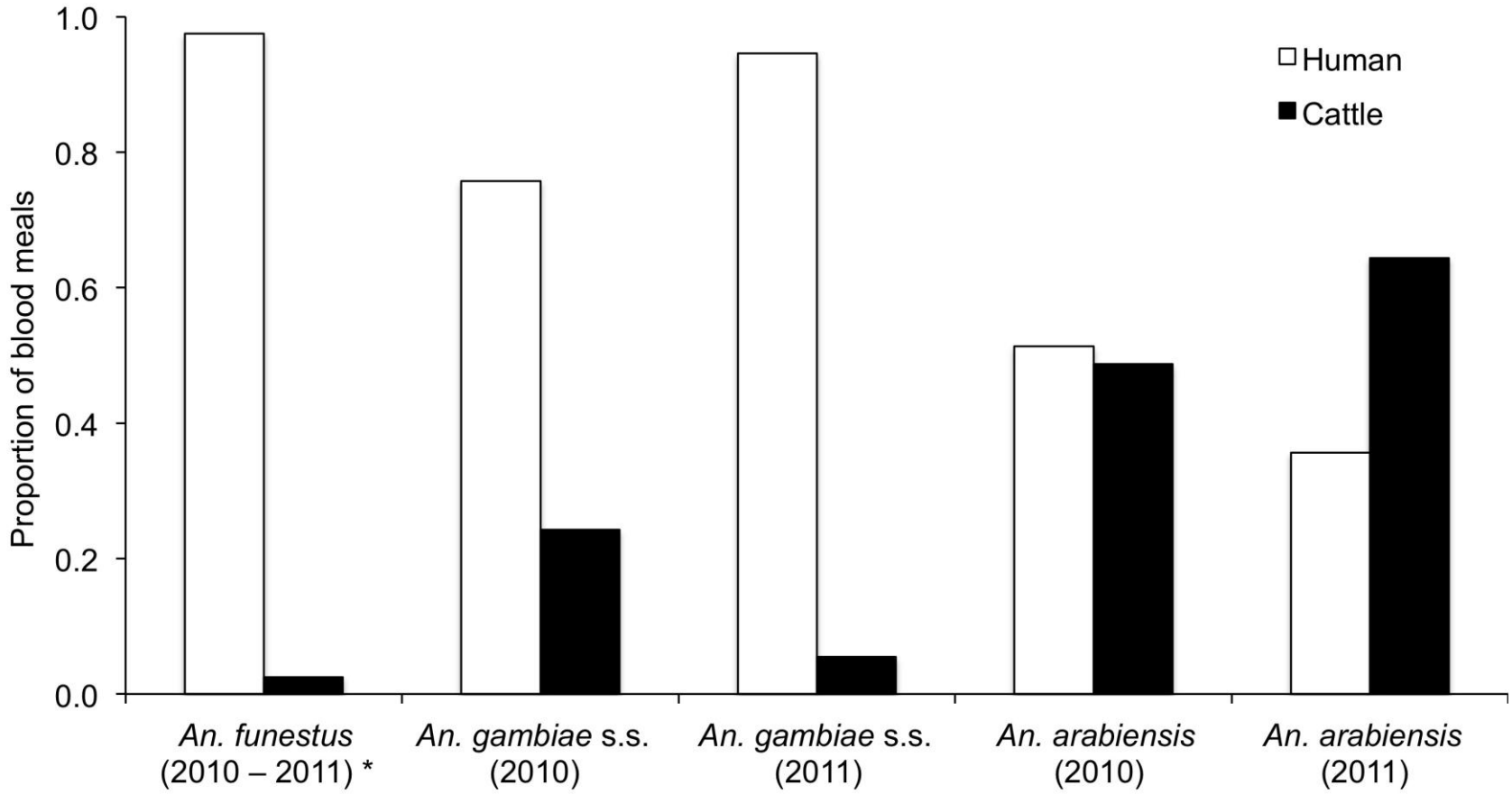


Figure 4.3: Number (No.) of *Anopheles* females collected per collector per night during human landing catch sampling shown by species and location (indoors/outdoors). Error bars are 95% confidence intervals. *Plasmodium falciparum* sporozoite rates as determined by enzyme-linked immunosorbent assays are shown as percentages above each species. *Anopheles gambiae* s.l. specimens were identified as members of the *An. gambiae* species complex according to morphological methods but could not be further differentiated by polymerase chain reaction.

