Integrated malaria vector control in different agro-ecosystems in western Kenya

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To the Imbahale’s (Alex, Evelyne, Nancy, Ann, Ruth and Lindah)
and the future generations.

Abstract

Malaria is a complex disease and its transmission is a function of the interaction between the *Anopheles* mosquito vector, the *Plasmodium* parasite, the hosts and the environment. Malaria control has mainly targeted the *Plasmodium* parasite or the adult anopheline mosquitoes. However, development of resistant parasites and mosquito vectors requires the development of other strategies, such as larval control, which can be integrated in the current control programmes. To develop a larval control programme, the local vector species and their breeding characteristics need to be known.

This thesis describes the results of a study on mosquito ecology, with an emphasis on malaria vectors in different agricultural settings within two highland sites (Fort Ternan and Lunyerere) and a peri-urban area (Nyalenda) in western Kenya, and the development of practical and effective mosquito larval control strategies. To provide information about the ecology of local vectors, a longitudinal two-year study on the population dynamics and breeding characteristics of local mosquito species and malaria prevalence was undertaken in the respective study sites. In addition, community perception and knowledge on malaria, causes of malaria and the control of mosquito vectors were established through a questionnaire. This information allowed for the development of small-scale mosquito larval control strategies combining source reduction, environmental manipulation through provision of shade and biological control using predatory fish (*Gambusia affinis*) and application of the biolarvicide, *Bacillus thuringiensis* var *israelensis* (*Bti*).

The main malaria vector species, *Anopheles gambiae* Giles *sensu stricto* and *An. arabiensis* Patton, were both present in all sites as larvae, while *An. funestus* Giles was only recorded in the highland villages. The majority (86%) of mosquito breeding sites were a result of human activities. *Anopheles arabiensis* was the main vector species in Nyalenda, *Anopheles gambiae s.s.* was dominant in Lunyerere while *An. funestus* was common in Fort Ternan. Lunyerere had the highest percentage (12.5%) of adult indoor resting *An. arabiensis* mosquitoes ever recorded in the western Kenyan highlands. Fort Ternan had the highest percentage (71%) of larval *An. arabiensis* ever recorded at such high altitude. *Plasmodium falciparum*, the main malaria parasite in the region, was present in the schoolchildren cohort examined and no significant differences in malaria prevalence were observed among the study sites.The inhabitants of the respective communities regarded malaria as a burden and they expressed a willingness to take part in mosquito control, although they did not know how this could be done. A pilot study of larval control strategies conducted in Nyalenda demonstrated the feasibility of environmental and biological control methods

in man-made, mostly agricultural, mosquito habitats. The larval control strategies that were applied in the highland villages led to complete elimination of both early and late instar mosquito populations and compared well with the effects of *Bti* application.

The results suggest that the larval control strategies developed in this study will contribute significantly to a reduction in adult mosquitoes and hence, malaria transmission. Larval control strategies need to be developed that take into account the breeding habits of the local vectors as well as the suitability of habitats for a given control strategy. An integrated approach using various larval control strategies that are locally available, can easily be adopted by the communities concerned. Community involvement in disease control will lead to knowledge on how the peoples' activities affect their health and this can empower them to take charge of their health.

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Chapter 1

General introduction

Susan S. Imbahale

Introduction

Malaria is one of the most serious public health problems in sub-Saharan Africa, where it kills between one and two million persons, mostly children under five years of age and pregnant women, every year (Snow & Marsh, 2002). In much of tropical Africa, *Plasmodium falciparum* is the dominant and most serious malaria parasite, responsible for high degrees of morbidity and mortality. Most of sub-Saharan Africa has stable endemic malaria because climatic conditions ideal for transmission coincide with the ranges of the malaria vectors. In East Africa the main malaria vectors are *Anopheles gambiae* Giles, *An. arabiensis* Patton, and *An. funestus* Giles. Recently, *An. coustani* has been documented as a potential malaria vector in East Africa (Geissbühler et al., 2009). Environmental conditions such as rainfall, temperature, humidity and topography including altitude, exposure and latitude determine the intensity of malaria risk, expressed as hypo-, meso-, hyper- and holo-endemicity (Gilles & Warrell, 1993). Extreme climatic events such as El Niňo or excessive rainfall may contribute to unusual epidemics (Bouma et al., 1994). Small increases in temperature in areas that experience low temperatures (highlands) can result in serious outbreaks where the population has developed little or no immunity (Patz et al., 2000). In stable malaria situations, transmission levels are characteristically high with little inter-annual variation, and collective immunity in the population is also high, thus epidemics are unlikely to occur. Here, prevalence rates are characteristically high, with most severe cases in infants. In unstable situations, transmission levels of infections vary from year to year and collective immunity is low, therefore, there is a high potential for epidemics. Low immunity (non-developed or impaired immunity) is the most significant predictor of malaria in those infected with *Plasmodium falciparum* and this phenomenon largely restricts the epidemics to areas with low herd immunity, which is mainly in the highlands in the tropics (Thomson & Connor, 2001).

High altitude areas in East Africa experienced infrequent outbreaks of malaria between the 1920s and the 1950s (Garnham, 1945) which was attributed to anomalous warming in the African region (I.P.C.C. 1998). From the 1960s to the early 1980s, malaria was not reported in the Kenyan highlands after a malaria eradication campaign. However, since 1988, frequent malaria epidemics have occurred here at elevations higher than 1,500 m above sea level (Hay et al., 2002; Malakooti et al., 1998), and by the year 2001, the epidemics had spread from 3 to 15 districts over a period of 13 years (Githeko & Ndegwa, 2001). In western Kenya, *P. falciparum* is the main parasite causing malaria. The primary malaria vectors include species in the *An. gambiae* complex and *An. funestus*. The two species of local importance in the *An. gambiae* complex are *An. gambiae* Giles *sensu stricto* (henceforth termed *An. gambiae*) and *An. arabiensis.*

Malaria epidemics have been associated with extreme weather events such as the El Niňo Southern Oscillation event of 1997 – 1998, which caused heavy rainfall and flooding in East Africa (W.M.O. 1999). Other mechanisms that have been hypothesized include increased travel from the malaria endemic Lake Victoria basin to the highlands (Garnham, 1948), degradation of the healthcare infrastructure (Malakooti et al., 1998), antimalarial drug resistance (Shanks et al., 2002), global warming (Lindsay & Birley, 1996; Martens et al., 2002) or increased climate variability (Zhou et al., 2004) and local malaria transmission as a consequence of land use changes (Lindblade et al., 2000a; Lindsay & Martens, 1998). Although drug resistance is mentioned, it can only aggravate malaria-induced morbidity and mortality but cannot initiate an epidemic (Githeko & Ndegwa, 2001). Climatic conditions, temperature, altitude, humidity, rainfall, flooding and phenomena such as El Nino can work towards either increasing malaria infection by creating more mosquito breeding habitats, or decreasing malaria infection by splashing out water collected in small pools, thereby eliminating mosquito breeding habitats. Climatic conditions influence the development, reproduction and survivorship of anopheline mosquitoes and malaria parasites (Zhou et al., 2004). At Londiani, situated at 2286 - 2377 m above sea level, *An. gambiae* is present but the mean temperature never reaches the critical figure for effective transmission of the parasites (Gilles & Warrell, 1993). The domestic habits of *An. gambiae* could be the contributing factor to its survival at such heights. *Anopheles gambiae* mosquitoes spend most of their life in human habitations, which are 3–5°C higher in temperature than the prevailing outside temperatures (Bodker et al., 2003; Garnham, 1945; Koenraadt et al., 2006). These indoor conditions may prove conducive to transmission in these otherwise *Plasmodium*-hostile environments (Reiter, 2001). A model developed by Zhou et al. (2004) predicts that during an epidemic, the most severe malaria cases come from highlands' human populations, which have not been regularly exposed to malaria infections.

Biology of anopheline malaria vectors in the highland areas

Mosquitoes (Diptera, Culicidae) of the *An. gambiae* and *An. funestus* complexes transmit the majority of malaria cases in the sub-Saharan Africa. *Anopheles gambiae* and *An. arabiensis*, are widespread over the African continent, although the latter species extends into more arid areas (Coetzee et al., 2000). Observations of the larval habitats of *An. gambiae* have noted a preference for temporary, sunlit pools (Gillies & Coetzee, 1987; Gillies & De Meillon, 1968) whereas *An. arabiensis* appears to exploit permanent, artificial habitats such as rice fields (Githeko et al., 1996a). However, consistent differences in habitat use by *An. gambiae* or *An. arabiensis* have not been observed and both species have often been found occupying small, sunlit, transient habitats (Minakawa et al., 1999; Gimnig et al., 2001; Koenraadt et al., 2004).

Populations of *An. arabiensis* survive the dry season better, while populations of *An. gambiae* peak shortly after onset of the rainy season (Githeko et al., 1996a). In Tanzania, *An. arabiensis* was common during the short rains and just before the long rains, whereas *An. gambiae* predominated during and just after the long rains (White et al., 1972a). *Anopheles gambiae* is highly anthropophilic, meaning that it almost exclusively bites humans, whereas *An. arabiensis* is more opportunistic, meaning that it may feed on humans as well as on animals, depending on the availability of both hosts (Githeko et al., 1996b). *Anopheles funestus* has a similar distribution over Africa as *An. gambiae* but it prefers to breed in large, permanent bodies of water with emergent vegetation (Gillies & De Meillon, 1968; Klinkenberg et al., 2003) and populations generally peak after those of *An. gambiae.*

Garnham (1948) attributes the incidence of malaria at high altitude to the gradual development of the country. Motor and rail transport brought up the mosquito from the low-lying hyper-endemic areas; it created numerous breeding sites and introduced a large, infected mosquito population. In the Kenyan highlands all the malaria has been largely caused by *An. gambiae* and a typical epidemic usually begins in late May or early June, following the "long rains", reaches its peak in June or July and is on the decline by August, with year-to-year variability. *Anopheles gambiae* shows increase in density only after the long rains whereas *An. funestus* density is seen to vary in direct proportion to the proximity of the permanent breeding grounds rather than rainfall (Garnham, 1929), but their habitats do not differ much in the adult stage. In Mumias, a high altitude site and a large scale sugar-cane growing zone in western Kenya, Shililu et al. (1998) found malaria transmission intensity to be low but perennial with the main vectors being *An. gambiae* and *An. funestus*, both highly anthropophilic. Balls et al. (2004) in their study in the Usambara Mountains, Tanzania, reported that altitude played an important role in determining malaria infection due to its effect on temperature. It is possible that temperature simply governs vector densities through a direct physiological effect on larval development time (Bodker et al., 2003; Paaijmans et al., 2009). In the Amani hills in Tanzania, deforestation is thought to have created new habitats for effective vectors, and the elimination of shade produced a marked change in local climate (Reiter, 2001), whereas in the Ugandan highlands, the elimination of papyrus has created a habitat for *An. gambiae* and *An. funestus*, leading to increased transmission (Lindblade et al., 2000a). *Anopheles gambiae* were collected in greater proportions along cultivated swamps and transmission was entirely due to this species. The increased malaria transmission has been attributed to increased local temperatures near cultivated swamps, combined with occasional excessive precipitation.

Do environmental changes create an opportunity for malaria transmission?

Environmental changes, either natural or through human intervention, alter the manner in which vectors and their parasites breed, develop and transmit disease (see Table 1.1). Deforestation and land-use changes, human settlement, commercial development, construction of roads, water control systems (dams, canals, irrigation systems and reservoirs) often lead to the creation of conducive breeding grounds for efficient malaria vectors (Patz et al., 2000; Norris, 2004).

Table 1.1: Malaria risk factors in the highlands and factors that might alter ma-

Risk factors	**Causes of change**
Anopheline vectors present	Land use change, incl. irrigation and urbanization
Suitable temperature for vectors	Temperature increase (climate change, deforestation)
Suitable temperature for parasites	Temperature increase (climate change, deforestation)
Parasite reservoir	Drug resistance; change in population density
Rainfall	Intensity of rainfall
Humidity	Intensified rainfall; higher/lower mean temperature
Habitat (land use)	Deforestation, new cultivation practices (e.g. irrigation, increased usage of land for farming)
Human population	Increased farming, urbanization
Urbanization	Lack of opportunities in rural areas, market garden farming, small-scale irrigated agriculture

These environmental changes either singly or in combination with climatic conditions affect parasites and insect vectors or in turn the incidence and prevalence of parasitic diseases (Patz et al., 2000; Norris, 2004).

Variation in climatic conditions has a profound effect on mosquito biology and on the development of malaria parasites [notably *Plasmodium falciparum*] (Gilles & Warrell, 1993). Hence climate's influence on the transmission of the disease and on its seasonal incidence. Most important factors are temperature and humidity. Malaria parasites cease to develop in the mosquito when the temperature is below 16° C. The best conditions for the development of *Plasmodium* in the mosquito and transmission of the infection are when the mean temperature is within the range of 20 to 30° C, while the mean relative humidity is at least 60%.

In the East African highlands, climate was always considered the limiting factor for malaria transmission whereas the adjacent valleys and plains were highly malarious. Ecological explanations for the observed changes in malaria epidemiology in the highlands are often lacking, possibly because the interactions between the mosquito vectors, the *Plasmodium* parasite and their environment are poorly understood.

The role of agriculture in malaria transmission

Agricultural water resource development, deforestation due to agriculture, logging and fuel wood, wetland cultivation, creation of urban market gardens, and land use changes for agricultural purposes in the highlands all expand habitats for vectors of diseases including malaria (Keiser et al., 2005a; Patz et al., 2004; Yasuoka & Levins, 2007). Although water projects using irrigation can lead to increased agricultural production, on the other hand they create suitable conditions for the propagation of mosquito populations (Diuk-Wasser et al., 2007; Hawkes & Ruel, 2006). Significant agricultural practices such as irrigated or non-irrigated urban and peri-urban agriculture, wetland cultivation, clearing of tropical forest lands and its associated deforestation, and agricultural encroachment on highlands have been the primary drivers of malaria transmission in the affected areas (Minakawa et al., 2005; Lindblade et al., 2000a; Patz et al., 2004; Sattler et al., 2005).

Problem definition and research objectives

Malaria is a complex disease and its severity is a function of the interaction between the *Anopheles* mosquito vector, the parasite, the hosts and the environment. Malaria control typically targets *Plasmodium* parasites or *Anopheles* vectors. Parasite control is now being undermined by the rapid spread of parasite resistance to once-effective antimalarial drugs (Trape, 2001). It is therefore unrealistic to make malaria control exclusively dependent on clinical care. The most effective method of malaria control remains the interruption of mosquito-host contact (Lindsay et al., 2002) through vector control strategies. Vector-directed control strategies have mainly targeted adult mosquitoes through indoor residual spraying (IRS) with potent insecticides and/or insecticide treated bed nets (ITNs) (Robert et al., 2000; Schellenberg et al., 2001; RBM, 2005). However, the rapid development of insecticide-resistant mosquitoes (N’Guessan et al., 2007; Ramphul et al., 2009) undermines current malaria control strategies and opens the way for introduction of alternative control measures. One alternative method would be through mosquito larval control as mosquitoes spend a considerable part of their life in the aquatic stage. However, for any control strategy to be effective, the ecology of the local vector in terms of the species responsible and characteristics of breeding habits needs to be known.

Once the ecology of the vector species is known, control strategies can be developed that are suitable for the area under study. This PhD study is about the ecology of mosquito vectors with emphasis on malaria vectors in different agricultural settings within two highland sites (Fort Ternan & Lunyerere) and a peri-urban area (Nyalenda) in western Kenya, (Figure 1.1) and the development of mosquito larval control strategies. The main objectives of this study were:

1) To establish the temporal and spatial dynamics of larval and adult malaria vectors and the prevalence of malaria in two highland villages and one peri-urban setting in Western Kenya.

2) To analyze the feasibility of applying environmental and biological methods of mosquito larval control in western Kenya, for the reduction of malaria risk.

The outline of this thesis is as follows: In **Chapter 2** a review of the farming systems, water management and malaria mosquitoes in Africa is provided. Examples of the effects of water management and agriculture on mosquitoes in general are provided. Later on it focuses on the available control measures with special emphasis on immature mosquito stages. **Chapter 3** presents the results of a longitudinal study on the ecology of anopheline and culicine larvae in distinct geographical and environmental settings (Figure 1.1) in western Kenya. In **Chapter 4** the dynamics of malaria transmission in various agricultural settings in western Kenya are provided. In this chapter, adult mosquito species and malaria parasite sporozoite rates are provided alongside malaria prevalence data. In **Chapter 5** the main focus is on the community perception of malaria in areas of different transmission levels in western Kenya. The goal is to find out if malaria is perceived as a problem by the communities and what knowledge the inhabitants have on the cause of malaria and its control. As a way to begin addressing the problem of a lack of research-based results on possible larval control strategies, small-scale field and laboratory trials of mosquito larval control using environmental manipulation and biological control are reported in **Chapter 6**. In **Chapter 7** the results of small-scale environmental management and biological control strategies implemented in two highland villages are reported. **Chapter 8** provides a summarizing discussion and recommendations for future research. The results of all chapters are discussed and integrated in the context of mosquito larval control in western Kenya.

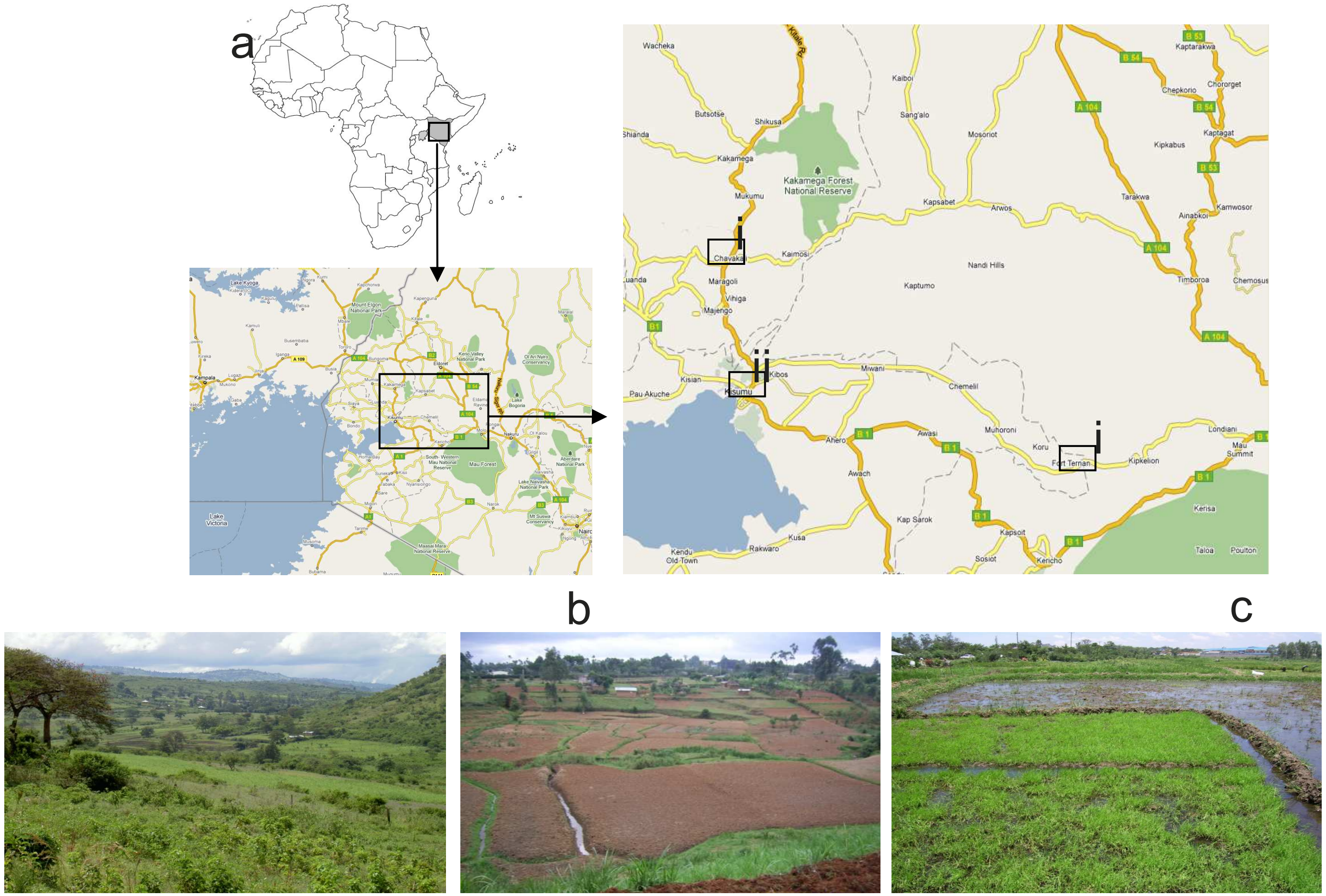

Figure 1: Map of Africa showing the location of Kenya (a), the study region (b) and the study sites (c). The pictures show the highland village of Fort Ternan (i), highland village of Lunyerere (ii) and peri-urban Nyalenda (iii). Courtesy of Google Maps and pictures by Susan Imbahale (2006)

Chapter 2

Farming systems, water management and malaria mosquitoes in Africa

Imbahale, S.S., Mukabana, R. and Takken, W.

Abstract

In the recent past, the effects of changes in land use on mosquitoes and mosquito borne diseases has received increasing attention. Changes related to agricultural development, deforestation and urbanization can either reduce or increase mosquito larval habitats that eventually affect vector densities and disease transmission. Malaria is still one of the most important diseases, especially in the tropics and subtropics and its control has mainly relied on parasite treatment and adult mosquito vector control by use of drugs and insecticides, respectively. However, these methods are hampered by the quick development of drug-resistant parasites and insecticide-resistant vectors. Consequently, there is a need to focus more on the control of immature mosquito stages, aiming at the development of control measures that are sustainable and can easily be adopted by the communities affected. We explore how different farming and water management systems affect mosquitoes, with an emphasis on malaria mosquitoes, and available larval control options that can be sustainably used in the tropics and subtropics.

Introduction

Malaria occurs in over 90 countries worldwide. According to the World Health Organization (W.H.O, 1996), 36% of the global population lives in areas with a risk of malaria transmission, 7% resides in areas where malaria has never been under meaningful control, and 29% lives in areas where malaria was once transmitted at low levels or not at all, but where significant transmission has been re-established. Malaria transmission occurs primarily in tropical and subtropical regions in sub-Saharan Africa, Central and South America, the Caribbean island of Hispaniola, the Middle East, the Indian subcontinent, South-east Asia, and Oceania (W.H.O, 1996). Malaria is mainly transmitted by female *Anopheles* mosquitoes and its geographic distribution is confined to climates that favor the extrinsic cycle of the malaria parasite in the mosquito vectors (Craig et al., 1999). The distribution of malaria vectors is dependent on the prevailing temperature, rainfall, humidity, vegetation, altitude, availability of blood hosts, distance from the breeding sites, type and condition of the larval habitat (Balls et al., 2004; Githeko et al., 2006; Koenraadt et al., 2003a; Minakawa et al., 2002a; Minakawa et al., 2002b). In Europe, *Anopheles atroparvus* (van Thiel), *An. labranchiae* (Falleroni), *An. messeae* (Swellengrebel & de Buck), *An. sacharovi* (Favr), and *An. superpictus* (Grassi) were historically associated with endemic malaria transmission (Kuhn et al., 2002). In present, malaria transmission is mainly reported in the tropics and subtropics, where the environmental conditions are highly suitable for the most efficient malaria mosquito vectors of *Plasmodium* parasites.

The *Anopheles gambiae* complex contains the world's most effective vectors of human malaria (Coetzee et al., 2000). This complex is considered to have seven morphologically indistinguishable sibling species, namely, *An. gambiae* Giles *sensu stricto, An. arabiensis* Patton*, An. quadriannulatus* A Theobald*, An. quadriannulatus* B Theobald*, An. melas* Theobald*, An. merus* Donitz and *An. bwambae* White. Mosquitoes (Diptera, Culicidae) of *An. gambiae sensu lato* (*An. gambiae sensu stricto* and *An. arabiensis*) and *An. funestus* Giles are responsible for the majority of malaria transmission in sub-Saharan Africa (Coetzee et al., 2000). *Anopheles gambiae s.s.* is the most efficient vector, mainly because of its highly endophilic and anthropophilic characteristics, which results in higher transmission rates than any of the other vectors mentioned (Githeko et al., 1996b). *Anopheles gambiae* is highly anthropophilic, biting almost exclusively humans, whereas *An. arabiensis* is more opportunistic and may feed on humans as well as on animals, depending on the availability of both hosts. These two species are sympatric; however, *An. arabiensis* is more widely distributed in drier low-altitude areas whereas *An. gambiae s.s.* prefers humid, higher-altitude areas. *Anopheles quadriannulatus* sub-species A occurs in South Africa while sub-species B is restricted to the Ethiopian highlands. *Anopheles bwambae* is uniquely distributed in Uganda, while *An. melas* is commonly found along the coastal zones of central and west Africa. *Anopheles merus* occurs in the coastal areas of southern and eastern Africa (Coetzee et al., 2000). In humans, malaria is caused by five species of protozoan parasites of the genus *Plasmodium*, namely *Plasmodium falciparum, P. vivax, P. ovale*, *P. malariae* and *P. knowlesi.* Variation in climatic conditions has a profound effect on the life of a mosquito and on the development of malaria parasites (notably *P. falciparum*), hence its influence on the transmission of the disease and on its seasonal incidence. The best conditions for the development of plasmodia in the mosquito and transmission of the infection are when the mean temperature is within the range of 20 to 30 ºC, with the mean relative humidity at least 60%. Generally, in warmer regions closer to the equator, *P. falciparum* predominates with transmission being more intense and year round (http://www.cdc.gov/malaria/distribution_epi/ distribution.htm).

In tropical and sub-tropical environments, conducive weather conditions, coupled with land use changes either through environmental management, modification and manipulation, are likely to have either a positive or negative effect on mosquito reproduction and survival (Birley & Lock, 1998). Human activities associated with settlement, agriculture, or other environmental alterations, such as road or dam construction may result in the creation of favorable larval habitats for malaria vectors (Kitron & Spielman, 1989). These infrastructural and agricultural developments, coupled with human population growth, more often have resulted in increases in mosquito vector species or disease prevalence that were not seen before the land use changes. In France, rice cultivation increased population densities of *An. hyrcanus* and *Culex modestus* (Poncon et al., 2007). In India, irrigation led to an increase in *An. annularis* and *An. culicifacies* mosquito densities (Singh & Mishra, 2000; Konradsen et al., 1998); however in that same country, small dams were found to reduce numbers of *An. fluviatilis* (Sharma et al., 2008). In Hawaii, forest fragmentation and agriculture increased *Culex quinquefasciatus* densities (Reiter & Lapointe, 2007). This review will focus on farming, urban development and forestry on malaria and mosquito densities in the African continent and the available intervention tools. Table 2.1 summarizes some of the studies that have been done (A) outside Africa and (B) within Africa, focusing on the impacts of different agricultural and water management practices on mosquito vectors and malaria.

Table 2.1: The effects of farming systems and water management on malaria vectors outside Africa (A) and within Africa (B). The effect is measured denoted with + (increase) and – (decrease) in either vector density or malaria prevalence as indicated in the column labeled 'measure'

A: Studies outside Africa

System	Measure	Species	Effect	Country	Reference
Intermittent irrigation	Adult mosquito densities	*An. anthropophagous* & *An. sinensis*	-	China	Qunhua et al. (2004)
Rice cultivation	Population densities	*An. hyrcanus* & *Culex modestus*	+	France	Poncon et al. (2007)
Agriculture & forest fragmentation	Adult mosquito density	*Culex quinquefaciatus*	+	Hawaii	Reiter & Lapointe (2007)
Small dams	Malaria	*An. fluviatilis*	-	India	Sharma et al. (2008)
Irrigation	Adult mosquito densities	*An. culicifacies*	+	India	Konradsen et al. (1998)
Canal irrigation	Larvae & adult density	*An. annularis*	+	India	Singh & Mishra (2000)

B: African studies

System	Measure	Species	Effect	Country	Reference
Irrigation	Malaria prevalence		-	Bukina Faso	Boudin et al. (1992)
Large dam irrigation	Malaria incidence		+	Cameroon	Robert et al. (1992)
Rice cultivation & irrigation	Adult densities	*An. arabiensis*	+	Cameroon	Antonio Nkondjio et al., (2008)
Poor environmental sanitation	Mosquito density & malaria infections	*An. gambiae* & *An. funestus*	+	Cameroon	Nkuo-Akenji et al. (2006)
Rice cultivation	Mosquito density	*An. gambiae s.s.*	+	Cote d'Ivoire	Doannio et al. (2002)
Rice cultivation	Adult mosquito density	*An. gambiae*	+	Cote d'Ivore	Koudou et al. (2007)
Rice cultivation	Vector densities		+	Cote d'Ivoire	Dossou-yovo et al. (1994)
Urban farming	Larval densities	Anophelines	+	Cote d'Ivoire	Mathys et al. (2006)
Maize cultivation	Malaria incidence	*An. arabiensis*	+	Ethiopia	Kebede et al. (2005)

Large Dam	Malaria rates	*P. falciparum*	+	Ethiopia	Lautze et al. (2007)
Dam development	Malaria	*P. falciparum*	+	Ethiopia	Yewhalaw et al. (2009)
Urban farming	Biting rates	*An. gambiae s.s.*	+	Ghana	Afrane et al. (2004)
Urban agriculture	Larval & adult densities	*An. gambiae s.s.*	+	Ghana	Klinkenberg et al., (2008)
Deforestation	enhanced survivorship and shorten development	*An. arabiensis*	+	Kenya	Afrane et al. (2006)
Deforested areas and cultivated swamps	Malaria vector	*An. gambiae s.l.*	+	Kenya	Munga et al. (2006)
Deforestation	Rapid sporogonic development	*An. gambiae s.s.*	+	Kenya	Afrane et al. (2007)
Deforestation	Vectorial capacity	*An. gambiae s.s.*	+	Kenya	Afrane et al. (2008)
Piped water Systems	Larval densities	*An. gambiae s.s.*	+	Kenya	Impoinvil et al. (2007)
Rice cultivation	Larval densities	*An. arabiensis*	+	Kenya	Mwangangi et al. (2007)
Abandoned fish ponds	Larval densities	*An. gambiae s.l.*	+	Kenya	Howard & Omlin (2008)
Planned rice irrigation	Malaria	*An. funestus*	-	Kenya	Muturi et al. (2008)
Rice cultivation	Malaria vector	*An. funestus s.l.*	+	Madagascar	Laventure et al. (1996)
Rice fields	Vector densities	*An. funestus*	+	Madagascar	Marrama et al. (1995)
Rice cultivation	Adult mosquito density	*An. gambiae s.s.*	+	Mali	Diuk-Wasser et al. (2007)
Rice cultivation in uncontrolled water depth plots	Larval & adult densities	*An. arabiensis & funestus*	+	Mali	Sogoba et al. (2007)

Waste stabilization ponds & wastewater irrigation	Malaria prevalence	N/A	+	Nigeria	Agunwamba. (2001)
Horticulture	Vector densities	Anopheline sp.	+	Senegal	Trape et al. (1992)
Market gardens wells	Vector densities	*Culex* spp	+	Senegal	Robert et al. (1998)
Irrigation	Adult mosquito density	*An. pharoensis*	+	Senegal	Faye et al. (1995)
Sugarcane and maize cultivation	Malaria prevalence	*An. arabiensis*	+	Tanzania	Ijumba et al. 2002)
Irrigation	Malaria & malaria vector density	N/A	-	Tanzania	Ijumba & Lindsay (2001)
"Matuta" agriculture, rice fields, shallow wells and irrigations channels	Larval densities	Anopheline sp.	+	Tanzania	Sattler et al. (2005)
Irrigated rice	Malaria prevalence	*P. falciparum*	+	Tanzania	Mboera et al. (2007)
Cultivated swamps	Malaria indices	*An. gambiae s.l.*	+	Uganda	Lindblade et al. (2000a)
Marshy areas	Adult mosquito density	Anopheline sp.	+	Uganda	Staedke et al. (2003)

Farming and malaria

Traditionally malaria has been viewed as a disease of the rural areas, very much associated with farming communities. In particular, there are changes along the rural to urban transect with respect to mosquito breeding (Lindsay et al., 1990). Generally the abundance of clean, sun-lit, and shallow bodies of water and corresponding malaria parasite transmission increases as the distance to the city center increases, which makes rural populations more vulnerable to increased contact with anopheline mosquitoes (Coetzee et al., 2000; Robert, 1992). In Africa much of the traditional malaria is associated with seasonal rains, which creates habitats suitable for *An. gambiae* and *An. funestus* breeding, which eventually leads to malaria transmission. In the last 50 years, agricultural development has taken off in Africa, and modernization of agricultural production systems including irrigation has caused a shift in malaria transmission in many areas.

Agricultural development may alter the local climate such that even small increases in temperature resulting from clearing of vegetation may increase larval survivorship, adult survivorship, reproduction and vectorial capacity, and therefore resulting in enhanced transmission (Lindblade et al., 2000b, Yasuoka & Levins, 2007; Norris, 2004; Afrane et al., 2006).

More often irrigation schemes in Africa have created conducive habitats for malaria vector species such as *An. gambiae* and *An. arabiensis,* which often leads to increased malaria transmission (Coluzzi, 1992; Antonio Nkondjio et al., 2008; Mboera et al., 2007). In some countries, dams were built to support agricultural production, but their presence also encouraged mosquito breeding. For example in Ethiopia, large dams and maize cultivation were found to lead to increased malaria transmission (Lautze et al., 2007; Kebede et al., 2005). However, irrigation does not always lead to increase malaria transmission as shown by Ijumba & Lindsay (2001) in Tanzania. In addition, studies by Muturi (2008) and Qunhua (2004), have shown that intermittent irrigation led to a reduction in malaria vector densities in Kenya and China respectively.

Urban development and malaria

An estimated 200 million people live in urban malaria endemic areas in Africa (Keiser et al., 2004). Donnelly et al. (2005) predict that by 2025, 800 million people will live in urban communities in sub-Saharan Africa. Most cities in the South are expanding rapidly and provide large markets for natural products, which may be produced in peri-urban areas that are also sinks for the city's wastes (Birley & Lock, 1998). The peri-urban zone can be broadly characterized as a mosaic of different land uses inhabited by communities of varying economic status, in a state of rapid change with a lack of infrastructure and a deteriorating environment (Birley & Lock, 1998). Urbanization is occurring rapidly in sub-Saharan Africa and may have a significant impact on the epidemiology of malaria, while the contributing factors seem complex and are not fully understood (Robert et al., 2003). It is has been found that many urban centres experience water pollution problems, which is thought to inhibit the development of anopheline larvae, resulting in fewer *Anopheles* mosquitoes (Barbazan et al., 1998; Chinery,1994). Most studies report the presence of *An. gambiae* in peri-urban areas, where the larvae of the mosquitoes are found in clean standing water, such as drainage channels or in rice fields. Highly polluted sites, rich in organic material are less preferred, although occasionally these may harbor anopheline mosquitoes (Awolola et al., 2007; Keating et al., 2004; Sattler et al., 2005). Malaria vectors have been studied in quite a number of African cities and evidence is rapidly accumulating that the urban poor are at far higher risk from malaria than previously acknowledged (Keiser et al., 2004).

In Dakar, Senegal, malaria vectors were found strongly associated with horticulture practiced at the edge of the city (Trape et al., 1992). In Kumasi, Ghana and several cities in the Ivory Coast, the higher densities of anopheline vectors was found to be associated with agriculture (Afrane et al., 2004; Klinkenberg et al., 2008; Matthys et al., 2006). By contrast, no such association between malaria vectors and agriculture was found in Dar es Salaam, Tanzania (Sattler et al., 2005). Keating and others (2003) observed that in both Kisumu and Malindi, Kenya, most larval sites were human-made, with the highest numbers of aquatic habitats observed in the unplanned, poorly-drained stratum in Kisumu, and the unplanned, poorly drained and peri-urban strata in Malindi. It was suggested that at high human population densities, the amount of open space and vegetation for adult and larval mosquito development will be extremely limited, which could in turn limit mosquito breeding (Keating et al., 2003). Although the intensity of malaria transmission might be lower in urban than in rural areas of Africa, transmission and parasite prevalence may be heterogeneous within densely populated urban areas, clustering near mosquito breeding sites (Smith et al., 2004; Thompson et al., 1997).

Forestry and malaria

Deforestation is a dramatic land-use change that leads to a reduction of forest canopy and an increased exposure to sunlight, which could substantially affect the survivorship and development time of larval and adult mosquitoes (Afrane et al., 2007). Deforestation and land transformation influence malaria mosquitoes, especially larval and adult survivorship, reproduction and vectorial capacity, through changing environmental and microclimatic conditions such as temperature (average, variability), sunlight (amount, duration), humidity, water condition (distribution, temperature, quality, turbidity, current), soil condition, and vegetation (Yasuoka & Levins, 2007). In the western Kenya highlands, experimental field studies by Tuno et al. (2005) and Munga et al. (2005) showed that only 2–6% of *An. gambiae* larvae developed into adult stages in the forested area while 49–65% larvae survived to the adult stage in the deforested area. Both studies concluded that the higher larval survivorship in aquatic habitats in the deforested zone was due to increased water temperatures. In their analysis, Yasuoka & Levins (2007) showed that deforestation and agricultural development are favorable for sun-loving species, allowing the species to increase in or invade deforested areas that have conducive water temperature and perhaps better food conditions. Tree canopy reduces the amount of solar radiation reaching larval habitats, consequently lowering the water temperature of larval habitats surrounding the forests and thereby resulting in unsuitable conditions for mosquito breeding. Munga et al. (2006) found that the annual average water temperature was 2.4 °C lower in natural habitats located in the forested area than in farmlands in western

Kenya. In the Ivory Coast, the deterioration of forests caused by demographic pressure over the past 30 years has led to elevated entomologic indices, at levels never observed before (Nzeyimana et al., 2002).

