Ecology and epidemiology of integrated malaria vector management in Dar es Salaam, Tanzania

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Yvonne Geissbühler
aus Lauperswil, BE

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Summary

Malaria remains one of the major contributors to the global burden of disease with approximately 70% of the clinical malaria attacks occurring in sub-Saharan Africa. Sub-Saharan Africa has the highest risk as ideal climatic conditions for transmission coincide with occurrence of some of the most efficient malaria vectors, namely *Anopheles gambiae* s.s., *Anopheles arabiensis* and *Anopheles funestus*. Even though it is estimated that by the year 2030 more than 50% of the African population will live in towns and cities, relatively little is known about urban malaria epidemiology, larval ecology and adult mosquito behaviour. Although integrated malaria control programs including environmental management and larviciding have proven successful before the Global Eradication Campaign started in 1955, they were neglected after the invention of DDT. Lately interest into these control measures has revived but it remains to be determined whether they are feasible and cost-effective in urban Africa.

The overall goal of the research presented in this thesis was to enhance current understanding of urban malaria epidemiology and ecology and to take an in-depth look at the effectiveness of larviciding with *Bacillus thuringiensis* (Bti) in the context of the Urban Malaria Control Program (UMCP) in Dar es Salaam, Tanzania. Our findings are based on data derived from the first 3 years of the UMCP, where data collection started in March 2004. The project area includes 5 wards in each of the 3 municipalities which consist of 67 *mitaa* covering an area of 55 km² in which 611,871 people lived during the population census of 2002. Achieving the UMCPs objectives fundamentally relies on three component activities: 1) Mapping and surveillance of potential *Anopheles* breeding sites, 2) Monitoring of adult mosquito densities, and 3) Household surveys with questionnaires and blood smears testing for malaria parasite infection. In the third year of the UMCP, beginning in March 2006, the routine application of...
the microbial larvicides Bti in open habitats and Bs in closed habitats was initiated in 3 of the 15 wards in the study area, adding to existing interventions such as bednets, house screening, ceiling boards, repellents, spray and coils. At the same time a detailed survey of mosquito biting behaviour, human behaviour and domestic protection measures was conducted in 12 Ten Cell Units (TCU), the smallest subunit of local government in Tanzania, which presented the highest An. gambiae s.l. densities during the early period of the UMCP surveillance system. Human landing catch (HLC) was conducted in 216 houses on an hourly basis indoors and outdoors from 6 pm till 7 am and residents were interviewed about their sleeping behaviour, where they spend their evenings and what kind of preventive measures against malaria they use. Personal protection of an insecticide treated net (ITN) was evaluated using an extension of a recently developed mathematical model.

Overall An. gambiae s.l. exhibited a classical hourly biting pattern. In contrast one of the complex’s component sibling species, namely An. arabiensis, had an early biting peak before 10 pm. Both sibling species, namely An. gambiae s.s. and An. arabiensis, as well as An. funestus and An. coustani were highly exophagic. This behaviour led to a reduced personal protection against exposure to An. gambiae s.s. by ITNs which conferred 59% reduction of exposure in Dar es Salaam compared to 70% in rural Tanzania. An. arabiensis is a vector of only modest importance in Dar es Salaam which is fortunate because ITNs only conferred 38% protection against exposure to this species of mosquito. ITNs conferred slightly less protection against exposure to malaria vectors in good quality houses. This is mainly because people living in good houses tend to spend more time indoors before they go to bed.

An. gambiae s.l. is the most important vector in Dar es Salaam , responsible for an EIR (entomological inoculation rate) of 1.00 infectious bites per person per year whereas An.
funestus has an EIR of 0.13. Surprisingly, An. coustani also acts as a notable vector in Dar es Salaam with an EIR of 0.20 infectious bites per person per year. Malaria transmission is seasonal with two peaks of malaria prevalence during and after the two rainy seasons. Malaria prevalence was only related to EIR in children under 5 years of age, with a classical age-prevalence distribution similar to most of rural Africa. Malaria prevalence steadily declined from 2004 onwards as the use of window screenings, ceiling boards and more effective drugs like amodiaquine and artemisin-based drugs increased. ITNs (prevalence reduction estimate 20%, 95% CI 0%-36%; P=0.060; year 1) and ceiling boards (prevalence reduction estimate 22%, 95% CI 3%-38%; P=0.026; year 2) conferred modest personal protection and reduced malaria prevalence by approximately one fifth. By comparison, a much greater reduction (prevalence reduction estimate 50%, 95% CI 20%-64%; P=0.002) of malaria prevalence was achieved by larviciding with Bti. This was mainly achieved through major reductions of An. gambiae during July and August when most of the sporozoite infected mosquitoes were caught, combined with all-year-round suppression of the secondary vectors, namely An. funestus and An. coustani. This major achievement was only possible through the novel surveillance and staff management procedures developed by the UMCP to enable effective community based implementation in a decentralized manner. Standards of the surveillance improved greatly after the onset of the program with realized reaction times to vector surveillance at observations being one day, week and month at ward, municipality and city level, respectively.

These results of changing biting behaviour of the main malaria vectors in urban settings and the therefore lower but still useful personal protection offered by ITNs call for additional complementary vector control methods such as environmental management or larviciding. The UMCP demonstrated that major reductions in malaria prevalence can be achieved
through routine application of microbial larvicides with its new practical management and surveillance system. As these represent the early results of the program, we expect substantial improvement with time and investment. Here we demonstrated for the first time since before the Global Eradication Campaign era, a success story of a malaria control program integrating larviciding, which could be easily adapted by other African cities as a cost-effective option for malaria prevention.
Zusammenfassung


Insgesamt besitzen *An. gambiae* s.l. ein klassisches, stündliches Stechverhalten. Im Gegensatz dazu stach die Mehrheit der *An. arabiensis*, einer Geschwisterart dieses Mückenkomplexes, vor 22 Uhr. Beide Geschwisterarten, nämlich *An. gambiae* s.s. und *An. arabiensis*, sowie *An. funestus* und *An. coustani* stachen vor allem im Freien (exophagic) und nicht innerhalb der Häuser. Dieses Verhalten führte zu einem reduzierten persönlichen Schutz durch ein ITN gegen Stiche von *An. gambiae* s.s.. ITNs bieten deshalb in Dar es Salaam nur 59% Schutz gegen Mückenstiche, wohingegen sie im ländlichen Tanzania 70% Schutz bieten. *An. arabiensis* ist glücklicherweise nur von mässiger Bedeutung in Dar es Salaam, wenn man in
Betracht zieht, dass ein ITN gegen diese Mückenart nur 38% Schutz bietet. In Häusern mit
guter Qualität bieten ITNs etwas weniger Schutz gegen Mückenstiche von Malariaüberträgern
als in Häusern mit vergleichsweise geringerer Qualität. Der Hauptgrund dafür ist, dass
Menschen, die in relativ guten Häusern leben, dazu tendieren, mehr Zeit drinnen zu
verbringen bevor sie ins Bett gehen.

An. gambiae s.l. ist der wichtigste Malariaübertrager in Dar es Salaam und verantwortlich für
eine entomologische Inokulationsrate (EIR) von 1.00 infektiösen Stichen pro Person pro Jahr,
wohingegen An. funestus eine EIR von 0.13 hat. Überraschenderweise stellt An. coustani mit
0.20 infektiösen Stichen pro Person pro Jahr einen beachtenswerten Vektor in Dar es Salaam
dar. Die Malariübertragung hat mit jährlich zwei Höhepunkten der Malariaprävalenz während
und nach den zwei Regenzeiten einen saisonalen Charakter. Malariaprävalenz war nur in
Kindern unter 5 Jahren durch die EIR bedingt, und die Alters-Prävalenzverteilung war wie in
den meisten Teilen des ländlichen Afrika klassisch. Die Malariaprävalenz hat seit 2004 stetig
abgenommen, während der Gebrauch von Mückengittern an Fenstern, Raumdecken und
effektivere Medikamente wie Amodiaquine und auf Artemisinin basierende Medikamente
zugemommen haben. ITNs (Prävalenzreduktionsschätzung 20%, 95% CI 0%-36%; P=0.060;
Jahr 1) und Raumdecken (Prävalenzreduktionsschätzung 22%, 95% CI 3%-38%; P=0.026;
Jahr 2) boten beschränkten persönlichen Schutz und reduzierten die Malariaprävalenz um
etwa ein Fünftel. Im Vergleich dazu wurde mit der Applikation des Larvizid Bti eine viel
größere Reduktion von Malariaprävalenz erreicht (Prävalenzreduktionsschätzung 50%, 95%
CI 20%-64%; P=0.002). Dies wurde hauptsächlich durch eine bedeutende Reduktion von An.
gambiae im Juli und August erreicht, in den Monaten, in denen auch die meisten Mücken mit
Sporozoiten gefangen wurden, und anderseits durch eine ganzjährige Unterdrückung von
den sekundären Vektoren, An. funestus und An. coustani. Dieser bedeutende Erfolg war nur
möglich durch die neuen Kontroll- und Personalmanagementmethoden, welche durch das
Zusammenfassung


1. Introduction

1.1 Global burden, geographical distribution and life-cycle of malaria infections

Malaria is one of the major contributors to the global burden of disease and a significant impediment to the socioeconomic development in poor countries (Sachs and Malaney 2002; WHO 2004). Malarial disease in humans is caused by 4 different species of *Plasmodium* parasites, namely *P. falciparum, P. vivax, P. ovale* and *P. malariae*. By far the most pathogenic of these, *P. falciparum* is mainly prevalent in sub-Saharan Africa, Papua New Guinea and Haiti. *P. vivax* accounts for most other cases of malaria in humans and is most common in Central and South America, North Africa, the Middle East and the Indian subcontinent. *P. ovale* is mainly found in West Africa and *P. malariae* is widely distributed but mainly found in Africa (White 2003). Between 300 and 660 million clinical attacks, caused by *Plasmodium falciparum*, occur globally (Snow et al. 2005) which results in at least a million deaths (Hay et al. 2004). Over 80% of deaths occur in Africa (Roll Back Malaria Partnership 2005). Around 70% of the clinical attacks occur in sub-Saharan Africa with the main part of the reminder occurring in south East Asia (Snow et al. 2005). Sub-Saharan Africa has such high malaria incidence because ideal climatic conditions for transmission coincide with the presence of efficient malaria vector mosquitoes such as *Anopheles gambiae* Giles, *An. arabiensis* Patton and *An. funestus* Giles (Kiszewski et al. 2004).