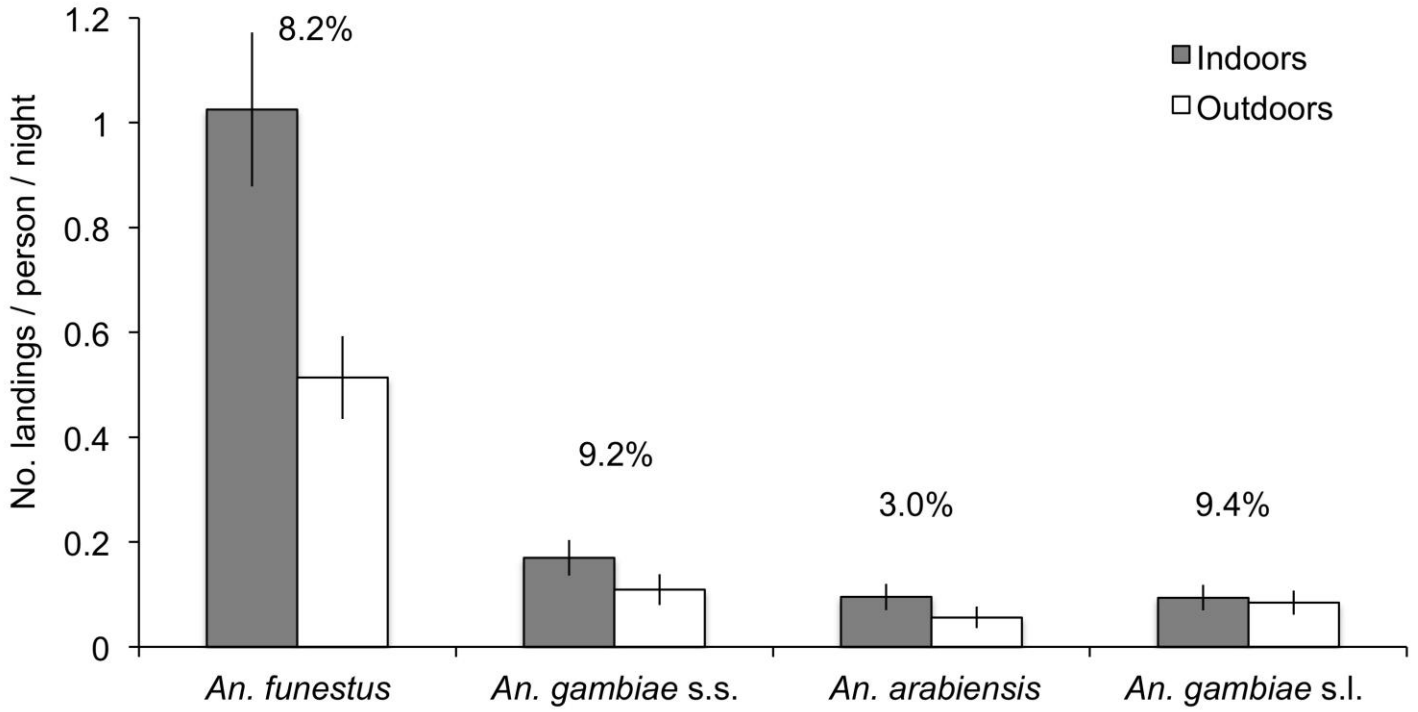


Figure 4.4: (A) Mean number of *Anopheles* females collected per sample in June through July from 1993 through 2011 using bed net traps (BNT; 1993-1997), light traps (LT; 2002-2008), and human landing catch (HLC; 2011). (B) Mean number of *Anopheles* females collected, adjusted for comparison to HLC sampling using the relative rates of BNT to HLC (1993-1997) and LT to HLC (2002-2008). (C) Proportion of total *Anopheles* females collected identified as either *Anopheles funestus* or *Anopheles gambiae* s.l. Error bars indicate 95% confidence intervals. Data were not available from 1998-2001, 2009, and 2010.