Strategies available for malaria control

Malaria is a complex disease and its severity is a function of the interaction between the *Anopheles* mosquito vector, the parasite, the hosts and the environment. Malaria control typically targets *Plasmodium* parasites or its mosquito vectors by use of drugs, indoor residual spraying (IRS) and insecticide-treated bednets (ITN's) (Chanda et al., 2008; Mufunda et al., 2007; Nabarro, 1999; Nyarango et al., 2006; Otten et al., 2009; Owusu-Agyei et al., 2007; Sievers et al., 2008). The control of malaria through immature mosquito control has not received much attention in Africa in recent years, even though great success was reported with this method more than half a century ago (Utzinger et al., 2001; Utzinger et al., 2002; Fillinger & Lindsay, 2006; Keiser et al., 2005). The control of immature mosquito populations is advantageous because the larvae are usually concentrated in focussed areas, relatively immobile, and occupy a minimal habitat area compared with highly mobile adult mosquitoes (Floore, 2006). Several larval control programmes in China, India and Sri Lanka have also been successful in reducing malaria transmission through immature mosquito control (Liu et al., 2004; Qunhua et al., 2004). In this review, we highlight the control of larval stages of mosquitoes with emphasis on the African malaria vectors in different farming and water management systems that may lead to the creation of suitable larval habitats. Advances in biological control, use of larvicides and environmental management for larval mosquito control suitable for the tropics and subtropics will be reviewed and the application of these technologies will be discussed in the perspective of sustainable, effective tools for malaria control.

Mosquito larval control and current technologies

Mosquito larval control options include chemical, mechanical, biological, environmental and water management methods. In the early 1960's to 1980's, oils and films were reported to be effective larvicides and pupicides when either sprayed or poured on the water, as many mosquito species had developed resistance to the larvicide alternatives of organochlorine and organophosphate (OP) larvicides (Floore, 2006). Larvicidal oils and films act by reducing surface tension, making it difficult for the larva, pupa, or emerging adult to attach to the water's surface, with the consequence that they drown. Examples of OPs and chlorinated hydrocarbons (e.g., DDT, fenthion, malathion, ethyl parathion, temephos, chlorpyrifos, methoxychlor). However only malathion and temephos are currently available for use as larvicides. Insect growth regulators (IGRs) prevent the normal maturation process of an insect, by interfering with the development of the adult or may lead to sterility or deformation of the adults if they emerge. Some IGRs act as chitin inhibitors, blocking the synthesis of chitin in the larval stage.

However most IGRs are toxic to juvenile stages of crustaceans (Floore, 2006). The use of chemicals such as oils, films, organophosphates, organochlorines and IGRs is mainly restricted to environments that are not sensitive. There is only little information on the use of these insecticides in Africa, while many trials using these chemical products have been done in the United States of America. However, with the continued use of chemicals, there is a threat of the development of insecticide resistance in vectors, hence calling for the development of alternative vector control tools that are sustainable. Existing knowledge shows the success of biological control methods, bio-insecticides and environmental (water) management for mosquito larvae control. These control measures can be used singly or in combination to reduce and/or eliminate mosquito vectors. We shall now focus on the three larval control options as they seem suitable for the tropics and subtropics where the environment is sensitive and highly heterogeneous.

Biological control methods: Biological control refers to the use of natural enemies and biological toxins directed towards aquatic stages. The most commonly used biological control methods for mosquitoes are the larvivorous fish *Gambusia affinis* (Baird & Girard) and *G. holbrooki* (Rose, 2001). *Gambusia* species can be reared in large numbers and released into mosquito breeding sites, where they may feed on many kinds of insects in addition to mosquito larvae (Homski et al., 1994; Floore, 2006). *Gambusia affinis* has been accepted as an effective mosquito control strategy (Gilles & Warrell, 1993; Homski et al., 1994; Bence, 1988). This fish has been widely used due to the fact that it seeks its food at the water surface, and because it is very prolific; it thrives under a large variety of conditions (Krumholz, 1948) and is able to tolerate a wide temperature range (Otto, 1973) which makes it suitable in the tropics and subtropics. Other means include the use of predators such as dragonfly nymphs and other indigenous aquatic invertebrate predators such as *Toxorhynchites* spp. mosquito larvae that prey on other mosquito larvae and pupae (Gilles & Warrell, 1993). Other fish species of potential use as biological control agents include *Tilapia zilli* Gervais (Asimeng & Mutinga, 1993), *Aphanis dispar* Boulenger (Homski et al., 1994) and *Oreochromis niloticus* Linnaeus (Howard et al., 2007). Fish can be very useful control agents when used in habitats such as dams, irrigation canals, ponds and in unplanned rice cultivation areas where water is present throughout. Other biological control methods include entomopathogenic fungi, nematodes and even viruses. However, none of these agents is currently being used or tested for the control of larval stages of anopheline mosquitoes.

Microbial insecticides: The spore-forming bacteria *Bacillus thuringiensis* var. *israelensis (Bti)* and *B. sphaericus* (*Bs*) belong to the most effective anti-mosquito microbials. Both are very selective agents against mosquito and midge larvae and the prospect of wide-scale environmental side-effects from the use of this biocide seems negligible (Floore, 2006; Takken & Knols, 2009).

The insecticidal properties of these bacteria are due primarily to insecticidal proteins produced during sporulation. In *Bti*, the key proteins are Cyt1A, Cry11A, Cry4A and Cry4B, whereas *Bs* produces a single binary toxin commonly referred to as Bin (Federici et al., 2003). These toxins work by attacking the midgut epithelium cells of mosquito larvae, causing the larvae to stop feeding within hours, and eventually killing them within 24 to 48 hours (Floore, 2006). Both *Bti* and *Bs* are usually applied directly to water, either temporary or permanent, that forms a mosquito larval habitat in any farming system (vegetable, rice, or maize), catch basins, swamps, or flood plains. Results from field trials in western Kenya with *Bti* water dispersible granules (WGD) showed that only a very low dosage of 200g/ha (2700 international toxic unit per mg [ITU/mg]) is required to effectively suppress late instars and resulting pupae despite low residual effect (Fillinger et al., 2003; Fillinger et al., 2004). Insecticide resistance against *Bti* biological pesticide is unlikely to develop because Bti has four protein toxins while *Bs* only contains one toxin (Takken & Knols, 2009; Federici et al., 2003). Although these products have achieved moderate commercial success in developed countries, their high cost deters the use in many developing countries (Federici et al., 2003).

Environmental (water) management: This is the planning, organization, implementation and evaluation of deliberate changes of environmental factors with the aim of preventing the propagation of vectors and reducing human-vector pathogen contact (Beales & Gilles, 2002). According to the World Health Organization (WHO), environmental vector management comprises: 1) environmental modification, which aims to create a permanent or long-lasting effect on land, water, or vegetation to reduce vector habitats; 2) environmental manipulation, which produces temporary unfavorable conditions for the vector; and 3) modification or manipulation of human habitation or behavior, which reduces human-vector contact (Keiser et al., 2005b). These alternatives can be used singly or in an integrated manner to achieve the best results. In Zambian copper mining communities, multiplicity of interventions centered at environmental management was applied, which included vegetation clearance, modification of river boundaries, draining swamps and flooded areas, regular application of oil to open bodies of water and finally applying gauze screens to houses to stop adult mosquitoes from entering houses (Utzinger et al., 2001). There is no uniform environmental management recipe that is appropriate in all settings; specifications for environmental management vary with local ecosystem structure (Keiser et al., 2005b). In Table 2.1, most studies cited report an increase in mosquito densities and/or malaria associated with rice cultivation and irrigation. As water is necessary for irrigation, the best control method should conserve water and hence complete drainage may not be feasible in some situations. Intermittent irrigation (alternate wet-dry irrigation) systems have been widely applied in China, Indonesia and parts of India and have proved efficient in mosquito control (Liu et al., 2004; Qunhua, 2004).

The use of larval predators, microbial larvicides and environmental manipulation on larval habitats could serve as a good option for larval control.

Environmental manipulation by using vegetation for mosquito control through its effects on the water temperature could be another option. In western Kenya, studies on deforestation and swamp reclamation have been shown to create malaria vector breeding habitats (Munga et al., 2006; Minakawa et al., 2005), which contributes positively to malaria transmission. Studies by Munga et al. (2006) showed that *An. gambiae* larvae were only collected from habitats located in the deforested and reclaimed swampy areas while those in forested and natural swamp areas recorded no *An. gambiae* larvae. In the southwestern highlands of Uganda, maximum and minimum temperatures were significantly higher in communities bordering cultivated swamps than in those near natural swamps, which led to an increase in malaria vector breeding that consequently led to an increase in malaria transmission (Lindblade et al., 2000a). A likely explanation for this development is that high canopy cover in forests and the tall grasses in swamps reduce the amount of solar radiation reaching larval habitats, which results in lower water temperatures (Munga et al., 2006; Lindblade, 2000a), that slows down larval development (Bayoh & Lindsay, 2003; Bayoh & Lindsay, 2004). Consequently reforestation programmes and the planting of plants that provide heavy shade on potential larval habitats, such as Napier grass and arrow roots grown in farmlands need to be explored.

Conclusion

Activities associated with farming, urban development and deforestation have often been reported to lead to an increase in mosquito vector populations. Many studies report an increase in mosquito vector numbers and/or incidence of malaria with rice cultivation, irrigation, poor sanitation, large dams and deforestation. However, not all farming activities result in increases in mosquito vectors. Areas under farming with intermittent irrigation report a reduction in malaria and/or mosquito vectors. Urban malaria control can benefit from good planning and the involvement of all stakeholders in the planning of development activities. Many studies in the recent past report a reduction in malaria disease incidence mainly by the use of insecticide treated bednets (ITN's) and indoor residual spraying (IRS), which aim at adult mosquito control. However, if these methods are integrated with larval control measures, we expect a drastic reduction in disease prevalence and vector densities. There is no one suitable control measure that is suitable in all environments because of temporal and/or spatial variation in environmental factors and the breeding habits of the local vectors species. As a result, vector control measures should be adopted depending on the ecology of the local mosquito vector species. If any control measure is to be sustainable, there is a need for the involvement of the local communities in the control activities so that people become aware of how their activities affect their health.

Chapter 3

A longitudinal study on the ecology of aquatic stages of malaria mosquitoes in distinct geographical and environmental settings in western Kenya

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Abstract

As mosquito larval ecology can be complex, there is need to develop a rational framework for undertaking larval ecological studies. We examined the suitability, stability and productivity of larval habitats in two highland villages and a lowland site in western Kenya to assess the possibilities of implementing an integrated approach to larval mosquito control under distinct ecological settings. Suitable larval habitats were classified according to their stability as either temporary or permanent aquatic habitats. The productivity of temporary and permanent habitats was quantified by carrying out longitudinal larval and pupal sampling for a period of two years. Ambient temperature, rainfall and relative humidity were measured in each study site. *Anopheles gambiae sensu latu* larvae were recorded from all the study sites. *Anopheles arabiensis* (93%) was more abundant in the lowland, Nyalenda area whereas among the highland villages Fort Ternan had more (71%) of this species. In Lunyerere *An. gambiae sensu stricto* was the most abundant comprising of 93% of the total *An. gambiae s.l.* larvae. Larvae of anopheline mosquitoes were present in both temporary and permanent habitats with monthly variations dependent on habitat type, vegetation cover on the habitat and the amount of rainfall. Among all the suitable habitats identified, drainage canals, hoof prints and burrow pits were productive for both larvae and pupae depending on the location. Eighty six percent of all habitats recorded during the study were man-made habitats. Grassy habitats were more preferred habitats and thus more productive compared to habitats with other vegetation types. Rainfall was an important meteorological factor associated with the abundance of mosquito larvae. In conclusion, both temporary and permanent habitats are equally suitable and productive for mosquito breeding and should both be given equal attention when implementing larval control programme.

Key words
Mosquito larvae, habitat type, vegetation, environment, rainfall, highland, lowland, western Kenya.

Introduction

In western Kenya, two sibling anopheline mosquito species, *Anopheles arabiensis* Patton and *An. gambiae* Giles *sensu stricto* (hereafter referred to as *An. gambiae*), both belonging to the *An. gambiae sensu lato* complex, and *An. funestus* Giles, are the principle vectors of malaria. Although *An. gambiae* is usually the predominant species in environments with high humidity and *An. arabiensis* is more common in zones with less rainfall, both species occur sympatrically across a wide range of tropical Africa (Coetzee et al., 2000, Petrarca et al., 1998). On a finer spatial scale, adult *An. gambiae* were collected more frequently in the foothills of western Kenya, while *An. arabiensis* was more abundant on the plains that received less rain (Minakawa et al., 2002b, White, 1972b).

Human activities act as a means of constant evolutionary challenge because they cause environmental changes to which anopheline mosquitoes must respond by developing a highly dynamic vector-host relationship (Mwangangi et al., 2007). Environmental alterations due to deforestation, swamp reclamation mainly for agriculture, excavation of sand and stones for building and brick making, poor drainage and vegetation clearance may lead to an increase in larval habitats of malaria vectors such as *An. gambiae* (Carlson et al., 2004, Fillinger et al., 2004, Minakawa et al., 2002a, Minakawa et al., 2005). Larvae of this anopheline species are commonly found in clear, sunlit pools of water in small depressions such as foot or hoof prints, the edges of bore holes and burrow pits, roadside puddles formed by tire tracks, irrigation ditches and other artificial water (Gillies & De Meillon, 1968, White, 1972b, Gimnig et al., 2001, Minakawa et al., 1999, Mutuku et al., 2006a). Anopheline malaria mosquito vectors have also been found breeding in polluted water rich in organic matter (Keating et al., 2004, Awolola et al., 2007, Sattler et al., 2005), in large bodies of water such as flood plains (Majambere et al., 2008) and in lake water especially when there are fluctuations in water level as it occurred in Lake Victoria (Minakawa et al., 2008).

Agricultural areas represent moist, disturbed environments that can sustain both immature and adult mosquitoes (Afrane et al., 2004). Water storage and irrigation are often necessary to sustain agricultural activity within the urban context. Therefore, urban farming may be increasing the availability of potential habitats for *Anopheles* mosquitoes (Keating et al., 2004). In the Ugandan highlands, the elimination of papyrus swamps has created a habitat for *An. gambiae* and *An. funestus*, leading to increased malaria transmission (Lindblade et al., 2000a). The increase in malaria transmission was attributed to the increased local water temperatures near cultivated swamps in combination with occasional excessive precipitation that favoured *An. gambiae* breeding (Lindblade et al., 2000a). In the highlands of western Kenya, *An. gambiae* emerged from habitats with agricultural crops only on land that had been extracted from forests and swamps (Munga et al., 2006). The successful breeding of anopheline mosquitoes was thought to be caused by the higher temperatures in the cleared habitats compared to the forest. In the same highlands it has been found that large among-site variation exists in the abundance and temporal dynamics of malaria vector populations indicating that the risk of transmission strongly differs among sites (Ndenga et al., 2006).

The abundance of clear or turbid sun-lit, and shallow water bodies and corresponding malaria-parasite transmission, increases as the distance to the city centre increases, which makes rural populations more vulnerable to increased contact with anopheline mosquitoes (Coetzee et al., 2000, Robert, 1993). Likewise, the absence of a suitable habitat and increased water pollution generally inhibits the development of anopheline larvae in urban centers, resulting in fewer *Anopheles* mosquitoes (Barbazan et al., 1998, Chinery, 1995). Our knowledge of anopheline larval ecology is spatially limited and often insufficient to achieve effective vector control through means of larval control (Oaks et al., 1991, Fillinger et al., 2004). From existing literature, little is known about the habitats, abundance and distribution of the larvae of the main African malaria vectors. Systematic research on the larval ecology of the main malaria vectors in Africa is still limited and often represents short periods (less than 12 months) of data collection, frequently considering mosquito-infested habitats only (Fillinger et al., 2004). The main objective of this study was to find out the suitability, stability and productivity of larval habitats in two highland villages and one lowland peri-urban area in western Kenya. The results of this study will provide baseline data for each of the study sites that will be important in finding a suitable and effective mosquito larval control measure.

Materials and methods

Study area

The study was undertaken in two highland villages (Fort Ternan and Lunyerere) and a lowland peri-urban area (Nyalenda) in western Kenya from March 2006 to April 2008. Fort Ternan and Lunyerere are almost at the same altitude but they differ in topography, vegetation cover and land use while lowland Nyalenda was chosen for comparison purposes. Lunyerere and Fort Ternan are about 40 and 70 km from Kisumu, respectively, while Fort Ternan and Lunyerere are 115 km apart. Western Kenya has a bimodal type of rainfall with long rains from April to June and short rains between November and December with yearly variability. Fort Ternan (0°12’S and 35°20’E) is a rural village, with approximately 2,000 inhabitants in the Kericho district located on the slopes of the Nandi hills, in western Kenya lying between an altitude of 1480-1650 m above sea level. The area is hilly with sharp, V– shaped valleys that normally drain water into a river at the bottom of the valley it rains. Typical to this area is the traditional practice of large scale farming of cash (sugarcane) and food (maize, vegetables) crop and livestock keeping. The Fort Ternan area has not yet undergone many land use changes. Lunyerere (0°06’N and 34°43’E) is a small village with approximately 4,000 inhabitants, located in Vihiga District, on the western side of Kakamega Forest, about 5 km North of the equator. The area under study has an altitude ranging from 1460-1580 m. The district is hilly and cold, characterized by undulating hills that often form basin-shaped valleys that are prone to flooding, offering excellent mosquito breeding habitats. Farming for both cash (tea) and food crops (maize, vegetables) is the main economic activity. Lunyerere was, until recently, characterised by forest and natural (mostly papyrus) swamps in the valley floor, which have been transformed into crop-land in the past 20 years. Characteristic to this area is the underground seepage of water that is always present, offering good breeding habitats for mosquitoes the whole year round. Nyalenda (0°06’S and 34°46’E, 1100 – 1200m altitude) is a low-income peri-urban area located on the Eastern outskirts of Kisumu city, with approximately 26,000 inhabitants. Kisumu is situated in the lowlands (approx. 1100 m) on the northeastern tip of Winam Gulf, an inlet of Lake Victoria. The Nyalenda area is flat, and has a natural source that produces abundant quantities of water used for irrigation of small scale agriculture and commercial tree nursery. About 10 years ago nearly 70% of Nyalenda was a natural swamp; however, the expansion of Kisumu town led to development activities in the area. The area is occupied by low-income population and due to population pressure and the need for a food supply, it has now been transformed into rice, vegetable and horticultural farming under irrigation. The area has an unplanned sewerage system that occasionally drains into the swamp, polluting it.

Environmental factors

Larval habitat characterization - A preliminary survey of suitability of habitats for mosquito breeding was done in March 2006, by searching for areas where water stagnates in the respective study sites was done in. Suitability was determined by the presence of all immature stages of mosquitoes. The bodies of water identified were then classified according to their nature: river fringe (habitats formed along the river when water level in the river drops), temporary pool, drainage channel, erosion pit (deep holes resulting from the action of water flowing down hill), tap (leaking taps and damaged pipes were grouped together), hoofprint, tire-track, rice paddy and burrow pit (in this case burrow pit refers to holes made by or for animals). Vegetation was broadly grouped into five groups namely: algae, grass, papyrus reeds, agricultural crops (crops used by the farmers either as food, such as arrowroot, rice and vegetables or as fodder, such as Napier grass) and others to cover for any other vegetation types that have not been listed. Habitats without any vegetation were grouped under 'no vegetation'. Vegetation was measured visually by estimating the percentage of the larval habitat covered. The larval habitats were finally grouped according to their stability into temporary habitats and permanent habitats. Temporary habitats held water for a short period of time (untill approximately 2 weeks after the rainy season had ended) and stem mainly from rain showers; when rain ceased these habitats dried out. The permanent sites, on the other hand, held water for a longer period of time (approximately 2-3 months after the rains) and hence were more stable. In each site 10, temporary and 10 permanent habitats were selected for weekly larval mosquito sampling. The permanent habitats remained in the same location throughout the sampling period while temporary habitats changed depending on the availability of water. To determine productivity, the habitats were examined once per week for the presence of aquatic stages of anopheline and culicine mosquitoes.

Meteorological data - Automatic weather stations were installed, one at Fort Ternan Health centre (1550 m altitude), one at Lyanaginga Health Centre (1500 m altitude) about 30 km from Lunyerere and a third station at the Kenya Medical Research Institute (KEMRI), Centre for Global Health Research, Kisian (1100 m altitude) about 17 km from Nyalenda. The weather stations measured temperature and humidity at 2 m above ground (ventilated probe; Vaisala, Finland) and precipitation (rain gauge, Eijkelkamp, The Netherlands) throughout the study period. The weather variables were recorded on a 21x Microdatalogger (Campbell Scientific Inc., UK) at an interval of 15 minutes from March 2006 to April 2008. Technical problems were experienced with the weather station installed at KEMRI between May to November 2006 and from June to October 2007. For the period the data presented were obtained from the Kenya Airports Authority, Kisumu airport. Relative humidity (between May to November 2006 and June to October 2007) is reported as the mean of two values collected in the morning (06.00 hours) and mid afternoon (12.00 hours). In the highland sites during the month of November 2007, the rain gauges experienced technical problems and hence the data for this period are excluded from the analysis.

Ecology

Larval sampling - Weekly surveys were conducted to establish the availability of stagnant water, habitat characteristics and larval densities from March 2006 to April 2008 in all habitats identified. Mosquito larval sampling was done in all habitats identified, by the dipping method using a standard dipper [350 ml] (Service, 1993). Both anopheline and culicine larvae were sampled and the stage of larval development recorded as either 1st-2nd instars (early), 3rd-4th (late) instars or pupae. A portion of the late instars were immediately preserved in 90% absolute ethanol and taken to the laboratory at the Kenya Medical Research Institute (KEMRI), Kisumu for identification under a compound microscope according to the keys of Gillies & Coetzee (1987). Microscopically identified *An. gambiae* late instars were then preserved individually in Eppendorf tubes containing absolute ethanol for further sibling analysis using polymerase chain reaction (PCR) analysis (Scott et al., 1993).

Data analysis - Data were recorded, and entered into a database. Relative humidity and temperature data were averaged and rainfall data were pooled for each month. General linear model (GLM) multivariate analysis of variance was used to check for any differences in densities of larvae in different habitats and in habitats with different vegetation cover. Post-hoc Tukey HSD tests were used to find out any differences in larval and pupal abundance among the different habitats as well as among habitats with different vegetation cover. Paired samples t-tests were done to check for any differences in larval abundance within the temporary and permanent habitats. Among the meteorological factors measured, only rainfall was used for further analysis. GLM, multivariate analysis of variance was done to find the effects of weekly rainfall on larvae and pupal abundance between the temporary and permanent habitats. Cross-correlation analysis with a time lag of up to 5 weeks was then used to address the correlation of weekly larval and pupal densities with rainfall.

Results

Larval habitat types and immature anopheline occurrence - In general, hoof prints, temporary pools, tire tracks, containers and paddies were temporary in nature while drainage canals, erosion pits, river fringes, burrow pits and livestock watering taps were permanent in nature (Table 3.1). In the highland villages of Fort Ternan and Lunyerere, livestock watering taps (25.9%) and drainage canals (89.2%) were the most encountered larval habitats, respectively. In the lowland area of Nyalenda, drainage canals (56.1%) and rice paddies (38.5%) represented more than 94% of all available larval sites (Table 3.1). Drainage canals, hoof prints and temporary pools were present in all the study sites. Erosion pits were present in the highland villages, whereas paddies and burrow pits were only found in lowland Nyalenda. Considering all habitat types recorded for the entire study period in all study sites, 86% of all habitats were as a result of human activities hence referred to as man-made habitats. Temporal variation in distribution of habitat types with an overall frequency of 25% and above from March 2006 to March 2008, are shown in Appendix 1.
Analysis of variance showed that in Fort Ternan, the densities of early and late instars of anophelines recorded varied significantly

(F = 16.70; df = 6; p<0.001 and F = 21.42; df = 6; p<0.001 respectively) depending on the habitat type. Higher densities of early and late instars were recorded in animal hoof prints in this area. In Lunyerere, the habitat type had a significant impact on densities of anopheline early instars (F = 2.44; df = 4; p<0.05) and pupae (F = 2.89; df = 4; p<0.05). In this area, drainage canals had slightly higher densities of immature anophelines compared to other habitats shown in Figure 1. Whereas, in Nyalenda habitat type influenced densities of early (F = 13.71; df = 4; p<0.001) and late anopheline instars (F = 4.31; df = 4; p = 0.002) only. Slightly higher densities of early and late instars were found in burrow pits than in drainage canals, rice paddies and temporary pools.

Vegetation cover and mosquito larvae - Habitats that had grass, algae, other vegetation, as well as those without vegetation were present in different frequencies among the sites (Table 3.2a). Papyrus reeds (swamp) and agricultural vegetation were present in Lunyerere and urban Nyalenda but not in Fort Ternan. The majority of larval habitats sampled, 66.4, 66.2 and 63.1% for Fort Ternan, Lunyerere and Nyalenda, respectively, had grass growing in them.

Occurrence of larvae and pupae in habitats with different vegetation cover differed depending on the area under study. Table 3.2b shows the mean numbers of larvae and pupae sampled with the number of dips taken for the entire sampling period. In Fort Ternan analysis of variance only found late instars of anophelines to be significantly affected by vegetation type (F=6.713; df=3; p<0.001) but not early instars and pupae. Tukey HSD tests showed that grassy habitats recorded more late instar anophelines than habitats without any vegetation (p=0.006, C.I. 0.005 – 0.045) and habitats with other vegetation types (p=0.005, C.I. 0.003 – 0.024).

In Lunyerere, vegetation in larval habitats had significant effects on all the immature anopheline stages occurrence. Early instar (F=23.067;df=5; p<0.001), late instars (F=20.760; df=5; p<0.001) and pupae (F=7.967; df=5; p<0.001) of anopheline showed variation among the different habitats depending on vegetation. Grassy habitats had significantly higher early instar densities (p<0.001, C.I. 0.1103 – 0.2432), late instar (p<0.001, C.I. 0.042 – 0.096) and pupae (p=0.001, C.I. 0.02 – 0.07), when compared to habitats without vegetation in Lunyerere (Table 3.2b). The densities of larvae and pupae in habitats without vegetation were not different from those in habitats that had algae, agricultural crops or papyrus reeds (p>0.05).

In Nyalenda, analysis of variance found vegetation type in larval habitats to have significant impacts on densities of early instar anophelines

(F = 3.183; df = 5; $p<0.05$) but not on that of late instars or pupae. Habitats with papyrus reeds had more early instar larvae than those with agricultural crops (p = 0.005; C.I. 0.0245– 0.2141) and grassy habitats (p = 0.017; C.I. 0.0078- 0.1307) (Table 3.2b).

Table 3.1: Frequency of habitat type occurrence and their distribution (%) in Fort Ternan, Lunyerere and Nyalenda during the 2 years of weekly sampling

Habitat name	**Habitat type**	**Fort Ternan (%)**	**Lunyerere (%)**	**Nyalenda (%)**
Drainage canal	Permanent	250 (14)	1754 (89.2)	1082 (56.1)
Erosion pit	Permanent	316 (17.7)	131 (6.7)	
Hoof print	Temporary	244 (13.6)	49 (2.5)	21 (1.1)
River fringe	Permanent	198 (11.1)		
Taps	Permanent	464 (25.9)		
Temporary pool	Temporary	78 (4.4)	30 (1.5)	45 (2.3)
Tire track	Temporary	239 (13.4)		
Container	Temporary		3 (0.2)	
Rice paddy	Temporary			762 (39.5)
Burrow pit	Permanent			20 (1.0)
Totals		1789 (100)	1967 (100)	1930 (100)

Table 3.2a: Dominant vegetation in the larval habitats in Fort Ternan, Lunyerere and Nyalenda

Dominant vegetation	**Fort Ternan (%) n=1789**	**Lunyerere (%) n=1967**	**Nyalenda (%) n=1930**
None	5.8	9.5	5.9
Grass	66.4	66.2	63.1
Algae	1.3	3.6	3.4
Others	26.4	0.2	18.5
Papyrus reeds		10.9	5.4
Agricultural crops		9.6	3.4

n = the total number of habitats encountered during the 2 year period of weekly sampling

Larval dynamics in temporary and permanent larval habitats – Anopheline larvae were recorded more in temporary habitats than in permanent habitats in Fort Ternan and Nyalenda (Figures 3.1b, 3.1c and 3.3b, 3.3c respectively). However, in Lunyerere both temporary and permanent habitats showed a similar trend in larval densities (Figure 3.2b and 3.2c). Culicine larvae were more abundant in temporary habitats in Fort Ternan whereas in Lunyerere and Nyalenda they were recorded more from permanent habitats (Table 3.4). Overall, culicine immatures were more abundant in Nyalenda than in Fort Ternan and Lunyerere (Table 3.4).

Table 3.2b: Mean number of anopheline larvae and pupae per dip sampled in habitats with different vegetation in Fort Ternan, Lunyerere and Nyalenda during the 2 year study period.

Site	Vegetation	No. of dips	Early instars	Late instars	Pupae
Fort Ternan	None	2,610	0.04	0.02	0
	Algae	1,220	0.05	0.02	0
	Grass	36,127	0.07	0.04	0
	Others	17,960	0.07	0.03	0
Lunyerere	None	7,510	0.03	0.01	0.01
	Algae	3,290	0.09	0.02	0.03
	Agricultural crop	10,580	0.10	0.04	0.03
	Grass	57,380	0.20	0.08	0.05
	Others	110	0.77	0.13	0.04
	Papyrus spp	11,700	0.06	0.02	0.02
Nyalenda	None	6,905	0.09	0.02	0.01
	Algae	6,540	0.13	0.01	0.01
	Agricultural crop	3,970	0.05	0.01	0
	Grass	119,590	0.10	0.01	0
	Others	29,084	0.10	0.01	0
	Papyrus spp.	5,940	0.17	0.01	0

Table 3.4: Mean (±SD) number of culicine larvae and pupae per dip sampled in temporary and permanent habitat types per site

Site	Habitat type	No. of dips	Early instars	Late instars	pupae
Fort Ternan	Permanent	30,725	0.07 ± 0.13	0.07 ± 0.14	0.01 ± 0.08
	Temporary	27,200	0.15 ± 0. 18	0.14 ± 0.18	0.02 ± 0.07
Lunyerere	Permanent	52,540	0.15 ± 0.30	0.14 ± 0.28	0.05 ± 0.18
	Temporary	38,080	0.15 ± 0.63	0.12 ± 0.30	0.04 ± 0.09
Nyalenda	Permanent	113,250	0.40 ± 0.75	0.19 ± 0.36	0.01 ± 0.03
	Temporary	58,889	0.71 ± 1.19	0.36 ± 0.62	0.02 ± 0.05

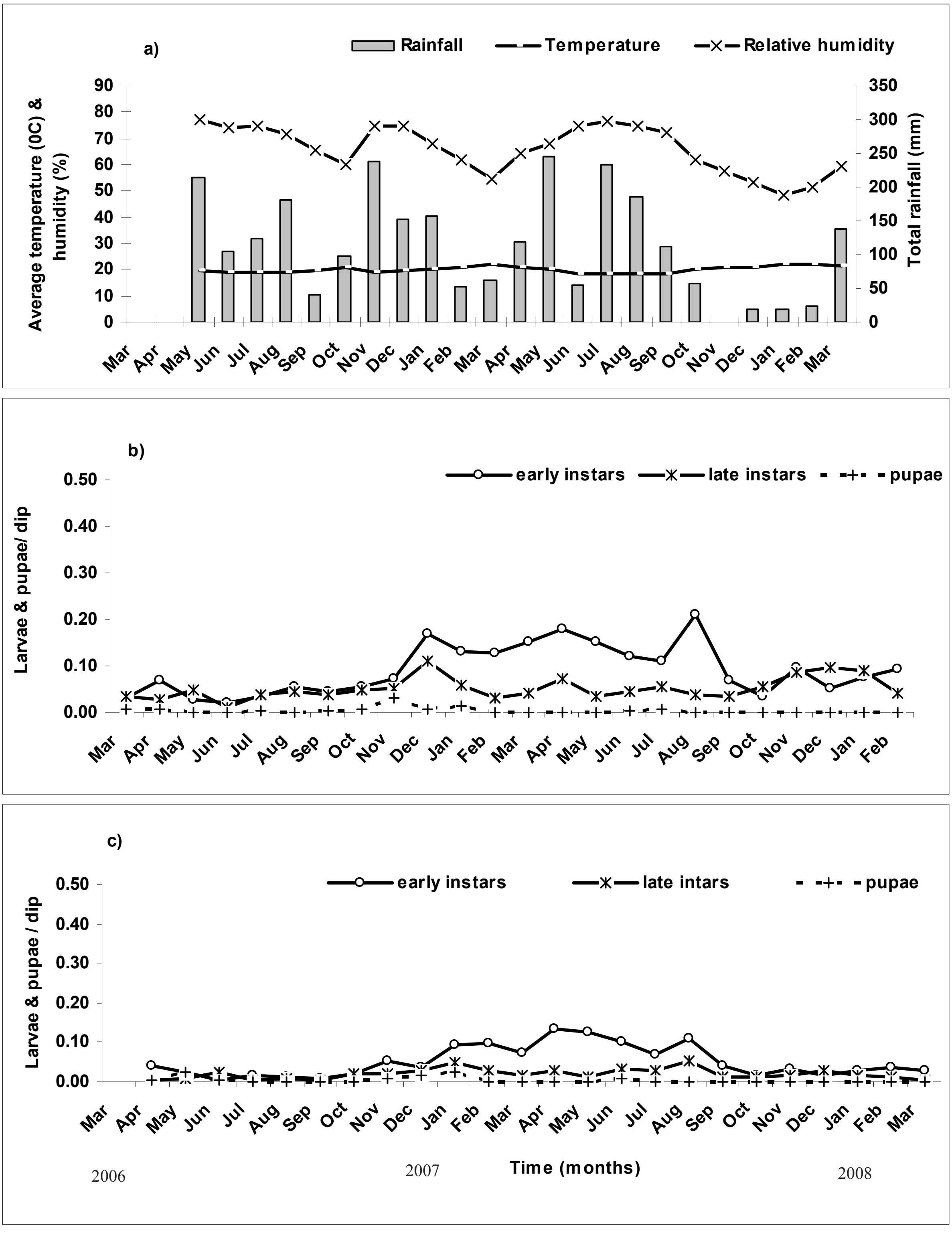

Figure 3.1: Weather data and larval population dynamics at Fort Ternan location a) Monthly average temperature, min and max relative humidity and total rainfall. b) Monthly anopheline larval and pupal dynamics from temporary and c) Permanent habitats from March 2006 to March 2008.

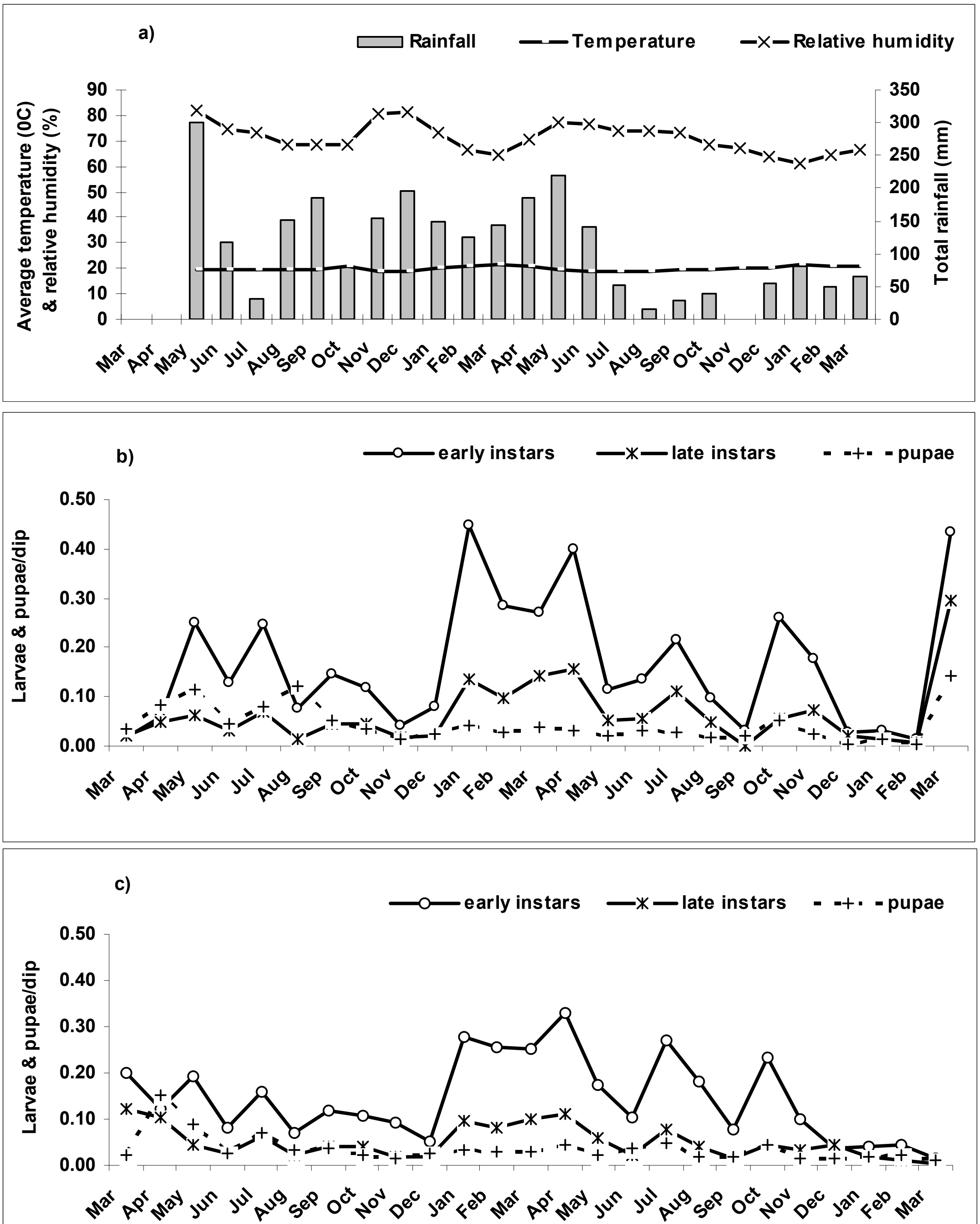

Figure 3.2: Weather data and larval population dynamics at Lunyerere location a) Monthly average temperature, min and max relative humidity and total rainfall. b) Monthly anopheline larval and pupal dynamics from temporary and c) Permanent habitats, from March 2006 to March 2008.