Malaria is one of the oldest diseases of mankind, with human-adapted species appearing to have evolved along with us (Qari et al. 1996; Bourgon et al. 2004). Over the millennia, seasonal fevers have been associated with living close to marshy areas, hence the name *malaria*, meaning bad air (Coluzzi and Corbellini 1995). Malaria used to be widespread even
in northern Europe and most of North America but was eliminated from these temperate areas in the 20th century (Bruce-Chwatt 1984). In other areas of modest transmission, including the middle East, China and India, the malaria burden has dropped (White 2003) and the global population at risk decreased from 77 % at the turn of the 20th century to 48 % at the turn of the 21st century (Hay et al. 2004) (Figure 1).

In Tanzania between 14 to 18 million malaria cases and 100,000 to 125,000 deaths occur per year. Malaria accounts for 40 % of outpatient attendances (MOH 2002) and is caused mainly by *Plasmodium falciparum* (Clyde 1967; DHS, Tanzania 2005) which is also the most common malaria parasite worldwide (Roll Back Malaria Partnership 2005).

Malaria is a vector-borne disease caused by a pathogen that is transmitted by female mosquitoes of several species from the genus *Anopheles*. The malaria parasite life cycle involves two hosts, namely humans and mosquitoes (Box 1).
Early clinical symptoms of mild malaria commonly include headache, muscular ache, vague abdominal discomfort, lethargy and lassitude. The fever which typically follows is accompanied by shivering, mild chills, worsening headache and loss of appetite. These symptoms can be caused by all four *Plasmodium* species but most cases of severe malaria are caused by *P. falciparum*. Typical symptoms of severe malaria are acidosis, severe anaemia, renal failure, pulmonary oedema, convulsions, splenomegaly, respiratory distress, impaired consciousness, hypoglycemia and jaundice often leading to death with the four last symptoms being the best prognostic indicators (Marsh et al. 1995; White 2003). Cerebral malaria and severe malarial anaemia are the main two “syndromes” leading to death (Marsh 1992).
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**Box 1.** Life cycle of malaria parasite (*Plasmodium*).
(Source: [http://www.cdc.gov/malaria/biology/life_cycle.htm](http://www.cdc.gov/malaria/biology/life_cycle.htm))

During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates sporozoites into the human host 1. Sporozoites infect liver cells 2 and mature into schizonts 3, which rupture and release merozoites 4. After this initial replication in the liver (exo-erythrocytic schizogony A), the parasites undergo asexual multiplication in the red blood cells (erythrocytes) (erythrocytic schizogony B). Merozoites infect red blood cells 5. The ring stage trophozoites mature into schizonts, which rupture releasing merozoites 6. Some parasites differentiate into sexual erythrocytic stages (gametocytes) 7. The gametocytes, male (microgametocytes) and female (macrogametocytes), are ingested by an *Anopheles* mosquito during a blood meal 8. The parasites’ multiplication in the mosquito is known as the sporogonic cycle C. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes 9. The zygotes in turn become motile and elongated (ookinetes) 10, which invade the midgut wall of the mosquito where they develop into oocysts 11. The oocysts grow, rupture, and release sporozoites 12, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites 1 into a new human host perpetuates the malaria life cycle.
1.2 Epidemiology of malaria

1.2.1 General

Malaria epidemiology is mainly dependent on the occurrence of efficient malaria vectors, climatic favorability for mosquito breeding as well as for parasite development, and the co-occurrence of the human host. The density of the later was recently found to be the critical factor for determining malaria risk when favorable climatic conditions and efficient vectors are present (Moffett et al. 2007). Of the nearly 400 anopheline species worldwide, 80 can transmit malaria and 45 are considered significant vectors (Gillies 1988; Molineaux et al. 1988). In Sub-Saharan Africa, there are two major malaria vectors: *Anopheles funestus* and the *An. gambiae* complex with *An. gambiae sensu stricto* Giles (*An. gambiae s.s.*), *An. arabiensis* Patton, *An. merus* Donitz in East Africa and *An. melas* Theobald in West Africa. Of localized importance are *An. nili* Theobald and *An. moucheti* Evans (Gillies and DeMeillon 1968). Major vectors were defined to be competent if they frequently contain sporozoites, tend to feed on human hosts (anthropophagic) and are more abundant than other anophelines (Kiszewski et al. 2004). Further species belonging to the *An. gambiae* complex are *An. quadriannulatus* Theobald and *An. bwambae* White. *An. quadriannulatus* occurs only in north-eastern and southern Africa and is not considered a malaria vector due to its exophilic and zoophagic behaviour. Also *An. bwambae* is of minor importance as it is only found associated with geothermal fresh water streams in the Rift valley in western Uganda (Service and Townson 2002).

Additional to the above-mentioned criteria for being an efficient malaria vector, malaria transmission mainly depends on the longevity of the anopheline mosquito vector as the mosquito has to survive sporogony (the time required for sporozoite parasite development in
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the mosquito) and after that survive another few days in order to infect human hosts (MacDonald 1957; Gillies 1988). Rates of development differ and are characteristic of each Plasmodium species, from time of gametocyte ingestion to the time when sporozoites are found in the salivary gland (Beier 1998). Sporogony is mainly temperature dependent with sporogonic development of Plasmodium falciparum taking approximately 9 days at 30°C, 10 days at 25°C and 23 days at 20°C (Beier 1998). Adult mosquito survival is dependent on blood feeding behaviour, availability of hosts, sugar feeding behaviour and environmental factors including availability of breeding sites (Killeen et al. 2004; Minakawa et al. 2006; Killeen and Smith 2007; Manda et al. 2007), humidity and temperature (Lindblade et al. 2000). An. arabiensis seems to have greater survival ability at high temperatures than An. gambiae s.s. (Kirby and Lindsay 2004). Even though sporogony occurs more rapidly at high temperatures, high mortality rates of anophelines at temperatures above 32°C have been reported while at low temperatures sporogony is slower and mosquito survival is low (Craig et al. 1999). Therefore ideal climatic conditions for stable malaria transmission are temperatures between 22°C and 32°C with monthly rainfall of approximately 80mm for at least five months per year. Temperatures below 18°C are considered unsuitable for transmission (Craig et al. 1999). The importance of mosquito longevity has been recognized since the first mathematical models of Ross in 1911 and this fundamental point of practical relevance for vector control has also been evaluated in more recent mathematical models (Ross 1911; MacDonald 1957; Killeen et al. 2000; Killeen et al. 2001; Smith and McKenzie 2004; Le Menach et al. 2005; Gu et al. 2006; Le Menach et al. 2007). Other important ecological and behavioural traits of the principal malaria vectors which have an impact on vector control are their biting time, if they bite indoors or outdoors (endophagic or exophagic), if they tend to rest indoors or outdoors (endophilic or exophilic), if they prefer animal or human hosts (zoophagic or anthropophagic),
their flight range as well as their preferred larval habitats (Gillies and DeMeillon 1968; Elliott 1972; White 1974; Gillies and Coetzee 1987; Service 1997; Pates and Curtis 2005).

1.2.1.1 Larval ecology

Oviposition and hence larval breeding site preference often varies substantially between mosquito species, even when they are closely related, for example the M and S form of *An. gambiae* s.s. The former was shown in Mali to be least abundant in puddles whereas the latter was least abundant in swamps (Edillo et al. 2006). *An. gambiae* s.l. mainly prefer shallow, open, sunlit habitats like rice fields, borrow pits and stagnant water such as pools, puddles and hoof prints (Gillies and DeMeillon 1968; Gillies and Coetzee 1987; Service 2000). They often utilize small temporary pools due to higher water temperature and less predation (Holstein 1954; Service 1971; Minakawa et al. 1999; Gimnig et al. 2001; Minakawa et al. 2001; Minakawa et al. 2004). *An. funestus*, in comparison, prefers shade and is therefore found in more or less permanent water bodies with vegetation such as marshes, river edges or rice fields with mature plants providing shade. *An. merus* and *An. melas* in contrast breed in brackish lagoons, ponds, swamps, pools and puddles with 50 – 75% seawater. The other two members of the *An. gambiae* complex and *An. funestus* generally prefer clean and unpolluted waters and are absent from habitats contaminated with faeces or containing rotting plants (Gillies and DeMeillon 1968; Service 2000). Different physical parameters like proportion of light and shade, temperature and water movement as well as chemical factors like alkalinity, PH, dissolved oxygen, nitrate and dissolved solids determine preferential breeding sites. All factors may have an effect on the quality of the breeding site, but normally only a few are important for a specific species (Muirhead-Thomson 1951). In a study in The Gambia *An. arabiensis* was mainly found in rice fields with alluvial soil whereas *An. melas* was found in hoof prints and habitats with high salinity (72% seawater), even *An. gambiae* s.s. was found in
quite brackish water (30% seawater) (Bogh et al. 2003). A study in Mali showed that the proportion of light and shade in rice fields led to high densities of *An. gambiae* s.s. during the first half of rice development whereas in the second half *An. funestus* was predominant (Klinkenberg et al. 2003). The presence of different vegetation types is typically associated with the presence of different *Anopheles* species (Bogh et al. 2003; Fillinger et al. 2004; Minakawa et al. 2004). However, this phenomena may also occur due to the effects of different vegetation types on local water temperatures (Haddow 1943). Although it can also be due to additional food sources as for example, proximity to maize enhanced development of *An. arabiensis* in studies conducted in Ethiopia (Ye-Ebiyo et al. 2000; Ye-Ebiyo et al. 2003). In Kenya several studies have found artificial and natural habitats equally productive and with no habitat preference for *An. gambiae* s.s or *An. arabiensis* (Minakawa et al. 1999; Gimnig et al. 2002; Fillinger et al. 2004) but *An. gambiae* s.l. mainly preferred farmlands and pastures (Munga et al. 2006) whereas *An. funestus* was mainly found in swamps and pastures (Minakawa et al. 2005).