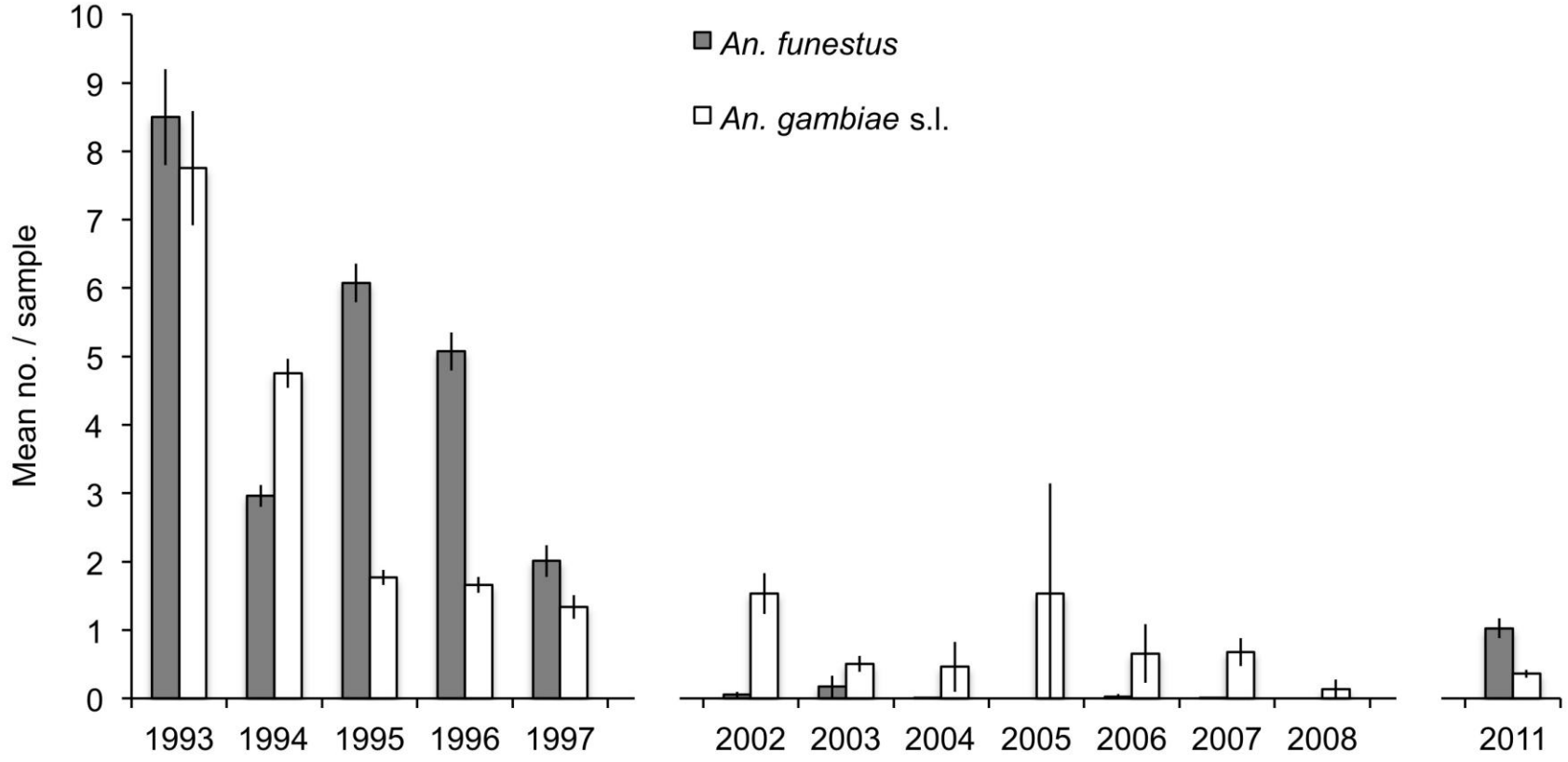


Figure 4.4 (cont'd)