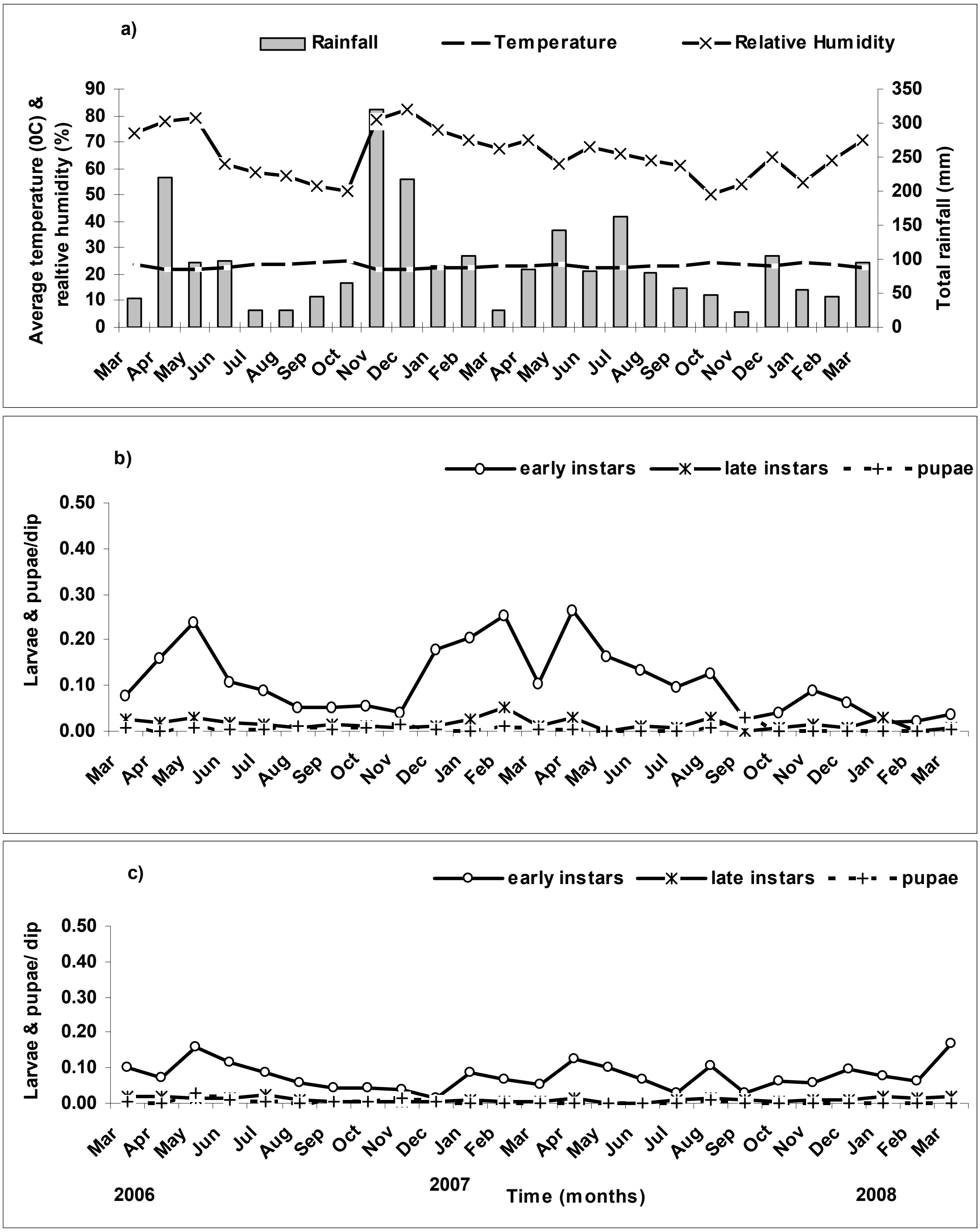

Figure 3.3: Weather data and larval population dynamics at Nyalenda location
a) Monthly average temperature, average relative humidity and total rainfall
b) Monthly anopheline larval and pupal dynamics from temporary and
c) Permanent habitats, from March 2006 to March 2008.

Meteorological variables and anopheline larval and pupal densities - The mean monthly air temperature did not show much variation during the study in all study sites (Figures 3.1a, 3.2a and 3.3a). Between the three sites, the months of November and December 2006 received the highest rainfall, and 2006 was a wet year compared to 2007. Overall, there is a general bimodal pattern of rainfall depicted in all sites (Figures 3.1a, 3.2a and 3.3a). Although we recorded the highest amounts of rain in November and December 2006, low numbers of anopheline larvae and pupae were recorded. The months in which we recorded a high relative humidity also showed higher amounts of rainfall and led to increases in larvae and pupae the following month. Overall, Nyalenda had a higher average temperature with slightly less rainfall than Fort Ternan and Lunyerere, which were relatively similar in temperature with high amounts of rainfall (Table 3.3). Lunyerere had a high average relative humidity when compared to Fort Ternan and Nyalenda. Analysis of variance showed significant site differences in temperature and relative humidity ($p<0.001$) while total rainfall did not show any significant differences among the sites ($p>0.05$) studied.

Table 3.3: Mean (±SE) values of monthly minimum, maximum and average temperature, rainfall and relative humidity (RH) in each study site

Site	Variable	Mean	Range
Fort Ternan	Temperature	19. 98 ± 0.24	18.07 – 22.01
	Rainfall	114.26 ± 15.21	
	RH average	65.99 ± 1.79	48.34 – 76.99
	RH minimum	50. 37 ± 3.34	27.78 – 82.25
	RH maximum	86.44 ± 1.11	74.88 – 93.40
Lunyerere	Temperature	19.89 ± 0.16	18.90 – 21.29
	Rainfall	124.76 ± 17.03	
	RH average	71.19 ± 1.15	61.19 – 81.79
	RH minimum	47.30 ± 1.59	31.57 – 61.19
	RH maximum	90.19 ± 0.55	85.85 – 95.33
Nyalenda	Temperature	23.16 ± 0.18	21.62 – 25.19
	Rainfall	93.80 ± 14.30	
	RH average	65.34 ± 1.83	50.19 – 82.0

Effects of rainfall on anopheline larvae and pupae - In Fort Ternan total weekly rainfall showed significant impacts on early instars in both temporary (F = 14.786; df = 1; $p<0.001$) and permanent (F = 5.880; df = 1; $p<0.05$) habitats. Cross correlation analysis showed that with no time lag weekly rainfall significantly led to high densities of early instars in both temporary and permanent habitats.

With a 2-week time lag early instars increased in densities with increase in rainfall while late instars increased with a 3-week time lag in both permanent and temporary habitats. Pupae showed an increase in density with rainfall ($p>0.05$) at a time lag of 0 and 4 weeks.

In Lunyerere analysis of variance only showed pupae to be significantly (F = 5.970; df = 1; p = 0.016) affected by the amount of rainfall. However, cross-correlations of rainfall and early instars showed a positive correlation ($p>0.05$) at a time lag of 3 and 5 weeks while late instars showed a positive correlation at a 5-week time lag. The pupal densities showed an increase immediately when it rained ($p>0.05$) when no time lag was applied.

In Nyalenda, rainfall did not show any relationship with anopheline early instars and pupae in permanent and temporary habitats. Rainfall was important for late instars in temporary (F = 5.470; df = 1; p = 0.022) and permanent habitats (F = 5.874; df = 1; p = 0.017). Cross-correlations with rainfall showed that for late instars in temporary habitats, at time lag 0, increase in rainfall led to significant ($p<0.05$) decrease in their densities while permanent habitats did not show any relationship with rainfall.

***Anopheles* species composition –** Of all *An. gambiae sensu lato* collected in Fort Ternan, Lunyerere and Nyalenda and analyzed by PCR, 29, 93 and 7%, respectively, were *An. gambiae sensu stricto* and 71, 7 and 93% were *An. arabiensis*. *Anopheles arabiensis* was the main malaria vector species in Nyalenda and Fort Ternan while *An. gambiae s.s.* was most abundant in Lunyerere. *Anopheles funestus* was present only in the highland villages but much more abundant in Lunyerere as opposed to Fort Ternan, where only a few individuals were found (Table 3.5). *Anopheles coustani* (Laveran) was present in all sites while *An. christyi* (Newstead & Carter) was only found in Fort Ternan. Other anophelines collected include varying proportions of *An. marshalli* (Theobald), *An. garnhami* (Edwards), *An. implexus* (Theobald) and *An. squamosus* (Theobald). Seasonal and temporal differences were observed among anopheline species abundance. *Anopheles gambiae* and *An. arabiensis* were recorded more often between the months of March to July and in October and November in all sites coinciding with the long and short rains.

Table 3.5: *Anopheles* species composition from a subsample of late anopheline instars collected during sampling in the three study sites

Species	**Fort Ternan (%)**	**Lunyerere (%)**	**Nyalenda (%)**
Anopheles gambiae sensu stricto	12 (4)	289 (51)	18 (5)
Anopheles arabiensis	29 (9)	21 (4)	231 (60)
Anopheles funestus	4 (1)	154 (27)	0 (0)
Anopheles coustani	83 (25)	29 (5)	46 (12)
Anopheles christyi	18 (5)	0 (0)	0 (0)
Other non vector anophelines	183 (56)	79 (14)	90 (23)

Anopheles coustani was more encountered from February to April and from September to December. *Anopheles christyi* was recorded more in the months of May, September and December.

Discussion

This study has revealed that drainage canals, hoof prints, tire tracks, rice paddies and watering taps, either leaking or broken with a constant water flow, were important man-made mosquito breeding habitats in the areas studied. In contrast, natural water bodies were poorly represented among the available mosquito habitats even in the rainy season. Drainage canals were present among the three sites for different purposes, in Lunyerere and Fort Ternan canals were dug to drain away unwanted water and for Lunyerere specifically to drain water from the reclaimed swampy land to allow for farming. However, in the lowland area of Nyalenda drainage canals were mainly used for irrigation purposes, drainage of excess and/or waste water and occasionally the water was polluted with human waste. This study supports the findings of many other studies that have documented the importance of man-made habitats, in the creation of small sunlit pools favourable for mosquito breeding (Fillinger et al., 2004, Minakawa et al., 2005, Mutuku et al., 2006a). However, in the three study sites there seemed to be no specific breeding habitat type for anopheline mosquitoes; females may oviposit in almost any water that is likely to be available as previously noted by Holstein (1954).

Our results in Fort Ternan and Nyalenda show that temporary habitats were more preferred breeding habitats for both anopheline and culicine mosquitoes. Conversely, in Lunyerere anopheline larvae occupied temporary and permanent habitats equally. These results are in line with the findings of Fillinger et al. (2004) that semi-permanent and permanent habitats can be suitable for proliferation of both anophelines and culicines similar to temporary habitats. The abundance of clean, sun-lit, and shallow bodies of water makes rural populations especially vulnerable to increased contact with anopheline mosquitoes (Coetzee et al., 2000, Keating et al., 2004), but may vary depending on the area under investigation. Of the highland villages studied, though they were at similar altitudes, we recorded more vector species in Lunyerere and in higher abundance than in Fort Ternan. In Lunyerere the mosquito breeding habitats were in a broad, flat, valley floor, which until recently was a natural swamp forest. Hence, Lunyerere experiences underground seepage of water that ensures the presence of small pools of water that are good mosquito breeding habitats all year round. By contrast, the Fort Ternan valley is narrow with steep slopes, causing a rapid drainage of water unless human activities create or construct water-retaining structures. Nyalenda was a natural drainage area, which in the last decade was turned into an agricultural area linked to the urban growth of Kisumu, creating many variable water bodies suitable for mosquito larvae.

Our results show that habitats that had grass growing in them had more immature mosquitoes than habitats with other vegetation types and open habitats. These results are in line with the findings of Fillinger and others (2004), who found anopheline larval density to be positively associated with the occurrence of tufts of low vegetation such as grass. These findings were true for both highland habitats studied and it could be that grass offered protection to mosquito larvae from being swept away by water or predation. Grass could have also been convenient in offering adult mosquitoes a shady resting site although this study did not investigate the role of grass as a resting place for gravid mosquitoes and more research is needed on this topic. In Nyalenda, habitats containing papyrus reeds were more important for both anopheline and culicine larvae than those without any vegetation. Previous studies have shown that areas that are under natural, undisturbed papyrus swamps were unsuitable for anopheline breeding in Uganda (Goma, 1960) and western Kenya (Munga et al., 2006). However, once the natural state of *Papyrus* sp. is disturbed, as in Nyalenda and Lunyerere, mosquito breeding can to take place and consequently may lead to an increase in malaria.

In Nyalenda, where on many occasions the water was polluted with human waste and other debris, *An. arabiensis* was more abundant than any other anopheline species.

This supports the hypothesis that anopheline species can adapt to breeding in environments rich in organic waste, as was observed in urban Dar es Salaam (Sattler et al., 2005) but contrary to the findings of Robert et al. (2003). The presence of rice paddies could partly explain why *An. arabiensis* was common in Nyalenda, a species that was found to exploit permanent artificial habitats such as rice fields (White, 1972b, Githeko et al., 1996a).

This study found *An. arabiensis, An. gambiae* and *An. coustani* to be present in the different sites. *Anopheles arabiensis* comprised 71% of *An. gambiae s.l.* collected from Fort Ternan. This is the highest proportion that has ever been recorded in the highlands of western Kenya. Previous studies in western Kenya highlands report the presence of *An. gambiae s.s.* but not *An. arabiensis* at a species that is present in the lowlands (Githeko et al., 2006; Ndenga et al., 2006; Koenraadt et al., 2006). The presence of *An. arabiensis* in Fort Ternan and Lunyerere can be attributed to the slow changes in land use, such as deforestation activities that lead to changes the micro-climatic conditions favoring the survival of this species at such high altitude areas. *Anopheles funestus* was only found in the highland villages while *An. christyi* was only present in Fort Ternan. This is in line with previous studies by Koenraadt and others (2006) who reported the presence of *An. christyi* from the same area. The vector status of *An. christyi* has not been resolved, although Garnham (1948) suggested that the species might occasionally be implicated as a malaria vector. *Anopheles coustani* was present in all the study sites and this species has recently been reported as a possible malaria vector in East Africa (Geissbühler et al., 2009).

The abundance of clean, sun-lit, bodies of water in Lunyerere and Nyalenda provide productive habitats however, it is not the case in Fort Ternan but for different reasons. One possible explanation for our findings is that Lunyerere and Nyalenda are reclaimed swamps and this land use change has contributed significantly to the suitability of habitats for anopheline mosquito breeding. In addition, there is a lot of maize farming in Lunyerere and partially in Nyalenda; maize pollen produced during flowering could be a good source of nutrition for mosquito larvae, hence favoring larval development (Ye-Ebiyo et al., 2000, 2003). Fort Ternan has not yet undergone major land use changes in the recent past although large scale maize farming is a traditional practice. In addition, in Fort Ternan, mosquito breeding was found not to be directly related to crop farming but rather related to livestock holding, as most anopheline larvae were collected from cattle hoof prints. However, with the increasing population in the highlands, enhanced human activities including deforestation, farming and livestock rearing are likely to create more vector habitats

(Chen et al., 2006; Minakawa et al., 2004; Minakawa et al., 2005). The preference of culicine mosquitoes for turbid water is coherent with their known breeding habitat preferences, as they breed successfully in rather polluted environments such as blocked drains and septic tanks (Chevase et al., 1995), unlike anophelines.

In this study, it was found that rainfall affected the abundance and distribution of anopheline and culicine aquatic stages differently depending on the location. Anopheline larvae were observed to increase steadily with increase in rainfall with or without time lags in Fort Ternan and Lunyerere, similar to the findings of Garnham (1929) and Koenraadt (2004). However, experiments by Paaijmans et al. (2007) showed that precipitation flushed, ejected and killed a significant proportion of *An. gambiae* larvae in different stages of development in a semi-natural environment. It is possible that in Nyalenda, due to occasional flooding after it rained, majority of mosquito larvae were flushed away hence no positive correlation was found between larvae and rainfall at this site.

The current study was done under natural conditions and external factors could have played an important role in the colonization and growth of mosquito larvae in the respective habitats. Factors such as water turbidity, nutrient content in the water, cannibalism, predation of immature stages, parasitism, pathogens, competition, water temperature and plant-odours that could have either repelled or attracted female mosquitoes during oviposition (Service, 1971; 1973; 1977; Okogun et al., 2005; Koenraadt & Takken, 2003b; Schneider et al., 2000; Paaijmans et al., 2009), were not controlled which could have played a role in the results obtained. Studies of the ecology of larval anopheline mosquitoes are methodologically challenging and the current study had several limitations. Quantitative sampling was problematic because the standard dipping technique adapted for this study was unsuitable in small habitats and consequently the density of mosquito larvae and pupae may have been underestimated (Mutuku et al., 2006b; Service, 1993). According to our results productivity of a habitat is expressed as the density/abundance of larvae or pupae (Minakawa et al., 1999, Gimnig et al., 2001, Service, 1971; 1973; 1977) and not on the numbers of pupae or adult mosquitoes that emerged from a habitat (Gu et al., 2008; Mutuku et al., 2006b). Although many habitats might record more larvae, not all larvae develop into pupae and adults. However, spatial models have shown the importance of non-productive habitats as part of transmission foci in facilitation of mosquito movement in search of a blood meal and aquatic habitats (Carter et al., 2000; Le Menach et al., 2005).

We have shown that mosquito larval abundance depends on the suitability, stability and productivity of the larval habitat. Suitability of any given habitat may be influenced by the vegetation type within the larval habitat and the land use in the area under study. Most of the mosquito larval habitats in the studied areas were a result of human activities. Permanent and temporary larval habitats were found suitable for breeding of mosquitoes; hence both should be given equal attention when implementing a larval control programme. Rainfall was an important meteorological factor associated with the abundance of mosquito larvae. The results obtained in this study will help in the development of integrated mosquito larval control options in the respective study sites. An integrated larval control approach such as water and environmental management measures, biological control options like predatory fish/larvae are highly feasible (Takken & Knols, 2009). In addition, the use of microbial larvicides, especially in temporary habitats and in areas where water can not be drained away would be feasible (see Chapter 7).

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Chapter 4

Dynamics of malaria transmission in different agricultural settings in western Kenya

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Abstract
Malaria endemicity and risk vary from one region to another and even from one village to another, depending on local environmental conditions. To understand the disparity in malaria transmission in western Kenya, we investigated malaria vector dynamics and malaria prevalence in three different agricultural settings, involving two highland villages (Fort Ternan and Lunyerere) and a lowland peri-urban area (Nyalenda) of Kisumu city. Fort Ternan is characterized by traditional highland agriculture, Lunyerere by farming in a recently-cleared highland forest zone and Nyalenda by irrigated farming in a peri-urban setting. In each area, adult mosquito collections in local houses and malaria parasite prevalence studies in children aged 2-10 years were done monthly from March 2006 to April 2008. Meteorological data were collected by automated weather stations. Anopheline mosquitoes were examined for *Plasmodium falciparum* infections. The malaria vectors *Anopheles gambiae sensu stricto*, *An. arabiensis* and *An. funestus* were all present in the highland villages, but the first two species in markedly higher densities in Lunyerere than in Fort Ternan. Considering species abundance in the highland villages, *An. funestus* was the main vector species in Fort Ternan. Of the *An. gambiae s.l*. from Lunyerere *An. gambiae s.s.* was most abundant accounting for 87.5% while *An. arabiensis* comprised of 12.5 % . *Anopheles arabiensis* was the dominant species in Nyalenda, where *An. funestus* was not found. Sporozoite rates varied among the study sites: no infectious mosquitoes were found in Fort Ternan, in Lunyerere sporozoite rates had a mean 15.2 ranging from 0-100% while Nyalenda had a mean of 0.28 ranging from 0-4%. The entomological inoculation rates were estimated to be 0, 109 (95% C.I. -1.73 – 19.92) and 24 (95% C.I. 2.01 – 6.42) infective bites annually in Fort Ternan, Lunyerere and Nyalenda, respectively. The mean malaria prevalence rate observed in school children aged 2-10 years was 5.2% in Fort Ternan, 7.0% in Lunyerere while Nyalenda had a mean prevalence of 4.1%. There was no correlation between mosquito abundance and malaria prevalence, however this can be explained by the differences in species composition. *Anopheles arabiensis* may contribute little to transmission due to its exophagic and exophilic characteristics. We conclude that Lunyerere was exposed to the highest malaria risk, followed by Nyalenda and Fort Ternan, respectively.

Key words
Anopheles gambiae sensu stricto, *An. arabiensis, An. funestus*, malaria, highland, lowland, agriculture, Kenya

Introduction

There are large among-site variations in the abundance and temporal dynamics of malaria vector populations indicating that the risk of parasite transmission differs among sites (Ndenga et al., 2006). Even in one topographic area mosquito vectors and malaria infections may not be distributed homogeneously and some households within the same area have a higher malaria incidence than others (Brooker et al., 2004; Carter et al., 2000; Munyekenye et al., 2005). Many factors may be responsible for this spatial heterogeneity of malaria vectors and transmission intensity such as land use and land cover changes, topography, house building materials and design and the level of household protection measures against mosquitoes (Balls et al., 2004; Githeko et al., 2006; Lindsay et al., 2002; Lindsay & Snow, 1988; Munga et al., 2006; Ye et al., 2006) and in most cases it is difficult to identify the factor that contributes most to these variations. In many African highlands malaria resurgence has been attributed largely to the rise in drug-resistant parasites (Omar et al., 2001), although other factors are also likely to be important, such as poor health systems (Lindsay & Martens, 1998), land use changes such as deforestation and swamp reclamation (Lindblade et al., 2000a; Munga et al., 2006), population growth and migration (Martens & Hall, 2000) and climate variability (Abeku et al., 2003; Githeko et al., 2000).

In western Kenya, malaria is predominantly a rural disease and the main malaria vectors are *Anopheles gambiae* Giles *sensu stricto, An. arabiensis* and *An. funestus* (Githeko et al., 1996b). *Anopheles gambiae* generally increases in density after the start of the long rains while *An. funestus* density is seen to vary in direct proportion to the proximity of permanent breeding grounds rather than rainfall (Garnham, 1929). In the adult stage these anopheline species share many of the same habitats. In the Usambara Mountains, Tanzania and in western Kenya, Balls et al. (2004) and Githeko et al. (2006) reported that altitude plays an important role in determining malaria infection due to its effect on temperature. It is possible that temperature simply governs vector densities through a direct physiological effect on larval development time (Depinay et al., 2004). Land use changes such as deforestation and swamp reclamation are thought to have created new habitats for effective vectors, and the elimination of shade has produced a marked change in local climate and microclimate (Lindblade et al., 2000a; Munga et al., 2006) leading to increased vector survival and subsequently malaria transmission. Over the past four decades, land use changes such as deforestation and swamp cultivation have widely occurred in western Kenya and these are now thought to be a major contributing factor to the abundance of breeding habits and the survival of malaria vectors. The ever-increasing human population and the need for food security place large pressure on land use and threaten the survival of undisturbed natural forests and swamps. The current study was undertaken to investigate the dynamics of malaria transmission, including both vector and pathogen, and prevalence in three different agricultural settings in western Kenya that are undergoing rapid change.

Materials and methods

The study was carried out in western Kenya in two highland villages, Lunyerere and Fort Ternan, and the lowland peri-urban Nyalenda, a suburb of Kisumu city. Fort Ternan (0°12’S and 35°20’E) is a rural village in Kericho district located on the slopes of Nandi hills lying between 1480-1650 m. The area is hilly with sharp, V–shaped valleys with high rainfall favoring agriculture. Typical to this area is traditional, large scale agriculture mainly for cash (coffee, sugarcane) and food (maize, vegetables) crop cultivation as well as cattle rearing. Lunyerere (0°06’N and 34°43’E) village is located in Vihiga District, on the eastern side of the Kakamega forest, about 5 km north of the equator, with an altitude ranging from 1460 and 1580 m. Lunyerere is under adapted agriculture for both cash (tea) and food crops (maize, vegetables) through the action of deforestation and swamp clearance. The area is characterized by basin shaped valleys that are prone to flooding offering excellent mosquito breeding habitats. Nyalenda (0°06’S and 34° 46’E, 1100 –1200 m) is a peri-urban area located on the outskirts of Kisumu city. Kisumu is situated on the northeastern tip of Winam Gulf, an inlet of Lake Victoria. Nyalenda was previously a swamp, deriving its water from natural wells, but due to an increase in population in urban Kisumu, farming for food crops has been encouraged as a way of ensuring food security for the expanding population. Nyalenda is now under irrigated peri-urban agriculture growing rice, maize and vegetables.

Meteorological data

Automatic weather stations were installed, one at the Fort Ternan Health centre, the other one at Lyanaginga Health Centre about 30 km from Lunyerere and another at the Kenya Medical Research Institute (KEMRI), Centre for Global Research, Kisian, about 17 km from Nyalenda. The weather stations measured temperature and humidity at 2 m above ground (ventilated probe; Vaisala, Finland) and precipitation (rain gauge, Eijkelkamp, The Netherlands) throughout the study period. The weather variables were recorded on a 21x Microdatalogger (Campbell Scientific Inc., UK) at an interval of 15 minutes from March 2006 to April 2008. For Lunyerere and Fort Ternan all variables were measured as expected. In Kisian, however, the weather station experienced technical problems for several months hence humidity data are not available for Nyalenda. As a proxy we have used the average relative humidity data from the Kenya airports authority based at Kisumu airport, midway between Nyalenda and Kisian.

Adult mosquito sampling: Ten houses were selected in Lunyerere and Nyalenda for adult mosquito sampling, while in Fort Ternan 20 houses were selected: 10 near the valley bottom and 10 on the higher slopes, hereafter referred to as sentinel houses. This was done to account for the large variation in altitude in this area. Most of the sentinel houses consisted of mud walls and thatched roofs, while a few had iron sheet roofs and cemented walls. In each site, adult mosquitoes were collected monthly from the sentinel houses by Centres for Disease Control (CDC) battery-operated light-traps (Model 512; John W. Hock Company, Gainesville, FL, USA) and pyrethrum spray catches (PSC). The CDC light trap catches preceded the PSC catches by 24 h throughout the study. Light traps were installed in the sentinel houses near the foot end of the bed, next to an untreated bed net (Mboera et al., 1998) and operated from 18.00 to 06.00 hours in each house. One day after the CDC light trap collections, PSCs were made using simple flit-guns to spray inside closed rooms with 2% pyrethrum extract synergized with piperonyl butoxide in kerosene (Service, 1993). Ten minutes were allowed before closed rooms were re-entered and the mosquitoes were collected from the sheets that had been laid out in the rooms. Female anopheline mosquitoes collected were then classified microscopically based on their gonotrophic stage as unfed, blood fed, half gravid and gravid (Gillies & Coetzee, 1987; WHO, 1975). Although culicine mosquitoes do not transmit malaria, mosquitoes of this genus are mainly nuisance biters and were also recorded during the sampling. Mosquito sampling took place in the same sentinel houses throughout the study. In any event such as abandoning of the houses by occupants, an adjacent house replaced the original one. Verbal consent was sought from the head of each household at the start of the study. Pyrethrum spray catches catches began in March 2006 while CDC light trap collections began later in July 2006.

Species identification: Vector species were first morphologically identified under a dissecting microscope. *Anopheles funestus* and species belonging to the *An. gambiae* complex were stored on silica gel at room temperature pending further analysis. Members of the *An. gambiae* complex were identified to the species level using the polymerase chain reaction method (PCR) (Scott et al., 1993). One leg or wing of adult female *An. gambiae* was used to determine sibling species by means of PCR (Scott et al., 1993). This was done at the Walter Reed Army Institute Laboratory based in Kisumu, Kenya.

***Plasmodium falciparum* sporozoite infection rates in mosquitoes**: The head and thorax of each female *An. gambiae* and *An. funestus* were separated from the abdomen, individually placed in a 1.5 ml Eppendorf microcentrifuge tube, processed for circumsporozoite (CS) protein as described by Beier et al. (1990) at the Walter Reed Army

Laboratory based in Kisumu, Kenya.

Monthly prevalence of malaria parasites in children

A house-to-house population survey was done in each study location to find out households with children aged between 2 and 10 years. These children were then enrolled to form a study cohort of 100 children. In Fort Ternan children were enrolled from houses located in the valley and up the valley. Consent was sought from the parents / guardians before the child was enrolled in to the study. Each child was then given a unique code that was used to track the same child throughout the study from June 2006 to April 2008. Blood samples were collected monthly by the standard finger-prick method, thick and thin smears were prepared on labeled slides (Gilles & Warrel, 1993). Core body temperature of the children was measured with a Braun Thermoscan® (Frankfurt, Germany) ear probe thermometer and each child was tested with a fresh sterile ear plug. The thin and thick blood smears were air dried. Thereafter the thin and thick smears were fixed in methanol and stained in 4% Giemsa for 30 minutes. An experienced technician examined the slides under 1,000 magnification by using oil immersion to identify and count the parasite species. Random checks were carried out on the slide counts (to include at least 10% of all slides) by independent microscopists to ensure quality control. Parasite density was scored against 200 leukocytes when the slide was positive; otherwise, the whole slide was carefully scanned before being declared negative. An individual was considered positive if malaria parasites were detected in the blood smear. Any child that was clinically ill at the survey date was taken to the nearest public health facility for treatment free of charge. A child was considered clinically ill if he/she had fever (a core body temperature of $\geq$ 38.0 °C) and malaria parasites identified from blood smear. Malaria prevalence studies commenced in Fort Ternan in June 2006 while in Lunyerere and Nyalenda we began in January 2007.

Ethical considerations

Institutional ethical clearance was given by the Kenya Medical Research Institute (KEMRI) and Wageningen University and Research Centre (WUR), The Netherlands, protocol approval numbers 1121 and 512. In addition, permission was obtained from the community elders and home owners.

Data analysis

Non-parametric tests were used to test for the differences in monthly adult mosquito collections (Wilcoxon signed ranks test) and malaria prevalence (Kruskal-Wallis test) among the study sites. Only female mosquitoes were included in data analysis as they are responsible for disease transmission. Cross correlations were done between monthly weather variables and female adult mosquito collections. Univariate analysis of variance was used to test for any site differences in malaria prevalence. For graphical representations log (n+1) of a number was used (Southwood & Hendersen, 2000). Sporozoite rate calculations were based on the total anopheline female catch from CDC light traps and PSC collections. However, for entomological inoculation rate (EIR) calculations, only CDC light trap catches for January to December, 2007 were used. The EIR was then calculated using the alternative method provided by Drakeley et al. (2003). Analysis was done using SPSS version 15.0 (SPSS Inc, Chicago, IL, USA). Due to political instability in the country between December 2007 and February 2008, we were unable to continue working in our study sites for the months of January, February and March, hence data for adult mosquito sampling and malaria prevalence for this period are not available in some sites.

Results

Effect of meteorological variables on female mosquito densities and malaria prevalence

The mean monthly temperature over the 24 months (May 2006 to April 2009) of study was 20.0±0.2, 20.0±0.2; 23.2±0.2 for Fort Ternan, Lunyerere and Nyalenda respectively, while the mean relative humidity was 66.0±1.8, 71.2±1.2 and 65.3±1.8, respectively. The total rainfall received during the 24 month study period from May 2006 to April 2009 was 2740, 2870 and 1553 mm for Fort Ternan, Lunyerere and Nyalenda respectively. Figure 4.1 shows the monthly dynamics of rainfall and relative humidity in the study areas. Peaks in rainfall are seen in the months of April, May and June, while higher amounts of rainfall were received in November and December 2006 in all sites. The potential correlation between monthly total rainfall, relative humidity and air temperature with numbers of anopheline and culicine adult mosquito collections were investigated. In Fort Ternan, relative humidity, temperature and rainfall did not show any relationship with female anopheline adult densities. However, in Lunyerere average maximum relative humidity was positively correlated with adult anopheline mosquitoes at 1 month time lag. While in Nyalenda average relative humidity and rainfall showed positive correlation with anopheline mosquitoes at zero and 1 month time lag, respectively. Culicine female mosquitoes did not show any correlation with rainfall, relative humidity nor air temperature. Cross correlations of monthly malaria prevalence with rainfall, humidity and temperature data did not show any significant relationship ($p>0.05$).

Adult mosquito densities in the three study sites

A total of 422 anophelines and 13,218 culicines were caught during the study period. Of the anophelines, 28.9% were male 71.1% female, while for the culicines this was 25.8 and 74.2%, respectively.

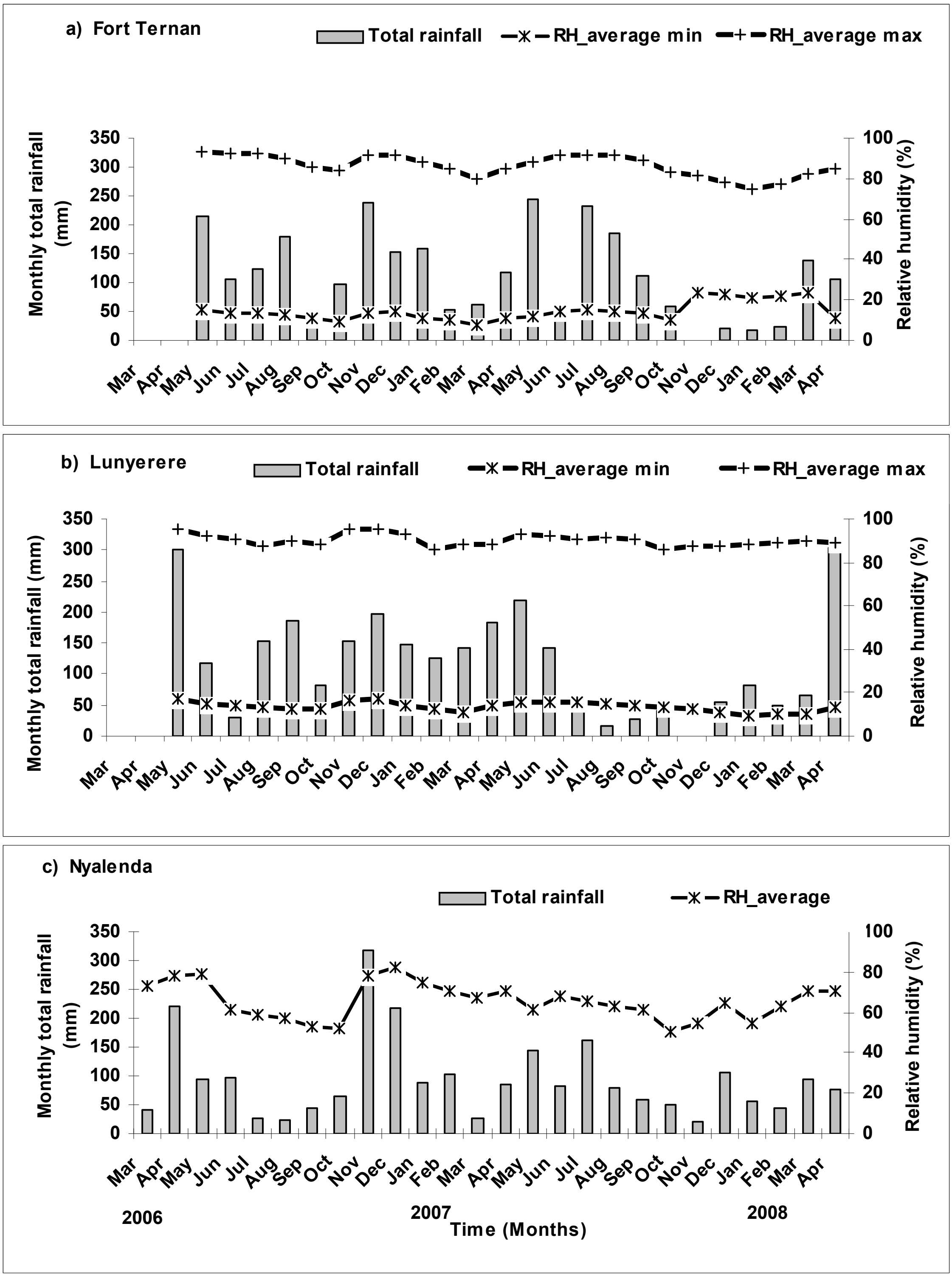

Figure 4.1: Monthly total rainfall, maximum and minimum or average relative humidity from March 2006 to April 2008, for a) Fort Ternan, b) Lunyerere and c) Nyalenda

Considering each group, 7, 39 and 54% of anophelines, and 6, 5 and 89% of the culicines were collected from Fort Ternan, Lunyerere and Nyalenda, respectively. Figure 4.2a and 4.2b show the monthly dynamics of female *Anopheles* and culicine mosquitoes in the respective study areas. Among all the study sites, culicine mosquitoes were more abundant compared to anophelines (Figure 4.2a and 4.2b). Overall, when all the female anophelines per house in all the study sites were combined, the CDC light traps caught more mosquitoes compared to PSCs (Table 4.1). The mean number of mosquitoes per house collected with the PSC was 0.234 (95% CI 0.15 – 0.32) while that of the CDC light trap was 0.345 (95% CI 0.26 – 0.43).

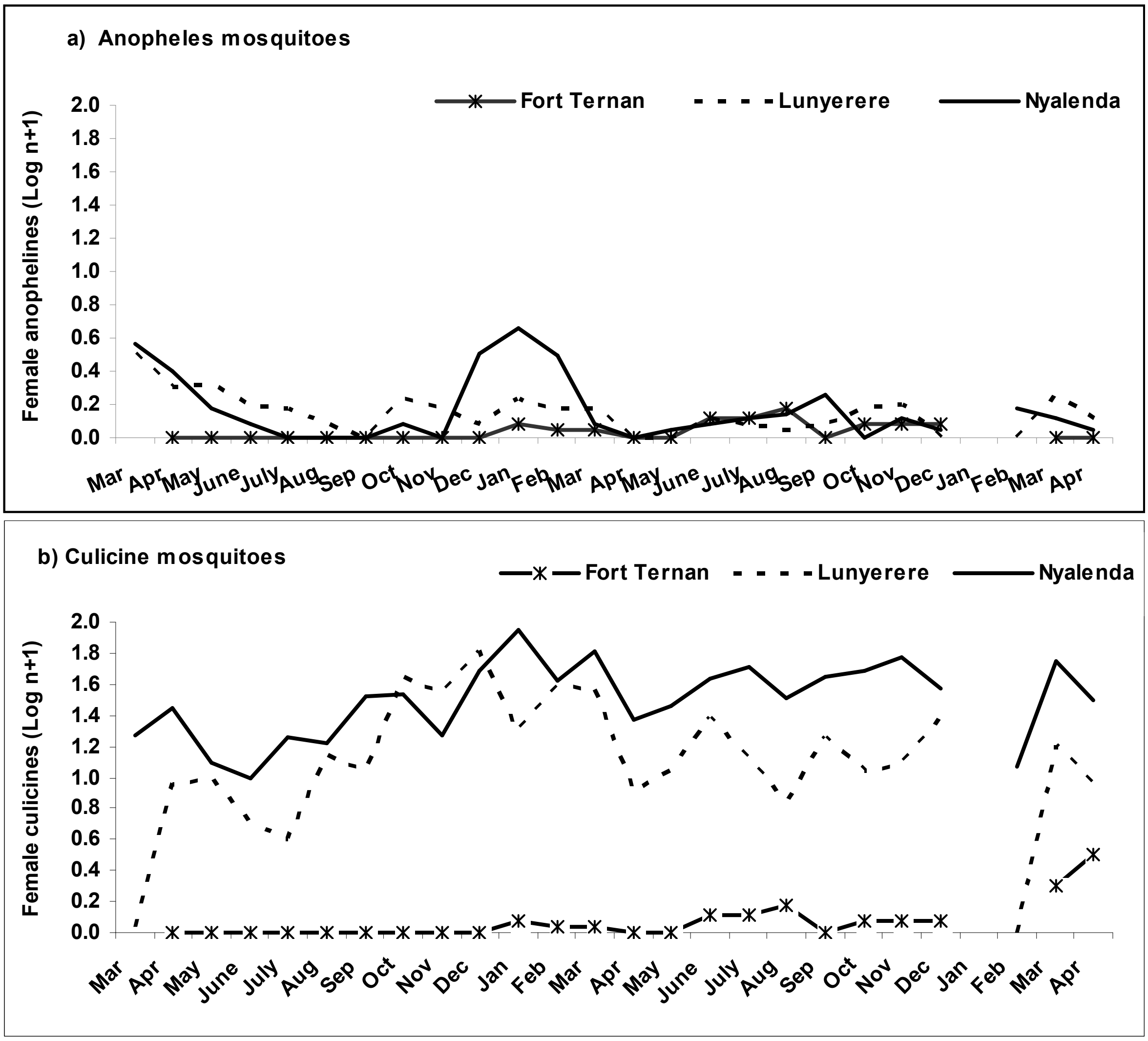

Figure 4.2: Mean monthly densities (Log [n+1]) of female (a) *Anopheles* spp. per house, (b) culicine mosquitoes per house from March 2006 to April 2008.