Larval development undergoes three stages: egg, four different instars of larvae and pupae. Under optimal climatic conditions larval development from egg to adult takes around six days (Gillies and DeMeillon 1968). Recent laboratory results showed that optimal climatic conditions balance optimal temperatures for larval survival with optimal temperatures for quick development, with the former being lower than the latter (Bayoh and Lindsay 2003, 2004). This occurs because there is a linear relationship between water temperature and larvae maturation time, while larval survival rates are non linear and reach saturation at high temperatures (Hoshen and Morse 2004). This can also help to explain the lower larval abundance in the highlands of East Africa (Minakawa et al. 2002; Minakawa et al. 2006).
1.2.1.2 Adult mosquito behavioural ecology and implications for control

Adult mosquito densities are seasonal and normally follow rainfall patterns, however this differs both across and within countries. For example in equatorial zones with two wet seasons like Tanzania there are usually two annual peaks in *An. gambiae* s.l. density (Gillies and DeMeillon 1968; Smith et al. 1993; Charlwood et al. 1995; Takken et al. 1998; Kulkarni et al. 2006; Oesterholt et al. 2006). *An. funestus* density begins to increase in the middle of the rainy season and peaks in the early part of the following dry season (Gillies and DeMeillon 1968; Smith et al. 1993). In some parts of Africa these two vector species seasonally replace each other in this manner (Gillies and DeMeillon 1968; Cohuet et al. 2004). Although rainfall creates many breeding sites, if it is heavy it can also flush out pools and reduce larval densities (Gillies and DeMeillon 1968). Due to its dependence of larval habitat abundance, permissive temperatures and humidity, malaria transmission is also seasonal. It has been shown that two rainfall seasons can actually complement each other by intensifying and prolonging the transmission season. Furthermore irrigation activities dampen seasonality by creating perennial breeding habitats independent of rainfall (Faye et al. 1993; Dolo et al. 2004; Mabaso et al. 2007). As transmission is most directly dependent on the density of older sporozoite infected mosquitoes, rather than overall vector population size, there is inevitably a time lag between peak mosquito densities and intensity of transmission. The reason is that during peak mosquito abundance the vast majority of mosquitoes are young and therefore not yet infectious. When densities decline, the mean age of mosquitoes and therefore also the proportion which are sporozoite infected increases (Charlwood et al. 1995; Shiff et al. 1995; Shililu et al. 2004; Kulkarni et al. 2006).

In rural African settings where the bulk of research has thus far been conducted, decreasing mosquito abundance is usually observed further away from the major breeding sites. This has
been most easily demonstrated in areas where the major breeding site was a river, large swamp or rice field (Faye et al. 1993; Lindsay et al. 1993; Lindsay et al. 1995; Ribeiro et al. 1996; Thomas and Lindsay 2000; Minakawa et al. 2002; Diuk-Wasser et al. 2005; Cano et al. 2006; Bogh et al. 2007). Nevertheless some paradoxical observations have been made showing lower malaria prevalence closer to rice fields and rivers than further away (Lindsay et al. 1991; Boudin et al. 1992; Thomas and Lindsay 2000; Ijumba and Lindsay 2001; Diuk-Wasser et al. 2005). Recent models suggest that this phenomena is due to water bodies further away from the main breeding site which may even be unsuitable for larval development but act as a oviposition site from which infected mosquitoes reinitiate the search for blood (Le Menach et al. 2005), thus resulting in the proportion of infectious mosquitoes increasing with the distance from their location of actual emergence (Smith et al. 2004).

As mentioned above, the biting behaviour of malaria vectors can have implications for vector control. \textit{An. gambiae} s.l. and \textit{An. funestus} are considered to be endophagic and endophilic (Gillies and DeMeillon 1968; Gillies and Coetzee 1987). \textit{An. gambiae} s.l. and \textit{An. funestus} typically bite between midnight and 4am but continue until just after sunrise (Haddow 1942; Haddow et al. 1947; Gillies and DeMeillon 1968; Surtees 1970; Dukeen and Omer 1986; Maxwell et al. 1998; Dossou-Yovo et al. 1999). In some rural areas in Africa and its adjacent islands \textit{An. gambiae} s.l. (either \textit{An. gambiae} s.s., \textit{An. arabiensis} or species not resolved) as well as \textit{An. funestus} were found to be exophagic (Charlwood et al. 2003; Laganier et al. 2003; Wanji et al. 2003; Afolabi et al. 2006). In recent years \textit{An. arabiensis} was also found to be exophilic in some rural areas, although in Tanzania this behaviour was seasonality dependent and it was partially due to zoophilic behaviour (Shililu et al. 2004; Kulkarni et al. 2006). Exophilic \textit{An. gambiae} s.s. were also found in Sao Tomé but most of the outdoor resting mosquitoes were dogophilic (Sousa et al. 2001). In two different regions in Ethiopia where
An. arabiensis is the main vector it was found to bite early in the night, mainly before people went to bed (Abose et al. 1998; Yohannes et al. 2005). This shift of biting time was most probably induced by the long-term application of DDT as 40 years ago An. gambiae s.l., in one of the regions, was observed to mainly bite after 11pm (Rishikesh 1966). Similarly in Zimbabwe, after eight years of insecticide spraying more An. gambiae s.l. (sibling species within this complex were not resolved in that study) were caught biting outdoors than indoors whereas before the intervention there was no difference (Muirhead-Thomson 1960a, 1960b). Whether these behavioural changes are heritable behavioural traits or due to the exitorepellent properties of DDT or other insecticides are difficult to distinguish (Roberts et al. 2000). The influence of insecticide-treated nets (ITN) (Lengeler 2004; Roll Back Malaria Partnership 2005; Roll Back Malaria Partnership 2005), improved housing (Lindsay et al. 2002; Lindsay et al. 2003), and other personal protection methods (Rozendaal 1997; Snow et al. 1998; Rowland et al. 2004) upon mosquito feeding behaviour has been discussed qualitatively but not quantitatively. Reduced indoor biting was reported due to ITNs and impregnated curtains throughout Africa (Carnevale et al. 1988; Magesa et al. 1991; Karch et al. 1993; Mbogo et al. 1996; Faye et al. 1998; Cuzin-Ouattara et al. 1999; Maxwell et al. 1999; Ilboudo-Sanogo et al. 2001; Takken 2002). Additionally, improved housing, especially mosquito-proof screening, closed eaves, ceilings and sealed frames for windows, can reduce indoor biting rates (Lindsay and Snow 1988; Lindsay et al. 1995; Lindsay et al. 2002; Lindsay et al. 2003). Recent studies also suggest a change in biting pattern may occur due to the use of personal and household protection (Braimah et al. 2005; Pates and Curtis 2005). Nevertheless continued and more extensive quantitative surveillance of biting behaviour will be required so that vector control strategies remain appropriately responsive to such challenges.
1.2.1.3 Clinical epidemiology

In the past, criteria used to classify the malaria transmission level were based on parasitological and clinical data such as average splenomegaly rates and prevalence of parasitemia (Snow and Gilles 2002) but it is increasingly recognized that malaria incidence and prevalence itself is mainly influenced by the intensity of exposure to transmission. Exposure to transmission is typically recorded and expressed as the entomological inoculation rate (EIR), defined as the number of infectious bites a person receives per per year or other relevant unit of time (Beier et al. 1999). An area with an EIR of 1 infectious bite per person per year results in modest prevalence and incidence rates and is described as hypoendemic whereas an EIR of 100 or more infectious bites per person per year is typically classified as holoendemic with very high rates of infection and disease. Apart from EIR, a number of non-entomological factors have an impact (Koram et al. 1995; Clarke et al. 2001; Mensah and Kumaranayake 2004) but these are interrelated and therefore difficult to dissect analytically (Bates et al. 2004). In areas with intense transmission, new born children are relatively protected against malaria infection for approximately three months due to passive immunity acquired from the mother (Fried et al. 1998). After that period, infants and children become highly susceptible to severe clinical manifestations of malaria and the overwhelming burden of morbidity and mortality falls upon this age group (Marsh 1992; Snow et al. 1997; Baird 1998; Snow and Marsh 2002; WHO/UNICEF 2003; WHO 2005; Marsh and Kinyanjui 2006; Lengeler et al. 2007). If children survive past the age of five years, after being repeatedly inoculated with sporozoites and therefore exposed to pathogenic asexual blood-stages, they acquire a state of semi-immunity which protects them from the severest outcomes of malaria. This occurs primarily through the suppression of parasite densities without necessarily shortening the duration of infection (Molineaux et al. 1988; Rogier and Trape 1995; Collins and Jeffery 1999; Molineaux et al. 2002; Maire et al. 2006; Smith et al. 2006). For this reason,
malaria prevalence in adults in highly endemic areas is often relatively low whereas the majority of young children are infected (Hoffman et al. 1987; Beier et al. 1994; Snow and Marsh 2002). However, prevalence in semi-immune adults and older children is probably underestimated as low-density infections are undetectable by microscopy (O'Meara et al. 2007).

1.2.2 Urban malaria in Sub-Saharan Africa

Most malaria research in Africa has historically focused on rural areas with intense transmission but the growing importance of urban settings is now increasingly recognized (Lines et al. 1994; Robert et al. 2003; Keiser et al. 2004; Donnelly et al. 2005; Hay et al. 2005; Wang et al. 2005). It is estimated that by the year 2030 more than 50% of the African population will live in towns or cities (UN 2004). Urban areas differ from rural settings in that exposure to transmission is typically lower and access to diagnosis, treatment and preventive measures is much better (Lines et al. 1994; Robert et al. 2003; Keiser et al. 2004; Donnelly et al. 2005; Hay et al. 2005; Wang et al. 2005). Here I describe the distinctive features of malaria ecology and epidemiology in urban Africa and highlight key knowledge gaps which existed before these studies, many of which still remain.

1.2.2.1 Larval ecology

Urban larval ecology differs from rural ecology in the sense that many of the natural habitats are destroyed by constructions of buildings, paving of roads and footpaths and pollution of standing water (Keating et al. 2003). On the other hand, new potential breeding sites are created by human activities such as the establishment of shantytowns with open sand pits and burrows as well as urban agricultural activities (Castro et al. 2004). The overall balance of these two opposing processes results in increasing habitat availability as population density
increases up to the point where physical space becomes limiting and habitats are both scarce and frequently disturbed (Keating et al. 2003). In Brazzaville, Congo anopheline mosquitoes were found breeding in ditches, gutters and tire tracks (Trape and Zoulani 1987) and in an newly urbanized area in western Kenya they were also commonly found in man made habitats such temporary pools of water and tire tracks (Khaemba et al. 1994) which is similar to rural areas. Urban agriculture also poses a problem for which there are many documented examples. Market garden wells in Dakar, Senegal were important breeding sites for An. arabiensis (Robert et al. 1998). In recent years more research effort was directed towards urban agriculture and matuta (a type of agriculture where plants are grown on top of small ridges), rice fields and irrigated vegetable fields and irrigation wells were identified as major Anopheles breeding sites in several settings (Afrane et al. 2004; Sattler et al. 2005; Matthys et al. 2006; Vanek et al. 2006). Another important contrast to rural larval ecology is that although aquatic-stage Anopheles mosquitoes are usually associated with relatively clean water, increased Anopheles breeding in domestic artificial containers and polluted waters such as pit latrines, was observed in Accra, Ghana over 20 years ago (Chinery 1984). More recent studies confirm that An. gambiae s.l. has adapted to urban settings by ovipositing and developing in a variety of polluted water bodies including oxidation ponds for sewage and hospital waste (Jacob et al. 2005; Sattler et al. 2005; Matthys et al. 2006). More detailed and contemporary knowledge of the evolving larval ecology of malaria vectors in urban settings is clearly needed if effective larval control is to become a sustained reality in African cities.