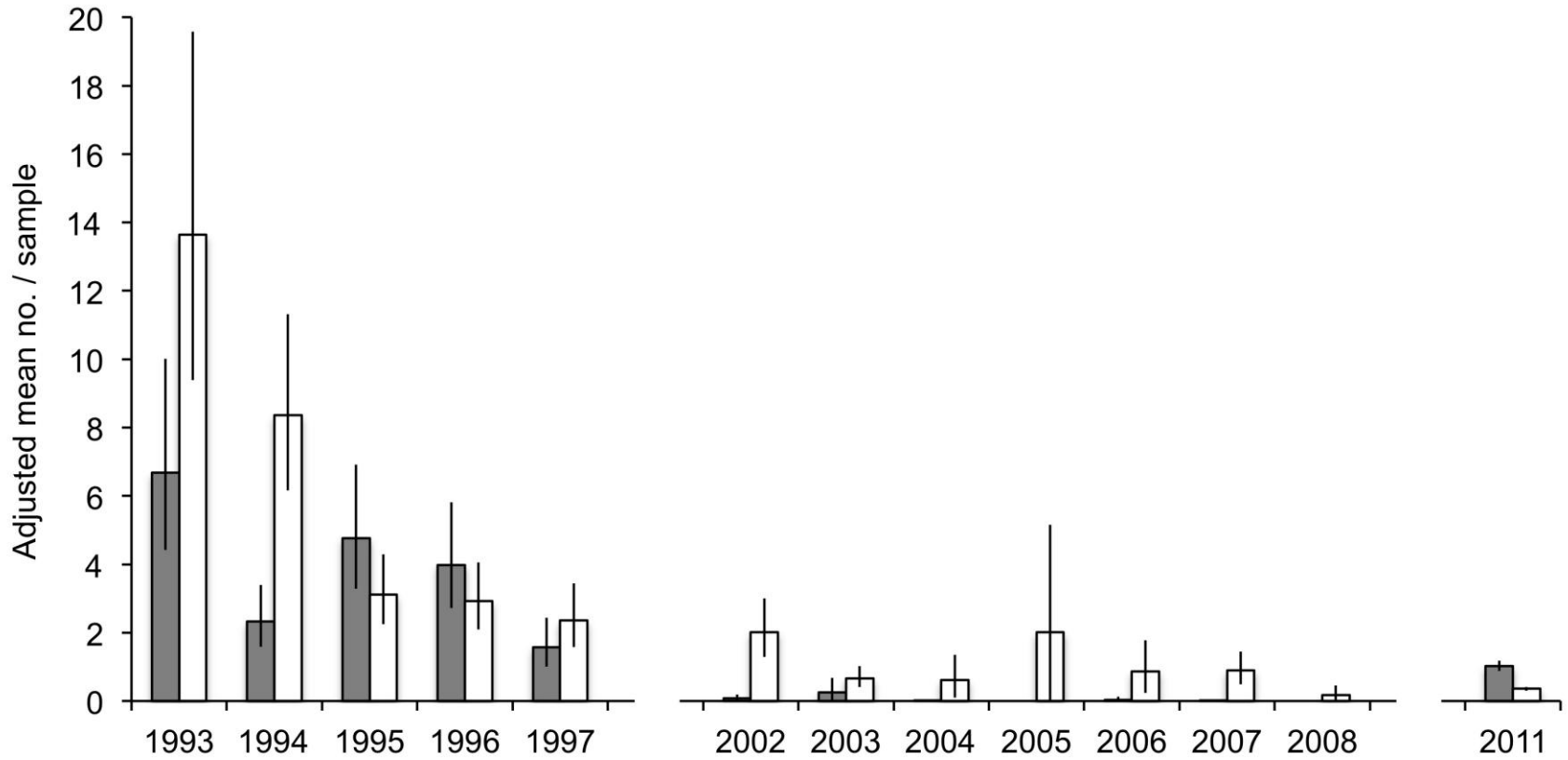


Figure 4.4 (cont'd)