Table 4.1: Monthly mean (± SE) adult female anopheline mosquitoes collected per house from March 2006 to April 2008 collected by PSC and CDC light traps

	Fort Ternan		**Lunyerere**		**Nyalenda**	
Month	**PSC**	**LTC**	**PSC**	**LTC**	**PSC**	**LTC**
Mar	0		2.60±1.86		6.10±2.41	
Apr	0		1.50±0.69		1.70±0.37	
May	0		1.20±0.89		0.60±0.22	
June	0		0.60±0.34		0.22±0.15	
July	0		0.50±0.40	1.10±0.55	0	0
Aug	0	0	0.22±0.22	0.20±0.20	0.10±0.10	0.20±0.13
Sept	0	0		0.10±0.10	0	0
Oct	0	0		0.70±0.30	0	0.20±0.20
Nov	0	0	0.10±0.10	0.40±0.22	0	0
Dec	0	0	0.20±0.20	0.20±0.13	0.70±0.26	1.60±0.64
Jan	0.15±0.08	0	0.50±0.27	1.00±0.39	0.30±0.21	4.20±1.45
Feb	0	0.10±0.10	0.50±0.40	0.10±0.10	0.20±0.20	2.10±0.72
Mar	0	0.10±0.10	0	0.70±0.50	0.20±0.20	0.40±0.32
Apr	0	0.10±0.10	0	0	0	0
May	0.05±0.05	0.14±0.14	0	0.40±0.27	0.10±0.10	0.40±0.22
June	0	0.30±0.15	0	0.30±0.21	0.20±0.20	0.20±0.13
July	0	0.30±0.30	0	0.33±0.24	0.10±0.10	0.30±0.21
Aug	0	0.50±0.34	0	0	0	0.30±0.21
Sept	0	0.30±0.21	0.20±0.13	0.10±0.10	0	0.90±0.41
Oct	0.10±0.07	0	0.40±0.31	0.30±0.21	0	0
Nov	0	0.20±0.13	0.10±0.10	0.50±0.17	0	0.30±0.21
Dec	0.05±0.05	0.10±0.10	0	0	0	0.10±0.10
Jan						
Feb			0.00	0	0.10±0.10	0.40±0.31
Mar	0	0	0.80±0.36	0.33±0.24	0	0.30±0.21
Apr	0	0	0.40±0.31	0	0.10±0.10	0.10±0.10
Total	5	16	75	42	63	99
Mean no. / hse/ night	0.02 ± 0.01	0.08 ± 0.03	0.30 ± 0.11	0.22 ± 0.05	0.25 ± 0.12	0.47 ± 0.18

When CDC light trap collections were compared to PSC collections done at the same time, light trap catches were higher (positive ranks) than the PSC (negative ranks) indicating that light traps caught more adult mosquitoes on most occasions than the PSC (Table 2).

Table 4.2: Comparison between CDC light trap and PSC catches of female anopheline and culicine mosquitoes showing negative (a), positive (b) and tied (c) ranks

Ranks	**Species**	**Ranks**	**N**	**Mean rank**	**Sum of ranks**
CDC LT vs. PSC	Anophelines	Negative (a)	5	4.9	24.5
		Positive (b)	14	11.82	165.5
		Ties (c)	3		
	Culicines	Negative (a)	4	3.25	13
		Positive (b)	17	12.82	218
		Ties (c)	1		

a)Light trap catches < PSC ; (b) Light trap catches >PSC; (c) Light trap catches

Mosquito species composition

Six anopheline species were identified from the study areas: *An. gambiae s.s.*, *An. arabiensis*, *An. funestus*, *An. coustani, An. garnhami* and *An. christyi* (Table 4.3). *Anopheles gambiae s.s.* and *An. arabiensis* were present at all sites, but in varying proportions. In Fort Ternan, during 840 collection nights, only two adult *An. gambiae* were collected: one *An. gambiae s.s.* and one *An. arabiensis*. Of the total *An. gambiae s.l.* tested by PCR in Lunyerere, 42 were *An. gambiae s.s.* (87.5%) while the rest (12.5%) were *An. arabiensis*. In Nyalenda, 92% were *An. arabiensis* while the remaining 8% was *An. gambiae s.s. Anopheles funestus* was only recorded in Lunyerere and Fort Ternan and not in the irrigated area of Nyalenda. Other *Anopheles* spp. collected are *An. christyi* and *An. garnhami* from Fort Ternan while *An. coustani* was present in all sites (Table 4.3). Culicine species collected include *Culex* spp., *Mansonia* spp. and *Coquilletidia* spp.

Malaria sporozoite rates and the entomological inoculation rate

In Fort Ternan, none of the adult anophelines tested by ELISA were found to be infected by *Plasmodium falciparum* sporozoites. Conversely, in Lunyerere *P. falciparum* sporozoite rates were recorded more often and in 2007 all *P. falciparum* sporozoite rates recorded were all ≥ 50% (Figure 4.2c). In Nyalenda sporozoite-positive mosquitoes were recorded on two occasions

Table 4.3: *Anopheles* species composition in the study sites

Species	Fort Ternan (%)	Lunyerere (%)	Nyalenda (%)
Anopheles gambiae s.s.	1 (3)	42 (66)	8 (7)
Anopheles arabiensis	1 (3)	6 (9)	93 (85)
Anopheles funestus	15 (41)	11 (17)	0 (0)
Anopheles coustani	3 (8)	2 (3)	6 (6)
Anopheles christyi	7 (19)	0 (0)	0 (0)
Anopheles garnhami	8 (22)		
Other anophelines	2 (5)	3 (5)	2 (2)

(March 2006 and January 2007) and the highest was 4.3% recorded in March 2006. Entomological inoculation rates were 0, 109 (95% CI. -1.73 – 19.92) and 24 (95% CI. 2.01 – 6.42) infective bites/ year for Fort Ternan, Lunyerere and Nyalenda, respectively from January to December 2007. Figure 4.2c shows the monthly EIR and spozoite rate records from all sites.

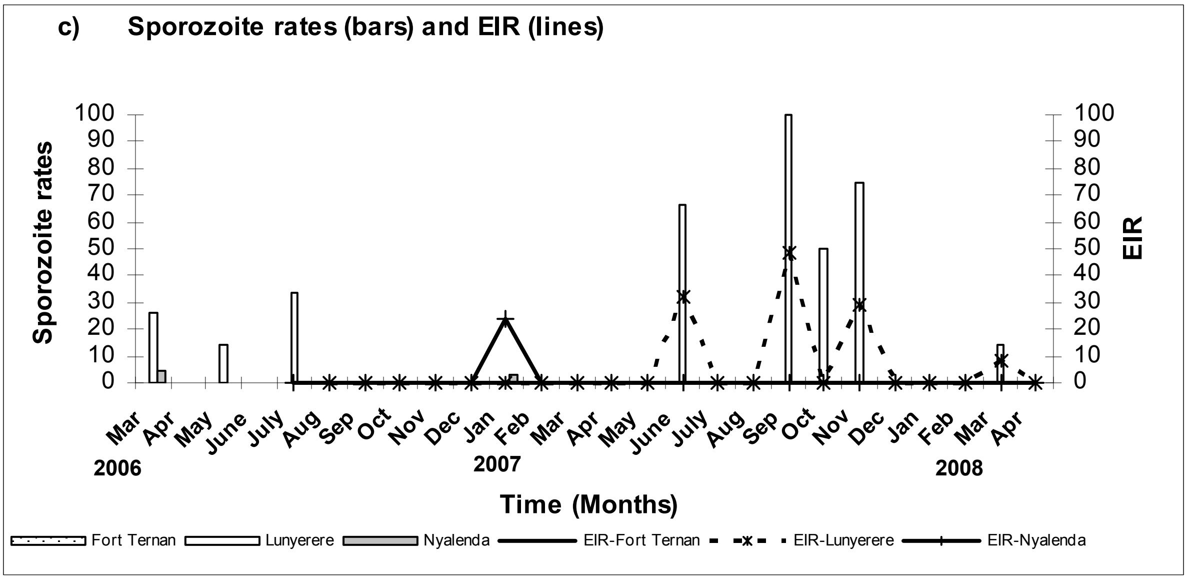

Figure 4.2c: The sporozoite rate of *Anopheles* spp. (bars) and entomological inoculation rate (lines) in the study area from March 2006 to April 2008.

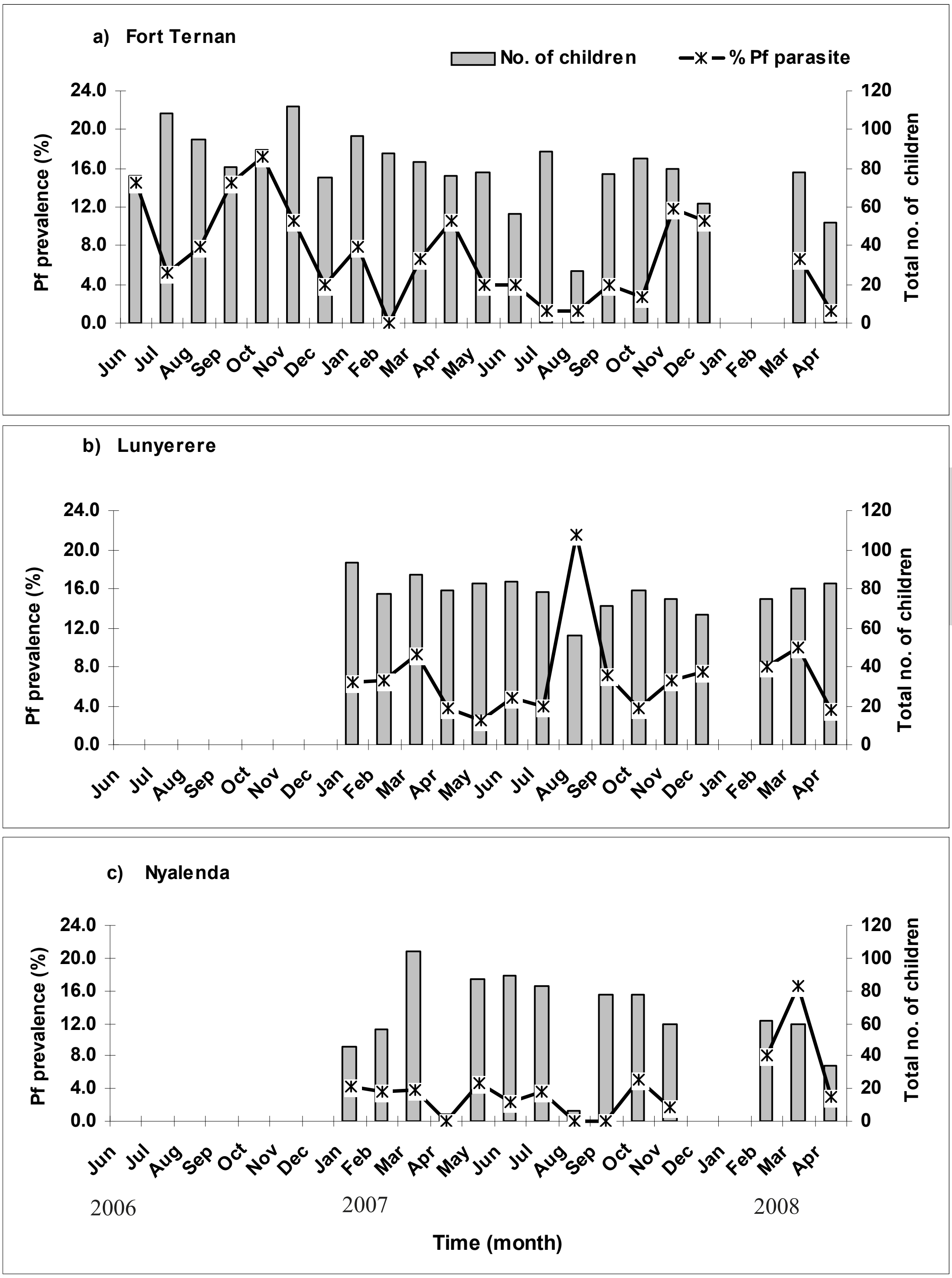

Figure 4.3: Monthly *Plasmodium falciparum* (*Pf*) prevalence and numbers of children examined in a) Fort Ternan b) Lunyerere and c) Nyalenda

Seasonality in malaria prevalence in the three study sites

In Fort Ternan, Lunyerere and Nyalenda 1665, 1167 and 849 blood slides were read for the entire study period, respectively. *Plasmodium falciparum* was the only malaria parasite identified from the study population. *Plasmodium falciparum* parasite prevalence in Fort Ternan had a mean of 5.2% (95% CI. 2.87 – 7.47). In Lunyerere this was 7.0% (CI. 4.79 – 9.23) while in Nyalenda the mean prevalence was 4.1% (CI. 1.76 – 6.35) for the entire study period. Monthly malaria prevalence was found not to be significantly different ($p>0.05$) in each of the study sites. In Fort Ternan prevalence rates ranged between a minimum of 0% and maximum of 17% (Figure 4.3). Small peaks are observed in the months of June, September and October 2006, and April, November and December 2007. In Lunyerere where adapted agriculture is being practiced *P. falciparum* prevalence ranged between a minimum of 2 and maximum of 21% and the highest prevalence was recorded in August 2007. However, in the irrigated area of Nyalenda, prevalence ranged between 0 and 17%, with highest prevalence recorded in March 2008. Analysis of variance comparing the three sites did not show significant differences in malaria prevalence rates ($F = 1.79$; $df = 2$; $p = 0.18$). Figure 4.3 shows in more detail the monthly parasite prevalence and the total number of children examined in each of the study sites. Overall, the turn-out of children during the surveys was poor in Nyalenda when compared to Lunyerere and Fort Ternan, especially during the long school holidays in the months of April, August and December.

Discussion

Our results clearly show the heterogeneous distribution of vectors and consequently malaria transmission in western Kenya. Although Lunyerere and Fort Ternan are both highland villages at similar altitudes, these sites exhibit markedly different dynamics of mosquito densities, malaria prevalence and malaria risk expressed as the entomological inoculation rate. These differences may be explained by availability of breeding habitats as defined by the topography, terrain characteristic and human activities such as swamp reclamation and deforestation. Lunyerere is characterised by undulating hills that give way to relatively shallow, basin shaped valleys that until recent years consisted of papyrus swamps and forests, have now been transformed to farmland. The changes in land use appear favourable to *An. gambiae s.s*. and *An. arabiensis* (Chapter 3), which are efficient malaria vectors. The 12.5% proportion of *An. arabiensis* recorded in Lunyerere is the highest ever to be recorded in the Kakamega area. Previous studies done in nearby villages did not record the presence *An. arabiensis* (Githeko et al., 2006; Ndenga et al., 2006). The presence of this species in Lunyerere can be attributed to the changes in land use changes which alter the temperature, humidity and rainfall patterns within the area, giving way to establishment of new species in an area.

In Fort Ternan, though hilly, the valleys formed are sharp and V-shaped, draining any stagnant water into the river further downhill. It is possible that the breeding habitats along the river were conducive for *An. funestus* breeding, the only adult anopheline vector collected in substantial numbers from the sentinel houses. However, larval collections show that both *An. gambiae* and *An. arabiensis* are also present in Fort Ternan (Chapter 3 & Koenraadt et al. 2006). In contrast to the highland villages, in the lowland peri-urban area of Nyalenda *An. arabiensis* was the predominant malaria vector. Our results are in line with previous studies that have shown *An. arabiensis* to survive better under drier conditions and lowlands than *An. gambiae* which is predominant in the highlands (Minakawa et al., 2002b; White et al., 1972a). In addition, *An. arabiensis* has also been found to dominate in environments with large water bodies such as irrigated fields (Mwangi & Mukiama, 1992), which could explain its abundance in Nyalenda. Longitudinal larval sampling in the same study sites showed *An. arabiensis* larvae to be present in Fort Ternan in high proportion (71%) that has not been recorded (Chapter 3). It is possible, however, that in the highland villages the ambient conditions are less suitable for *An. arabiensis*, leading to relatively long development times, which in most cases does not lead to adult mosquitoes (Koenraadt et al., 2006). Hence, only one specimen of this species was collected as adult, comparable with previous studies in these highlands (Koendraadt et al., 2006). The presence of *An. christyi* in Fort Ternan was also reported by Koenraadt et al. (2006), as well as previously by Garnham (1945), and may indicate special conditions that favour this mosquito species in this highland habitat. On the other hand, *An. coustani* was present in all sites, the vector potential of this species has been reported recently (Geissbühler et al., 2009).

The presence of all three malaria vectors in Lunyerere and Fort Ternan confirms that malaria can potentially be transmitted locally in these highland areas. *Anopheles gambiae s.s.* is highly anthropophilic and endophagic hence is a very efficient vector of malaria. *Anopheles arabiensis* differs dramatically from *An. gambiae s.s.* and *An. funestus* by its striking variation in anthropophily and its high degree of exophilic and exophagic behaviour (Fornadel & Norris, 2008; Gillies & Coetzee, 1987; Okello et al., 2006). Lunyerere had the highest proportion of indoor resting *An. arabiensis* ever recorded in the western Kenya highlands. However, in Fort Ternan very low numbers were caught resting indoors, and it could be that after taking a blood meal they rest outdoors or that most of the biting took place outdoors. Studies have found blood-fed *An. arabiensis* with mixed or double blood meals collected inside houses (Bogh et al., 2001; Fontenille et al., 1997; Githeko et al., 1994; Kent et al., 2007), demonstrating its wide range of blood hosts (Garrett-Jones et al., 1980).

Among our study population, Lunyerere had high sporozoite and entomological inoculation rates and consequently, the human population in Lunyerere is at a higher risk of malaria than Fort Ternan and Nyalenda. In Lunyerere, *An. gambiae s.s.* was responsible for 75% and *An. funestus* for 25% of malaria transmissions. Our results support the findings of Githeko et al. (2006), who report *An. gambiae s.s.* to have higher sporozoite rates of malaria transmission compared to *An. arabiensis* and *An. funestus*. *Anopheles funestus* is mainly considered to be of secondary importance in malaria transmission, even though it has a high degree of anthropophily (Gillies & De Meillon, 1968). We recorded a substantial number of *An. funestus* from Fort Ternan but none was found to be infected with parasites. It is possible that the species recorded might have been a non-vector sibling of the *An. funestus* species complex (Gilles & Coetzee, 1987; Gillies & De Meillon, 1968). We did not carry out PCR analysis to identify the specific sibling species within the *An. funestus* complex. Our results show no records of sporozoite rates in Fort Ternan although malaria parasites were present in the cohort of children studied and no significant differences in malaria prevalence was observed among the three study sites. Previous work in Fort Ternan suggested that malaria transmission was by mosquito vectors that originated from the neighbouring lowlands (Koenraadt et al., 2006), which would explain the situation of malaria without adult anophelism in this village. However, the presence of all three malaria vectors and the presence of *Plasmodium falciparum* parasites in children from Fort Ternan confirm that malaria may be transmitted locally and there is a possibility that we could have missed parasite-positive mosquitoes. Koendraadt et al. (2006) show that throughout a 36 month period of CDC light trap collections (360 collections), one out of three *Anopheles gambiae s.l.* individuals collected was parasite-positive. Together with our data, this is a strong indication that malaria is transmitted locally, however, the numbers of mosquito vectors are below the threshold that can be sampled. It could also be true that adult mosquitoes in this area seldom rest indoors, and, thus, future studies should consider incorporating both indoor and outdoor mosquito catches. *Anopheles arabiensis* was the main malaria vector in the irrigated Nyalenda area, with a considerable contribution from *An. gambiae s.s*. Our results show that both *An. arabiensis* and *An. gambiae s.s.* contributed equally (50% each) to malaria transmission in Nyalenda. All adult mosquito collections were done indoors and due to the exophilic and exophagic behaviour of *An. arabiensis*, we may have missed blood-fed and hence *P. falciparum* sporozoite-positive mosquitoes in the study sites.

The EIRs from our study population show distinct differences among the study sites with the rural village of Lunyerere having a higher EIR in contrast to Fort Ternan where the EIR was zero throughout the study period. The peri-urban Nyalenda had an EIR (24) lower than Lunyerere and the value lies within the expected EIR for urban areas of sub-Saharan Africa (Hay et al., 2001). Our results show that villages just a few kilometres apart such as Lunyerere and Fort Ternan, can have different sporozoite transmission rates, even though no significant differences were found in malaria prevalence.

We found malaria prevalence in our study population to range from low to moderate with no significant differences among the three cohort study populations from January 2007 till March 2008. In this study, microscopy was the standard method used to examine malaria parasites in a cohort of children throughout the study period. Although microscopy still remains the standard diagnostic method for malaria parasites (Ohrt et al., 2007), recently, a number of studies have shown that microscopy may fail to detect low parasitemia levels that are common in asymptomatic individuals as compared to polymerase chain reaction [PCR] (Baliraine et al., 2009; Dal-Bianco et al., 2007; Franks et al., 2001; John et al., 2005). Asymptomatic individuals are able to sustain malaria transmission (Baliraine et al., 2009) and thus the failure of microscopy to identify such individuals could mean that our results are an underestimate of the real situation. There were distinct differences in vector species and the sporozoite rates among the sites. We therefore would expect differences in malaria prevalence, but our results did not find any significant difference in malaria prevalence among the sites. This may indeed be attributed to asymptomatic individuals and to overcome this underestimation, future studies on malaria prevalence should consider using both PCR and microscopy for more accurate results. In addition, the turn-out of children in the Nyalenda was poor compared to Lunyerere and Fort Ternan, this could have led to overestimation of prevalence rates in this area. However, historical records of malaria prevalence in the highlands of western Kenya were previously higher (Githeko & Ndegwa, 2001) than what is reported in this study. The reduction in malaria prevalence has been attributed to the adoption of the roll back malaria initiative (RBM, 2005) by the Kenyan government since the year 2006, which scaled up the use of insecticide treated bednets (ITNs) (Noor et al., 2007).

Climatic factors such as air temperature, rainfall and relative humidity have been associated with the dynamics of malaria vector population as well as with the spread of the disease (Ye et al., 2007). The average minimum relative humidity, average maximum relative humidity and rainfall were found to correlate differently with anopheline mosquitoes depending on the area. The mean temperature did not show any correlation with anopheline mosquito densities nor malaria prevalence in our study. Our results show that variations in malaria risk are dependent on the biology of local vector species, which is dependent on the climate of the area, amongst other factors. On the other hand, local vector densities are controlled by the availability of breeding habitats as defined by the local topography and terrain characteristics, and the farming activities such as swamp reclamation and irrigation.

The findings in this study found the irrigated Nyalenda to be at a lower malaria risk than Lunyerere village, which has basin shaped valleys where farming is practised on reclaimed land that was previously a natural swamp. Previous studies have shown valley bottoms in highland areas to be "malaria transmission hot spots" (Balls et al., 2004; Bodker et al., 2003; Ernst et al., 2006; Githeko et al., 2006). Other studies in highland areas focusing on the impacts of land use changes on mosquito vector densities and malaria transmission, provide evidence that deforestation and swamp reclamation lead to changes in microclimatic conditions that favor malaria vector breeding and consequently increased malaria transmission in the affected areas (Lindblade et al., 2000a; Munga et al., 2006).

Based on data from this study malaria transmission in western Kenya is heterogeneously distributed, both temporally and spatially, depending on the availability of mosquito breeding habitats, topography and land use of the area under study. The species and adult anophelines mosquito varied among the sites studied. In Fort Ternan *An. gambiae* and *An. arabiensis* were recorded only once during the 24-month sampling period, *An. funestus* was present, though in relatively low densities compared to Lunyerere. In Lunyerere, *An. gambiae s.s* was the dominant species comparable to the findings of other studies in western Kenya highlands (Githeko et al., 2006; Ndenga et al., 2006). In the lowland peri-urban area *An. arabiensis* was the most abundant species throughout the study. In western Kenya, *An. gambiae s.s, An. arabiensis* and *An. funestus* are all efficient vectors of malaria (Githeko et al., 1996b) hence their presence in any area indicates the possibility of local malaria transmission.

In conclusion, our results have shown that land use changes associated with swamp reclamation and irrigation lead to good breeding grounds for *An. gambiae s.l.* an efficient malaria vector in western Kenya. The presence of parasite-infected children in all the study sites provides evidence of local malaria transmission, although in Fort Ternan the mosquito density was too low to explain the relatively moderate levels of malaria prevalence. Longitudinal studies on malaria and nuisance mosquito dynamics are few and this study was implemented to provide baseline information that would be useful on future planning for integrated malaria mosquito control activities. An integrated programme consisting of adulticiding measures such as indoor residual spraying, insecticide impregnated bed-nets, mosquito larval and habitat control (Shililu et al., 2003; Killeen et al., 2002a; Takken & Knols, 2009) are needed to significantly reduce malaria transmission.

Acknowledgements
We thank the community members of Fort Ternan, Lunyerere and Nyalenda for their support during the study. We want to particularly thank all the children that were involved in this study for their co-operation. We are grateful to the field assistants, Samuel Akoto, Tedd Omondi, Hilary Yegon and the research team, Annette Obukosia, Nicholus Juma and David Madahana for their tireless support in the field and laboratory. We thank David Abuom and John Kamanza of Walter Reed Army, Kisumu, for running the PCR and ELISA analysis. Ron van Lammeren and Marcel Dicke are thanked for helpful comments and suggestions on the draft version of this chapter.We are grateful to the Diorapthe Foundation for financial support.

Chapter 5

Community perception of malaria in areas having different transmission levels in western Kenya

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Abstract

In order to involve communities in larval source management (LSM) for the control of disease vectors, the value of man-made aquatic habitats for the community should be well understood. People should understand the contribution of these habitats to disease transmission. We used structured questionnaires in 30 households in two rural highland sites and one peri-urban site in western Kenya, to find out if people were aware of the larval stages of malaria mosquitoes and if they associated man-made habitats with mosquito proliferation, as well as to assess how important these habitats are for peoples' daily life. Malaria prevalence was monitored in these communities for 12 months to assess the malaria situation prior to the knowledge, attitude and practice survey. These sites differed in topography, vegetation cover and land use, and hence were expected to exhibit different malaria transmission levels. Malaria transmission was moderate to low with 5.5 and 6.6% in the rural Fort Ternan and Lunyerere and 3.2% in the peri-urban Nyalenda. Nevertheless, findings show that many people (70%) perceived malaria to be an important health risk and were willing to participate in malaria vector control but only few respondents (42%) actually recognised that the major breeding sites for malaria mosquitoes are those that they create themselves in their immediate vicinity. Many respondents (46%) did not only associate mosquito bites with malaria but felt that a number of factors, e.g., weather, food and lack of hygiene cause malaria. Most importantly, 36% of all respondents did not associate mosquitoes with malaria transmission. Knowledge depended on the education level and the area residence. People with a formal education and people living in the peri-urban community were more likely to associate mosquitoes with malaria.

Theoretical knowledge about drainage of stagnant water for malaria control was well known by the respondents; however, hardly anyone practiced it. Community members need the water derived from these habitats for domestic use and thus willingness to destroy such habitats was limited. In order to encourage LSM for malaria control, more education is required on where malaria vectors breed and acceptable strategies need to be developed in close collaboration with the communities that depend on these sources of water. Rural communities expressed greater willingness to participate in vector control compared to peri-urban citizens.

Key words

Malaria, mosquito, larval source management, community participation, rural, peri-urban, Kenya

Introduction

Malaria remains the largest burden upon health and economic growth of many tropical nations, particularly in Sub-Saharan Africa (Girardin et al., 2004; Sachs & Malaney, 2002). Control strategies for African malaria mosquitoes largely involve methods that kill or repel adult mosquitoes and the treatment of parasites using drugs. Adulticiding strategies include promoting the use of insecticide-treated bed nets (ITNs) and indoor residual spraying (IRS). The use of ITNs has been shown to increase child survival substantially in studies done in several sites in Africa (D'Alessandro et al., 1995; Schellenberg et al., 2001; Eisele et al., 2005; Fegan et al., 2007; Noor et al., 2007; Gosoniu et al., 2008). Kenya has made great progress with ITN coverage increasing rapidly from 7% in 2004 to 67% in 2006 (Fegan et al., 2007). In 2006, when free mass ITN distribution was initiated, this led to a 44% reduction in child mortality with near perfect equality between the poor versus least poor in four districts in Kenya (Noor et al., 2007). These results are encouraging and make further efforts to achieve further reduction worthwhile. Treatment and mosquito control currently depend on few compounds and thus are vulnerable to the emergence of compound-resistant parasites and mosquitoes (Greenwood et al., 2008). At present several mosquito species have developed resistance to a wide variety of insecticides and the current widespread use of bed nets and re-introduction of indoor residual spraying is likely to enhance this further (Coetzee et al., 1999; Hemingway & Ranson 2000; Hemingway et al., 2002; Nauen, 2007). The choice of vector control depends on the magnitude of the malaria burden, the feasibility of timely and correct application of the required interventions. The World Health Organization (WHO) recommends a systematic approach to vector control based on evidence and knowledge of the local situation, an approach called Integrated Vector Management (IVM). An IVM approach takes into account the available health infrastructure and resources and integrates all available and effective measures, whether chemical, biological, or environmental thus encouraging an integrated approach to disease control (http://who.int/malaria/vectorcontrol.html). Concerns about the development of insecticide resistance, environmental impact of insecticides like DDT, rising costs and logistical constraints of IRS have stimulated renewed interest in larval control (Walker & Lynch, 2007). Recent studies in rural areas of western Kenya have demonstrated that larvicides can reduce the abundance of malaria mosquito larvae and adult females by >90% (Fillinger & Lindsay, 2006; Fillinger et al., 2009). Furthermore, vector control with microbial larvicides and ITNs combined, resulted in a 2-fold reduction in new malaria infections compared with ITNs alone indicating that the addition of anti-larval measures to ITN programmes can provide substantial additional protection against malaria parasites (Fillinger et al., 2009)

An individual's health is strongly influenced by society and the environment (Hawkes & Ruel, 2006). Factors that affect individual's health directly and indirectly include income, access to food, clean water and land, health services and environmental changes (Hawkes & Ruel, 2006). Human activities associated with settlement, agriculture, or other environmental alterations may increase larval habitats of anopheline malaria vectors (Kitron & Spielman, 1989; Minakawa et al., 2002a; 2005; Munga et al., 2006; 2007; Afrane et al., 2006). Environmental alterations through deforestation or swamp reclamation mainly for agriculture, excavation of sand and stones for building and brick making, poor drainage and vegetation clearance readily promote the proliferation of mosquito vectors like *Anopheles gambiae sensu lato* which prefers open sunlit water bodies (Fillinger et al., 2004; Carlson et al., 2004). As the human population continues to rise, there is increased land pressure for settlement and agriculture, which calls for innovative approaches for disease control working together with the concerned population to address alterations in the environment. People are responsible for creating, maintaining, or using *An. gambiae s.l.* larval habitats (Mutuku et al., 2006a). Consequently, people need to be involved in the development of any intervention targeting these sites. Sustainable elimination of larval habitats or rendering of such habitats unsuitable for larval development needs horizontally organized, community-based programmes with local resources, and preferably integration of malaria control with broader public health efforts such as sanitation (Kitron & Spielman 1989; Utzinger et al., 2001). Community involvement in larval source management (LSM) requires a good understanding of the value and risks of created aquatic habitats for the community and the communities understanding of their contribution to disease transmission. Only then can educational programmes be designed to teach the population about the sources and prevention of mosquitoes. A major challenge is to motivate people to assume responsibility for controlling mosquitoes in and around their homes (Service, 1993), responsibilities often assumed to be that of the government (Brieger, 1996; Mutuku et al., 2006a; Yasuoka et al., 2006).

The work presented here is part of a series of studies recently undertaken in three different settings at increasing altitude from (1) lowland peri-urban setting to (2) rural highland villages (one village has more historically endemic malaria transmission while the other is more epidemic prone) in western Kenya with the overall goal of developing LSM strategies targeted at the local conditions suitable for community participation. Here we compare malaria prevalence and people's perceptions of malaria transmission and control in order to inform further studies on how to design interventions tailored to the local situation. The main objectives were to investigate if people are aware of the larval stages of mosquitoes and if they associate man-made habitats with mosquito proliferation, and to find out how important these sites are for people's daily life.

Materials and methods

Study area

The study was implemented in the Western province of Kenya. Three study areas with different ecology were chosen along a transect of increasing altitude. The sites differ in topography, vegetation cover and land use, which all may contribute to the local ecology of malaria vectors and transmission. The lowest site was a suburb of Kisumu town (Nyalenda) at Lake Victoria (0°06'S and 34°46' E, 1100—1200 m above sea level) with a population of approximately 26,600 people. The land surface area is flat, provided by a natural source of water used for irrigation of small scale agriculture. The other two sites were rural highland villages; Lunyerere (0°06' N and 34°43' E) in Vihiga District at 1460—1580 m and Fort Ternan (0°12' S and 35°20' E) in Kericho District at 1480—1650 m above sea level. Lunyerere has approximately 4,000 inhabitants. The village is situated between undulating hills that form large basin-shaped valleys that collect water runoff frequently in extended swamps. Houses are widespread over a large area. In the last decades the area used to be covered by forests that have now been taken up for farmlands. In contrast, Fort Ternan a small village with about 2,000 inhabitants, is located on the slopes of Nandi hills, an area that is characterized by steep slopes forming sharp V-shaped valleys providing less room for standing water. The main economic activity of this area is mixed farming with coffee, maize and sugarcane plantations. The area has been largely deforested since the start of commercial farming 100 years ago. Lunyerere and Fort Ternan are about 40 km and 75 km from Kisumu, respectively, while the distance between Fort Ternan and Lunyerere is about 115 km.

Climate

Western Kenya has two rainy seasons with the 'long rains' falling between April and June and 'short rains' in October to November, nevertheless with high variability from year to year. The quantity of rainfall (mm) was measured with an automated rain gauge (Eijkelkamp, The Netherlands; opening at 0.9 meters height; threshold 0.201 mm) installed at Fort Ternan health facility, Lyanaginga health facility about 30 km from Lunyerere and for the peri-urban Nyalenda an automated rain gauge was installed at the Kenya Medical Research Institute (KEMRI) about 17 km from Nyalenda.

Malaria prevalence survey

Malaria prevalence was monitored in all three study sites for 12 months to assess the malaria situation prior to the knowledge, attitude and practice (KAP) survey. In western Kenya children below 10 years of age are at a high risk of malaria infection. A house-to-house population survey was done in each study location to find out which houses were having children aged between 2 to 10 years. Children falling within the age window were then identified and enrolled to form a study cohort of 100 children. Consent was sought from the parents or guardians before the child was enrolled into the study. Each child was then given a unique code that was used to track the same child throughout the study from January to December 2007. Blood samples were collected monthly by the standard finger-prick method, and thick and thin smears were prepared on labeled slides (Gilles & Warrell. 1993). Any child that was clinically ill at the survey date was taken to the nearest public health facility for treatment free of charge. The thin and thick blood smears were air dried, then fixed in methanol and stained in 4% Giemsa for 30 minutes. An experienced technician examined the slides under 1,000 oil immersion to identify and count the parasite species. Random checks (to include at least 10% of all slides) were carried out on the slide counts by independent microscopists to ensure quality control. Parasite density was scored against 200 leukocytes when the slide was positive; otherwise, the whole slide was carefully scanned before being declared negative. An individual was considered positive if malaria parasites were detected in the blood smear.

Knowledge, attitude and practice (KAP) survey

A semi-structured questionnaire was used for data collection. In each of the three study communities, 30 households were selected randomly from the houses in which children had been enrolled for the malaria prevalence study. The household head, his spouse, or a member of the household who was ≥ 18 years of age was interviewed. One questionnaire was administered to one household per compound. Interviews took place in March 2008, and were conducted in private to reduce the influence from other people.

The survey was semi-structured with open and multiple-choice questions to obtain information regarding villagers': 1) socio-demographic background and their health problems; 2) living environment and housing conditions; 3) knowledge about malaria mosquitoes' life cycle; 4) actions taken for mosquito and disease prevention; and 5) attitudes and perceptions towards mosquito control and their willingness to actively participate.

Ethical considerations

Institutional ethical clearance was given by the Kenya Medical Research Institute (KEMRI) and Wageningen University and Research Centre (WUR), The Netherlands, protocol approval number 1121 and 512. In addition permission was obtained from the community elders. Individual interviews were only started after the purpose of the study had been explained to the participant and a consent form signed.