### 1.2.2.2 Adult mosquito behavioural ecology

Malaria transmission intensity is generally lower in urban areas but clearly depends on the degree of urbanization (Trape and Zoulani 1987, 1987; Lindsay et al. 1990; Coene 1993; Robert et al. 2003). Urbanization can also change the species composition of mosquito
populations. For example, in Dar es Salaam, Tanzania, *Anopheles* densities declined whereas *Culex* densities increased (Bang et al. 1977). Furthermore the distribution of seasonal and permanent breeding sites is highly localized and mosquito dispersal is limited by high availability of blood meal hosts, leading to patchy, heterogeneous transmission at particularly fine scales (Trape and Zoulani 1987, 1987; Trape et al. 1992; Service 1997; Eisele et al. 2003; Killeen et al. 2003; Castro et al. 2004; Keiser et al. 2004). Therefore malaria prevalence and incidence also tend to decrease further away from these breeding sites (Trape 1987; Trape et al. 1992; Thompson et al. 1997; Staedke et al. 2003). This occurs largely because mosquitoes tend not to disperse far from their breeding sites when blood meals and aquatic habitats are in close proximity (Trape et al. 1992; Service 1997; Minakawa et al. 2002; Killeen et al. 2003).

Very little is known about biting behaviour of malaria vectors in urban areas. To our knowledge, biting intensities at different times of the night and at different indoor versus outdoors locations had never been studied in urban settings prior to recent reports from Lagos, Nigeria where *Anopheles arabiensis* appear to be exophagic (Oyewole and Awolola 2006). This behaviour did not appear to be associated with the use of protective measures such as ITNs, ceiling boards or window screening. Nevertheless, in some other African cities reduced indoor biting due to ceiling boards and window screenings has been observed (Lindsay et al. 1990; Trape et al. 1992; Adiamah et al. 1993). As cities often have large areas with relatively good housing and relatively high coverage with personal protective measures such as ITNs, repellents and coils (Evans 1994; Lines et al. 1994; Stephens et al. 1995; Curtis et al. 2003; Lines et al. 2003; Wang 2006; Wang et al. 2006) this could conceivably force changes in epidemiologically relevant behavioural patterns of vector mosquitoes, as already demonstrated in some rural areas (Lines et al. 1987; Njau et al. 1993; Jaenson et al. 1994; Bogh et al. 1998; Curtis et al. 1998; Knols and Takken 1998; Maxwell et al. 2002; Maxwell et
al. 2003; Pates and Curtis 2005). Changing biting behaviour is highly relevant to vector control success because domestic personal protection measures such as ITNs which act indoors only, are likely to be less effective if primary vectors mainly bite before people go to bed or mainly bite outdoors. In the case of exophagic behaviour, even window screening and ceiling boards would confer less protection (Killeen et al. 2006).

1.2.2.3 Clinical epidemiology

Urban areas are generally characterized by lower EIRs and therefore lower transmission, thus malaria prevalence is lower in urban settings compared to rural settings with similar climatic conditions. Parasite prevalence in urban areas never exceeded 75% (Omumbo et al. 2005). Low EIRs due to urbanization are caused by increased population densities which lead to a lower mosquito emergence rate per person. There are simply more people to bite for a given number of mosquitoes, so each person is bitten less (Killeen et al. 2000; Smith et al. 2004). It was recently elucidated using detailed transmission models (Ross et al. 2006; Ross et al. 2006; Smith et al. 2006) that such lower exposure levels lead to a lower level of immunity in the population as a whole, as well as to higher prevalence, morbidity, mortality and infectiousness in older age groups (Trape et al. 2002; Robert et al. 2003; Keiser et al. 2004; Donnelly et al. 2005; Hay et al. 2005; Wang et al. 2005). Although this was validated in several urban settings (Trape 1987; Yohannes and Petros 1996; El Sayed et al. 2000; Klinkenberg et al. 2005; Wang et al. 2005), others exhibit a classical age-prevalence distribution typical of rural areas with infection and disease burden concentrated in younger children (Modiano et al. 1998; van der Kolk et al. 2003; Matthys et al. 2006; Wang et al. 2006).

Malaria incidence and prevalence is not only influenced by transmission intensity but also by non-entomological parameters which are often quite different in urban settings. Education
level of the head of the household and socioeconomic status as well as traveling to rural areas with higher transmission levels all influence malaria incidence and prevalence (Ng’andu et al. 1989; Koram et al. 1995; Mensah and Kumaranayake 2004; Klinkenberg et al. 2006; Ronald et al. 2006; Wang et al. 2006; Wang et al. 2006). Poverty, lack of education and travel to rural areas can all increase risk of contracting malaria by influencing what protective measures and curative drugs inhabitants can afford and use (Stephens et al. 1995; Govere et al. 2000; MacIntyre et al. 2002; Doannio et al. 2004). All these factors are highly interrelated and therefore difficult to dissect analytically (Bates et al. 2004) but nevertheless further insight is needed, highlighting the need for ambitious, detailed and extensive studies which evaluate the social, economic, behaviourable, ecological and epidemiological determinants of malaria in an integrated and interactive fashion.

1.3 Malaria control

Due to growing concerns of governments across the world, but particularly in Africa, about the continuing and increasing burden of malaria, the Roll Back Malaria (RBM) campaign was initiated in 1998. Cornerstones of the RBM are to provide access to prompt diagnosis and effective treatment, especially for the most vulnerable groups of young children and pregnant women and to promote the use of insecticide treated bednets as a mean of prevention (Roll Back Malaria). The Abuja declaration was signed in the year 2000 by most African countries, committing to intense efforts in support of RBM with the overall goal of halving malaria mortality by 2010 (WHO 2003). In Tanzania these goals were integrated into the National Malaria Medium Term Strategic Plan (NMMTSP) in 2002, with the specific target of reducing mortality and morbidity due to malaria in all regions of the country by 25% by 2007 and by 50% by 2010 (MOH 2002).
1.3.1 Vector control for malaria control: Strategic options available today

1.3.1.1 Insecticide treated nets (ITN) and indoor residual spraying (IRS)

The effect of ITNs is threefold. On the one hand they offer personal protection by acting as a physical barrier between mosquitoes and the person sleeping under the net. On the other hand they also reduce indoor biting by a combination of increased mosquito mortality which is caused by the insecticidal properties and the reduction of mosquito house entry caused by their excito-repellent properties (Lines et al. 1987; Lindsay et al. 1991; Miller et al. 1991).

These two properties combined lead to good protection (Lengeler 2004, 2004) but even bigger reduction in transmission and therefore exposure can be attained at the community level where high population coverage is achieved (Maxwell et al. 2002; Hawley et al. 2003; Killeen and Smith 2007; Le Menach et al. 2007). Community-level effects which even benefit unprotected individuals are attained by reducing the density (Carnevale et al. 1988; Magesa et al. 1991; Robert and Carnevale 1991), survival (Carnevale et al. 1988; Magesa et al. 1991; Robert and Carnevale 1991), human blood indices (Bogh et al. 1998; Charlwood et al. 2001) and feeding frequency of malaria vectors (Charlwood et al. 2001). Indoor residual spraying works in the same way by decreasing house entry and reducing the survival of the mosquitoes.

It has a strong community effect which contributes to reductions malaria prevalence (Kouznetsov 1977; Mabaso et al. 2004; Nyarango et al. 2006; Kleinschmidt et al. 2007; Sharp et al. 2007). The greatest sustained success in Africa thus far achieved with IRS has been in South Africa (Mabaso et al. 2004) but growing resistance of malaria vectors to available insecticides like pyrethroids is a major cause for concern and an increasing threat to such essential and effective programs (Corbel et al. 2007; N'Guessan et al. 2007; Sharp et al. 2007). Alternative vector control methods like larviciding and environmental management may have to be reconsidered as front-line options wherever they may prove to be appropriate (Utzinger et al. 2001; Killeen et al. 2002; Utzinger et al. 2002; Keiser et al. 2005).
1.3.2 Larval control

1.3.2.1 Environmental management in integrated vector control programs

A number of approaches to environmental management exist with distinctive advantages, disadvantages and potential applications (Rozendaal 1997; Utzinger et al. 2001; Keiser et al. 2005). One approach is environmental manipulation which refers to activities that reduce larval breeding sites through temporary changes in the aquatic environment. This includes activities like changing water levels in reservoirs, flushing streams or canals, providing intermittent irrigation to agriculture fields and flooding or temporarily de-watering man-made or natural wetlands. An alternative approach is environmental modification which involves a physical change, often long-term, to potential mosquito breeding areas designed to prevent, eliminate or reduce vector habitat (Walker 2002). The advantage of environmental management is that it is non-toxic, cost-effective, long-lasting and sustainable (Utzinger et al. 2001; Keiser et al. 2005) but its greatest limitation is usually affordability. Most success stories of malaria control programs incorporating environmental management and effectively reducing morbidity and mortality were implemented before the Global Eradication Campaign (1955 – 1969) which mainly relied on IRS with dichlorodiphenyltrichloroethane (DDT) (Keiser et al. 2005). Nevertheless, only four programs were implemented in Africa during the pre-DDT era using different kinds of environmental management like drainage, filled marshes, modification of river boundaries and vegetation management (Ross 1907; Gilroy and Bruce-Chwatt 1945; Kitron 1987; Utzinger et al. 2001; Utzinger et al. 2002).

Environmental management has great potential for urban settings as demonstrated in Dar es Salaam, Tanzania where construction and cleaning of anti-malarial drains continued even throughout the eradication era, although it was eventually neglected in the wake of the economic crisis in Tanzania during the 1970s and 1980s (Bang et al. 1975; Bang et al. 1977; Kilama 1991, 1994; Yamagata 1996; Castro et al. 2004). Recent theoretical studies suggest
that drastic reductions in EIR can be achieved by environmental management and therefore environmental management should gain more attention (Killeen et al. 2000; Gu et al. 2006).