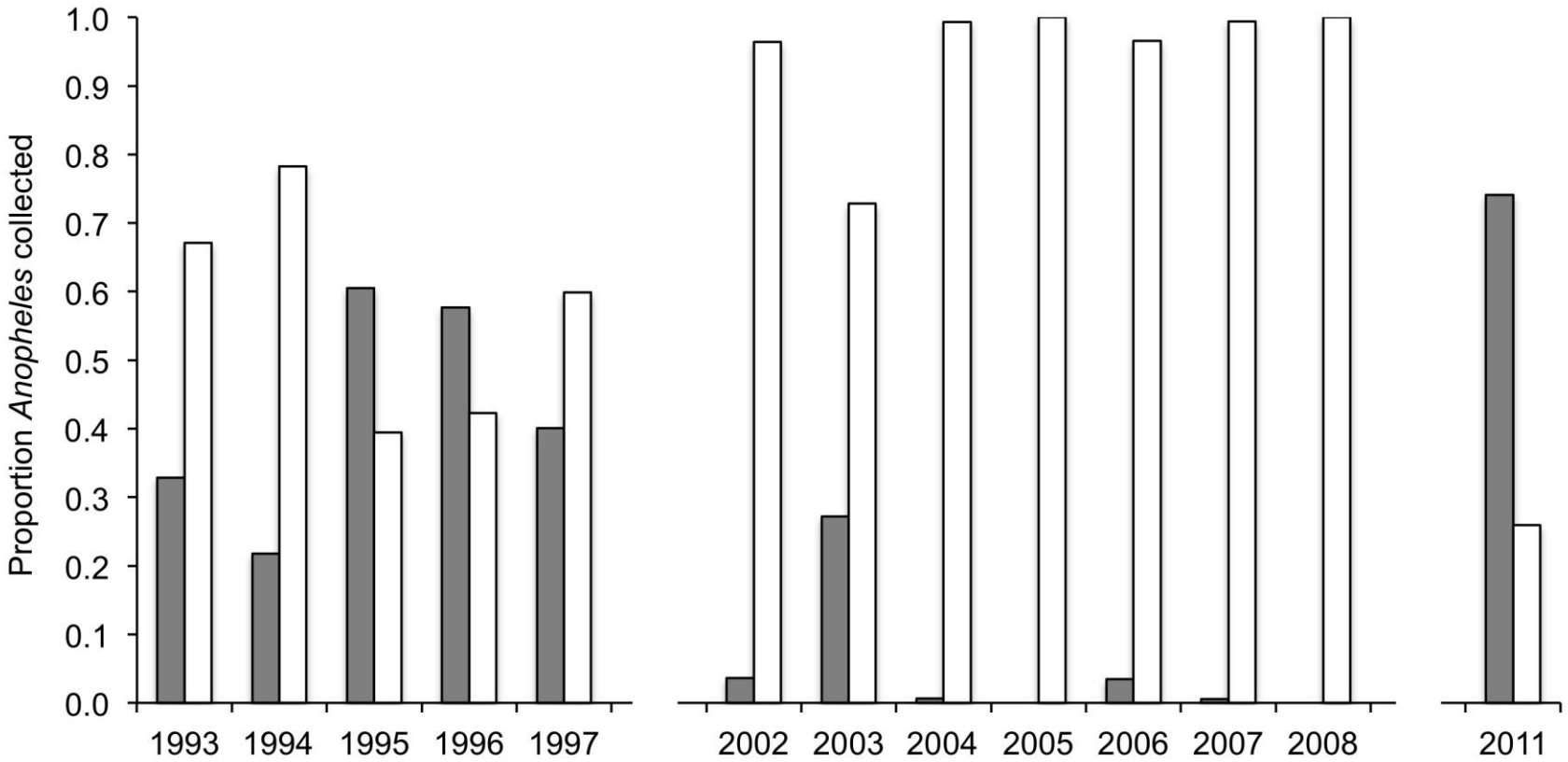