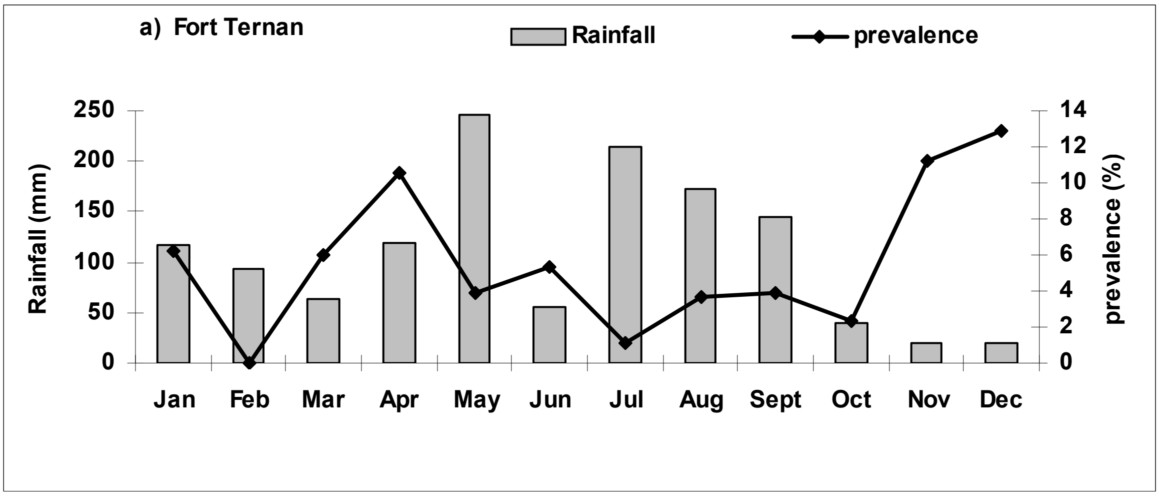
Data analysis

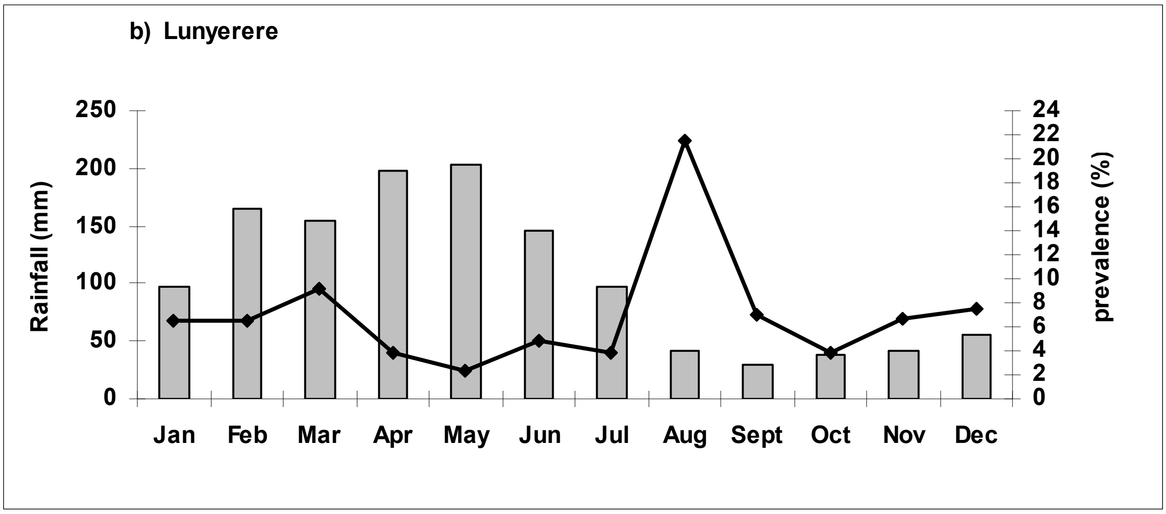
The open ended part of the semi-structured questionnaire was coded after completion of the survey. All data were entered in Excel sheets and analysed using the statistical software for social scientists (SPSS) Version 15.0. Analysis of the outcome of variables was performed excluding non-responders or missing data points. A basic socio-economic indicator was developed based on information on the type of house owned. The lowest indicator was associated with "traditional" grass-thatched houses made up of mud, followed by "semi-permanent" houses with iron sheet roofs with either mud or cemented walls. The highest indicator was associated with "permanent houses" made of iron sheet or tile roofs, brick or cemented walls, with or without glass windows. Each interviewed household was grouped according to these indicators. Chi-square tests (χ^2) were used to examine whether the distribution of individuals / households among the categories of one variable is independent of their distribution among the categories of the other. Binary logistic regression analyses in a backward stepwise approach were used to explain variations in responses to questions.

Results

Seasonality in malaria prevalence in the three study sites

In Fort Ternan, Lunyerere and Nyalenda 898, 929 and 691 blood slides were read for the entire study period, respectively. The overall malaria-parasite prevalence rate in Fort Ternan was 5.5% and in Lunyerere 6.6% and not significantly different ($p>0.05$). Both villages exhibited large seasonal differences in prevalence as shown in figure 5.1. In the peri-urban setting of Nyalenda only 3.2% of all blood smears taken were parasite positive. Here malaria prevalence ranged between 0 and 5% and did not show distinct monthly differences. In Lunyerere, the highland site between 1460 to 1550 m altitude, one extended rainy season was observed during the study period between February and July 2007 (Figure 5.1). The highest prevalence rate (22%) occurred in August one month after the heavy rains had ended. In the high altitude site in Fort Ternan, between 1480 to 1650m, malaria prevalence fluctuated between a low of 0-1% and a high of 10-13%. The prevalence rate did not correlate well with the rainfall during the two rainy seasons (April-May and July-September) observed in this site.





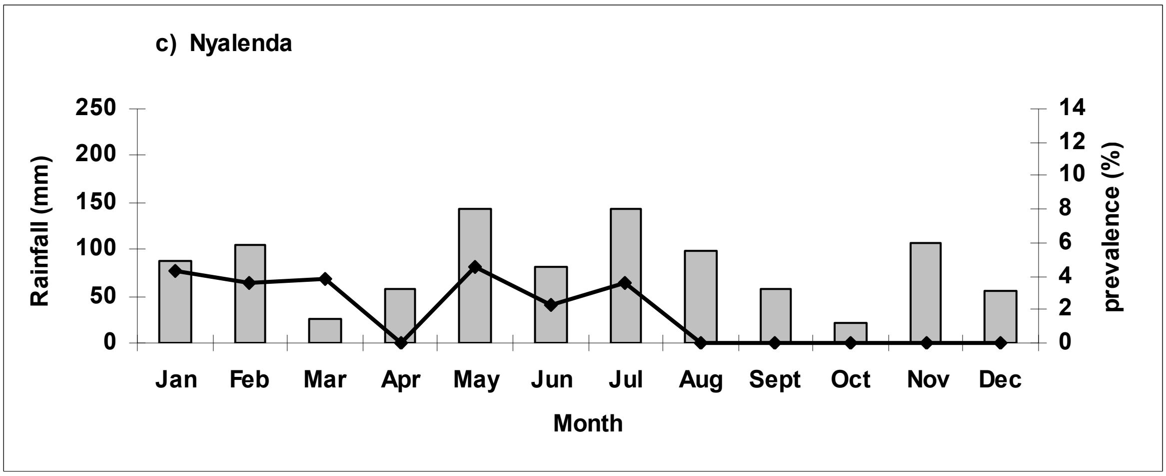


Figure 5.1: Seasonality in malaria prevalence and rainfall in rural Fort Ternan a), rural Lunyerere b) and peri-urban Nyalenda c) respectively.

Socio-demographic characteristics per site

In total 90 respondents, 30 per site, were interviewed; among them 50 were females and 40 males. The average age of the respondents varied among the locations (Table 5.1); it was in Fort Ternan 27.5 years (95% C.I. 23.8–31.2); in Lunyerere 35.9 years (95% C.I. 24.7–47.1) and in Nyalenda 39.5 years (95% C.I. 32.6–46.4). Nevertheless, the average age of household heads was similar in all sites (Fort Ternan 43.9, 95% C.I. 38.2–49.5; Lunyerere 44.4 95% C.I. 37.8-51.1, Nyalenda 50.2, 95% C.I. 42.1–58.3). The majority of the respondents had attended primary school (62%), whereas only 24% had attended secondary school and 12% had had no formal education. There were no differences among the sites considering the education level of the respondents (Table 5.1). The average number of people per household was higher in the rural highland sites with 7.7 in Fort Ternan (95% C.I. 6.8–8.6) and 7.3 in Lunyerere (95% C.I. 3.7–11.0). In Nyalenda an average of 5.6 people lived in one household (95% C.I. 4.0–7.2). The average number of children was 5 in Fort Ternan (95% C.I. 4.2-6.0), 5.6 in Lunyerere (95% C.I. 2.1–9.0) and only 2.7 in Nyalenda (95% C.I. of 1.4–4.1).

There were significant differences between the income generating activities of rural and peri-urban dwellers. The probability of encountering farmers in the peri-urban was reduced by 96% compared to rural dwellers (Odds Ratio [OR] =0.038; 95% CI. 0.008–0.178; p<0.001). Eighty seven percent of household heads in Fort Ternan were farmers and hence most of the family income was generated from farming activities. In Lunyerere people lived on a wider range of activities, 31% of the households generated income from farming, 23.3% were casual labourers and 20% earned their income from business activities, whereas in peri-urban Nyalenda 76.7% of the households were involved in business activities (Table 5.1).

There were highly significant differences in the socio-economic status between locations ($\chi^2 = 52.267$; df = 2; $p < 0.001$), with the majority of people in Fort Ternan living in grass and mud houses whereas these were not found in Lunyerere and Nyalenda (Table 5.1).

Health problems in the population

The respondents were given an open question about the health problems that affected them most, ranking them from the most serious to the least serious. Interestingly, in all three study communities, malaria was perceived the most serious health threat. Seventy percent of the respondents ranked malaria 1st, 20% ranked it 2nd while only 10% ranked it 3rd. Typhoid fever was perceived to be the second most important disease with 9% respondents ranking it 1st, 37% ranking it 2nd

Table 5.1: Summary of the socio-demographic characteristics of the study

Characteristics		N	Fort Ternan (rural)	Lunyerere (rural)	Nyalenda (peri-urban)
Total interviewees			30	30	30
Sex of house head (χ^2 = 3.88;df =2; p >0.05)		90			
	Male		26	20	25
	Female		4	10	5
Age of respondent (χ^2=48.05;df=6;p<0.001)		89			
	<25 years		17	2	0
	26 to 35 years		6	4	11
	36 to 45 years		5	6	12
	≥ 46 years		2	17	7
Educational level of respondent (χ^2=12.07;df=6;p>0.05)		90			
	None		0	6	5
	Primary		25	15	16
	Secondary		5	8	9
	College		0	1	0
Number of household members (χ^2=19.747; df=6; p<0.05)		90			
	1 to 3		0	4	10
	4 to 6		11	13	13
	7 to 9		15	7	6
	≥ 10		4	6	1
Number of children (χ^2=12.717; df=6;p<0.05)		85			
	1 to 3		11	11	19
	4 to 6		13	10	7
	7 to 9		4	6	0
	≥ 10		2	2	0

Occupation of house head (χ^2=30.942; df=10;p<0.001)	58			
None		0	2	1
Business		2	2	3
Farmer		23	2	2
Small scale business		1	0	3
Formal employment		1	4	3
Casual laborer		3	4	2
Main household income source (χ^2=61.878;df=10;p<0.001)	89			
None		0	1	1
Casual labor		2	7	2
Farming		26	9	3
Business		1	6	23
Dependants (children)		0	3	0
Salary and / or wages		1	3	1
Socio-economic indicator (χ^2 =51.267; df=2; p<0.001)	90			
Traditional housing		15	0	0
Semi-permanent housing		14	26	22
Permanent housing		1	4	8

and 15% ranking it 3rd. Feeling unwell was associated with stomach problems, flu or cold and discomforts including amoeba infections, back ache, head ache, eye and ear problems were mentioned as well (Figure 5.2).

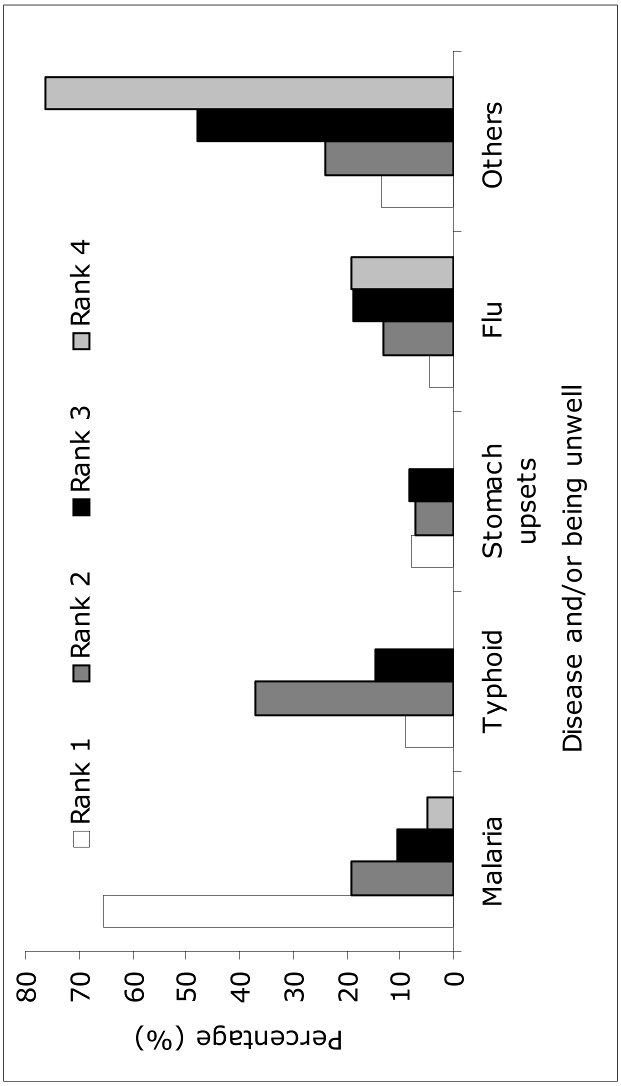


Figure 5.2: Ranking of disease threats or being unwell in decreasing order as perceived by the respondents

The age group that was perceived to be most frequently sick with malaria differed between locations ($\chi^2 = 32.197$; df = 6; $p<0.001$). In Fort Ternan 67% of the respondents felt that 5 to 12 year old children were most affected, while in Lunyerere the majority (47%) said those above 18 years and 53% of all respondents from the peri-urban, Nyalenda felt children below the age of 5 years were most frequently sick.

Knowledge and beliefs on how one catches malaria

In response to the open question "***how does one catch malaria*?"**, mosquito bites was the most frequently responded cause, but notably only accounting for 36.3% of all responses. Other factors that respondents associated with catching malaria had to do with weather conditions, e.g., cold weather or being rained on (32.7%). Eating sugary, fatty or oily food accounted for 17% of all responses and living under untidy conditions like in a dusty or bushy environment or lack of general hygiene was mentioned in 6.4% of all responses as a cause for malaria. Notably, 12% of all respondents admitted to having no idea of how one catches malaria (Table 5.2).

Table 5.2: People's knowledge on how one catches malaria

Cause of malaria	Frequency (N=171)	Percent (%)
Mosquito bite	62	36.3
Bad weather	56	32.7
Bad food	29	17.0
Untidy/unhygienic	11	6.4
No idea	13	7.6

Knowledge is defined as information, understanding and skills gained through education or experience while belief is to think that something is true although you are not completely certain. To distinguish knowledge from beliefs the responses were grouped in four categories as shown in Table 5.3. Off all respondents, 22% believed that a mosquito bite was the only way to be infected with malaria, the majority though felt that a number of factors (e.g., weather, food, hygiene) causes malaria including mosquito bites (46%). Most importantly, 36% of all respondents did not know that mosquitoes are responsible for the transmission of malaria.

Table 5.3: Perceived causes of malaria

Causes /site	Fort Ternan N=30 (%)	Lunyerere N=30 (%)	Nyalenda N=30 (%)
Mosquito bite only	3 (10)	3(10)	15 (50)
Mosquito bite and others	10 (33)	15(50)	6(20)
Others	17 (57)	7(23)	3(10)
Don’t know	0 (0)	5(17)	6(20)

Binary regression showed that the knowledge depended on the education level and the residence of the respondent. People with a formal education were significantly more likely to associate mosquitoes with malaria. Furthermore, peri-urban residents were 3 times more likely than their rural counterparts to attribute malaria to mosquito bites (Table 5.4).

Further analysis showed that mentioning mosquitoes as the only cause of malaria were dependent on the sex and residence of the respondent. Male respondents and peri-urban residents were more likely to attribute malaria to mosquito bites only (Table 5.5).

Knowledge of mosquito control

Eighty eight percent of all the respondents felt that it was possible to control mosquitoes, while the remaining 12% did not believe that it would be possible. Reasons listed for the latter were that mosquitoes are creatures of God just like humans; no one can control weather conditions; breeding sites are always present; mosquitoes are only seen at night; and mosquitoes are there to bring malaria and bilhazia.
When asked ***how can mosquitoes be controlled,*** responses varied depending on the location ($\chi^2 = 22.849$; df = 12; $p<0.05$). Respondents from Fort Ternan were more likely (OR = 0.17; C.I. = 0.05-0.56; p = 0.002) to suggest draining of stagnant water and clearing the bushes while in Lunyerere respondents suggested to clear bushes and use personal protection (bed nets, burning of mosquito coils). Peri-urban residents preferred personal protection measures followed by indoor insecticide spraying and/or drainage of stagnant water. Table 5.6 lists the methods that the respondents perceived as useful for mosquito control. The most frequently mentioned methods were bush clearing (23.8%), drainage of stagnant water (22.9%) and personal protection measures like using bed nets (15.2%) and indoor spraying (13.3%). Of all the respondents, 8.6% had no idea on how to control mosquitoes.

Table 5.4: Factors associated with the correct knowledge of mosquitoes being involved in malaria transmission

Variable	**Category**	**OR**	**Sig**	**95% CI**
Education level	No formal education		0.025	
	Primary education	10.852	0.007	1.941 – 60.690
	Higher education	8.592	0.020	1.394 – 52.956
Location	Rural – Urban	3.037	0.038	1.065 – 8.660

Table 5.5: Factors associated with mosquitoes as the only cause of malaria

Variable	Category	OR	Sig	95% CI
Gender	Female-male respondent	5.839	0.005	1.711 – 19.922
Location	Rural - urban	8.325	0.000	2.552 – 27.155

Variables entered on step 1: education level, respondents age and gender, socio-economic status, location

Table 5.6: Respondents knowledge on mosquito control

Method	Frequency (N = 105)	Percentage
Bush clearance	25	23.8
Draining stagnant water	24	22.9
Bed nets	16	15.2
Indoor spraying	14	13.3
No idea	9	8.6
Sanitation (filling up of holes / burying tins)	5	4.8
Mosquito coils	4	3.8
Mosquitoes are there to stay, were created by God	4	3.8
Medication	2	1.9
Oil application	2	1.9

Table 5.7:Practiced mosquito prevention

Method used	Frequency (N=193)	Percentage
Bed nets	66	34.2
House eaves closed	51	26.4
Mosquito coils	23	11.9
Indoor residual spaying (IRS) by MOH	18	9.3
Bush clearance	16	8.3
Fumigation (smoke/cow dung/flowers)	9	4.7
Closing doors and windows early in the evening	4	2.9
Drainage of stagnant water	2	1
Medication	2	1
Oil application	2	1
Sanitation (burying tins, filling holes)	2	1

Practiced mosquito prevention behaviour

Interestingly, when exploring the practiced mosquito prevention behaviour hardly anybody implemented any drainage of stagnant water or other measures targeting the larval stages of mosquitoes (Table 5.7).

The use of mosquito nets and other personal protection measures were most common. Environmental management targeted primarily the clearing of bushes. In peri-urban Nyalenda, 100% of the interviewed households possessed at least one bed net, in contrast, only 73.3% in Lunyerere and only 46.7% in Fort Ternan ($\chi^2 = 21.818$; df = 2; $p<0.001$). In other words the probability of finding a bed net in Fort Ternan was reduced by 87% as compared to the other study sites (OR = 0.135; C.I.= 0.048-0.378; $p<0.001$). Regression analyses did not show any association between the age and sex of the household head, the socio-economic status of the household, educational level of the household head or knowledge on mosquitoes as malaria vectors association with bednet possession. Among those that possessed a bed net, every person in the household used a net in 44.6% of the households, in 15.5% only the parents used a net and in 9.2% only children used a net. The remaining 30.8% possessed a bed net but nobody was using it. 25.6% of the households burned mosquito coils for mosquito control. Peri-urban households were six times more likely to burn mosquito coils than the rural dwellers (OR = 6.5; C.I.= 2.32-18.25; $p<0.001$). Closed eaves, which protect from mosquitoes entering the house, were found in 56.7% of all household, whereas 43.3% had open eaves. Notably, residents from Lunyerere were more likely to live in a house with closed eaves than residents in Fort Ternan (OR = 0.2; C.I.= 0.06-0.66; $p=0.008$) and Nyalenda (OR 0.12; C.I.= 0.03-0.39; $p<0.001$). Regression analysis revealed that these differences observed in the use of bed nets, mosquito coils or house construction in different locations were independent of the education level, age and gender of the household head or income source and socio-economic status of the household.

Knowledge on mosquito breeding sites

In preparation for mosquito control interventions targeting the larval stages of mosquitoes in the three study locations the communities' knowledge on immature stages was explored. The respondents were asked if they knew any mosquito breeding habitat close to their houses. Sixty-nine of all the respondents said yes, there were breeding habitats close (within 100 meters) to their houses while 21 said mosquito breeding site could not be found close by. Responses were significantly different in the three locations ($\chi^2 = 10.062$; df = 2;$p<0.05$); residents from Fort Ternan were far more likely (42%) to suggest that mosquito breeding sites were in close vicinity to homes than residents from the other locations (OR 14.5, 95% C.I. = 1.84-114.28; p = 0.011).This is despite the fact that in Lunyerere, there were far more breeding habitats given the shape of the valley while in lowland Nyalenda water almost stagnates everywhere.

When asked to name the habitats where malaria mosquitoes lay eggs, 23.1% of the respondents believed that they do so in bushes and 17.1%

in plantations of coffee, maize or bananas; 16.2% thought they breed in rivers and in water from leaking taps. However, of all the responses, only 12% identified stagnant water and pools as oviposition sites (Table 5.8). Residents from the peri-urban location were far more likely (OR 21.59, 95% C.I.= 2.67–174.72) to associate water with mosquito breeding than their rural counterparts, who frequently believed that bushes and plantations were responsible for the development of mosquitoes. This was independent of age groups, sex, and education level of the respondent.

On further enquiry, trying to find out if community members had any knowledge on the aquatic life stages of the mosquito, only 18.9 % of the respondents could describe what a mosquito larva looked like, the majority of the respondents had no idea. Regression analysis revealed that peri-urban residents were 11 times more likely to describe mosquito larvae (OR 11.67; C.I. = 2.86-47.63; p<0.001) than the rural dwellers. Furthermore, male respondents were five times more likely to know what larvae look like (OR = 5.41; C.I. = 1.26-23.3; p = 0.02) compared to females. Education level, age or the knowledge of water as larval habitats was not associated with knowing the larval stage.

Table 5.8: Perceived mosquito breeding habitats

Name	**Frequency (N=117)**	**Percent**
Bushes and shrubs	27	23.1
Plantations (coffee, maize, banana)	20	17.1
Leaking taps and river	19	16.2
Stagnant water	14	12
Sewerage, toilets, pit latrines	12	10.3
Containers	6	5.1
Water pool	5	4.3
Drainage channels	4	3.4
Swamp	4	3.4
Slaughter house	2	1.7
Brick pits	2	1.7
Water	2	1.7

Amongst the respondents who associated mosquito development with water, 55.6% said that mosquito breeding habitats were important to them in many ways as (1) the water was used for domestic purposes (77.1%) e.g. washing clothes, and utensils and for drinking water and (2) irrigation purposes (10.4%) like watering crops; (3) in addition canals were dug for water drainage (10.4%). There were significant site differences ($\chi^2 = 20.610$; df = 2; $p<0.001$) with more respondents from Fort Ternan (48%) and the peri-urban site (38%) saying the habitats were important while many in Lunyerere (57.5%) said the habitats were not important. Other than location, no other explanatory variables could be found for these differences in perception of habitat importance based on the responses.

Willingness to participate in mosquito control

Independent of the location, the majority of the respondents (93.3%) expressed their willingness to participate in mosquito control measures. Reasons provided for the willingness to participate were to stop mosquito bites, stop breeding of mosquitoes and stop malaria infections. When asked "**what can you imagine doing for mosquito control?"**, there were significant site differences in the responses ($\chi^2 = 48.078$; df = 2; $p<0.001$). The majority of residents (56.7%) from the peri-urban Nyalenda, though willing, had no idea of what they could do; 60% of the respondents from Fort Ternan, 33.3% from peri-urban Nyalenda and 11.1% from Lunyerere said they would be willing to assist by draining stagnant water. Fort Ternan residents were 92-94% more likely to drain stagnant water than residents from Nyalenda (OR = 0.08; C.I. = 0.022-0.298; $p<0.001$) and Lunyerere (OR = 0.059; C.I. = 0.015-0.222; $p<0.001$), respectively. A good number of respondents, namely 33.3% from Lunyerere, 23.3% from Fort Ternan and 10% from peri-urban, Nyalenda were willing to assist by clearing bushes. An additional 37% from Lunyerere imagined spraying houses with insecticides for mosquito control. Overall 90.9% of the respondents believed that breeding habitats could be removed. When asked how, 65.9% said by drainage whereas 18.8% said by bush clearance. On further analysis age, sex, education, location and income source of the respondent or household head did not determine what the respondents imagined doing during mosquito control.

The respondents were also asked whom they felt should take the lead in mosquito control. Again, there were significant differences among the sites ($\chi^2 = 62.555$; df = 8; $p<0.001$), with 86.7% of respondents in Fort Ternan believing the community should take lead in getting rid of potential mosquito breeding habitats. The majority (58.6%) in the peri-urban Nyalenda considered mosquito control to be the government's responsibility. However residents of Lunyerere believed the responsibility lies with the house owners (31%), the community (27.6%) and the respondents themselves (27.6%) as shown in table 5.9. Regression analysis showed that peri-urban dwellers were 10 times more likely (OR = 10.23; C.I. = 2.80-37.33; $p<0.001$) to believe that it is the government's role to control mosquitoes than the rural counterparts.

Table 5.9: Responsibility for vector control

Characteristics	Fort Ternan N= 30 (%)	Lunyerere N= 29 (%)	Nyalenda N= 29 (%)
Community	26 (86.7)	8 (27.6)	11 (37.9)
Government	0 (0)	4 (13.8)	17 (58.6)
House owners	3 (10)	9 (31)	0 (0)
My self	0 (0)	8 (27.6)	0 (0)
No idea	1 (3.3)	0 (0)	1 (3.4)

Discussion

The most striking result from the survey was that malaria was considered the major disease threat to people in all three communities, despite parasite surveys confirming that malaria transmission was moderate to low in the context of the African situation. Respondents did not clearly define younger children as the most frequently sick, which might be an indication that what is perceived by the community as malaria is in fact some other febrile illness (Mwanziva et al., 2008). Even here, in these traditionally low-transmission areas, there is, as seen elsewhere (Deressa et al., 2007; Brieger et al., 2001), a strong feeling that every disease is malaria and if not then it is typhoid, a clear indication of lack of knowledge on health and diseases.

Our study clearly shows that there are significant differences in knowledge and perception of malaria between peri-urban and rural dwellers. Our results are comparable to other studies that found distinct differences between urban and rural dwellers concerning knowledge about mosquitoes, mosquito avoidance behavior, malaria control and bed-net use (Agyepong & Manderson, 1999; Kumar & Gururaj, 2005; Sanjana et al., 2006; Lipowsky et al., 1992). Peri-urban residents knew more about mosquitoes and their role in malaria transmission could describe the larval stages and knew the breeding habitats. This was independent of their educational or socio-economic status. Two reasons might be responsible for this: (1) peri-urban residents have more exposure to educational media such as television, radio, posters and sign posts, and

(2) peri-urban residents are exposed to a large amount of nuisance non-malaria mosquito biting. Although the nuisance is most likely associated with bites of *Culex quinquifasciatus* (Birley & Lock, 1998) the inhabitants nevertheless are aware that mosquitoes are the cause of malaria. Increased awareness of these insects led to the use of prevention measures. Consequently, far more peri-urban dwellers possessed and used a bednet than their rural counterparts. In the rural highland villages, many possessed a bednet but conspicuously few used it. In contrast to peri-urban sites, the highland sites have relatively little nuisance biting and seasonal transmission, therefore bed net use might not have priority in these communities. It is for this reason that the rural communities are more vulnerable to mosquito bites and hence malaria infection, thus calling for alternative methods to complement vector control. Control aimed towards the aquatic stages of mosquito, such as environmental management through drainage of stagnant water, provision of shade on potential larval habitats, biological methods such as the use of predatory fish and application of larvicides might reduce malaria transmission (Keiser et al., 2005b; Fillinger & Lindsay 2006; Utzinger et al., 2001; Utzinger et al., 2002; Takken & Knols, 2009) community-wide and does not depend on individuals applying it.

Interestingly, men were more equipped with the correct knowledge on the role of mosquitoes in malaria transmission and what mosquito larvae looked like, than women. Women tended to believe in a whole variety of reasons for family members catching malaria, possibly because of lack of exposure and the fact that they spend more time at home taking care of children/family. Causes of malaria were not only associated with mosquitoes but frequently with a combination of local beliefs such as eating sugary, fatty or oily foods. Community beliefs were found to be similar to those found elsewhere in Kenya and Africa (Opiyo et al., 2007; Aikins et al., 1993; Mboera et al., 2007). Notably, 36% of all respondents did not know that mosquitoes are responsible for the transmission of malaria. If such a large number of people still do not know how malaria is transmitted, there is a need for education, especially in the rural settings, with perhaps more attention paid to women.

One third of the respondents associated breeding of mosquitoes with bushes and coffee, sugarcane or maize plantations rather than stagnant water, a misinformation that is persistent in all communities in Africa. Despite many educators calling for a reform of this information (Opiyo et al., 2007; Mukabana et al., 2006), it persists as common knowledge. Those that associated mosquitoes with water, frequently only associated them with rivers and leaking taps, but hardly perceived any of the man-made habitats to be breeding sites of mosquitoes. Only few actually recognized that the major breeding sites are those that they create themselves in their immediate vicinity. If that knowledge is lacking, then we cannot expect that people take preventive action. This becomes even more significant when we realize that 86% of all mosquito breeding habitats in the three study areas are man-made (Chapter 3). The majority of respondents believed that bush and grass clearance around the compound would be the best option, none of which has been reported for controlling malaria in Africa. Bush clearance is a good option for the control of shade-loving mosquito species; however, the main malaria vector in the study area is *An. gambiae,* which thrives best in open, sun-exposed habitats (Gilles & De Meillon, 1968; Ghebreyesus et al., 1999; Lines et al., 1994; Afrane et al., 2004). This misconception seems to be emerging from both primary and secondary schools syllabi within the country, where the main primary message is the encouragement of bush and grass clearance around houses for malaria prevention, although vegetation does not serve as oviposition and resting site for *An. gambiae* (Opiyo et al., 2007).

Although the majority of respondents were able to list a number of malaria control measures, only few of these were practiced. Knowledge about drainage of stagnant water was well known by the respondents. However, hardly anyone implemented it. Drainage of stagnant water is one of the environmental management (EM) strategies that can be successful if adopted by communities, as they are more familiar with their surroundings (van den Berg & Takken, 2007) and it is affordable and sustainable. In the respective study locations, the major breeding habitats are drainage channels, open drains, paddies and animal hoof prints, mainly man-made as a result of agricultural activities (Chapter 3), mostly limited in number, confined and easily accessible. Significantly the community members pointed out that the water derived from these sites is important to them for domestic purposes. Therefore, willingness to destroy or avoid creating such habitats might be limited, as they are important for their livelihoods. We therefore need to develop control methods in collaboration with the communities that are acceptable to them, such as ensuring that drains do not hold stagnant water, or the use of predatory fish in drains that must keep water or shading by plants such as Napier grass or arrow roots planted along drainage channels (Chapter 6). In areas where EM might not be feasible, larvicide measures can be employed especially in temporary habitats that are common during the rainy periods. Nevertheless, vector control programmes should be integrated with other public health and development activities (Townson et al., 2005) with more emphasis on the development of Integrated Vector Management (IVM) strategies to complement existing methods.

It was observed that rural houses were more likely to have closed eaves, which ensured that mosquitoes had no access to the house (mosquito proof). Lindsay et al. (2002) illustrated the importance of mosquito proofing on reducing the incidence of mosquito biting and malaria, and that simple modifications to the design of indigenous houses protect people from mosquitoes and malaria. More efforts need to be made to promote this intervention.

The majority of the respondents were willing to participate in mosquito control measures, as stopping mosquito bites and mosquito-borne disease was a high priority for them. Despite their willingness, there were great differences regarding who should really take the lead in the control measures against mosquitoes. In the rural villages, the community expressed more willingness to take the initiative while the peri-urban community made the government responsible for it. This idea of the community relying on authorities to come to their aid has been observed elsewhere (Brieger, 1996; Yasuoka et al., 2006) and therefore even if beliefs and perceptions about the disease were modified, there would still be barriers to cross as the technical solutions to the problem appeared to lie with the government and not in the community domain (Brieger, 1996). In peri-urban places, the population is normally diverse and many people do not know each other; people move frequently and so they hardly work together. As a consequence, many do not see the need to invest much in disease control thus leaving it to the authorities. In contrast, rural communities usually have traditional social structures such as communal work and village meetings, and people are more permanently settled, making it easier to know each other and work together. These traditional structures make it easier to channel interventions at the grassroot level in the rural than the peri-urban setting, a major drawback to many peri-urban vector control programmes. Community-based malaria control programmes have shown great successes. In the Tigray region of Ethiopia, acceptance of responsibility by the community led to efficient treatment at the village level, thus reducing disease burden (Ghebreyesus et al., 1996). In urban Dar es Salaam decentralization of health systems led to successful community-based LSM programmes with assistance from government, local and international scientists (Geissbühler et al, 2009). The WHO has also recognized this and in response, proposals to address integrated-community based interventions for urban populations were recently invited, as a way to involve and encourage communities to take the lead in health matters affecting them (http://www.who.int/malaria/vectorcontrol.html).

In conclusion, this study has shown that although communities are aware of malaria being a problem, many, especially in remote rural environments, do not associate it with mosquitoes. Hence, many are not aware that their activities encourage mosquito breeding. On the other hand, there is a strong knowledge base in the community that elimination of stagnant water is an important malaria control tool but no activities are geared towards implementation, which shows that there has been very little effort made to date to include EM in national policies. Evidence-based knowledge and institutional support may help to utilise communities for vector control more efficiently, as there is a clear willingness to participate and room for integration of other interventions despite personal protection measures. Innovative and community-based approaches for EM that take into account the peoples' needs and livelihoods will be most suitable.

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Chapter 6

Strategies of controlling malaria mosquito larvae using environmental management and biological control: a case study of Nyalenda, western Kenya

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Abstract

Malaria mosquitoes spend a considerable part of their life in the aquatic stage, rendering them vulnerable to interventions directed at aquatic habitats. Recently, successes of mosquito larval control have been reported using environmental and biological tools. Here, we report on a study in which the effect of shading by plants and the simultaneous use of biological control agents were investigated for their effect on the development and survival of anopheline and culicine mosquito larvae in man-made natural habitats in western Kenya. Laboratory and field trials in these man-made habitats consisted of environmental manipulation using locally available plants, the introduction of predatory fish and/or the use of *Bacillus thuringiensis* var. *israelensis* (*Bti*) in various combinations. Man-made habitats provided with shade from different crop species produced significantly fewer larvae than unshaded habitats. In particular, larval populations of the malaria vector *Anopheles gambiae* were affected. Predatory fish and *Bti* on their own were ineffective, but when applied simultaneously, larvae of *An. gambiae* were no longer found, and no pupae of anophelines were recorded. It is concluded that the integration of environmental management techniques using shade-providing plants and predatory fish and/or *Bti* can be an effective and sustainable tool for the control of malaria and other mosquito-borne disease vectors, and that these techniques can easily be adopted by local communities.

Key words
malaria mosquitoes, *Anopheles gambiae*, shading, Napier grass, arrow root, rice, *Bacillus thuringiensis* var *israelensis*, *Gambusia affinis,* larval control, Kenya

Introduction

Development activities that entail clearing of forests and/or swamps for timber, agriculture, human settlement and road construction have often led to the creation of suitable breeding sites for malaria mosquitoes (Lindblade et al., 2000a; Munga et al., 2006; Norris, 2004; Walsh et al., 1993). Irrigated fields and areas with vegetable crops are ecologically good breeding sites for anopheline larvae (Chapter 3; Klinkenberg et al., 2003; Matthys et al., 2006; Mwangangi et al., 2008) and indirectly lead to sustained levels of malaria transmission (Chapter 4). In western Kenya, the gradual increase in human population has put pressure on land available for farming and as a consequence, areas that were previously natural swamps and forests have been transformed into agricultural fields that now provide habitats conducive to mosquito breeding (Munga et al., 2006).

Reversing the increasing trend in the breeding of malaria vectors due to changes in land use presents opportunities for development of sustainable control programmes for mosquito vectors. One way of adapting to changes in land use and preventing the transmission of mosquito-borne disease may be achieved through the control of immature mosquitoes. The control of immature mosquito populations is advantageous because the larvae are usually concentrated, relatively immobile, and occupy a minimal habitat area compared to adults (Killeen 2002b; Floore, 2006). Several larval control programmes in China, India and Sri Lanka have shown great success in controlling mosquitoes through good water management practices (Liu et al., 2004). In Africa, malaria prevention through immature mosquito control has not received as much attention as adult mosquito control (Killeen et al., 2002). However, successful larval mosquito control in Africa through environmental management and application of larval insecticides was reported already more than half a century ago (Keiser et al., 2005b; Kitron & Spielman, 1989; Utzinger et al., 2002), and there is currently renewed interest by the scientific community around the world to assess the feasibility of these methods of disease control (Fillinger & Lindsay, 2006; Geissbühler et al., 2009; Majambere et al., 2008; Shililu et al., 2003; Vanek et al., 2006).

Mosquito larval control can be achieved through environmental (water) management, biological and chemical control and the use of insect growth regulators (Rose, 2001; Floore, 2006). Environmental management entails modification and manipulation of the environment, and modification or manipulation of human habitation or behaviour to prevent propagation of vectors in order to reduce human vector pathogen contact (Keiser et al., 2005; Beales & Gilles, 2002). However, specifications for environmental management vary with local ecosystem structure and hence there is no uniform environmental management recipe that is appropriate in all settings (Keiser et al., 2005b; Shiff, 2002). Biological control methods directed against mosquitoes mostly refer to the use of natural enemies such as predatory fish and invertebrate predators and toxins produced by microbial agents (Rose, 2001; Becker, 2003; Floore, 2006).