**1.3.2.2 Chemical and biological larval control in integrated vector control programs**

Unlike environmental management control measures relying on chemical or biological larvicides don’t change the natural habitats of the mosquitoes but rather directly kill larvae through the use of insect-specific toxins. Traditional surface-layer treatments, the prototypes of which are mineral oils, are still used to a modest extent although much more advanced and environmentally friendly formulations are used (Beales and Gillies 2002). Environmentally hazardous chemicals such as Paris Green (copper acetoarsenite) and DDT were replaced decades ago by organophosphates such as temephos and malathion, which are considered vastly superior in terms of safety and environmental impact. More recently, insect growth regulators (IGRs) and biological methods including larvivorous fish, some protozoans, fungi as well as bacteria, notably *Bacillus thuringiensis* var. *israelensis* (*Bti*) and *Bacillus sphaericus* (*Bs*) have come into widespread use globally and may have applications in Africa (Yapabandara et al. 2001; Walker 2002; Yapabandara and Curtis 2002). The most successful larval control program which has been documented is the eradication of *An. gambiae* from Brazil using *Paris Green* as a larvicide (Soper and Wilson 1943; Killeen et al. 2002). Toxic *Paris Green* can now be replaced with safe and environmentally friendly *Bti* and *Bs* and I suggest that the time has come to evaluate the potential of larviciding in appropriate African settings such as cities and towns. Both *Bacillus* species function as stomach poisons in the mosquito larva midgut. The lethal effect is caused by toxins on the bacterial spore coat. Formulations of *Bti* use dead spores whereas formulations of *Bs* use live spores which have the potential to self-propagate within the cadavers of their mosquito victims (Charles and Nicolas 1986; Pantuwatana et al. 1989; Sutherland et al. 1989; Hougard 1990; Karch et al. 1990).
1990; Matanmi et al. 1990; Skovmand and Bauduin 1997). *Bti* is substantially cheaper than *Bs* but has to be applied on a weekly basis and is not effective in all types of habitats. *Bti* requires clean water to be effective, whereas *Bs* can be used successfully in water which is organically polluted (Walker 2002; Lacey 2007). *Bti* and *Bs* effectively kill African malaria vector mosquito larvae under both laboratory and field conditions (Fillinger et al. 2003; Shililu et al. 2003; Fillinger and Lindsay 2006; Majambere et al. 2007; Shililu et al. 2007). Furthermore they reduced adult mosquito densities and therefore transmission in selected African settings (Fillinger and Lindsay 2006; Shililu et al. 2007) and therefore have great potential for prevention of malaria in Africa.

1.3.2.3 The potential of integrated vector management in contemporary Africa

As described above, most vector control programs that included larval control were implemented before the Global Eradication Campaign (1955 – 1969) which overwhelmingly relied on IRS with DDT (Killeen et al. 2002; Killeen et al. 2002; Keiser et al. 2005). The impact of microbial larvicides and other forms of larval control against African malaria vectors has been demonstrated in qualitative terms (Soper and Wilson 1943; Shousha 1948; Watson 1953; Louis and Albert 1988; Kitron and Spielman 1989; Sabatinelli et al. 1991; Fletcher et al. 1992; Gopaul 1995; Julvez 1995; Ragavoodoo 1995; Rozendaal 1997; Barbazan et al. 1998; Utzinger et al. 2001), and estimated using simulation models (Gu and Novak 2005; Gu et al. 2006; Killeen et al. 2006). Past successful programs showed that community participation, diverse and specialized skills in malaria epidemiology, entomology and vector ecology, decentralized management and stable and sustainable financing are of high importance (Killeen et al. 2002; Killeen et al. 2004; Keiser et al. 2005; Barat 2006). This was reinforced by a comparison with recent mosquito control programs (Impoinvil et al. 2007). In this context, after larval control options were neglected in Africa for almost 40
years, the Urban Malaria Control Program (UMCP) in Dar es Salaam, Tanzania, began implementing a community-based but vertically managed larval control program using microbial larvicides. Herein I describe a detailed evaluation of the impact on mosquito populations, malaria transmission and malaria risk of routine larviciding with environmentally-friendly microbial pesticides and existing standard vector control tools such as ITNs in the context of the UMCP in contemporary Dar es Salaam.
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Introduction


Introduction


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2. **Goal and objectives**

2.1 **Goal**

Enhance the current understanding of urban malaria epidemiology, mosquito behavioural ecology and their implications for implementing Integrated Vector Management (IVM) in urban Africa, using the Urban Malaria Control Program (UMCP) in Dar es Salaam, Tanzania as a model programmatic platform.

2.2 **Objectives**

1. To estimate the proportion of human exposure to malaria vectors which occurs outdoors.
2. To evaluate the impact of outdoor biting on personal protection offered by insecticide-treated nets (ITNs).
3. To determine whether ITNs confer less protection in higher quality houses.
4. To characterize seasonal variations in local mosquito densities as well as malaria prevalence and transmission intensity.
5. To evaluate the epidemiological impact of community-based application of microbial larvicides upon malaria prevalence in the context of a *de facto* IVM program incorporating multiple personal protection measures.
Goal and objectives
3. **Interdependence of domestic malaria prevention measures and mosquito-human interactions in urban Dar es Salaam, Tanzania**

Yvonne Geissbühler\(^1,2\)*, Prosper Chaki\(^2,3,5\), Basiliana Emidi\(^3,4\), Nicodemus J. Govella\(^2,3,5\), Rudolf Shirima\(^3\), Valeliana Mayagaya\(^2\), Deo Mtasiwa\(^3\), Hassan Mshinda\(^2\), Ulrike Fillinger\(^5\), Steven W. Lindsay\(^5\), Khadija Kannady\(^3\), Marcia Caldas de Castro\(^6\), Marcel Tanner\(^1\), Gerry F. Killeen\(^1,2,5\)

\(^1\)Swiss Tropical Institute, Department of Public Health and Epidemiology, Socinstrasse 57, P.O. Box, 4002 Basel, Switzerland
\(^2\)Ifakara Health Research and Development Centre, Co-ordination Office, Kiko Avenue, PO Box 78373, Dar es Salaam, Tanzania
\(^3\)Dar es Salaam City Council, Dar es Salaam, Tanzania
\(^4\)University of Dar es Salaam, Dar es Salaam, Tanzania
\(^5\)School of Biological and Biomedical Sciences, South Road, Durham DH1 3LE, UK
\(^6\)Department of Population and International Health, Harvard School of Public Health, 655 Huntington Avenue, Boston, MA 02115, USA

* Corresponding Author:

Yvonne Geissbühler, Swiss Tropical Institute, P.O.Box, 4002 Basel, Switzerland
Tel: +41 61 284 82 09; E-mail: Y.Geissbuehler@unibas.ch

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3.1  Abstract

**Background:** Successful malaria vector control depends on understanding behavioural interactions between mosquitoes and humans, which are highly setting-specific and may have characteristic features in urban environments. Here mosquito biting patterns in Dar es Salaam, Tanzania are examined and the protection against exposure to malaria transmission that is afforded to residents by using an insecticide-treated net (ITN) is estimated.

**Methods:** Mosquito biting activity over the course of the night was estimated by human landing catch in 216 houses and 1,064 residents were interviewed to determine usage of protection measures and the proportion of each hour of the night spent sleeping indoors, awake indoors, and outdoors.

**Results:** Hourly variations in biting activity by members of the *Anopheles gambiae* complex were consistent with classical reports but the proportion of these vectors caught outdoors in Dar es Salaam was almost double that of rural Tanzania. Overall, ITNs confer less protection against exophagic vectors in Dar es Salaam than in rural southern Tanzania (59% versus 70%). More alarmingly, a biting activity maximum that precedes 10pm and much lower levels of ITN protection against exposure (38%) were observed for *Anopheles arabiensis*, a vector of modest importance locally, but which predominates transmission in large parts of Africa.

**Conclusions:** In a situation of changing mosquito and human behaviour, ITNs may confer lower, but still useful, levels of personal protection which can be complemented by communal transmission suppression at high coverage. Mosquito-proofing houses appeared to be the intervention of choice amongst residents and further options for preventing outdoor transmission include larviciding and environmental management.
3.2 Background

Malaria and other vector borne diseases are major contributors to the global burden of disease and a significant impediment to socioeconomic development in poor countries [1]. It is estimated that 300 to 660 million clinical attacks of malaria occur globally [2] which result in at least 1 million deaths [3, 4]. Over 80% of these deaths occur in Africa [4]. Approximately 70% of clinical malaria attacks occur in sub-Saharan Africa with the vast bulk of the remainder occurring in south East Asia [4]. Sub-Saharan Africa has the highest incidence because ideal climatic conditions for transmission are exacerbated by some of the world’s most efficient malaria vectors, such as *Anopheles gambiae*, *Anopheles arabiensis* and *Anopheles funestus* [5].

While the bulk of malaria research in Africa has focused on rural areas, the growing importance of urban settings is increasingly recognized [6-11]. Transmission intensity is generally lower in urban areas but it is estimated that, by the year 2030, more than 50% of the African population will live in towns and cities [12] so improved understanding and evidence-based strategies for controlling urban malaria are needed. Urban areas differ from rural settings in that exposure to transmission is typically lower and access to diagnosis, treatment and preventative measures is much better [6-11]. As recently elucidated using detailed transmission models [13-15], such lower exposure levels lead to a lower level of immunity in the population as a whole, as well as to higher prevalence, morbidity, mortality and infectiousness in older age groups [6-10, 16]. Furthermore, the distribution of seasonal and permanent breeding sites is highly localized, leading to patchy, heterogeneous transmission at particularly fine spatial scales [7, 17-21]. Malaria prevalence and incidence tends to be much higher for residents living close to major larval habitats [19, 22-24]. This is because
mosquitoes tend not to disperse far from the breeding sites as blood meal and aquatic habitat resources are in close proximity to each other [19, 25-27]. This may even be true for water bodies which are not suitable for larval development but do act as oviposition sites [28], possibly resulting in the proportion of infectious mosquitoes increasing with the distance from their location of actual emergence [29]. Urban setting often have large areas with relatively good housing and relatively high coverage with personal protection measures such as ITNs, repellents and coils [11, 30-35] with the potential to force changes in epidemiologically relevant behavioural patterns of vector mosquitoes [36-49].

*Anopheles gambiae* and its sibling species *An. arabiensis* are the most important vectors of malaria in most parts of Africa, where they readily adapt to urban ecosystems by ovipositing and developing in atypical larval habitats such as domestic containers and polluted water bodies [50-52]. Although this species is most commonly found in artificial larval habitats, even in rural areas, this is particularly the case in towns and cities [51-57]. Despite the enormous importance of these mosquito species, relatively little is known about their feeding behaviour, and even less about their broader ecology, particularly in urban setting.