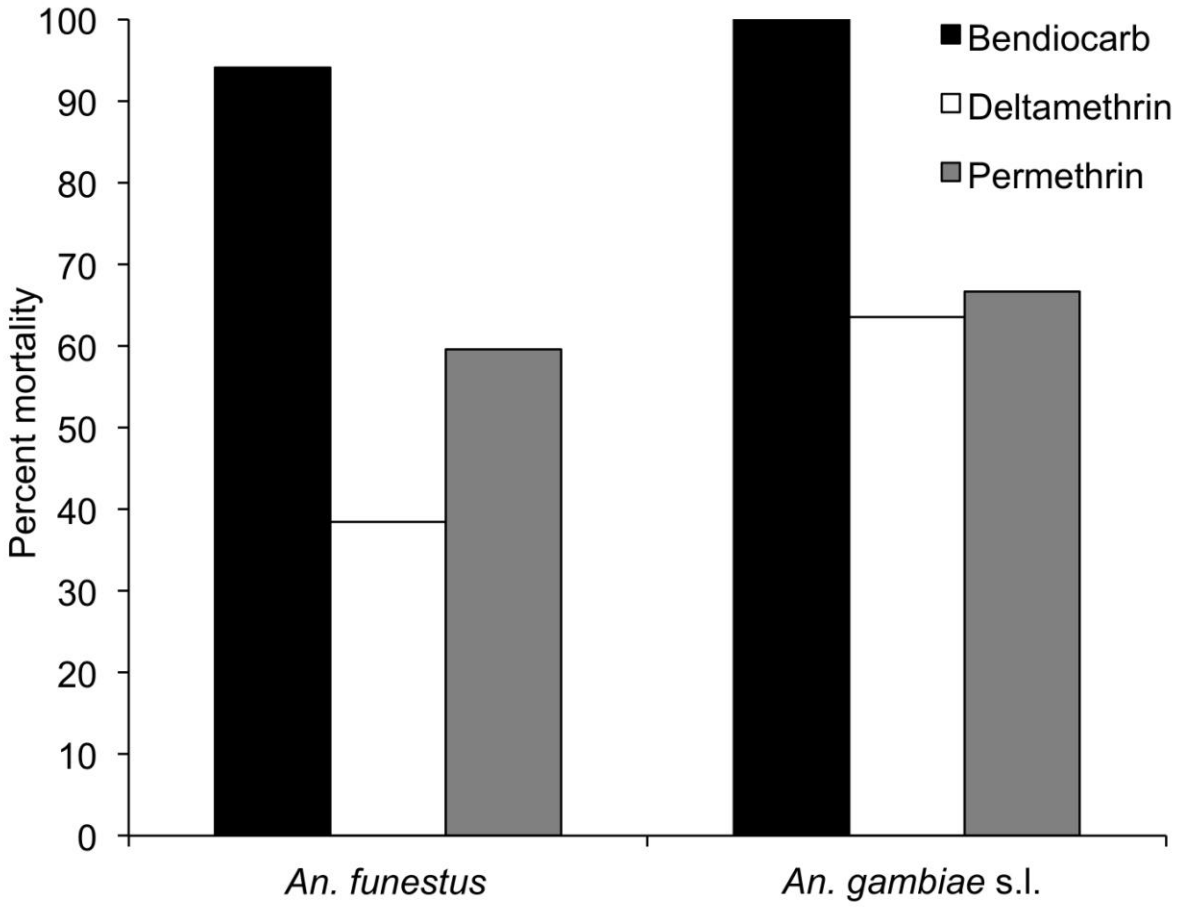