We recently found out that while members of communities affected by malaria are willing to take part in mosquito control activities (Chapter 5), there is lack of evidence-based research on locally-available strategies. A longitudinal study carried out in these same communities showed that larval populations of *Anopheles gambiae* Giles are continuously present (Chapter 3). In the current study, we investigated the potential of environmental manipulation and biological agents for controlling anopheline mosquito larvae. Using man-made habitats within a natural habitat, we conducted experiments using i) shade from locally available crops and plants, ii) larvivorous fish *Gambusia affinis* (Baird & Girard) and iii) the microbial insecticide *Bacillus thuringiensis* var. *israelensis (Bti)* in various combinations, to assess their impact on populations of mosquito larvae.

Material and methods

In the Kisumu region of western Kenya, two field trials were conducted in Nyalenda and one laboratory experiment at the Kenya Medical Research Institute (KEMRI).

Study site

The field study was conducted in Nyalenda (0°06’S and 34°46’E, 1100 – 1200 m above sea level), a peri-urban, low-income area in Kisumu city, western Kenya. The site represented a transformed swamp under irrigated agriculture. The main economic activities were subsistence agriculture with rice, maize, sweet potatoes and vegetables under cultivation. Commercial nurseries of ornamental plants and trees were also present. The area received a total annual rainfall of 1004 mm and experienced mean annual relative humidity of 64% and air temperature of 23 °C in 2007, with small seasonal variations. The area receives short seasonal rains in the months of October through December, while long rains occur between March and May with year-to-year variation in intensity. Laboratory studies were conducted in a screenhouse at the Centre of Global Health Research (CGHR), KEMRI, Kisian. The physical location of KEMRI’s CGHR, Kisian is located 13 km north-west of Kisumu city.

Mosquito colony

Anopheles gambiae Giles *sensu stricto* larvae (Iguhu strain), which were used in the laboratory experiment, were maintained at the KEMRI insectaries at Kisian. Larvae were fed 0.5 mg brewer’s yeast per larva

(Pharmadass Ltd., Harrow, UK) daily.

Fish colony

A colony of *Gambusia affinis* (Cyprinodontiformes: Poeciliidae) was initiated from wild-caught samples with the help of staff of the Kenya Marine and Fisheries Research Institute (KEMFRI), Kisumu city. The mosquito-fish colony was maintained in a screenhouse under natural conditions at KEMRI, Kisian. The fish were fed on locally made fish food supplement obtained from KEMFRI. Adult fish were used for laboratory and field experiments.

Manipulation of mosquito breeding habitats through shading

This study was conducted for a period of 17 weeks from 9 March to 29 June 2007. Thirty-six (1 by 1 by 0.5 m) plots were created as mosquito larval habitats by building a shallow dyke (0.2 m) around each plot (Figure 6.1). Habitats were planted with one of four locally grown plant species: Napier grass (*Pennisetum purpureum*), arrow root (*Maranta arudinacea*), papyrus reeds (*Cyperus* spp.) and rice (*Oryza sativa*) and each plot/plant combination was replicated six times. One additional plot series of rice was added and left unweeded to find out if removal of weeds had any effect on mosquito larvae. A sixth series of habitats was left unplanted (control). The habitats filled naturally with water by seepage or rainfall. Weeding was done once per month in all habitats except in the unweeded rice habitats to remove any other plant species that would cause unforeseen effects on the experiment. From week one, larval sampling was done once per week using the standard dipping method with a 350 ml mosquito scoop (Bioquip, Gardena, CA, USA) as described by Service (1993). A maximum of 10 dips was taken from each plot and the larvae collected were separated based on morphological characteristics and counted as anophelines and culicines. The larvae were recorded either as early instars (L1 and L2) or late instars (L3 and L4). Late instar anopheline larvae were identified to species level microscopically using existing identification keys (Gillies & Coetzee, 1987).

Biological control of mosquito larvae

Laboratory investigations of *Bti* and *Gambusia affinis*

This study was done for a period of 8 weeks from the month of November to December 2007. The main goal was to estimate the number of fish and the quantity of *Bti* required for effective control of mosquito larvae. Six different treatments were randomly administered as *Bti* 1 day, *Bti* 3 days and *Bti* 5 days (*Bti* was put in water, left to stay for 1, 3 and 5 days before the larvae were introduced), *Bti* and fish, *Bti* only and fish only while one series was left untreated to act as a control. Each treatment was replicated 25 times. Small plastic washbasins, 27.5 by 17.3 by 10 cm were used, and filled with 2 l of water collected from the Nyalenda field site to a depth of 3 cm. Sixty larvae consisting of 30 early (L1 and L2) and 30 late (L3 and L4) instars were randomly dispensed using a rubber pipette. Equal numbers for each larval control option were randomly allocated into the different plastic basins containing larvae. In total, 9000 laboratory-reared larvae of *An. gambiae s.s.* were used. The optimum *Bti* dosage and concentration of 5 mg/l of water for *Bti* was determined based on the existing literature (Fillinger et al*.,* 2003). Preliminary trials were done with different numbers of adult fish, which were offered 60 larvae (mixed larval stages of development) and we found that four adult fish were able to consume 60 larvae in 24 h. Different sizes of adult fish, ranging from 4 to 7 cm in length were used, to cater for differences in predation resulting from effect of size. The number of living larvae present after introducing the treatments in different washbasins was recorded after 24 and 48 h of exposure.

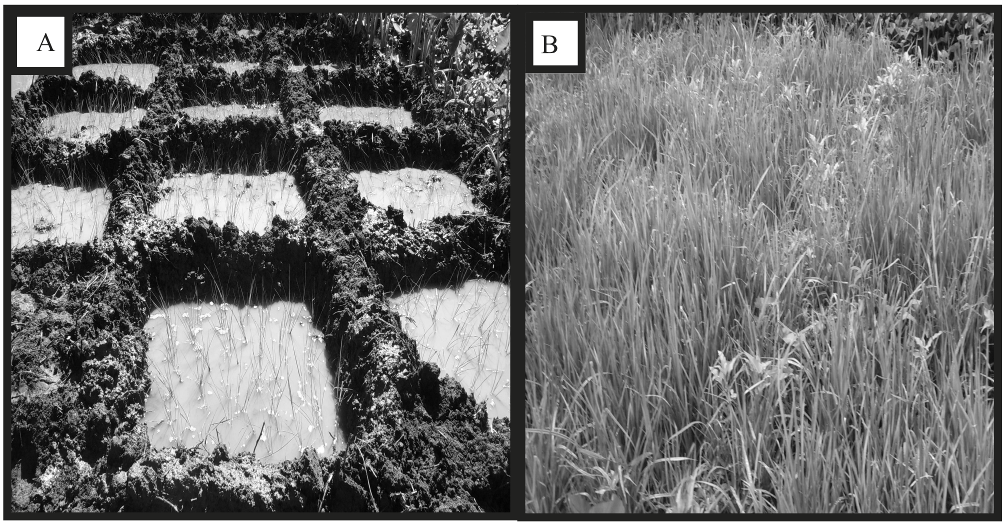

Figure 6.1: Experimental set up in the field showing the man-made habitats (A) with young transplanted rice and (B) with mature rice plants

Biological control of mosquito larvae under field conditions

This study was done for a period of 13 weeks from 4 February to 2 May 2008. Thirty 1 by 1 by 1 m plots were created as mosquito larval habitats by building a 30 cm shallow dyke around each plot (Figure 6.2). Six treatments were randomly administered as follows, *Bti, Bti* + fish in full required amount, *Bti* + fish at half the required amount of each, fish introduced once, and fish introduced fortnightly, while one pond series was left untreated to act as a control. Each treatment was replicated five times.

Figure 6.2: Experimental set up of man made habitats used for biological control studies in the field

In the man-made habitats provided with fish, an extension hole measuring 30 by 30 by 30 cm was dug to provide a hiding place for the fish whenever the water level was reduced to minimal levels, in order to avoid dehydration. The average quantity of *Bti* applied was determined by calculating the volume of water present in the site before treatment was administered. Granule formulation of *Bti* was broadcasted into each sampling site at the rate of 5 mg/l of water. The total number of mixed sizes of mosquito fish (4 to 7 cm) used was based on the feeding rate of four mosquito fish per 60 mosquito larvae per day (from laboratory investigation in experiment 2). This was also used as the minimum number of mosquito fish applied in the respective habitats. For the duration of the experiment, treatments were repeated at 14-day intervals, each time on the same day of the week, with exception of the once-only introduction of predatory fish. Larval sampling of mosquitoes was done 24 h after treatment and thereafter regular sampling of mosquito larvae was conducted twice weekly using the standard dipping method with a 350 ml mosquito scoop (Bioquip, Gardena, CA, USA) as described by Service (1993). A maximum of 10 dips was taken in each plot and the larvae collected were separated into anophelines and culicines and counted. The larvae were recorded either as early instars (L1 and L2), late instars (L3 and L4) or pupae. Late instar anopheline mosquitoes were microscopically identified to species level using identification keys from Gillies & Coetzee (1987).

Data analysis

Data analysis was done using SPSS 15.00 for windows (SPSS Inc, Chicago, IL, USA). The non-parametric Kruskal-Wallis test was used to determine differences in mosquito larval densities among the habitats. The General Linear Model (GLM), multivariate analysis with Bonferroni correction was used for pair-wise comparisons of larval densities to find out any differences in habitats provided with crop cover and among different treatments. The post-hoc Dunnets test was used to find out any statistical differences among the different treatments in comparison to the control.

Results

Manipulation of mosquito breeding habitats through shading

The mean weekly numbers of larvae and pupae are shown in Table 6.1a. Young anophelines (L1 and L2) were abundant in all habitat types but the number of late stage larvae (L3 and L4) were fewer in most habitats except in the controls and Napier grass-covered habitats. Anopheline larval densities exhibited weekly variations within habitats with different treatments. No larvae were found in the 8th, 11th, 12th and 17th week and only few were found in weeks 1 and 3 (Table 6.1a). In the 12th week, the whole field site was flooded due to heavy rains, and the subsequent weeks many young larvae were recorded. Substantial numbers of early instars were found in the control, papyrus reed, arrow root and weeded-rice habitats during the 2nd, 5th, 6th, 13th and 16th week. More late instars were observed in plots with weeded rice, papyrus reeds and the control habitats during the 5th, 10th, 13th and 16th week. The effect of crop type was significant only on early instar anophelines as well as early and late instars of culicines (in all these cases, $p<0.05$).

Overall, culicines were more abundant than anophelines (N = 2445; 71% culicines, 29% anophelines) with the early instars being the majority during the entire sampling period (Table 6.1b). Early and late instars of both culicines and anophelines were distributed differently ($p<0.05$), except for late instar anophelines, whose numbers did not differ significantly among the habitats ($p>0.05$).

Pairwise comparisons between habitats with different crops showed that the abundance of early instars of anopheline mosquitoes was not different among the habitats of unweeded rice, weeded rice and arrow root. The densities of early instars of culicines were differently distributed ($p<0.05$) among habitats with different shade plants whereas for the late instars, significant differences were observed in Napier grass, papyrus reeds and unweeded rice habitats. Overall, early and late instar culicines were most abundant in the control habitats and the lowest numbers were recorded in the habitats with papyrus and unweeded rice plants. Culicine pupae were recorded more frequently in

Table 6.1a: Weekly mean numbers of anopheline larvae and pupae in man-made habitats under environmental manipulation for a period of 17 weeks.

Stage	Treatment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
L1-L2	**Control**	**0.80**	**9.20**	**0**	**0**	**2.40**	**2.20**	**2.20**	**0**	**1.20**	**3.60**	**0**	**0**	**4.60**	**0.80**	**1.20**	**4.20**	**0**
	Papyrus	**0**	**0**	**1.00**	**0.6**	**4.00**	**10.00**	**0**	**0**	**2.00**	**3.4**	**0**	**0**	**6.80**	**2.40**	**0.80**	**2.60**	**0**
	Napier	**0**	**1.6**	**0**	**0**	**0**	**0**	**0.6**	**0**	**0**	**0**	**0**	**0**	**1.20**	**4.40**	**2.40**	**1.00**	**0**
	Rice, weeded	**0**	**0**	**0.80**	**1.40**	**4.80**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**8.20**	**0**	**4.40**	**7.60**	**0**
	Rice, Un-weeded	**0**	**0**	**0.40**	**0.60**	**2.40**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**4.0**	**0**	**0**	**8.40**	**0**
	Arrow root	**0.60**	**0**	**0**	**0.20**	**0**	**4.40**	**0**	**0**	**0**	**0**	**0**	**0**	**9.0**	**0**	**0**	**12.40**	**0**
L3-L4	**Control**	**0**	**0.20**	**0**	**0**	**0.20**	**0.20**	**0**	**0**	**0.20**	**0.20**	**0**	**0**	**0.20**	**0**	**0.20**	**0.80**	**0**
	Papyrus	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.20**	**0.40**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Napier	**0**	**0.20**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.60**	**0**	**0.20**	**0.20**	**0**
	Rice,	**0**	**0**	**0.20**	**0**	**0.60**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.80**	**0.80**	**0**
	Rice, Un-	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.20**	**0**	**0**	**0**	**0**
	Arrow root	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.20**	**0**	**0**	**0**	**0**
Pupae	**Control**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2.00**	**0**	**0**	**0**
	Papyrus	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Napier	**0**	**0**	**0**	**0**	**0**	**0**	**0.20**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Rice, weeded	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Rice, Un-weeded	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Arrow root	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

habitats with papyrus reeds, arrow roots and Napier grass (Table 6.1b). *Anopheles coustani* was present in all habitats except the unweeded rice habitats, whereas almost 85% of the total *Anopheles gambiae s.l.* were recorded from the control (Figure 6.3).

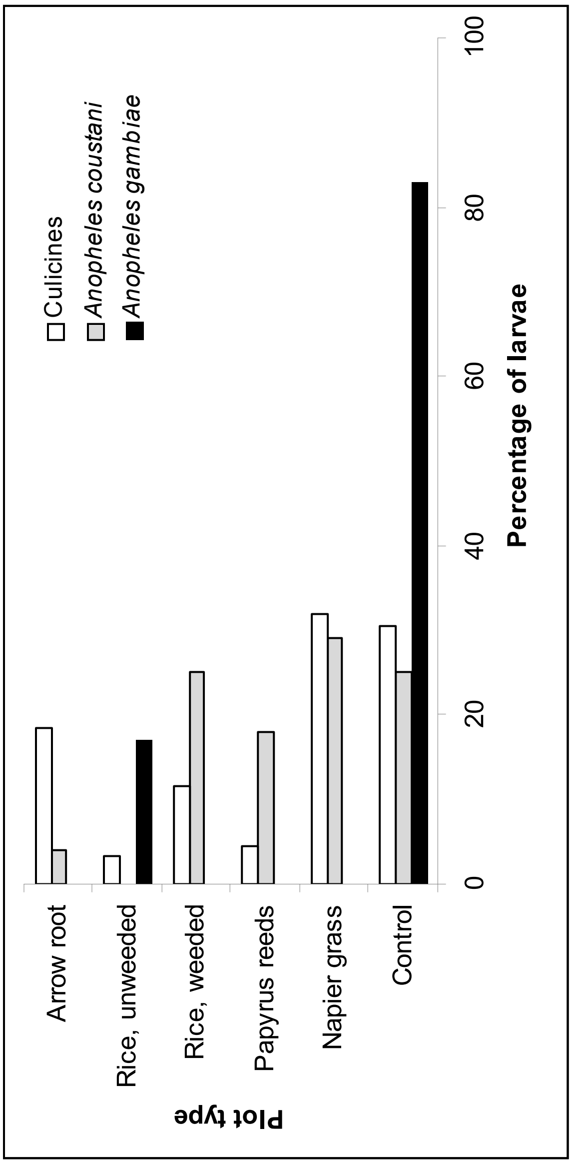

Figure 6.3: Anopheline and culicine late instar distribution in the control and in habitats under shade provided by different plants, expressed as a percentage of the total number of larvae collected. Habitats with unweeded rice and arrow roots were marginally significant when compared to the control ($p=0.05$). The late instar culicines in Napier grass habitats and the control were not significantly different ($p > 0.05$).

Table 6.1b: The mean (± SE) numbers of anopheline and culicine larvae and pupae per dip in the control plot and plots shaded by different plants during the study period.

Plant Cover	***Anopheles* early (L1/L2)**	***Anopheles* late (L3/L4)**	***Anopheles* pupae**	***Culex* early (L1/L2)**	***Culex* late (L3/L4)**	***Culex* pupae**
Control	1.91±0.38	0.13±0.05	0.12±0.12	8.09±1.18	2.95±0.60	0.08±0.03
Papyrus reeds	1.98±0.34	0.04±0.03	0	0.72±0.20*	0.35±0.13*	0.19±0.12
Napier grass	0.6±0.18*	0.07±0.03	0.01±0.01	4.69±0.80*	2.22±0.48	0.15±0.06
Rice, weeded	1.35±0.32	0.09±0.04	0	3.12±0.58*	1.13±0.31*	0.04±0.02
Rice, unweeded	0.93±0.26	0.01±0.01*	0	0.75±0.24*	0.35±0.13*	0.01±0.01
Arrow root	1.56±0.41	0.01±0.01*	0	3.06±0.73*	1.46±0.34*	0.14±0.05

*Effects of plant type on larval abundance was significantly different from the control.

Efficacies of *Bacillus thuringiensis* var *israelensis* and *Gambusia affinis* in the laboratory

The numbers of larvae that survived after 24 and 48 h of exposure to different treatments were quite low (Figure 6.4). Treatment with *Bti* resulted in pupation in some 4th instars, however the pupae did not develop into adults. Univariate analysis of variance found significant differences among the treatments (F = 16.457; df = 4; $p<0.001$). Pairwise comparison of different treatments showed that larvae exposed to *Bti* + fish, *Bti* 1day, *Bti* 3 days and *Bti* 5 days were not statistically different ($p = 1.0$) from each other. However, apart from the control, treatment with fish recorded significantly more surviving larvae after 24 h when compared to those treated with *Bti* 1 day, *Bti* 3 days and *Bti* 5 days ($p<0.001$).

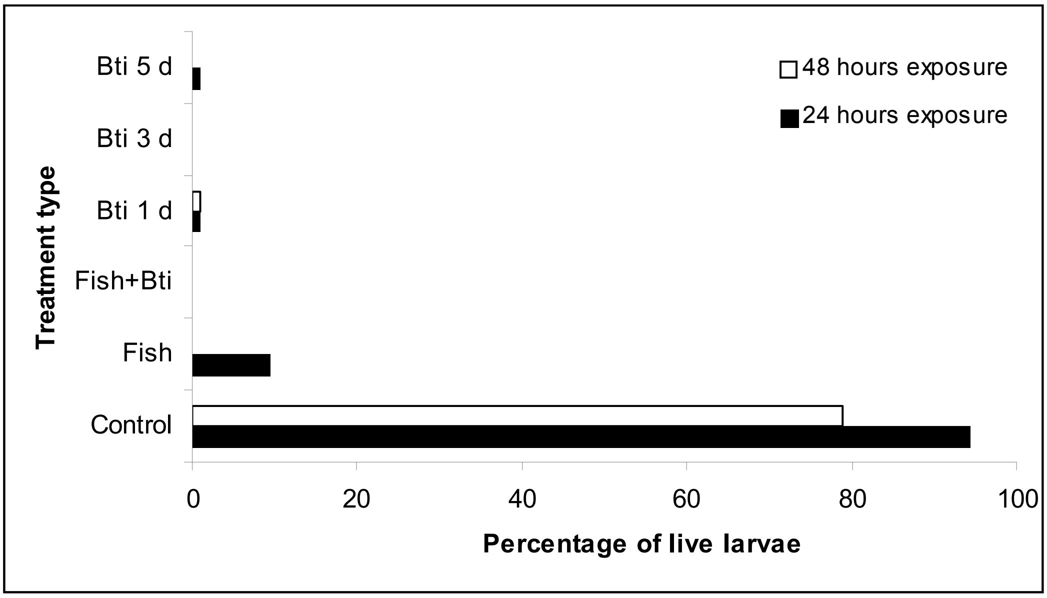

Figure 6.4. Percentage of *An. gambiae* larvae surviving after 24 and 48 hours of exposure to treatment with *Gambusia affinis* and/or *Bti* in the laboratory. Treatments with fish +*Bti*, *Bti* 1d, 3d and 5d were not significantly different at 0.05

Efficacy of *Bti* and fish in man-made habitats

Weekly variations in densities of anopheline larvae indicate that early and late instars were present throughout the study, with lowest numbers recorded at the end of the trial, i.e., 12th and 13th week (Table 6.2a). Few pupae were recorded in all treatments and habitats provided with either half or full amounts of *Bti* and fish together did not produce any pupa. More anopheline larvae were sampled from the control and in

Table 6.2a: Weekly mean numbers anopheline larvae and pupae in man-made habitats under biological control for a period of 13 weeks

Stage	**Treatment**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
L1-L2	**Control**	**3.60**	**2.80**	**2.40**	**5.50**	**3.20**	**1.20**	**3.93**	**5.30**	**2.33**	**2.70**	**1.07**	**0.50**	**0.80**
	Fish biweekly	**4.27**	**1.90**	**3.93**	**5.10**	**3.07**	**1.60**	**5.80**	**6.10**	**2.27**	**2.60**	**1.47**	**0.80**	**0.73**
	Fish once	**3.73**	**2.90**	**3.60**	**5.00**	**4.60**	**1.20**	**2.93**	**4.00**	**2.27**	**2.40**	**1.47**	**0.40**	**0.13**
	Bti	**3.13**	**4.40**	**1.73**	**5.50**	**1.87**	**2.20**	**2.87**	**4.70**	**2.40**	**2.60**	**0.40**	**0.50**	**0.47**
	Fish+*Bti* (half)	**2.67**	**2.00**	**1.67**	**4.10**	**2.20**	**0.50**	**2.53**	**4.10**	**1.60**	**1.40**	**0**	**0.30**	**0.13**
	Fish+*Bt*i (full)	**1.13**	**1.40**	**0.33**	**4.33**	**2.00**	**0**	**0.67**	**1.10**	**0.60**	**0.60**	**0.13**	**0**	**0**
L3-L4	**Control**	**0.13**	**1.30**	**0.53**	**2.30**	**1.73**	**0.10**	**1.20**	**1.30**	**0.87**	**0.60**	**0.07**	**0.10**	**0**
	Fish biweekly	**0.53**	**1.50**	**0.60**	**1.60**	**1.27**	**0.10**	**2.13**	**2.00**	**1.13**	**1.10**	**0.07**	**0**	**0**
	Fish once	**1.20**	**1.20**	**2.53**	**2.90**	**1.27**	**0**	**1.00**	**1.40**	**0.33**	**0.90**	**0**	**0**	**0**
	Bti	**0.07**	**1.70**	**0.67**	**1.80**	**0.60**	**0**	**0.07**	**1.60**	**0.93**	**0.60**	**0.07**	**0**	**0.07**
	Fish+*Bti* (half)	**0.47**	**1.30**	**0.40**	**0.80**	**0.67**	**0.10**	**0.47**	**1.00**	**0.73**	**0.70**	**0**	**0**	**0**
	Fish+*Bt*i (full)	**0.40**	**0.70**	**0.07**	**0.78**	**0.60**	**0**	**0**	**0.80**	**0.27**	**0.10**	**0**	**0**	**0**
Pupae	**Control**	**0**	**0**	**0**	**0.10**	**0**	**0**	**0**	**0**	**0.13**	**0**	**0**	**0**	**0**
	Fish biweekly	**0**	**0**	**0**	**0**	**0.80**	**0**	**0**	**0.20**	**0.13**	**0.20**	**0**	**0**	**0**
	Fish once	**0.07**	**0**	**0.80**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Bti	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.10**	**0**	**0**	**0**	**0**	**0**
	Fish+*Bti* (half)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Fish+*Bt*i (full)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

habitats with fish only; more culicine larvae were encountered in habitats treated with full amounts of *Bti* + fish (Table 6.2b).

Table 6.2b: The mean (± SE) numbers of anopheline and culicine larvae per dip in the control plot and in habitats treated with different treatments during the study.

Treatment type	***Anopheles* early (L1/2)**	***Anopheles* late (L3/ L4)**	***Anopheles* pupae**	***Culex* early (L1/L2)**	***Culex* late (L3/L4)**	***Culex* pupae**
Control	2.67±0.24	0.76±0.12	0.02±0.02	1.41±0.37	0.19±0.07	0.01±0.01
Bti	2.38±0.25	0.57±0.10	0.01±0.01	1.27±0.55	0.35±0.14	0
Fish bi-weekly	3.05±0.26*	0.90±0.12*	0.11±0.07	1.29±0.38	0.66±0.20	0
Fish once	2.67±0.23	0.96±0.13	0.08±0.07	0.59± 0.18	0.37 ±0.18	0
***Bti* + fish (half)**	1.73±0.21*	0.48±0.08	0	0.56±0.20	0.31±0.24	0
***Bti* + fish (full)**	0.87±0.18*	0.26±0.07	0	2.85±0.68	0.62±0.22	0

* significantly different from the control at 0.05 level

The effects of treatment type were significantly different from the control for early and late instar anophelines, as well as early instar culicines (in all cases $p<0.05$). Multivariate analysis of variance indicated that the effects of treatment on early ($p<0.001$) and late instars of anophelines ($p<0.001$) and early instars ($p = 0.001$) of culicines were significantly different. Pairwise comparisons using the Dunnets test showed that controls produced significantly more anopheline early instars than habitats containing fish + *Bti* ($p = 0.011$) and those provided with fish fortnightly ($p<0.001$). The control habitats were, however, not different ($p>0.05$) from habitats offered *Bti* only and where fish were introduced only once. Habitats in which mosquito fish were introduced on a fortnightly basis had the lowest numbers of anopheline early instars when compared to habitats with *Bti* only, fish + *Bti* and where fish were introduced once ($p<0.05$). Within the late instar group of anophelines, no significant differences ($p>0.05$) were found among habitats with fortnightly fish introductions when compared with *Bti* + fish and those with *Bti* only. However, early instars of culicines were recorded more in habitats with fortnightly fish introductions than in all other treatments. Considering anopheline species composition, *An. gambiae s.l.* was more recorded in habitats provided with fish alone, whereas more *An. coustani* were recorded from the plots provided with *Bti* alone (Figure 6.5).

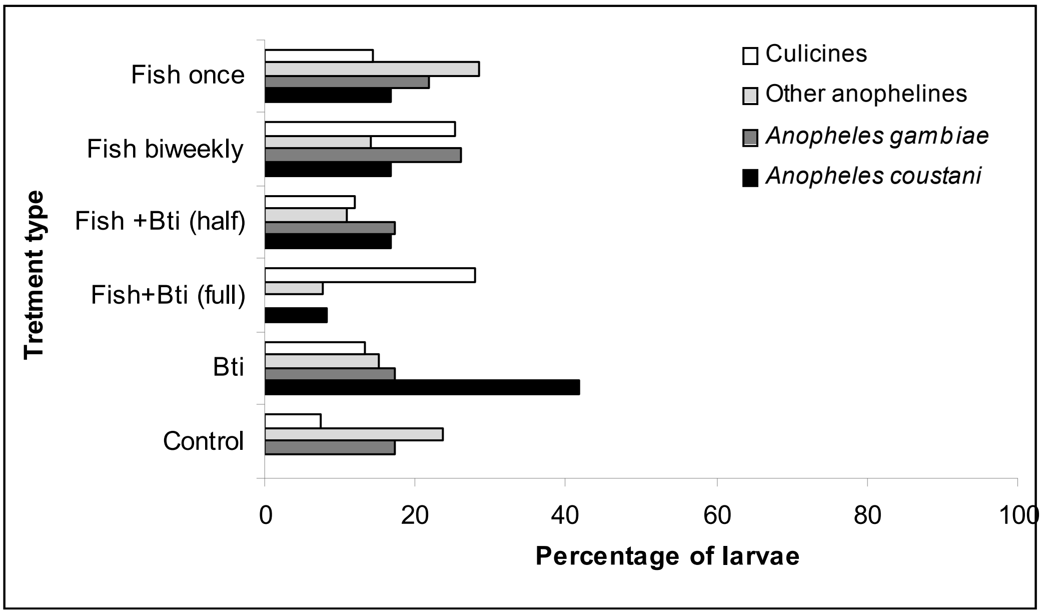

Figure 6.5: Anopheline and culicine late instars distribution in the control and in the plots under different treatments expressed as a percentage of the total number of larvae recorded.

Discussion

We have shown that simple strategies such as using locally available crops and plants to provide shade over mosquito larval habitats as well as using predatory fish and *Bti* as biological control agents are feasible options for the control of immature mosquitoes, including malaria vector species. All habitats provided with shade, i.e., those planted with arrow root, Napier grass, rice and papyrus reed, produced significantly fewer larvae than the controls. Habitats provided with shade did not have *An. gambiae s.l.* except for the unweeded rice, while *An. coustani*, a less important malaria vector, was recorded in all the habitats with exception of the unweeded rice habitats. Culicine mosquitoes did not thrive well in papyrus reeds, unlike early instars of anophelines that were frequently found in this habitat.These effects exceeded our expectations, as *An. gambiae* was never recorded from the treated sites except from unweeded rice, where it was found on one occasion. By contrast, *An. gambiae* was frequently present in the unshaded control sites. From our longitudinal studies in Nyalenda (Chapter 3 & 4), we established that *An. arabiensis* was the main species of the *An. gambiae* complex found in this area; hence, shading affected this malaria vector, which is recorded to thrive when exposed to sunny habitats (Gillies & Coetzee, 1987; Petrarca et al., 1998)

Our results show that as the crops planted in the habitats grew tall and had more leaf cover, the numbers of larvae recorded decreased, depending on the type of crop. Chandler & Highton (1975) observed that as rice grew taller and older, water depth and algal growth were reduced, whereas numbers of predators increased, leading to reductions in larval abundance. The same factors may explain the overall reduction in larval populations within habitats provided with crops or plants. Although we did not measure shade directly, we worked on the assumption that as the crops grew taller, increase in height was directly proportional to shade over the mosquito habitat. Our findings are in agreement with other studies that report that heavy shade provided by leafy emergent plants is negatively correlated with larval abundance in breeding sites and consequently leads to decreases in productivity of malaria vectors (Foley et al., 2002; Munga et al., 2005, 2006; Mwangangi et al., 2007). Previous studies carried out in Uganda showed that *An. gambiae s.l.* did not breed in the interior of papyrus swamps in their natural, undisturbed state (Goma, 1960). However, once the natural state of papyrus swamps is disturbed or drained and the land brought under crop cultivation, larvae were found (Goma, 1960). In our study papyrus reed seedlings were transplanted into man-made habitats, hence they were not in their natural state and a reasonable abundance of both anopheline and culicine larvae were recorded within the habitats. The main malaria vector *An. gambiae s.l.,* prefers open, sunlit pools of water, when these habitats are shaded; these pools of water become non-conducive for ovipositing females (Klinkenberg et al., 2003). This probably arises as a result of the action of shade on lowering the water temperature, which induces females to select sun-exposed sites (Tuno et al., 2005).

The total number of *An. gambiae s.l.* mosquito larvae recorded 24 h after treatment with *G. affinis* or *Bti* was significantly influenced by the type of treatment offered in both trials under laboratory conditions. However, in the man-made habitats to which different treatments were applied, no significant differences were observed among the late instar anophelines. Treatments involving both *Bti* and fish resulted in the greatest reduction in anopheline larval population densities when compared to habitats where only *G. affinis* was introduced. These results are comparable to the outcome of experiments conducted by Blaustein (1992) where *G. affinis* alone failed to control mosquitoes in experimental rice habitats. Our results indicate that the predatory effectiveness of mosquito fish on anopheline mosquito larvae diminished when introduced into the man-made larval habitats. This is in contrast to laboratory experiments, and could be attributed to other factors that we did not investigate, such as the fish preying on other aquatic organisms.

Bence (1988) reported that in nature, *G. affinis* fed on other mosquito predators, other alternative prey, and external food or invertebrate sources. In addition, Homski et al. (1994) found that higher turbidity in man-made habitats may have favored a higher abundance of invertebrates and reduced visibility of anopheline larvae for mosquito fish than in sites covered with emergent vegetation. Such feeding habits tend to divert the feeding ability of *G. affinis* on mosquito larvae, thus causing resurgence in mosquito larval density. This observation may account for the higher population densities of larvae in habitats where fish were the only control agents. Effective predation of mosquito fish may be enhanced by their long life span, high rate of proliferation and greater adaptability than other seasonal predators like tadpoles (Bence, 1988). Blaustein (1992) suggested that this control strategy would be enhanced by repeated introduction of mosquito fish so that they could cope with complex multivariate factors of the specific larval habitats. This is in line with our findings, in which higher larval population densities of anophelines were recorded in habitats where mosquito fish were introduced only once compared to where introduction of mosquito fish was repeatedly done at 2-week intervals. The findings on larval control options suggest that *G. affinis* and *B. thuringiensis* var *israelensis* (*Bti*), when used together in the right quantities to complement each other were more effective in reducing mosquitoes in man-made habitats than either agent alone.

Our results show a lower effect of larvicide than expected in habitats that were treated with *Bti* only, which contradicts the findings of Fillinger & Lindsay (2006) and Majambere et al. (2008), who report that microbial larvicides reduced anopheline larval density by 95%. Nevertheless, Fillinger & Lindsay (2006) concluded that the efficacy of *Bti* is greater if optimum quantities of *Bti* are applied on a weekly basis. In our study, *Bti* was applied once every 14 days, which matched with the larviciding studies carried out by Shililu (2003) in Eritrea. Occasional presence of heavy downpours may have caused water runoff and drifting of *Bti* granules from sampling sites and therefore a full dose might not have been achieved in all instances. Also, the persistence of *Bti* endotoxins may have reduced rapidly under the conditions in the field, hence showing no apparent effect on anopheline larval abundance. As previous studies clearly showed that *Bti* is non toxic to non-target organisms (Fillinger et al., 2003; Fillinger & Lindsay, 2006), we used this property of *Bti* to serve as a basis for integrating this product with *G. affinis* for increased efficacy of larval control.

In the current study, trials were done under field conditions in man-made habitats that were naturally colonized by mosquito larvae. Under these conditions, external factors were not controlled and could have played an important role in the colonization and growth of mosquito larvae in the respective habitats. The changes in water level and occasional flooding of habitats could not be avoided, as the sites were exposed to ambient conditions. Factors such as water turbidity, nutrient content in water, cannibalism, predation of immature stages, parasitism, pathogens, competition, water temperature and plant odours that could have either repelled or attracted female mosquitoes during oviposition (Service., 1971; 1973; 1977; Okogun et al., 2005; Koenraadt & Takken, 2003b; Schneider et al., 2000; Paaijmans et al., 2009) were not controlled and hence could have played a role in the results obtained. Habitats with few anopheline larvae recorded more culicine larvae while those that recorded more anophelines had fewer culicine larvae, suggesting selective oviposition behaviour among these mosquito families. Competition may have played a role in structuring larval populations, although we did not investigate this. In the current study, the standard dipping method was used to estimate mosquito larval and pupal densities, which may have underestimated larval abundance (Service, 1993; Mutuku et al., 2006b; Gu et al., 2008) and consequently, may have influenced the amounts of *Bti* and numbers of *G. affinis* used, leading to contrasting results.

In the western region of Kenya, the current human population pressure is blamed for the large changes in land use. For instance, areas that were previously natural swamps and forests have been transformed into agricultural fields. These agricultural developments have an impact on the ecological characteristics of the local mosquito vector in terms of density, local microclimate and malaria incidence (Minakawa et al., 2005b; Munga et al., 2005, 2006; Yasuoka & Levins, 2007). Communities in western Kenya are willing to take part in malaria control (Chapter 5) and to effectuate this, simple control strategies suitable for the local mosquito vectors need to be available as a way of adapting to the changes in land use. Our results show that locally available leafy plants could be of use for mosquito control especially in areas under traditional agriculture. Use of mosquito fish is another option that can easily be put into practice, especially in areas where water is always present. The effectiveness of biological larvicides for the control of African anophelines has already been demonstrated by several studies (Fillinger et al., 2006; Geissbühler et al., 2009; Majambere et al., 2007) and in areas where locally available solutions are not feasible and the water cannot be drained, then application of microbial larvicides could be the best option. Although our results are spatially and temporally limited, the option of using shade from locally available crops and mosquito fish seems an easily applicable alternative for the control of mosquito larvae This study was part of an ongoing project in agro-ecological settings in two highland villages (Lunyerere and Fort Ternan) and one peri-urban area (Nyalenda), where most larval habitats were man-made (Chapter 3). The field trials reported in this study were done in the peri-urban area to assess the best ways of immature mosquito control. For Nyalenda, we suggest larviciding and use of predatory fish while in the highland villages environmental (water) management and the use of biological control methods such as predatory fish seem promising. However, for better results, an integrated programme for *Anopheles* vector control that can be supplemented with existing adulticiding options is promising (e.g., Fillinger et al., 2009).

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Chapter 7

Integrated control of malaria mosquito larvae in two highland villages in western Kenya

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Abstract

In western Kenya, malaria is endemic and malaria control is an important public health measure. In two highland villages, the efficacy of locally available strategies for the control of larval stages of malaria vectors (anopheline species) was examined, with the view that these must be easily applicable by the affected communities at a local scale. Larval control strategies applied included environmental management through source reduction and vegetation management, and the use of larvivorous fish in comparison with the use of bio-larvicide, *Bacillus thuringiensis* var *israelensis* (*Bti*). All intervention methods used led to 100% reduction of late-instar anophelines in both temporary and permanent habitats, whereas in the control habitats mosquito larvae were present as before. Non-anopheline mosquitoes also disappeared. Most of these intervention strategies were applied in habitats that arose due to human activities. The proposed intervention strategies utilize locally available resources, are much based on inclusion in the farming system, and thus, can be applied by the farmers themselves. Involvement of the local communities in larval control activities is considered sustainable and can be valuable and effective in addition to currently applied malaria control strategies.

Key words

Anopheles spp., larval habitat, *Bacillus thuringiensis* var *israelensis* (*Bti*), drainage, *Gambusia affinis*, Arrow root, Kenya.