Furthermore, the influence of insecticide-treated nets (ITNs) [4, 58, 59], improved housing [60, 61] and other personal protection [62-65] methods upon their feeding behaviour has been discussed qualitatively but has yet to be evaluated in quantitative terms. There is one example of Zimbabwe, where after eight years of insecticide spraying more *An. gambiae* sensu lato (s.l.) (as sibling species within this complex were not resolved in that study) were caught biting outdoors than indoors whereas before the intervention there was no difference [66, 67]. In many places throughout Africa, a reduced indoor biting was reported due to ITNs and impregnated curtains [37, 39, 42, 45, 46, 68-71] through a combination of increased mosquito mortality caused by their insecticidal properties and the reduction of mosquito house entry.
caused by their excito-repellent properties [49, 72, 73]. Indoor biting rates of malaria vectors can be reduced by improved housing, specifically mosquito-proof screening, closed eaves, ceilings and sealed frames for windows and doors [19, 60, 61, 74-78] and some recent studies suggest changes in their biting patterns in response to personal or household protection measures [36, 79, 80]. However, only 20% (4/20) of the studies described in these papers have been carried out in urban areas so here the behavioural interactions between vector mosquitoes and their human hosts in the context of a large-scale integrated malaria control programme in Dar es Salaam, Tanzania are examined [52, 81].

In Dar es Salaam, the main malaria vectors are members of the An. gambiae species complex and An. funestus [82]. Dar es Salaam has a relatively high coverage with bednets and ITNs (91.8 % and 43.1%, respectively) [33]. In order to see if increasing ITN usage and house quality has influenced mosquito biting behaviour, a survey of behavioural interactions between mosquitoes and humans during the main rains of 2006 was undertaken. This study was also carried out in order to estimate the extent of protection against exposure to malaria transmission that is afforded to residents of Dar es Salaam by using an ITN and to evaluate the influence of housing quality upon this level of protection. Furthermore, the implication these behaviours have for malaria control in Dar es Salaam and elsewhere in Africa where similar trends are observed are discussed.

3.3 Methods

Study site

Dar es Salaam is situated at the shores of the Indian Ocean coast with a hot and humid climate which is ideal for mosquito proliferation and malaria transmission, satisfying the climatic
requirement for stable transmission of temperatures between 22°C and 32°C and a rainfall of around 80 mm per month for at least five months per year [83]. There are typically two rainy seasons: a main rainy season from March to June and a shorter, more erratic rainy season from October to December. Dar es Salaam has around 2.5 million inhabitants and covers a total area of 1,400 km² [84]. The city is divided into three municipalities; Temeke, Ilala and Kinondoni which collectively comprise 73 wards. Each ward is further subdivided into neighbourhoods known as mitaa (singular mtaa) which typically comprise between 20 and 100 mashina (singular shina) or Ten Cell Units (TCU). The TCU is the smallest subunit of local government in Tanzania which, in principle, comprises a cluster of 10 houses with an elected representative known as a mjumbe although in practice most TCUs include 20-30 houses and some may even exceed 100. This study was based within the project area of the ongoing Urban Malaria Control Programme (UMCP) implemented by the Dar es Salaam City Council [52, 81]. The main project area includes five wards from each municipality with a total of 67 mitaa. Overall, this study area covers an area of 55 km² with a total population of 609,514 people [84]. The houses surveyed here were located in five wards, eight mitaa (Figure 1).

For comparison, the results obtained in Dar es Salaam are contrasted with those obtained with similar methodology in the Kilombero Valley, a rural setting with intense perennial malaria transmission in southern Tanzania [85].
Figure 1. Wards included in the study area of the Urban Malaria Control Program in Dar es Salaam, showing the ten cell units (TCU) of the adult mosquito monitoring system as well as of the detailed survey.
Preliminary survey of the overall study site

For the purposes of routine monitoring and programme management, the UMCP surveys mosquito biting densities at 268 locations (four in each mtaa), distributed across the study area every four weeks. Initial trials proved that existing trapping technologies were not sufficiently sensitive to monitor the low densities of An. gambiae which occur across the study area. Therefore, outdoor human landing catch (HLC) [86] has been implemented as the standard sampling tool for adult mosquitoes as an interim measure until a suitable alternative is proven practical, effective and affordable. Once every four weeks at each location, HLC is conducted from 6 pm to 6am for 45 minutes of each hour, allowing 15 minute breaks for rest, hot drinks and snacks. All collected mosquitoes are identified morphologically to genus and, in the case of Anopheles to species complex level [87, 88]. Members of the Anopheles gambiae species complex are further resolved to sibling species level by polymerase chain reaction (PCR) [89]. The sporozoite infection status of each mosquito was determined by enzyme-linked immunoabsorbent assay as previously described [90].

HLCs between April and December 2005 were used to identify the primary vectors of malaria in Dar es Salaam and to test for variation by location in the distribution of An. gambiae biting activity across the night. Members of the An. gambiae species complex were identified as the major malaria vectors in Dar es Salaam (See Results) so only these species were considered in the following analysis and study design. The influence of location as a determinant of An. gambiae biting habits was tested by treating TCU unique ID for each sampled site as a fixed factor in a logistic model with the proportion of mosquitoes caught during typical sleeping hours of city residents (10pm to 6am; see results) as the outcome variable. This data set was also used to identify sites with the highest densities of An. gambiae s.l. for the detailed and intensive mosquito behavioural surveys described below.
Detailed surveys of mosquito biting behaviour

The 12 TCU in Temeke municipality and 2 TCU in Ilala municipality, which had the highest An. gambiae s.l. densities in the UMCP surveillance system, were selected for further, more detailed, surveys of the behavioural patterns of mosquitoes and humans. Informed consent was obtained from 216 houses in order to conduct HLC both indoors and outdoors. In each house, HLC was conducted for one night from 6 pm to 7 am as described above except that catchers switched between indoor and outdoor stations every hour in order to preclude biases resulting from variations in individual attractiveness [91-93]. These human landing catch surveys took place during 10 weeks of the main rainy season between April and June 2006. In order to estimate the biting rate for a full hour, total catches per hour were divided by 0.75.

Interview surveys of human behaviour and domestic protection measures

A brief interview was conducted with all household members present at the time of the interview. They were asked where they usually eat dinner, where they stay after dinner before going to bed, what time they go to bed and what time they typically get out of bed in the morning. Furthermore, they were asked which preventive measures, such as bednets or insecticides, they use to avoid mosquito bites. The quality of their houses, i.e. the quality of screening and availability of ceiling boards was examined in each household. In order to verify the sleeping and resting behaviours reported by residents during interviews, also surveys were conducted based on direct observation by walking through these TCUs once every hour of the night and counting the number of people seen outdoors. Direct observation surveys were conducted for three nights in each TCU. Once validated by direct observation (see results), the questionnaire reports were used to estimate proportion of the inhabitants in each of the three behavioural compartments (outdoor, indoor awake, indoor asleep) at each hour of the night.
Estimating the protective efficacy of ITNs in terms of reduced biting exposure

Data from the human and mosquito behavioural surveys described above were integrated to evaluate the interaction between them using an extension of a recently developed mathematical model [85]. EIR is the product of the biting rate experienced by humans exposed to a vector population and the sporozoite infection prevalence of that mosquito population [94]. The latter is only reduced by community-level impacts of malaria interventions [95, 96] so here personal protection purely in terms of biting rates and the impact that protective measures such as ITNs have upon them were estimated. First $B_{u,t}$, the mean biting rate experienced by an unprotected individual at each time of the night ($t$), based on the proportion of time spent outdoors multiplied by the outdoor biting rate at that time ($B_{o,t}$) plus the proportion of that hour spent indoors multiplied by the indoor biting rate at that time ($B_{i,t}$) was calculated. The main difference between this model and the one of Killeen et al. is that, because of the available information from the questionnaires, there was the possibility to divide the indoor compartment into being indoor but not asleep (and therefore not under a bednet) and being indoor and asleep (and, therefore, protected if using a bednet). The proportion of people sleeping or trying to sleep in bed and indoors ($S_t$) is not the same as the proportion of people staying indoors asleep or not asleep ($I_t$). If people are unprotected because they do not have a bednet, it only matters if they are indoors or outdoors and thus they experience the following biting rate:

$$B_{u,t} = B_{o,t} (1-I_t) + B_{i,t} I_t$$  \hspace{1cm} 1$$

The number of bites experienced per night, or nightly biting rate, for an unprotected non-user ($B_u$) can thus be calculated by summing the relevant biting rates for each hour:

$$B_u = \sum_{t=1}^{24} B_{u,t}$$  \hspace{1cm} 2$$

Note that an unprotected individual is defined as someone lacking any net whereas a protected individual is defined as someone regularly using an effectively insecticidal net. The nightly
biting rate of a protected individual \( (B_p) \) based on the combined nightly profiles of mosquito biting rate \( (B_{u,t}) \) over time \( (t) \), the protective efficacy of ITNs \( (P) \), which is assumed to be constant, and the behaviour of humans which results in fluctuating adherence of ITN users over the course of the night was modelled. As here a more detailed behavioural survey was taken into account, the nightly biting rate of a protected individual is calculated by multiplying the proportion of time spend outdoors at a certain time of the night by the outdoor biting rate at that time \( (B_{o,t}) \) plus the proportion of that hour being indoors but not asleep \( (I_t - S_t) \) multiplied by the indoor biting rate during that hour \( (B_{i,t}) \) plus the proportion of that time spent indoors being asleep under an ITN multiplied by the indoor biting rate at that hour \( (B_{i,t}) \) times the proportion of bites which can not be prevented by an ITN \( (1-P) \), as measured in experimental hut trials [44, 97, 98]. The effective adherence to ITN use at a given time of the night was assumed to be equivalent to the proportion of people sleeping at that time \( (S_t) \). This assumption allows us to express the overall effect of this interaction as follows:

\[
B_p = \sum_{t=1}^{24} B_{p,t} = \sum_{t=1}^{24} [B_{o,t}(1-I_t) + B_{i,t}(I_t - S_t) + B_{i,t} S_t (1-P)]
\]

Based on existing evidence from experimental hut trials [49, 97, 98], a conservative minimum protective efficacy level of 80% for ITNs \( (P = 0.8) \), equivalent to a relative exposure to bites of 20% when, and only when, actually sleeping under the net, was assumed. In this study, it was possible to take into account the proportion of people staying indoors or outdoors during waking hours and experiencing the corresponding biting rate. Furthermore, there was the possibility even to do the same for people living in different house quality who spent different amount of time in different compartments. During sleeping hours, people staying indoors were presumed sleeping under an ITN if available, whereas people sleeping outdoors were presumed not using a net and being fully exposed to the outdoor biting rate.
Taking the data for nightly human and mosquito behaviour profiles, the relative biting rate for ITN users which is equivalent to relative availability of protected individuals ($\lambda_p$) as previously defined (See equations 8 and 14 in reference [95]), could be estimated. $\lambda_p$ was calculated by comparing the total biting rate that protected individuals are exposed to ($B_p$) with that of non-users ($B_u$) who are unprotected:

$$\lambda_p = \frac{B_p}{B_u}$$

The true protective efficacy of an ITN ($P^*$) against transmission exposure is then calculated as the overall nightly reduction of biting rate:

$$P^* = 1 - \lambda_p$$

This estimate of protective efficacy differs from that previously reported from experimental hut trials as well as previous applications of this approach [85], because it allows for typical shortcomings in adherence resulting from the time people typically spend outside of their ITN indoor, as well as outdoors and even considering people staying or sleeping the whole night outdoors. Note, however, that this estimate is merely a comparison between the biting rates experienced by those who use an ITN and those who do not. It does not include the community-level protection of both groups when ITNs reach sufficient levels of coverage to reduce vector biting densities and sporozoite prevalence over large areas [95].