Figure 4.5: Percent mortality after 24 hours of wild collected *Anopheles funestus* and *Anopheles gambiae* s.l. adults from Asembo when exposed to permethrin, deltamethrin, or bendiocarb in one-hour World Health Organization tube bioassays.



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CHAPTER V: Conclusions

Within the last decade, there has been renewed interest in the regional elimination and global eradication of malaria (Roberts and Enserink 2007, Feachem et al. 2010, Alonso et al. 2011, World Health Organization 2011). For this goal to be attainable, we must understand the ecology of the local malaria vectors and apply vector control methods in ways that acknowledge and exploit their biological complexity (Ferguson et al. 2010). This dissertation contributes to such an understanding by describing the use of microdam-associated aquatic habitat by malaria vector larvae, which had not been previously investigated in western Kenya. Given that larvae of the primary malaria vector species in the region used these habitats during the dry season, microdam reservoirs could be important for the persistence of malaria vector populations through the dry season. Malaria control programs could exploit this finding by implementing larval source management (Fillinger and Lindsay 2011) at microdams in the dry season. Indeed, larval control could have the desirable effect of delaying the seasonal build up of adult populations as the rainy season commences (J. Miller, unpublished). Approaches could include habitat modification to make the habitats less suitable for larvae. For example, vegetation could be cleared away from the reservoir perimeters to discourage egg-laying and reduce survival of *An. funestus*. Additionally, microdam perimeters could be modified to reduce the formation of hoof print aggregations, the preferred habitat of *An. gambiae* s.l. near microdams. For example, the entrance to microdam reservoirs used by livestock could be covered with grass cuttings to prevent hoof prints from forming and filling with water. Furthermore, the application of a larvicide could complement habitat modifications at microdams. Finally, larval source management in the rainy season in

western Kenya should also include microdams, because *An. gambiae* s.l. larvae were relatively abundant during the rainy season in microdam-associated habitats sampled here.

This dissertation also provides detailed information about the relationship between malaria vector larval habitat locations and both landscape variables and accumulated precipitation. This information was used to model the locations of larval habitats across a relatively large area and throughout seasons with variation in precipitation. This has two potential applications. First, accurate larval habitat models can be used to investigate the relationship between larval habitat proximity to houses and the number of adult malaria vectors in those houses, across large areas where mapping all larval habitats is infeasible (Bogh et al. 2007). Such models can provide indirect evidence for determining the dispersal distances of adult malaria vectors (Thomas et al. 2013), which is one of the major gaps in our understanding of malaria vector ecology (Ferguson et al. 2010). Second, predictive models of larval habitat locations could potentially be used to assist in larval control efforts by allowing targeted management (Li et al. 2011, Nmor et al. 2013). A risk for this approach is that models fitted to data from a single geographic location may have limited generalizability (Strauss and Biedermann 2007). However, the sampling strategy employed here for model parameterization can be used in other geographic locations to allow malaria control programs to build models that are useful over larger areas.

Finally, this dissertation addresses two significant limitations of insecticide-treated bed nets (ITNs), including long-lasting impregnated nets, as a long-term, stand-alone method for malaria control. First, differences between ITN ownership at the

household-level and individual ITN use could have important implications for malaria transmission. ITNs have been shown to provide community-wide benefits, even for those not sleeping under ITNs, by reducing malaria vector population levels (Hawley et al. 2003b). However, the results presented here indicate that individuals living in houses with ITNs but not sleeping under ITNs are exposed to as many malaria vectors as individuals in houses without ITNs. Thus, while ITNs serve as a public good (Hawley et al. 2003a), they do not confer equal household-wide protection to those not sleeping under them. Second, the risk of malaria vector populations developing insecticide resistance is of serious concern. Resistance to the pyrethroids used in ITNs has been reported in multiple regions for *Anopheles gambiae*, *Anopheles arabiensis*, and *Anopheles funestus*, the three most important malaria vectors in Africa (Ranson et al. 2011). Insecticide resistance in one potential cause of the reemergence reported here of *An. funestus* to the highest relative abundance since the introduction of ITNs in an area of long-term ITN use. If ITNs are to remain a useful tool for malaria control, strategies to attenuate insecticide resistance development and complementary control methods for integrated vector management (World Health Organization 2004) must be employed immediately.

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