Introduction

In East Africa, malaria is endemic in many regions where climate and environment together present conditions suitable for malaria vectors. The main vector species, *Anopheles gambiae* Giles *sensu stricto*, *An. arabiensis* Patton and *An. funestus* Giles breed abundantly in small water puddles and pools, and their distribution is mostly determined by temperature (Lindsay & Martens, 1998). In the past, malaria and malaria parasite-positive vectors were not reported at higher altitudes. However, in the last decade an increasing number of cases of malaria in formerly malaria-free areas and highland areas have become common (Chen et al., 2006; Garnham., 1945; Lindblade et al., 2000a). Several hypotheses have been proposed to explain the increased malaria transmission in the highlands, including land-use changes, global climate changes, increased drug resistance, cessation of malaria control activities, and demographic changes (Lindblade et al., 2000a; Lindsay & Martens, 1998; Malakooti et al., 1998; Zhou et al., 2004). Cox (1999) estimated that 34 million individuals were at risk of malaria in the East African highlands. In these highlands, transmission is probably much more focal in its distribution than in many lowland areas, as breeding sites are more common in the valley floor than on the steep valley slopes (Balls et al., 2004; Chapter 3). In addition, recent studies report that human activities in these highlands have subsequently created potential mosquito breeding habitats (Lindblade et al., 2000a; Munga et al., 2006). In the Ugandan highlands, the elimination of papyrus swamps created a habitat for *An. gambiae* and *An. funestus*, leading to increased malaria transmission (Lindblade et al., 2000a). Increased malaria transmission was attributed to increased local temperatures near cultivated swamps, combined with occasional excessive precipitation that favoured *An. gambiae* breeding. In the highlands of western Kenya, *An. gambiae* was found only in farmland habitats but not in forest and swamp habitats (Munga et al., 2006). These differences in larval distribution were attributed to the fact that farmland habitats received more sunlight, and hence water temperatures were conducive for *An. gambiae* breeding. In Ethiopia, changes in land use and climate expose the highland areas to unexpected malaria epidemics, presumably due to expansion of environmental conditions suitable for malaria transmission (Das, 2004). The high endemicity of malaria in Africa favours good knowledge of the disease and its vectors, creating significant opportunities for its control (Hay et al., 2009).

Research on malaria in the highlands has mainly focused on the development of early-warning systems to identify when epidemics are expected (Ernst et al., 2006; Hay et al., 2003). The core idea behind these systems is that when parameters indicate a malaria epidemic is likely, resources can be channeled to the highlands to prevent or contain the epidemic (Ernst et al., 2006). However, in sub-Saharan Africa, malaria epidemics arise suddenly in mostly remote, disadvantaged settings without effective alert systems (Checchi et al., 2006). In resource-limited countries such as those of highland East Africa, an all-or nothing approach to interventions such as insecticide spraying or bednet distribution, often results in complete coverage for some areas and no coverage for others when funds run out (Ernst et al., 2006). Thus, regular vector control activities targeted at the malaria risk areas could be more cost effective than emergency interventions that often face delays in mobilization (Protopopoff et al., 2007). In addition, because full coverage of control measures is hardly achieved, the control of immature mosquitoes integrated with adult mosquito control measures could be highly feasible and sustainable in the long run.

In Kenya, for instance, since the implementation of the roll back malaria initiative (RBM, 2005) malaria control has been based on insecticide treated nets (ITNs), indoor residual spraying (IRS) and the use of antimalarial drugs for the treatment of malaria parasites. Following the adoption of RBM, there are indications that malaria morbidity and mortality is on a decline as a result of scaled use of ITNs (Noor et al., 2007) and increased availability of antimalarial medicines (Okiro et al., 2007). However, although there is a decline in malaria morbidity and mortality, the disease is still reported, and the development of insecticide resistance and drug-resistant parasites undermines the reliance on ITNs and medicines for malaria control. An integrated approach for malaria control would benefit from the inclusion of larval control. Results from a current study by Fillinger et al. (2009) indicate a two-fold reduction in malaria transmission through integrated control using microbial larvicides combined with the use of ITNs in western Kenya. Microbial larvicides have been proven efficient in the control of anopheline mosquito larvae and the reduction in adult mosquito densities (Fillinger & Lindsay, 2006; Geissbühler et al., 2009; Majambere et al., 2007). However, access to microbial larvicides is still a challenge for developing countries, thus calling for the development of alternative larval control strategies that can utilize locally available resources. In the current study, an integrated approach was used in the control of malaria mosquito larvae, with an emphasis on using various anti-larval strategies in comparison to application of microbial larvicides. The main goal was to provide results-oriented proof of control strategies that can be adopted by the affected communities using locally available resources.

Materials and methods

Study area

The study was implemented in the western province of Kenya. Two rural highland villages, Lunyerere (0°06′N and 34°43′E) in Vihiga District at

1460 to 1580 m and Fort Ternan (0°12’S and 35°20’E) in Kericho District at 1480 to 1650 m above sea level were selected for this study. Lunyerere has approximately 4,000 inhabitants. The village is situated between undulating hills that form large basin shaped valleys that collect water runoff frequently in extended swamps. Houses are spread over a large area. In contrast, Fort Ternan, a small village with about 2,000 inhabitants, is located on the slopes of Nandi hills, an area that is characterized by steep slopes forming sharp V-shaped valleys providing less room for standing water. Mosquito breeding habitats have been identified in both villages. In Fort Ternan, the main breeding habitats were livestock watering points, erosion pits and drainage canals. In Lunyerere these are drainage canals, erosion pits and hoof prints. The majority of these mosquito breeding habitats are formed as a result of human activities. A detailed description of the study area and the breeding habitats has been provided in Chapter 3.

Interventions

Larviciding: Water-dispersible and granulated formulations of commercial strains of *Bacillus thuringiensis* var. *israelensis* (*Bti*; Valent Biosciences Corporation, USA) was applied to all temporary habitats and selected permanent habitats (see Chapter 3) in Fort Ternan and Lunyerere from August and April 2008 to March 2009, respectively. Larvicide was broadcasted on the larval habitats at weekly intervals. The optimum dosage and concentration of 200g/ha *Bti* was used (Fillinger et al., 2003).

Predatory fish: A colony of *Gambusia affinis* (Cyprinodontiformes: Poeciliidae) was initiated from wild-caught samples with the help of the Kenya Marine and Fisheries Research Institute (KEMFRI), Kisumu. The fish colony was maintained at Fort Ternan, in man-made ponds under natural conditions. The fish population was left to establish from June to July 2008, and in August, introductions of adult fish were made into the selected permanent habitats, based on a laboratory-determined ratio of four fish per 60 larvae (Chapter 6). Adult fish were introduced into the respective habitats only once and left to increase in population naturally; re-introduction of fish into the habitats was done if the fish appeared to be missing.

Source reduction: Source reduction was achieved through drainage of canals, land levelling or by filling ditches with soil. Drainage was achieved by creating ditches to keep water moving in order to avoid any stagnant water that could provide a breeding site. Water was drained off by a natural gradient into a main canal, which flowed into a natural river. Canals subjected to drainage were checked weekly to remove any unwanted debris that could reduce or stop water movement. Habitats located along the river fringe were filled and levelled using stones and/

or soil to prevent any water from stagnating. In areas where water stagnated due to debris in the river, the debris was removed to allow for easy flow of water.

Environmental manipulation: This refers to activities that reduce larval breeding sites of vector mosquito through temporary changes to the aquatic environment in which the larvae develop (Walker & Lynch, 2007). Breeding habitats were provided with shade from arrow root (*Maranta arudinacea*) crops that were planted along selected water canals in Lunyerere to provide temporary non-conducive conditions for mosquito breeding. Arrow root seedlings were obtained locally from resident farmers at a small fee. Previous field trials showed that mosquito breeding habitats shaded by arrow root and Napier grass (*Cyperus* spp.) plants had significantly reduced immature anopheline mosquito populations (Chapter 6).

Implementation programme of interventions

Fort Ternan: This study was conducted for a period of 8 months, from August 2008 to March 2009. Twelve mosquito breeding habitats were identified from baseline data (Chapter 3). Three different intervention types were applied, i.e., larviciding, source reduction and introduction of predatory fish. Each intervention was replicated three times and a fourth series of habitats was left without any treatment (control). Interventions were offered depending on the suitability of the habitat, for example erosion pits were provided with predatory fish, leaking taps were treated with *Bti,* while habitats along the river fringe were either drained to allow a flow of water, filled with dirt or levelled to the ground.

Lunyerere: This study was conducted for a period of 12 months, from April 2008 to March 2009. Twenty-four breeding habitats were selected, and in this case all were drainage canals. Three different interventions were applied, i.e., environmental manipulation, larviciding and source reduction. Each intervention type was replicated six times and a fourth series of water canals was left untreated (control). The drainage canals filled naturally with water by underground seepage or rainfall.

Post-intervention population studies

The interventions ended in March 2009. From April through June 2009 larval habitats in the intervention zones were sampled to investigate the re-establishment of mosquito populations in the previously controlled zones.

Outcome measure

Larval density: Larval sampling was done once per week between Monday and Wednesday using the standard dipping method with a 350 ml mosquito scoop [Bioquip, Gardena CA, USA] (Service, 1993)**.** A maximum of 10 dips was taken from each habitat and the larvae collected were separated into anophelines and culicines, counted and recorded. The larvae were recorded either as early instars (L1 and L2) or late instars (L3 and L4). Late instar anopheline larvae were immediately preserved in 90% absolute ethanol and taken to the laboratory at the Kenya Medical Research Institute (KEMRI), Kisumu for taxonomic identification (Gillies & Coetzee, 1987).

Data Analysis

Results were analysed by comparing the weekly densities of younger and older instars of anophelines and culicines before and during the intervention period from March 2006 to March 2009. We also examined whether the mosquito densities in the control sites were different from habitats applied with different interventions. Comparisons of larval densities during the pre-intervention period and intervention period in the control habitats were done using an independent samples t-test. Presence or absence of larvae in the control and intervention zones was compared using χ^2 tests. The effect of different intervention strategies on larval densities in comparison to the control was done using multivariate analysis of variance in SPSS 15.0 for Windows.

Results

Longitudinal dynamics of mosquito larvae

Anopheline mosquito larvae: In Fort Ternan, a complete reduction of both early and late instar anophelines in temporary habitats (Figure 7.1a) and permanent habitats (Figure 7.2a) was observed, with few early and late instars recorded at the beginning of the intervention in August, September and in the month of October. In Lunyerere there was an immediate and complete reduction of both early and late instar larvae in both temporary (Figure 7.1b) and permanent (Figure 7.2b) habitats subjected to interventions. In Fort Ternan, significant differences in early ($p<0.001$; 95% CI. 0.026 – 0.046) and in late instar ($p<0.001$; 95% CI. 0.009 – 0.020) anopheline populations were observed before intervention and after intervention in the control zones. Contrary, in Lunyerere, no significant differences among early ($p = 0.25$) and late instar ($p= 0.92$) anophelines were observed in the population before the intervention and the control population during the intervention.

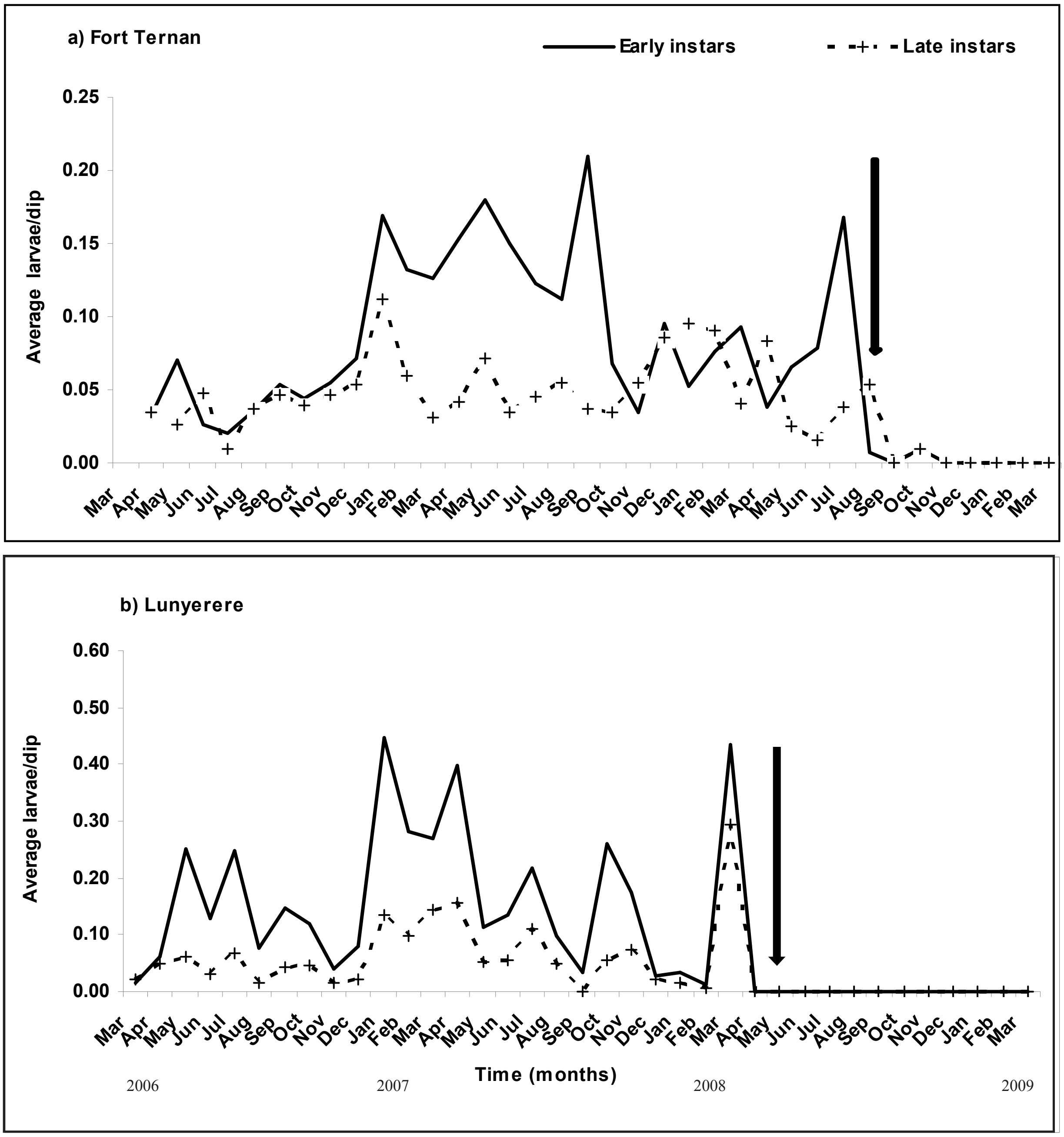


Figure 7.1: Monthly dynamics of anopheline larvae in temporary habitats from March 2006 to March 2008 in a) Fort Ternan and b) Lunyerere before and during intervention period. The black arrow indicates the start of interventions.

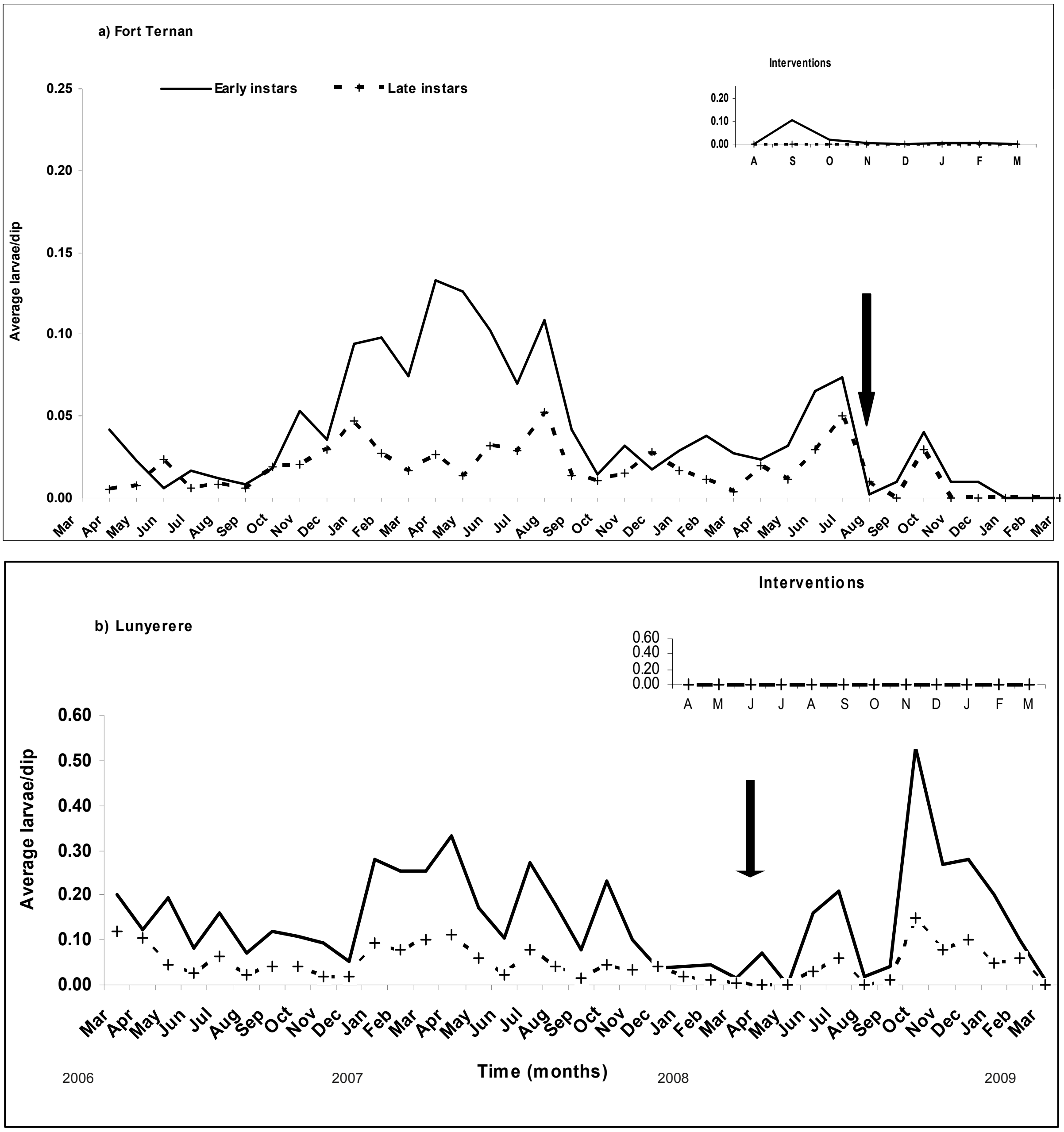
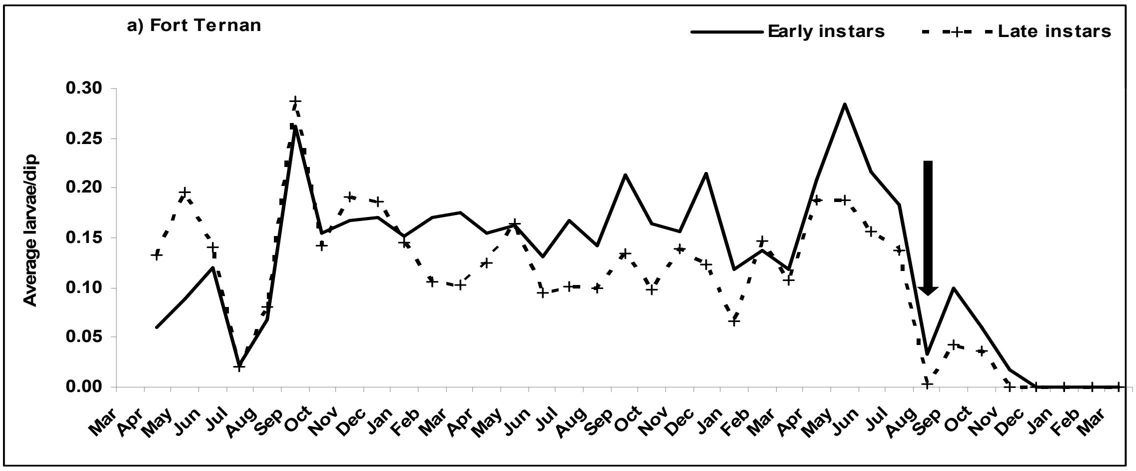
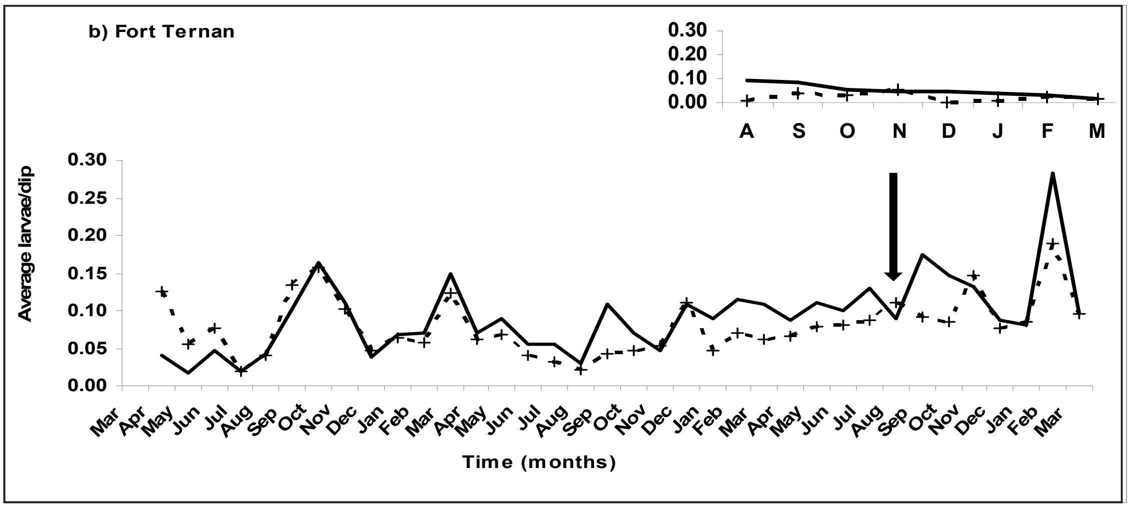


Figure 7.2: Monthly dynamics of anopheline larvae in permanent habitats in a) Fort Ternan and b) Lunyerere before and during the intervention period. The black arrow indicates the start of interventions. The graph (inset) at the top provides data on larval densities in the intervention zone, while as of August 2008 (Fort Ternan) and April 2008 (Lunyerere) the bottom graphs shows larval densities of the control zone.

Culicine larvae: In Fort Ternan, within the temporary habitats few early and late instars of culicine mosquitoes were recorded in September, October and November (Figure 7.3a) during the intervention compared to the pre-intervention period. In the permanent habitats, higher densities of early and late instar culicines were recorded in the control habitats during the intervention period than during the pre-intervention period, and in February 2009 the highest densities over the three year period was recorded (Figure 7.3b) were recorded. There were significant differences in densities of early instars (p = 0.002; 95% CI. -0.11 – -0.03) and late instars (p = 0.01; 95% CI. -0.07 – - 0.01) of culicine mosquitoes between the control and pre-intervention period in Fort Ternan. In Lunyerere no early or late instars of culicine mosquitoes were recorded from temporary (Figure 7.3c) habitats and habitats subjected to intervention from April 2008 to March 2009 (Figure 7.3d). However, substantial densities of both early and late instars of culicines were recorded from the control habitats. There were significant differences in early (p < 0.001; 95% CI. 0.07 – 0.12) and late (p < 0.001; 95% CI. 0.06 – 0.12) instar culicine populations were observed before intervention and after intervention in the control zones.





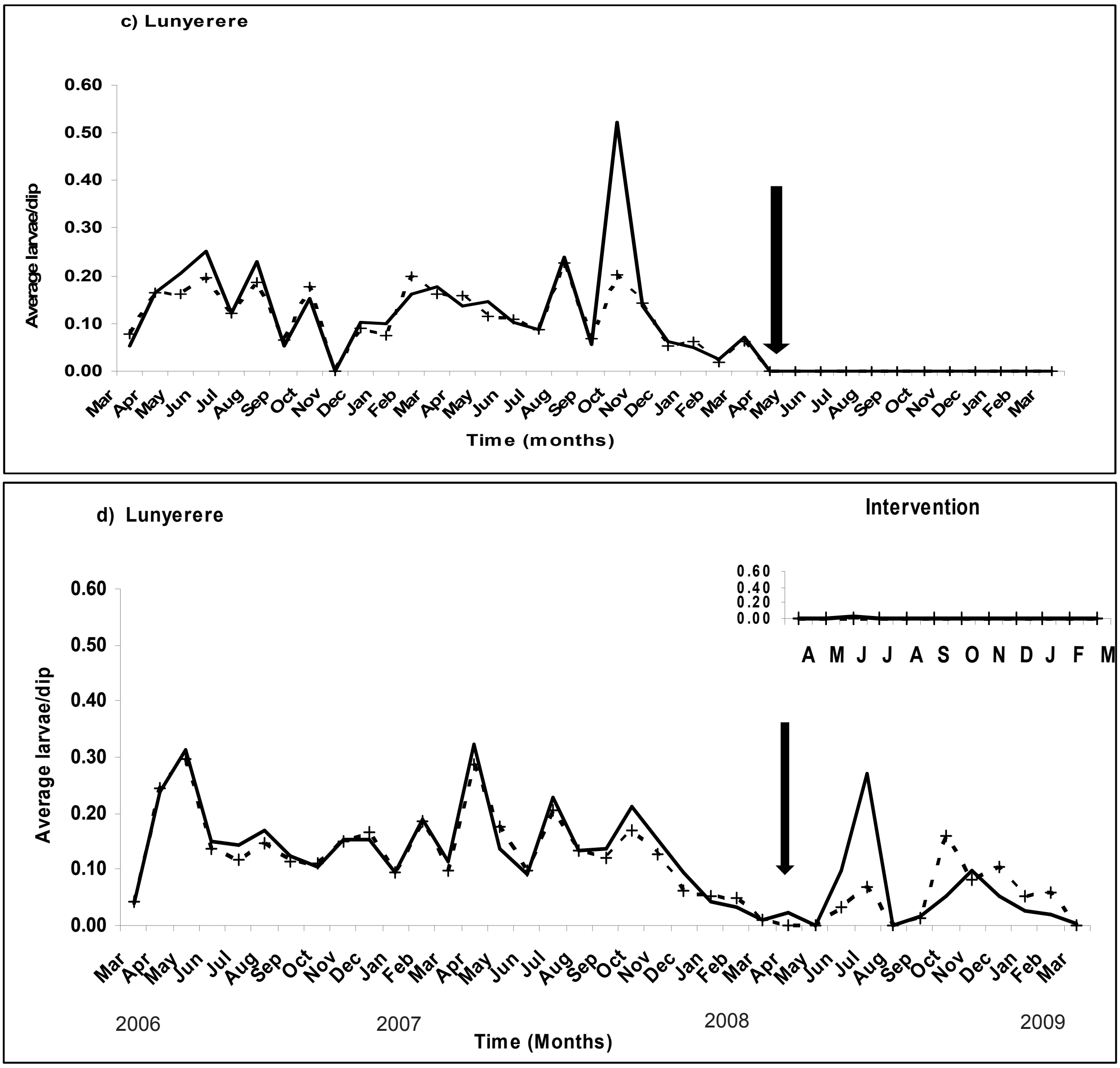


Figure 7.3: Monthly culicine larval dynamics in Fort Ternan in a) temporary and b) permanent habitats and in Lunyerere in c) temporary and d) permanent habitats. The black arrow indicates the start of interventions. The graph (inset) at the top provides data on larval

Impact of larval control strategies

In Fort Ternan during the intervention period, 34.2% of the control and 65.8% of the intervention habitats had early instars of anophelines present. However, the presence of early instars was not significantly different between the two groups ($\chi^2 = 1.819$; df = 1; $p = 0.177$). Considering the late instars, 80% were present in the control and 20% of intervention habitats and the two habitats were significantly different ($\chi^2 = 16.356$; df = 1; $p < 0.001$).

Figures 7.4a and 7.4b show the monthly larval densities during the control period and 100% reduction of late instar anophelines was achieved in habitats applied with predatory fish and drainage while habitats with *Bti* achieved 80%. However, the control habitats only recorded late instars in October and none were recorded during the remainder of the intervention period (Figure 7.4b). During the post-intervention period from April to June 2009, only early instars were recorded from control habitats and habitats treated with *Bti* and fish but no late instars were recorded.

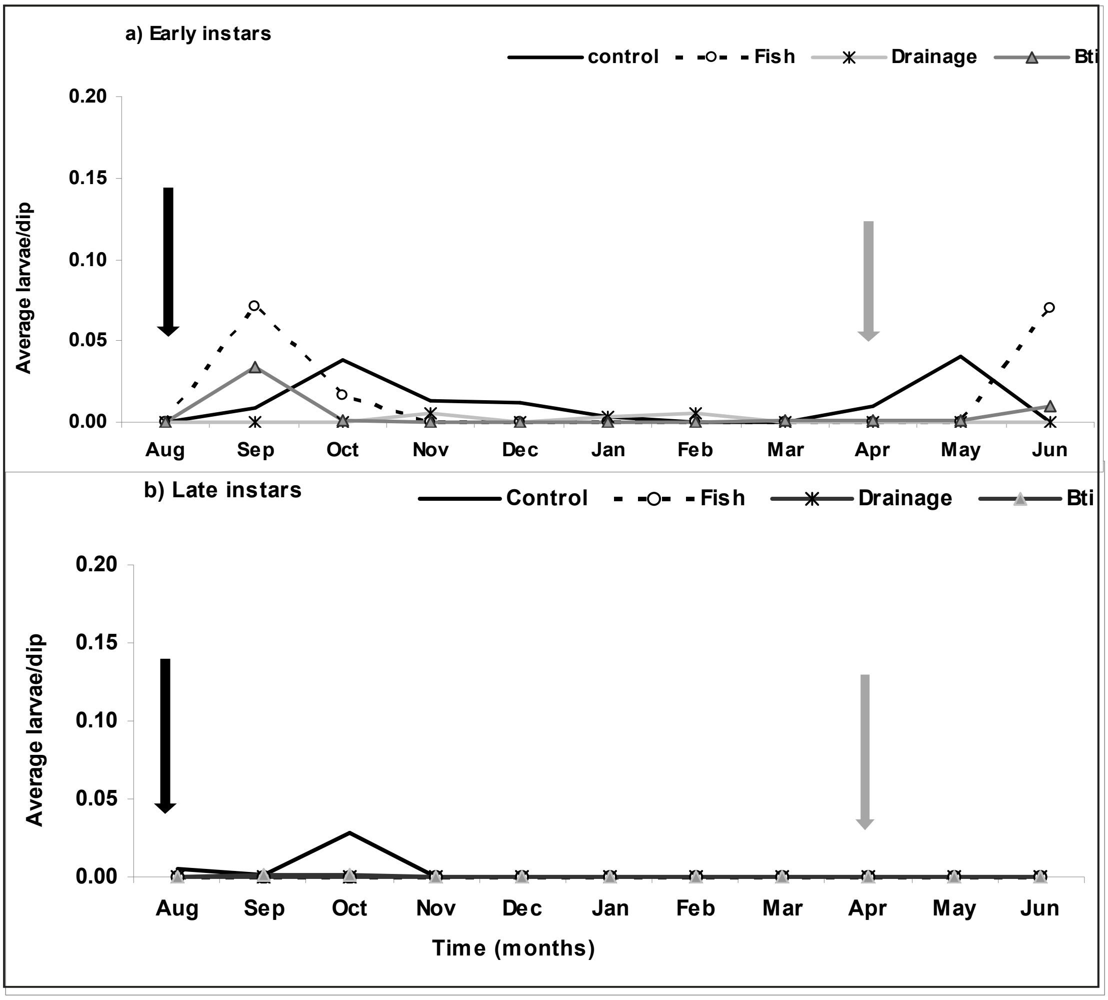


Figure 7.4: Monthly dynamics of (a) early and (b) late instar anopheline in permanent habitats subjected to interventions in Fort Ternan from August 2008 to July 2009. The black arrow indicates the start while the grey arrow indicates the end of interventions.

In Lunyerere there was a 100% reduction in early and late instars of anophelines in habitats where interventions were applied compared to the control habitats. The presence of early and late instars between the control and intervention habitats was significantly different (in both, $p < 0.001$). Both early and late instars were recorded from the control habitats only, as shown in Figures 7.5a and 7.5b. In the post-intervention period from April 2009 to June 2009, early and late instars of anophelines were recorded in the control habitats as well as those that were previously treated with *Bti* or under drainage during the interventions. Multivariate analysis of early and late instar anopheline densities showed significant differences ($p< 0.001$ and $p< 0.05$, respectively) between the control and habitats subjected to different interventions.

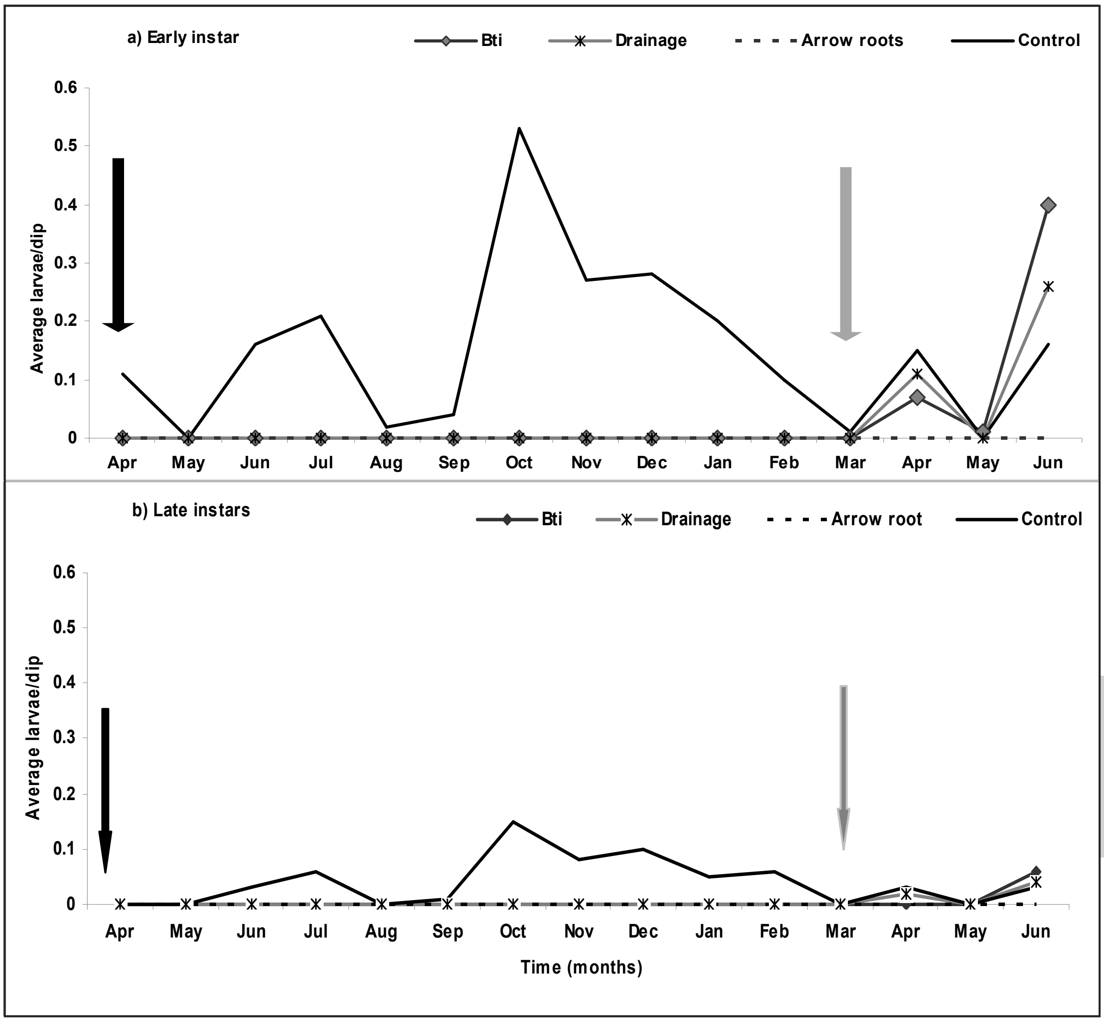


Figure 7.5: Monthly dynamics of (a) early and (b) late instar anopheline in permanent habitats subjected to interventions in Lunyerere from April 2008 to July 2009. The black arrow indicates the start while the grey arrow indicates the end of interventions.

Larval habitat dynamics
In Fort Ternan when it was dry or when it rained heavily, some of the habitats would dry up or get flooded. Figure 7.6 shows the distribution of the habitat condition, stagnant water, flooded or dry, during the intervention period. More habitats dried up between November and December 2008 and in January and March 2009. By contrast, in Lunyerere water was always present from underground seepage but no flooding was experienced during the intervention period.

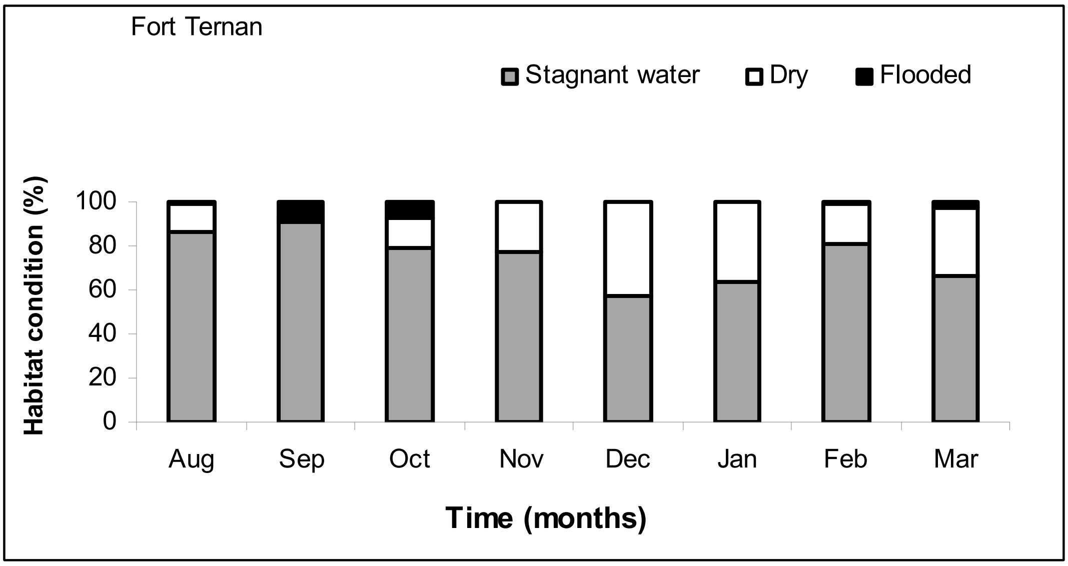


Figure 7.6: The distribution of larval habitats over categories stagnant water, flooded and dry in Fort Ternan from August 2008 to March 2009.

Species composition
A total of 30 and 163 anophelines late instars were collected from the control habitats in Fort Ternan and Lunyerere, respectively. Among these, 2 from Fort Ternan and 14 from Lunyerere were *An. gambiae s l.*. *Anopeles funestus* and *An. coustani* were only recorded in Lunyerere and the other mosquito species collected were non-vector anophelines.