Distinct and useful indicators with which to interpret the results of the above equations are the proportion of exposure which occur indoors and the proportion that occurs during sleeping hours. The proportion of bites that occur during the observed peak sleeping hours ($\pi_s$) for an unprotected individual can thus be calculated as the nightly biting rate experienced during these hours divided by the total nightly biting rate:

$$\pi_s = \frac{\Sigma_{t=10pm}^{6am} B_{u,t}}{\Sigma_{t=1}^{24} B_{u,t}}$$
Note that $\pi_s$ describes the proportion of human exposure during which an ITN is in use and is used as a key parameter for modelling the community- and individual-level effects of ITNs upon malaria transmission [95]. Overall, $\pi_s$ was usually calculated using median reported values of 10 pm to 6 am for the whole study area but was evaluated separately for individual houses or houses with different quality of screening and ceiling boards for some analysis.

The proportion of bites occurring indoors but while awake and, therefore, not protected by a bednet ($\pi_a$) can be calculated as the estimated number of bites estimated to occur indoors while awake, divided by the total number of bites estimated to occur both indoors and outdoors:

$$
\pi_a = \frac{\sum_{t=1}^{24} [B_{i,t} (I_t - S_t)]}{\sum_{t=1}^{24} [B_{o,t} (1-I_t) + B_{i,t} I_t]}
$$

The proportion of bites occurring indoors ($\pi_i$) for an unprotected individual can be calculated as the total number of bites estimated to occur indoors, divided by the total number of bites estimated to occur both indoors and outdoors. It should be noted that this equivalent to summing $\pi_a$ and $\pi_s$:

$$
\pi_i = \pi_a + \pi_s = \frac{\sum_{t=1}^{24} [B_{i,t} I_t]}{\sum_{t=1}^{24} [B_{o,t} (1-I_t) + B_{i,t} I_t]}
$$

**Ethical considerations**

All activities of the UMCP, including these field surveys are approved by the Medical Research Coordination Committee of the National Institute for Medical Research, Ministry of Health, Government of Tanzania (Reference numbers NIMR/HQ/R.8a/Vol. IX/279 and 324). No persons in high risk groups, namely people under 18 years or women of reproductive age, were recruited to conduct human landing catches. Furthermore, the human landing catchers were screened every week for malaria microscopic examination of thick smear peripheral
blood samples and treated with artemisinin-based combination therapy when diagnosis was positive.

3.4 Results and Discussion

Preliminary surveys of the entire study area
In the areas in Dar es Salaam which were covered by the urban malaria control programme (UMCP) during the first three rounds of the household surveys, bed net usage was quite high and mosquito-proofed houses were common with many being made of concrete or bricks with a corrugated iron roof (Table 1). Around half of the houses had a complete ceiling board and/or good screening although a small proportion of residents didn’t use any protection measures at all. The same was true in the TCUs which were selected for the more detailed study (Table 2). When compared to historical reports from Dar es Salaam, bednet usage had increased whereas the use of other protective measures had decreased [34]. In contrast, in the Kilombero Valley in southern Tanzania, where ITNs have been promoted since 1997, bednet use is currently approximately at the same level, but both treatment of these nets and the use of other protective measures (coil, spray or repellent) are higher in Dar es Salaam (Killeen et al, Unpublished). Bed net usage in two contemporary Kenyan cities in 2001 was slightly lower and it should be noted that while screening of houses was less common than in Dar es Salaam, use of personal protection measures was more common [99].
### Table 1

Characteristics of the houses and residents in all 15 wards of the study area in Dar es Salaam, Tanzania, during the first three rounds of household surveys from May 2004 until May 2006.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Houses</strong></td>
<td>3073</td>
<td>100</td>
</tr>
<tr>
<td><strong>Walls</strong></td>
<td>3073</td>
<td>100</td>
</tr>
<tr>
<td>Stone, cement, fired or concrete bricks</td>
<td>1684</td>
<td>54.4</td>
</tr>
<tr>
<td>Unfired bricks, sand, wood</td>
<td>1355</td>
<td>43.7</td>
</tr>
<tr>
<td>Corrugated iron sheets, mud, grass</td>
<td>59</td>
<td>1.9</td>
</tr>
<tr>
<td>Grass thatch, cardboard</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Roof</strong></td>
<td>3073</td>
<td>100</td>
</tr>
<tr>
<td>Tiles, cement, reinforced concrete</td>
<td>193</td>
<td>6.3</td>
</tr>
<tr>
<td>Corrugated iron sheets, asbestos</td>
<td>2868</td>
<td>93.3</td>
</tr>
<tr>
<td>Thatch, sticks, mud, grass, plastic sheets</td>
<td>11</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>Ceiling board</strong></td>
<td>3066</td>
<td>100</td>
</tr>
<tr>
<td>Whole house</td>
<td>829</td>
<td>27</td>
</tr>
<tr>
<td>Partly</td>
<td>554</td>
<td>18.1</td>
</tr>
<tr>
<td>None</td>
<td>1683</td>
<td>54.9</td>
</tr>
<tr>
<td><strong>Screening</strong></td>
<td>3057</td>
<td>100</td>
</tr>
<tr>
<td>Intact</td>
<td>684</td>
<td>22.4</td>
</tr>
<tr>
<td>With holes</td>
<td>1006</td>
<td>32.9</td>
</tr>
<tr>
<td>Incomplete</td>
<td>503</td>
<td>16.5</td>
</tr>
<tr>
<td>Glass windows</td>
<td>105</td>
<td>3.4</td>
</tr>
<tr>
<td>None</td>
<td>759</td>
<td>24.8</td>
</tr>
<tr>
<td><strong>Residents</strong></td>
<td>20289</td>
<td>100</td>
</tr>
<tr>
<td>Bednet coverage</td>
<td>20285</td>
<td>100</td>
</tr>
<tr>
<td>User</td>
<td>16883</td>
<td>83.2</td>
</tr>
<tr>
<td>Non-user</td>
<td>3402</td>
<td>16.8</td>
</tr>
<tr>
<td><strong>Treatment status of net</strong></td>
<td>16883</td>
<td>100</td>
</tr>
<tr>
<td>Treated in last 6 months</td>
<td>5194</td>
<td>30.8</td>
</tr>
<tr>
<td>Treated more than 6 months ago</td>
<td>66</td>
<td>0.4</td>
</tr>
<tr>
<td>Never treated</td>
<td>11623</td>
<td>68.8</td>
</tr>
<tr>
<td><strong>Other protection against mosquitoes</strong></td>
<td>20287</td>
<td>100</td>
</tr>
<tr>
<td>Coil</td>
<td>1245</td>
<td>6.1</td>
</tr>
<tr>
<td>Spray</td>
<td>2167</td>
<td>10.7</td>
</tr>
<tr>
<td>Repellent</td>
<td>307</td>
<td>1.5</td>
</tr>
<tr>
<td>None</td>
<td>16571</td>
<td>81.7</td>
</tr>
<tr>
<td><strong>Usage of at least 1 protection measure</strong></td>
<td>20289</td>
<td>100</td>
</tr>
<tr>
<td>Net, coil, spray, repellent</td>
<td>17437</td>
<td>85.9</td>
</tr>
<tr>
<td>None</td>
<td>2852</td>
<td>14.1</td>
</tr>
</tbody>
</table>
Table 2 Protection measures against mosquitoes in urban Dar es Salaam in the past and present, in rural Tanzania and in two Kenyan cities.

<table>
<thead>
<tr>
<th>Location</th>
<th>Net usage</th>
<th>Net treatment status</th>
<th>Window screening</th>
<th>Other protection measures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>N</td>
<td>In the last 6 months</td>
<td>More than 6 months</td>
</tr>
<tr>
<td>Urban Kenya and Tanzania</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dar es Salaam; 2006</td>
<td>78.8</td>
<td>1696</td>
<td>35.9</td>
<td>0.6</td>
</tr>
<tr>
<td>Dar es Salaam; 1994</td>
<td>62</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kisumu, Kenya; 2001</td>
<td>56</td>
<td>287</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Malindi, Kenya; 2001</td>
<td>69</td>
<td>332</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Rural Reference Site</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kilombero, Valley Tanzania; 2003</td>
<td>74.5</td>
<td>650</td>
<td>4.7</td>
<td>6.6</td>
</tr>
</tbody>
</table>

Data derived from the study presented here.

Any type of protection measures (spray, coil, herbal, physical)
A total of 1,388 *An. gambiae* s.l. (meaning members of the species complex as a whole in the absence of further identification to species by cytological or molecular methods) were caught in 1,650 catcher-nights, through routine monitoring activities of the UMCP during the preliminary survey of the entire study area (Figure 2). The majority of these proved to be *An. gambiae* (often referred to as *An. gambiae sensu stricto*): 75.6%, 21.3% and 3.1% of 1099 successfully amplified specimens proved to be *An. gambiae s.s.*, *An. arabiensis* and *Anopheles merus*, respectively. During the same preliminary surveys, only 55 *An. funestus* were caught, indicating that although it is usually a very efficient vector [87], its contribution to transmission in urban Dar es Salaam is minor. Nevertheless, sporozoite infection and local transmission within urban Dar es Salaam was confirmed for *An. gambiae s.s.* (0.24%; 2/831) and *An. funestus* (2.32%, 1/43), but not *An. arabiensis* (0.0%, 0/234) and *An. merus* (0.0%, 0/34). Estimates of actual transmission intensity and its spatio-temporal heterogeneity over longer, more representative time periods will be reported in detail elsewhere. The only other *Anopheles* species caught was *Anopheles coustani* (370), of which none were found to be sporozoite-infected, so it is thought to contribute little or no vectorial capacity as described elsewhere [87].