Discussion
The results obtained in this study indicate that the strategies used for targeting larval mosquitoes were highly effective in preventing the mosquitoes from breeding in the selected areas. An integrated approach using environmental management through source reduction and habitat manipulation, and the use of predatory fish showed great potential in controlling mosquito larvae in man-made habitats within the study area. Larval control strategies such as the use of arrow root

plants, predatory fish and drainage can easily be applied by the local communities for the control of mosquitoes in the respective study area as they compare well with the application of *Bti.* Culicine mosquitoes, which are vectors of filariasis and several arboviruses as well as being a severe nuisance, were also strongly reduced in the intervention sites. The methods tested in this study can be integrated in a malaria vector control programme.

Anopheline mosquito species breed in a variety of habitats; however, those created by human activity may be of particular importance for malaria transmission (Walker & Lynch, 2007). In the current study areas, *An. gambiae,* the principal malaria vector in the region, was found to occupy temporary habitats more often in Fort Ternan, whereas in Lunyerere both temporary and permanent habitats were used equally (Chapter 3). For this reason, interventions targeting larval control were applied to both temporary and permanent habitats in both study areas. Temporary habitats exhibit a low stability and because of their transient nature, the application of *Bti* was the most suitable strategy. *Bacillus thuringiensis* var. *israelensis* although efficient in controlling mosquito larvae, it is not locally available and hence not accessible by the community members. However, were there collaboration between research institutions and available funds, *Bti* could be locally produced and made accessible to communities. In Lunyerere, our results indicated 100% reduction of both early and late instar anophelines in habitats that were treated with *Bti* as well as habitats shaded by the leaves of arrow roots plants and those that were drained. The results from Fort Ternan indicate a 100% reduction in late-instar anophelines from habitats with *Bti*, predatory fish and drainage. The efficacy of *Bti* in reducing mosquito larval populations recorded in this study is comparable to previous studies of Fillinger et al. (2003) and Majambere et al. (2007), which showed a high efficacy of *Bti* as larval control agent.

The use of predatory fish for mosquito control has not been widely used in Africa; however, recently large scale trials using various species of larvivorous fish to control anopheline larvae have been reported in Ethiopia (Fletcher et al., 1992) and India (Gupta et al., 1992; Kumar et al., 1998). In a controlled study done in western Kenya, Howard et al. (2007) showed that edible fish has the potential to be used in mosquito control. Existing reports on the use of larvivorous fish for mosquito larval control shows that the most commonly used species in mosquito larval control is *Gambusia affinis* (WHO, 1982). A previous trial on the efficacy of *Gambusia affinis* in Western Kenya indicated that when used in combination with larvicides, efficiency of the fish was improved (Chapter 6). However, this seems to vary from one place to another, and in the current study we found that when predatory fish was used as a single option in erosion pits, few early instars were recorded, and late instars had disappeared from these habitats. We assume that female mosquitoes still oviposit in habitats with fish but it could be that the larvae were fed upon before reaching their late stages. The results of the current study point to the fact that the outcome of intervention strategies targeting any mosquito vector, is determined by the characteristics of the local vector and the suitability of breeding habitat for a given intervention type. Walker & Lynch (2007) suggest that a major factor determining the efficacy of larvivorous fish is the suitability of the fish species to the bodies of water in which the vector species breeds. Reports of field experiments evaluating the effectiveness of larvivorous fish are rare and to our knowledge, this is the first study to report on the effectiveness of *Gambusia affinis* for larval control in Western Kenya.

Environmental management through source reduction by draining or filling and levelling are mainly aimed at discouraging larval development and reducing the vector population densities. In this study, we applied larval source management and environmental manipulation through management of vegetation for mosquito control dependent on the local ecological requirements of the local vector. In Lunyerere, the main malaria vector is *An. gambiae s. s.*, a species that prefers open sunlit pools of water for breeding. The source of larval habitats was underground seepage that provided small pools of stagnant water in drainage canals, thus creating good breeding grounds (Chapter 3). Manipulation of the habitats by providing shade from arrow roots along drainage canals has proven to be efficient. Arrow root plants were selected for this study as they are locally available, and are mainly grown in areas with a regular supply of water. Source reduction in Lunyerere involved modification of the existing canals to increase water flow so that larvae would be flushed out. In Fort Ternan, however, good mosquito breeding habitats along the river fringe originated from animal hoof prints, which left depressions that filled with water and formed stagnant pools with debris blocking the flow of water along the river fringe. The intervention measure on the pools created by animal hoof prints consisted of filling them with small stones and soil and levelling them so that no possible depression was left for water to accumulate, while debris that blocked water flow was removed to allow for free flow of water. In both sites, no anopheline and culicine larvae were recorded in habitats subjected to drainage. A number of studies have reported the successes of malaria reduction and eradication through environmental management projects (De Castro et al., 2004; Kitron & Spielman, 1989; Utzinger et al., 2001).

Recent times have witnessed the success of integrated malaria control through the combination of ITNs and IRS as advocated by the Roll Back Malaria initiative (2005). Although ITNs and IRS are highly effective for the control of indoor biting and resting mosquitoes, they are vulnerable to vector avoidance (Killeen et al., 2002b). The primary goal of IRS and ITNs is the reduction of human-vector contact rather than reduction of the vector population. However, the reduction of the vector population would be preferable to achieve effective malaria control. Fillinger et al. (2009) have recently shown that the combined use of ITNs and larviciding resulted in a two-fold reduction in malaria transmission. Indeed, mathematical models of malaria transmission indicate that integrated control programmes that combine multiple interventions are more likely to succeed than programmes that promote only one intervention (Killeen et al., 2000; McKenzie & Samba, 2004). In addition, we envisage that broad mosquito control involving both anopheline and nuisance mosquito species will generally be accepted by participating communities rather than interventions targeted solely at anopheline mosquitoes. Our results are promising and due to the endemicity of malaria in Western Kenya, a sustainable malaria control programme that can be easily adopted by the local communities is required. In our previous study, we found that although the community members were willing to take part in larval source reduction, they lack evidence-based results on control strategies that can be used with the locally available resources (Chapter 5). As a first step towards achieving this goal, we have provided evidence-based field results from research on larval control strategies that can be utilized using locally available resources in comparison to the application of larvicides for mosquito larval control.

Most of the mosquito breeding sites were man-made, as the result of land use change and agricultural activities such as deforestation, swamp reclamation and animal husbandry (Chapter 3). The methods applied can be integrated in the activities of the farming communities. Involvement of these communities in these interventions is expected to lead to more sustainable malaria control than is currently the case (van den Berg & Knols, 2006; van den Berg & Takken 2008). An additional effect of the reported environmental changes is the likelihood of species succession, such as *An. arabiensis*, which is more recorded in lowland and hot areas, while a high percentage of *An. arabiensis* was recorded in Fort Ternan, a highland area (Chapter 3). Malaria control directed at larvae will be more effective than adult control as it intercepts all species concerned regardless of these changes.

We conclude that integrated management of mosquito larvae in the highlands of western Kenya can be highly effective, and should be considered for inclusion in malaria intervention strategies. Future studies should investigate to what extent the local community can participate in these interventions.

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Chapter 8

Summarizing discussion

Susan S. Imbahale

Summarizing discussion

Our knowledge of spatial dynamics of anopheline larval ecology is often insufficient to achieve effective vector control through means of larval control (Fillinger et al., 2004; Oaks et al., 1991). Little is known about the habitats, abundance and distribution of larvae of the main malaria mosquito vectors and most studies ignore variations in larval ecology caused by local environmental conditions. The research described in this thesis was a longitudinal study undertaken to establish the temporal and spatial dynamics of malaria mosquito larvae, adult mosquitoes and malaria prevalence in three different environments in western Kenya, and to analyze the feasibility of environmental and biological methods for mosquito larval control. Two highland villages (Lunyerere and Fort Ternan) and one peri-urban (Nyalenda) area next to the city of Kisumu were selected. The selected villages provide a good comparison of malaria-endemic sites. Although at almost similar altitude levels, the two highland villages are significantly different in terms of topography, land use and associated anthropogenic activities. The peri-urban lowland site selected provides a good example of how an increase in the urban population in Kisumu city and the need for food security for the expanding population has encouraged small-scale irrigated agriculture. Lunyerere and Nyalenda represent transformed swamp areas that are currently used for agriculture, whereas in Fort Ternan agriculture is an old practice that has not changed over generations. Although more emphasis is placed on malaria vectors, I also included other anopheline species and non-anophelines, which represent the nuisance biting culicine mosquitoes.

This chapter provides a synthesis of the preceding chapters on integrated mosquito larval control, starting with the identification of the mosquito species of local importance in malaria transmission, the mosquitoes' preferred breeding habitats and trials of environmental management and biological control strategies that would be useful for larval control in the respective study sites.

Overview of the major results

Larval and adult mosquito vector dynamics

In Western Kenya, the mosquito species *Anopheles gambiae sensu lato* (comprising two sibling species *An. arabiensis* Patton and *An. gambiae* Giles *sensu stricto*) and *An. funestus* Giles are the principle vectors of malaria (Coetzee et al., 2000). Recently *An. coustani* Laveran has been reported as a possible vector species of malaria in East Africa (Geissbühler et al., 2009). *Anopheles gambiae s.l.* prefers to breed in open sun-lit, shallow pools of water while *An. funestus* prefers more permanent bodies of water with emergent vegetation (Gillies & Coetzee, 1987; Klinkenberg et al., 2003). The findings of this thesis indicate that 86% of all the mosquito breeding habitats recorded were man-made (Chapter 3). The most productive (in terms of mosquito larval density) man-made habitats were drainage canals, hoof prints, tire tracks, rice paddies and watering taps (either leaking or broken) with a constant water flow. In Lunyerere, both temporary and permanent habitats were important for ovipositing female mosquitoes, while in Nyalenda and Fort Ternan temporary habitats were more preferred. In Lunyerere an area with natural seepage of underground water, the main habitats were drainage canals built to help in draining water out of the swampy land to allow for crop farming. The lack of maintenance of these canals has often led to the creation of small pools of stagnant water with or without grass, providing suitable oviposition sites for gravid female mosquitoes. All three main malaria vector species were present here*. Anopheles arabiensis* was the main species in Nyalenda, consistent with the reported distribution range of this sibling species; it prefers to breed in low and hot areas as opposed to *An. gambiae s.s*, which has been found to prefer cool, high altitude areas (Coetzee et al., 2000). Contrary to this expectation, based on larvae collected, *An. arabiensis* was the most abundant (71%) anopheline species in Fort Ternan, setting a new record in the Western Kenya highlands. Although larvae of *An. arabiensis* were frequently collected in this area, only one adult mosquito was collected from indoor sampling during a 24-month period (Chapter 4). Previous studies on larval development in Fort Ternan indicated that the larvae of both *An. gambiae* and *An. arabiensis* rarely complete their development, mainly due to low temperatures (Koenraadt et al., 2006). Hence, populations of adult mosquitoes may be small at all times and therefore constitute a paradox for malaria transmission, as the malaria prevalence rate in Fort Ternan averaged 15% at times (Chapter 4). *Anopheles arabiensis* prefers to rest outdoors and obtains blood from many hosts, ranging from humans to domestic animals and due to its outdoor characteristics, larval control would be more suitable for this species. In Lunyerere, the main vector species was *An. gambiae s.s.*, consistent with previous studies done in neighboring areas within western Kenya highlands (Githeko et al., 2006; Ndenga et al., 2006).

In Lunyerere, the main adult mosquito vector was *An. gambiae s.s., An. arabiensis* made up 12.5% of the total *An. gambiae s.l* collected. Previous studies in the same highlands did not report the presence of *An. arabiensis* (Githeko et al., 2006; Ndenga et al., 2006), hence this was the highest to be ever recorded. The aquatic stages of this species have recently been recorded in the Mount Kenya region (Chen et al., 2006). The presence of *An. arabiensis* in such high-altitude areas could be a result of human activities, such as deforestation and/or swamp reclamation, which impact on the micro-climatic conditions of breeding habitats and create environmental conditions that favour mosquito breeding and the invasion of new species. *Anopheles funestus* was more recorded as larvae in Lunyerere from habitats that had grass growing in them, whereas in Fort Ternan more adult mosquitoes of this species were recorded from houses that were within 500 m of the river; thus, all its breeding habitats were along the river fringe. Such areas formed pools of stagnant water and provided good livestock drinking points. Such areas were often cool due to shade provided by the tall trees along the river margin. In Nyalenda, an area where rice was grown, no *An. funestus* was recorded throughout the study. This is in contrast to other studies, in which *An. funestus* was commonly found in rice paddies during the late stages of rice development (Klinkenberg et al., 2003). The collective data demonstrated the widespread presence of larval stages of malaria vectors in all study sites and with sufficient ambient temperature is for parasite development, malaria will be transmitted locally.

Prevalence of *Plasmodium falciparum*

Malaria transmission in Lunyerere was caused by *An. gambiae s.s.* and *An. funestus,* while in Nyalenda *An. gambiae s.s.* and *An. arabiensis* contributed equally to disease transmission. The presence of *Plasmodium falciparum* parasites in mosquitoes in Lunyerere and Nyalenda explains the origin of malaria in the population as shown by the monthly cohort studies carried out among school children (Chapter 4). In contrast, none of the few adult *An. funestus* collected from Fort Ternan contained malaria parasites and hence it is difficult to explain the origin of malaria parasites that was present in the cohort of school children in this study. In a previous study, *P. falciparum* was identified from one of five adult anophelines collected in Fort Ternan (Koenraadt et al., 2006), providing proof of parasite transmission under low density adult mosquito populations. This could mean that the adult *An. funestus* collected from Fort Ternan were not malaria vectors but simply non-vector species within the *An. funestus* complex (Garros et al., 2004; Gillies & Coetzee, 1987; Gillies & De Meillon, 1968). However, the baseline larval data shows that *An. arabiensis* is present and this species could be important in malaria transmission. *Anopheles arabiensis* prefers to rest and bite outdoors. Therefore, we could have missed it during the indoor collections. On the other hand, there is a lot of movement between Fort Ternan and the neighboring lowland areas associated with trade, and it could be that infected mosquitoes are introduced either through transportation or through human migration. This needs to be addressed in future studies. *Anopheles coustani* was present in all sites; this species has recently been reported to be a potential vector of malaria (Geissbühler et al., 2009). We did not test for the presence of *P. falciparum* parasites in this species. The overall monthly mean malaria parasite prevalence in the cohort of school children from Fort Ternan, Lunyerere and Nyalenda was not significantly different. This result is surprising, as with the differences in adult species composition and sporozoite rates among the sites, it was expected that there would be differences in malaria prevalence, which turned out not to be true. In Fort Ternan, monthly malaria prevalence varied between 0 and 17% and did not correlate well with rainfall. In Lunyerere it ranged between 1 and 22% and the highest prevalence was recorded one month after the heavy rains ended, whereas in Nyalenda prevalence varied between 1 and 5% with no distinct monthly variations. The school children from the highland villages exhibited a high prevalence rates when compared to those in the peri-urban Nyalenda. The low monthly turnout of children from Nyalenda could have contributed to the low prevalence rates recorded (Chapter 4). In Chapter 5, results show that residents of peri-urban Nyalenda were more likely to use mosquito nets, hence protecting themselves from infectious mosquitoes and therefore the rural population is more vulnerable to malaria.

Community involvement in malaria transmission and its control

The majority of the mosquito larval habitats recorded were man-made and almost all were linked to agricultural activities (Chapter 3). This contributed to the disease burden by providing conducive breeding sites for the mosquitoes. However, a survey done in the current study revealed that many community members did not associate the breeding of mosquitoes with stagnant water or their farming activities (Chapter 5). One third of the respondents associated breeding of mosquitoes with bushes and plantations of coffee, sugarcane or maize and consequently many thought that mosquitoes would be controlled through bush clearance. Only few recognized that the major breeding sites are those that they create themselves in their vicinity. If this knowledge is lacking, then we cannot expect that people take any preventive action. Agricultural water resource development through irrigation can benefit health by increasing food yields and production; on the other hand, this also creates conditions suitable for propagation of insect vectors (Hawkes & Ruel, 2006) such as the case of Nyalenda. However, it is important to understand that irrigated areas are known to have higher densities of mosquitoes but this may not automatically lead to higher malaria incidence among the inhabitants, as explained by the paddies paradox (Ijumba & Lindsay, 2001). A similar situation was observed in peri-urban Nyalenda, with high densities of adult mosquitoes vectors collected but where the sporozoite rates and malaria prevalence were relatively lower.

Generally, men were more informed concerning the cause of malaria than women, especially in the villages. The potential impact of malaria on women engaged in agriculture, especially food production is substantial (FAO, 1996). In sub-Saharan Africa, women account for about 70% of agricultural workers and 60-80% of food crop producers for household consumption and sale (FAO, 1996). In addition, women also spend considerable time on taking care of sick adults and especially children. These findings emphasize the importance of capacity building especially targeting women, to increase knowledge on the causes of malaria and how their activities affect or create opportunities for vector-borne diseases.

Lunyerere provided a good example of land use change leading to disease risk, being a reclaimed natural swamp that is currently under crop cultivation. These land use changes have opened up the area, but the continued presence of underground seepage provides small pools of water, favorable to the most efficient malaria vector *An. gambiae* and the invasion of new species like *An. arabiensis*. Agriculture is the mainstay of rural communities and if these farming communities would understand the link between agriculture and health, this would be one step in solving the problem. Once these links are understood, then suitable control strategies need to be developed in combination with the affected communities. It became clear that, although community members that took part in this study were willing to take part in mosquito vector control, they did not know how they can help (Chapter 5). This study has addressed this issue by providing results on a proof of concept that can be adopted in the integrated control of malaria. Integrated mosquito larval control options such as environmental (water) management and biological control can be adopted by farming communities without interrupting the activities on their farm.

Integrated mosquito larval control strategies

The variation in topography and vector species composition in the two highland villages clearly show that intervention strategies are site specific, and all interventions studied are not necessarily suitable for all habitats at all locations. This calls for the development of site-specific larval control strategies that are applied depending on the habitat suitability. Information relating to habitat suitability was obtained from the baseline longitudinal larval data presented in Chapter 3. Peri-urban Nyalenda is relatively flat, with water present throughout the year for crop and commercial tree nursery irrigation. Because water is needed and consequently it cannot be drained away, application of bio-larvicides such as *Bacillus thuringiensis* var *israelensis* (*Bti*) with or without combination of larvivorous fish (Chapter 6) would be feasible. The predatory fish *Gambusia affinis* was found suitable as it was locally available and it thrived well under local conditions in the study areas. The transient nature of temporary habitats required a strategy such as the application of *Bti* which could be applied weekly as advised by Fillinger & Lindsay (2006). The effectiveness of *Bti* for the control of African anophelines has been demonstrated by several studies (Fillinger & Lindsay, 2006; Geissbühler et al., 2009; Majambere et al., 2007). In this study the efficiency of *Bti* is comparable to the findings of Fillinger & Lindsay (2006) and Majambere et al. (2008), who reported that larviciding reduced anopheline larval density by 95%. The *Bti* used in this study was imported as it is not locally available, hence the main limitation is the accessibility of this product to the local population. However, with available funds and collaboration among scientists supported by a good political will, *Bti* can be produced locally. Apart from the application of *Bti*, permanent habitats in the rural highland villages of Fort Ternan and Lunyerere were subjected to multiple mosquito larval control options. This was possible due to the distinct differences in topography and the nature of the breeding habitats.

The intervention strategies involved environmental (water) management through source reduction, habitat manipulation by provision of shade and the use of biological control through larvivorous fish (*Gambusia affinis*) in comparison to the application of *Bti.* In this case provision of shade was selected for Lunyerere because the main vector species, *An. gambiae,* prefers to breed in open, sunlit, temporary water pools (Gillies & Coetzee, 1987), and hence is not likely to breed in shaded habitats. Conversely, *An. funestus* thrives in permanent habitats with emergent vegetation. Arrow root plants were not foreseen to provide such a habitat because, as they grow they suck in water, leaving no stagnant water for ovipositing females. The arrow root plant produces root tubers that are a good source of carbohydrates. It was envisaged that because it is a source of food and can be utilized in controlling mosquito larvae, then the idea would easily be accepted by the farm owners.

One of the remarkable results was that all the intervention strategies applied led to complete elimination of the aquatic stages of both anophelines and non-anopheline species within habitats when compared to the controls (Chapter 7). A study by Stephens et al. (1995) found that the persistence of nuisance mosquitoes created dissatisfaction with an existing insecticide spraying programme for malaria control in two cities in Tanzania. By reducing aquatic stages for both vectors for malaria and nuisance biting mosquitoes, we envisage that mosquito bites were reduced or stopped and that the community members will be more willing to take part in vector control activities. In the present study, when the intervention strategies were stopped, mosquito larvae were immediately recorded anew from the respective habitats, implying that for the process to be effective, active management and co-ordination of activities is required at a local level. Vector control strategies developed with the collaboration of community members are envisaged to be sustainable and acceptable in the long term. In Sri-Lanka, Yasuoka et al. (2006) showed that the limitations of chemical use and reliance on unstable foreign aid raised awareness on the importance of community-based approaches that require no external funding and place no financial burden on the community.

In Sri-Lanka, Yasuoka et al. (2006) showed that the limitations of chemical use and reliance on unstable foreign aid raised awareness on the importance of community-based approaches that require no external funding and place no financial burden on the community. Source reduction through drainage and provision of shade using locally-grown arrow root crops and biological control using fish were as efficient as the application of *Bti*. Currently, *Bti* is not accessible by the local community and therefore alternatives such as source reduction, environmental manipulation and use of predatory fish can be adopted. The inhabitants of the respective communities were aware of the fact that source reduction (drainage of stagnant water) would be useful for mosquito control; however, it was not clear how many practiced it (Chapter 5). With proper institutional support and active management (monitoring and evaluation) of the intervention program, using social workers and village volunteers, source reduction can easily be applied in the respective villages. Environmental modification has been reported to be acceptable and cost effective in African urban and peri-urban areas, particularly if old drainage systems exist (De Castro et al., 2004; Utzinger et al., 2001). The control of aquatic stages of mosquitoes can be incorporated into the current integrated malaria control programmes that mainly target adult mosquitoes and treatment of malaria. Although spatially limited, this study provides evidence-based combinations of mosquito larval control strategies which can be explored on a large scale.

To foster progress toward agriculture-based food security and economic growth, developing countries such as Kenya, need to pay closer attention to the development of human capital through investment in education and training, health and sanitation (Asenso-Okyere et al., 2009). Addressing the malaria-agricultural interface requires a broad interdisciplinary and integrated approach that involves local communities and more than one public sector (van den Berg & Knols, 2006). There is a need to build bridges between sectors that currently tend to work in isolation (Singer & de Castro, 2007).

Conclusion

Sustainable disease control programs need to involve the concerned communities in their implementation. The community perceptions, current knowledge on the disease of interest and their cultural values with regard to the cause and control of the particular disease need to be considered. This study has shown that mosquito larval control through environmental management and biological control utilizing locally-available options compared well with the application of *Bti*. However, for this to be possible there is a need for active management with frequent monitoring and evaluation, which can be achieved with institutional support and village volunteers.

Future research needs to test the strategies developed on a large scale, with active involvement of communities and to develop other novel strategies that are applicable to transient mosquito habitats. However, for this to be practical there is a need for collaboration between the different institutions involved in malaria control so that a lasting solution can be developed.

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Samenvatting

Malaria kent een complexe etiologie, waarbij de interactie tussen de vector (muggen van het geslacht *Anopheles*), de eencellige parasiet *Plasmodium*, de gastheren en omgevingsfactoren bepalend zijn voor de transmissie ervan. Tot op heden heeft bestrijding van malaria zich met name gericht op *Plasmodium* en op volwassen *Anopheles* muggen. Ontwikkeling van resistentie van deze organismen tegen de gangbare bestrijdingsmethoden vraagt om een andere strategie, zoals het bestrijden van de *Anopheles* larven, eventueel geintegreerd met gangbare bestrijdingsmethoden. Goede kennis over lokale vectorcompetente muggen en hun broedplaatsen is essentieel voor de ontwikkeling van een bestrijdingsprogramma dat zich op muggenlarven richt.

Dit proefschrift beschrijft bevindingen van een studie naar de ecologie van met name muggen die een vector voor malaria kunnen zijn, in verschillende landbouwgebieden gelegen in twee hooglandgebieden (Fort Ternan en Lunyerere) en een peri-urbaan gebied (Nyalenda) in West Kenia. Om informatie te krijgen over de ecologie van locale malariavectoren, werd gedurende een tweejarig onderzoek enerzijds naar populatiedynamiek en broedeigenschappen van lokale muggensoorten gekeken en anderzijds naar het voorkomen van malaria onder de bevolking in de bovengenoemde locaties. Daarnaast werden de visie en kennis over malaria, oorzaken van malaria en eventueel reeds aanwezige preventieve maatregelen in de lokale gemeenschap onderzocht door middel van een enquête. De hierbij verkregen gegevens werden gebruikt voor het ontwikkelen en op kleine schaal testen van verschillende alternatieve bestrijdingsmethoden voor muggenlarven. Daarbij werd gebruik gemaakt van biologische bestrijding met een predator van muggenlarven: de vis *Gambusia affinis* en met het biologische bestrijdingsmiddel *Bacillus thuringiencis* var *israelensis* (*Bti*). Daarnaast werden broedplaatsen verwijderd (door middel van drainage of het opvullen met stenen) en minder geschikt gemaakt door het toevoegen van schaduw.

De belangrijkste vectorsoorten voor malaria, *Anopheles gambiae* Giles *sensu stricto* and *An. arabiensis* Patton, werden beide in alle locaties in het larvale stadium aangetroffen, terwijl *An. funestus* Giles uitsluitend in de hooglandlocaties werd aangetroffen. De meerderheid (86%) van de onderzochte broedplaatsen bleek ontstaan te zijn door menselijk ingrijpen. *An. arabiensis* was de dominante malariavector in Nyalenda terwijl *An. gambiae* s.s. dominant was in Lunyerere. Lunyerere kende tevens het hoogste percentage (12,5%) van binnenshuis rustende volwassen *An. arabiensis* muggen dat ooit in de hooglanden van West Kenya werd aangetroffen. In Fort Ternan werd het hoogste percentage (71%) van *An. arabiensis* larven geregistreerd dat ooit op zo'n grote hoogte is gevonden. Schoolkinderen in alle onderzoekslocaties waren besmet met de dominante malariaparasiet in de regio, *Plasmodium falciparum*. De prevalentie van deze parasiet verschilde niet significant tussen de drie onderzoekslocaties. Malaria werd door de plaatselijke bevolking van de onderzoeksgebieden als overlast beschouwd. Men was bereid deel te nemen in een bestrijdingsprogramma, maar gaf aan niet goed te weten hoe dit aangepakt kon worden. Een verkennende studie in Nyalenda liet zien dat bestrijding van muggenlarven door middel van habitataanpassingen en biologische bestrijding haalbaar is voor habitats die door menselijk toedoen zijn ontstaan, met name als gevolg van landbouwactiviteiten. De in de hooglandlocaties toegepaste bestrijdingsmethoden resulteerden in volledige uitroeiing van muggenlarven in zowel vroege als latere (=alle) ontwikkelingsstadia. Dit was vergelijkbaar met resultaten verkregen door middel van toepassing van *Bti*.

De in dit onderzoek verkregen resultaten suggereren dat met een op muggenlarven gerichte bestrijding een significante reductie in de aantallen volwassen muggen teweeg gebracht kan worden. Dit kan vervolgens leiden tot een verminderde overdracht van malaria op mensen. Bij het ontwikkelen van bestrijdingsmethodes gericht op muggenlarven, moet rekening gehouden worden met de aard van de lokale muggenbroedplaatsen en de geschiktheid van dergelijke broedplaatsen voor de beoogde bestrijdingsmethode. Een geïntegreerde aanpak van verschillende lokaal beschikbare methodes van larvenbestrijding, kan eenvoudig door de plaatselijke bevolking worden opgepikt. Betrokkenheid van de plaatselijke bevolking bij bestrijding van ziekten, zal hun bewustwording van de effecten van hun handelen op hun gezondheid vergroten. Dit zal hen in staat stellen om meer verantwoordelijkheid te nemen over hun eigen gezondheid.

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Francoise and family I take this opportunity to say thank you very much for always welcoming me at your home. I am grateful for the time we spent together and the very nice picnics we had. I also appreciate the time you set aside to look at my work with your editor's eyes. To Carla and family I am thankful for the good times we have shared since we first met in November, 2006. I always felt at home whenever we gathered. Thank you for the love you showed me and to Kristie and Jellan I hope we will continue with our good friendship.

To the Kenyan community at Wageningen University, thank you for ensuring we all felt at home despite the many miles away from Kenya. I enjoyed the good company you offered while touring around Holland and during the many Kenyan parties we held. I extend my appreciation to Drs. Evelyne Kiptot and Harrison Charo, who were very helpful at the beginning of my studies in 2005.

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Last but not least I would like to give thanks to my departed parents Mr. Hudson Imbahale and Mrs. Phanice Ajaya, for the wisdom, strength and courage you passed on to me at a younger age. Thank you for the education you provided and the discipline you instilled in me, I remember vividly what it meant to fail exams and bring home a bad report card. How I wish you were still here!.

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Susan S. Imbahale,

29th October, 2009.

Curriculum Vitae

Susan S. Imbahale was born in a small village in Maragoli, Vihiga District, Western Province, Kenya on the 30th of June 1978. She grew up and did her primary education at Mukumu Girls Primary School and thereafter in 1993 joined Butere Girls High for her Secondary School education. The successful completion of her Secondary School education earned her a chance in the University of Nairobi for a Bachelor of Science degree at Chiromo campus, in 1998. During her undergraduate studies she pursued chemistry and zoology and graduated in the year 2002. At the end of her undergraduate studies in June 2002, she undertook internship with the Kenya Wildlife Services (KWS) at the Aberdares national park's research centre based at Mweiga in Nyeri. In November 2002 she secured a scholarship to attend Tropical Biology Association (TBA) course on conservation of dry deciduous forest of Kirindy in Madagascar. This was followed by a two weeks training as an Earth Watch fellow on habitat restoration of the blue swallows in Limpopo province, South Africa. It was during this period that she developed interest in ecology. Thereafter she registered for a Master of Science degree in biology of conservation at the same campus in University of Nairobi from 2003 till early 2005. Her MSc thesis research focused on resource (herbage) utilization of the large migratory herbivores (wildebeest and zebra) of the Nairobi National Park, Kenya. Towards the end of writing her MSc thesis in 2004, she came in touch with Dr. Richard Mukabana, who introduced her to the world of mosquitoes and entomology at large. In September 2005 she enrolled for a sandwich PhD programme at the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC), Wageningen University. The PhD study integrated water management, agriculture and malaria mosquitoes in western Kenya, between Kenya Medical Research Institute (KEMRI) and Wageningen University with Prof. Willem Takken. Her initial months were spent at the Laboratory of Entomology, Wageningen University from September 2005 till February 2006. Thereafter she proceeded to carry out the field research from March 2006 to March 2009 in western Kenya based at KEMRI, Kisumu. She then returned to the Laboratory of Entomology, Wageningen University to write and compile this thesis. The results of her hard work is what you are holding and reading right now.

Correspondence: sueimbahale@yahoo.com

List of publications

Imbahale, S.S., Githaiga, J.M., Chira, R.M and Said, Y.M; 2008.Resource utilization by large migratory herbivores of the Athi-Kapiti ecosystem (African Journal of Ecology)

Imbahale, S.S., Fillinger, U., Githeko, A., Mukabana, W.R and Takken, W; Community perception of malaria and its control in three different transmission settings in western Kenya (Submitted**)**

Paaijmans, K.P., **Imbahale, S.S**., Thomas, M.B and Takken, W; Understanding the link between environmental temperature, malaria mosquito development and climate change (Submitted)

Julius, A., Soka, G and **Imbahale, S** (2002): Assessment of dispersal and predation of *Strychnos decussata* fruits and seeds by frugivorous tree visitors in Kirindy forest. http://www.tropical-biology.org/admin/documents/pdf_files/Madagascarabstracts/Mada_PLANTANDFORESTECOLOGY_07.pdf

To be submitted

Imbahale S.S., Mukabana, R. and Takken, W. Farming systems, water management and malaria mosquitoes in Africa

Imbahale S.S., Paaijmans, K.P., Mukabana, W.R., Githeko, A.K. and Takken, W. A longitudinal study on the ecology of aquatic stages of malaria mosquitoes indistinct geographical and environmental settings in western Kenya

Imbahale S.S., Mukabana, W.R., Githeko, A.K. and Takken, W Dynamics of malaria transmission in different agricultural settings in western Kenya

Imbahale S.S., Mweresa, C., Takken, W . and Mukabana, W.R. Strategies of controlling malaria mosquito larvae using environmental management and biological control: a case study of Nyalenda, western Kenya

Imbahale, S.S., Githeko, A., Mukabana, W.R and Takken, W Integrated control of malaria mosquito larvae in two highland villages in western Kenya.

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PE&RC PhD Education Certificate

With the educational activities listed below the PhD candidate has complied with the educational requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of Literature (5.6 ECTS)

- The larval ecology of Anopheles mosquitoes (2005)

Writing of Project Proposal (7 ECTS)

- The effects of water management systems on malaria mosquitoes in western Kenya (2005)

Laboratory Training and Working Visits (4.2 ECTS)

- Mosquito ecology; National Institute for Medical Research, Tanga, Tanzania (2007)
- Mosquito identification; Walter Reed Army Training Centre, Kisumu, Kenya (2007)

Post-Graduate Courses (5.3 ECTS)

- Basic and advanced statistics; PE&RC (2008)
- Analyzing farming systems and rural livelihoods in a changing world: vulnerability and adaption; PE&RC, Zimbabwe (2008)
- Expanding your scientific network; WUR Graduate Schools (2009)

Deficiency, Refresh, Brush-up Courses (5.6 ECTS)

- Ecological aspects of biointeraction; Entomology (2006)
- Spatial data infrastructure; Environmental Sciences (2005)

Competence Strengthening / Skills Courses (2.8 ECTS)

- Introduction to GIS; Environmental Sciences (2005)
- Geographical information tools; Environmental Sciences (2005)

Discussion Groups / Local Seminars and Other Scientific Meetings (7 ECTS)

- Vector group meetings; Laboratory of Entomology, Wageningen UR (2005- 2009)
- Climate and Human Research Laboratory meetings & presentations (2006-2009)
- PhD lunch discussion meetings; Laboratory of Entomology, Wageningen UR (2006 & 2009)

PE&RC Annual Meetings, Seminars and the PE&RC Weekend (1.5 ECTS)

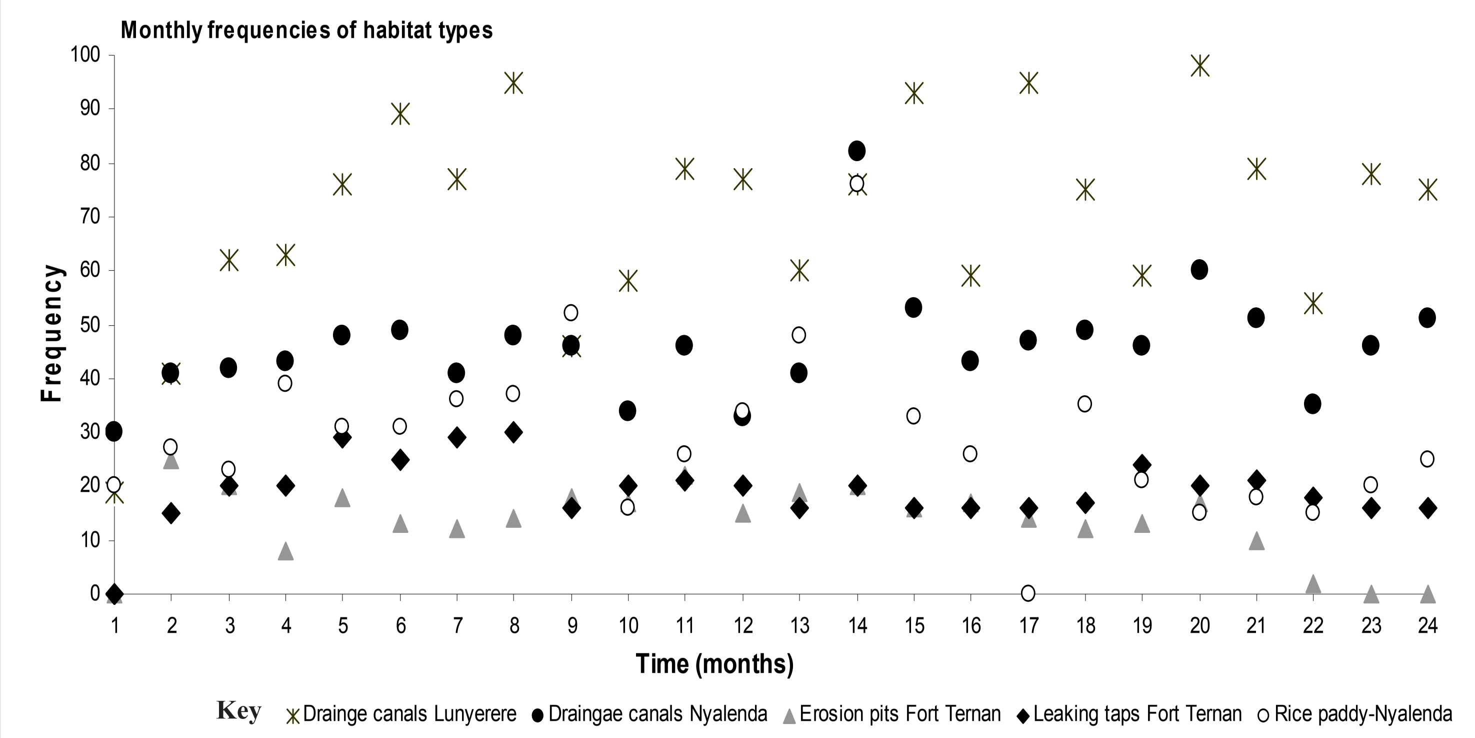
- PE&RC Introduction weekend (2007)
- Seminar: developments in malaria research in Zambia (2009)
- Seminar: ecological research in bio-control: previous and present practice (2009)

International Symposia, Workshops and Conferences (4 ECTS)

- International Congress of Entomology; Durban, South Africa (2008)
- Society of Vector Ecology Congress; Antalya, Turkey (2009)

Supervision of MSc Students (8 months; 2 students)

- Effects of different agricultural practices on malaria mosquitoes
- Effects of different water management systems on malaria mosquitoes



Appendix 1: Monthly variation in frequency of habitat types that represented more 25% and above in each study site from March 2006 to March 2008

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