*Anopheles gambiae s.s.* was by far the most important vector in the study area so all subsequent analysis focus upon this species and, to a lesser extent, *An. arabiensis*. Based on preliminary surveys of the total study area, location had no influence upon the proportion of *An. gambiae s.l.* bites which occurred between 10 pm and 6 am when residents of Dar es Salaam typically slept (*An. gambiae s.s.*: P=0.519 by logistic regression, N=72 locations, n=714 mosquitoes, *An. arabiensis*: P=0.398 by logistic regression, N=32 locations, n=133 mosquitoes). The great majority of the combined bites of these species occurred during sleeping hours ($\pi_s = 83.16\%$; equation 6). Subsequent detailed surveys of mosquito and
human behaviours therefore focussed upon the 14 TCUs with the highest *An. gambiae* densities observed during the preliminary site-wide surveys (Figure 1).

**Figure 2.** Hourly biting profile of *An. gambiae s.l.* based on averaged results of routine outdoor human landing catches from across the entire study area covered by the Urban Malaria Control Programme.

**Detailed focal surveys of household and personal protection**

A total of 2,153 people were living in these 216 houses at the time of survey, of whom approximately half were under the age of 22 (Table 3). All the TCU were either near a swamp or close to a depression with poorly functioning drains and most of these areas were partially flooded during the rains. Although these were mostly poorer, unplanned areas, half of the houses had intact screening or screening with small holes. Almost three quarters of these houses did not have a ceiling board and it was typically observed that the eaves of most houses in Dar es Salaam were accessible to mosquitoes. Although more than three quarters of residents slept under a net, only a third of these nets had ever been treated with insecticide. Very few residents reported using alternative protective measures such as repellents, mosquito coils or insecticidal sprays (Table 3).
Table 3 House characteristics and human behaviour traits (time period from February to June 2006) of the areas in Dar es Salaam where mosquitoes were sampled indoors and outdoors.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1 year</td>
<td>62</td>
<td>2.9</td>
</tr>
<tr>
<td>1-5 years</td>
<td>231</td>
<td>10.7</td>
</tr>
<tr>
<td>5-14 years</td>
<td>403</td>
<td>18.7</td>
</tr>
<tr>
<td>&gt;14 years</td>
<td>1457</td>
<td>67.7</td>
</tr>
<tr>
<td><strong>Ceiling board</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole house</td>
<td>37</td>
<td>16.6</td>
</tr>
<tr>
<td>Partly</td>
<td>27</td>
<td>12.1</td>
</tr>
<tr>
<td>None</td>
<td>159</td>
<td>71.3</td>
</tr>
<tr>
<td><strong>Screening</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>44</td>
<td>19.7</td>
</tr>
<tr>
<td>With holes</td>
<td>90</td>
<td>40.4</td>
</tr>
<tr>
<td>Incomplete</td>
<td>31</td>
<td>13.9</td>
</tr>
<tr>
<td>Glass windows</td>
<td>2</td>
<td>0.9</td>
</tr>
<tr>
<td>None</td>
<td>56</td>
<td>25.1</td>
</tr>
<tr>
<td><strong>Bednet usage</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>1695</td>
<td>78.8</td>
</tr>
<tr>
<td>&lt;1 year</td>
<td>53</td>
<td>96.4</td>
</tr>
<tr>
<td>1-5 years</td>
<td>213</td>
<td>92.6</td>
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<tr>
<td>1-5 years</td>
<td>322</td>
<td>79.9</td>
</tr>
<tr>
<td>&gt;14 years</td>
<td>1107</td>
<td>76</td>
</tr>
<tr>
<td><strong>Treatment status of net</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated in last 6 months</td>
<td>774</td>
<td>35.9</td>
</tr>
<tr>
<td>Treated more than 6 months ago</td>
<td>11</td>
<td>0.6</td>
</tr>
<tr>
<td>Never treated</td>
<td>1368</td>
<td>63.5</td>
</tr>
<tr>
<td><strong>Other protection against mosquitoes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coil</td>
<td>198</td>
<td>9.2</td>
</tr>
<tr>
<td>Spray</td>
<td>343</td>
<td>15.9</td>
</tr>
<tr>
<td>Repellent</td>
<td>158</td>
<td>7.3</td>
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<tr>
<td>None</td>
<td>1454</td>
<td>67.6</td>
</tr>
<tr>
<td><strong>Eating location</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indoor</td>
<td>783</td>
<td>74.1</td>
</tr>
<tr>
<td>Outdoor</td>
<td>270</td>
<td>25.6</td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
<td>0.3</td>
</tr>
<tr>
<td><strong>Dinner time</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before 7 pm</td>
<td>59</td>
<td>5.6</td>
</tr>
<tr>
<td>Between 7 and 8.30 pm</td>
<td>492</td>
<td>46.6</td>
</tr>
<tr>
<td>After 8.30 pm</td>
<td>505</td>
<td>47.8</td>
</tr>
<tr>
<td><strong>Resting location after dinner</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indoor</td>
<td>505</td>
<td>47.8</td>
</tr>
<tr>
<td>Outdoor</td>
<td>540</td>
<td>51.1</td>
</tr>
<tr>
<td>Other or don’t know</td>
<td>11</td>
<td>1.1</td>
</tr>
<tr>
<td><strong>Bedtime</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before 6 pm</td>
<td>3</td>
<td>0.3</td>
</tr>
<tr>
<td>Between 6 and 7 pm</td>
<td>18</td>
<td>1.7</td>
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<tr>
<td>Between 7 and 8 pm</td>
<td>48</td>
<td>4.5</td>
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<tr>
<td>Between 8 and 9 pm</td>
<td>117</td>
<td>11.1</td>
</tr>
<tr>
<td>Between 9 and 10 pm</td>
<td>312</td>
<td>29.5</td>
</tr>
<tr>
<td>Between 10 and 11 pm</td>
<td>379</td>
<td>35.9</td>
</tr>
<tr>
<td>Between 11 and 12 pm</td>
<td>125</td>
<td>11.8</td>
</tr>
<tr>
<td>After 12 pm</td>
<td>53</td>
<td>5</td>
</tr>
<tr>
<td>Don’t know</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Waking time</strong></td>
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<td></td>
</tr>
<tr>
<td>Before 4 am</td>
<td>4</td>
<td>0.4</td>
</tr>
<tr>
<td>Between 4 and 5 am</td>
<td>23</td>
<td>2.2</td>
</tr>
<tr>
<td>Between 5 and 6 am</td>
<td>173</td>
<td>16.4</td>
</tr>
<tr>
<td>Between 6 and 7 am</td>
<td>509</td>
<td>48.2</td>
</tr>
<tr>
<td>After 7 am</td>
<td>346</td>
<td>32.8</td>
</tr>
<tr>
<td>Don’t know/didn’t respond</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Sleeping location</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outdoor sleeping</td>
<td>56</td>
<td>5.3</td>
</tr>
<tr>
<td>Indoor sleeping</td>
<td>1000</td>
<td>94.7</td>
</tr>
</tbody>
</table>
**Human-mosquito behavioural interactions**

The reported and observed behaviours of humans were largely consistent (Figure 3A). The minor discrepancies can be explained as follows. Less people were observed than reported outdoors in the evenings and mornings, because it was not possible for us to enter all courtyards and some individuals may be elsewhere during these hours. More people were observed than reported to be outdoors towards midnight but, based on direct experience, this was attributed to the transition of people through the TCU who do not live there. The residents reported that shortly after 10 pm, 50% of the people had gone to bed and at around 6 am 50% of the people were still asleep. A small, but noteworthy, proportion of residents slept outdoors all night (Table 3), often citing heat and poor ventilation inside the house as their primary motivation.

During the intensive entomological study in the selected sites with high *An. gambiae* densities, 432 catcher-nights yielded 2,484 *An.gambiae* s.l., 63 *An. funestus*, 370 *An. coustani*, 41,290 *Culex*, 70 *Aedes* and 97 *Mansonidae*. Of the 2,027 *An. gambiae* s.l. which were successfully amplified, 83.9%, 15.9% and 0.2% were identified as *An. gambiae* s.s., *An. arabiensis* and *An. merus*, respectively. Only 0.41% (7/1700) of *An. gambiae* s.s. and 0.31% (1/322) and *An. arabiensis* were found to be infected with sporozoites. *An. gambiae* s.s., *An. arabiensis*, *An. funestus*, *An. coustani* and Mansonidae were all exophagic, meaning that they mainly bite outdoors [100] as evidenced by the proportion of mosquitoes caught outside being significantly greater than half (Figures 3 and 4). *Anopheles gambiae* s.l. is generally endophagic in rural Tanzania [36, 87, 101] and the proportion of *An. gambiae* s.l. caught outdoors was higher in Dar es Salaam than in Kilombero valley (Figure 4; 63 versus 34 %, respectively; $\chi^2 = 597.1$, P <0.001), considering only catches up to 6 am because the studies in Kilombero valley stopped at this time.
Figure 3. Human and mosquito behavioural patterns in Dar es Salaam, Tanzania. A. Number or proportion of time residents spend outdoors, comparing what they reported themselves with direct observations in the field. B. Mean numbers of *An. gambiae* s.s caught indoors and outdoors. C. Mean number of *An. arabiensis* caught indoors and outdoors. D. Mean number of *An. gambiae* s.l. caught indoors and outdoors.
In Dar es Salaam, the proportion of *An. arabiensis* caught outdoors was significantly higher than the proportion of *An. gambiae s.s.* caught outdoors ($\chi^2 = 23.4$, P-value < 0.001). *Culex* sp. and *Aedes* sp. exhibited neither exo- nor endophagic tendencies in Dar es Salaam.

**Figure 4.** Comparison of exophagic and endophagic behaviour of different mosquito species in urban and rural Tanzania. Degree of exophagy or endophagy is presented as the proportion of mosquitoes caught outdoors so that all mosquitoes with a proportion of outdoor biting significantly greater than 0.5 are considered to be exophagic and all below 0.5 are considered endophagic.

Hourly biting pattern almost exactly followed classically reported patterns of *An. gambiae s.l.* [87] with an increase of *Anopheles gambiae s.s.* densities towards midnight, and a second peak around 4 - 5 am, followed by a decline towards dawn (Figure 3B). In fact, the proportions of *An. gambiae s.s* mosquitoes caught during peak sleeping hours was greater in the city than in the rural area ($\chi^2 = 112.9$, P <0.001) with peak sleeping hours in Kilombero valley from 9pm to 5am and in Dar es Salaam from 10pm to 6am. As summarized in Figure 4,
biting activity was more intense outdoors than indoors throughout the night and was highest during sleeping hours (Figure 3B). *An. gambiae s.s.* constituted 84 % of *An. gambiae s.l.* and therefore dominates the shape of the curve for the pooled sibling species (Figure 3D).

Nevertheless, it is noteworthy that *An. arabiensis* had its peak biting time at 10 pm, when more than three quarters of the residents were still awake, and then slowly declined towards the morning (Figure 3C).

Combining the human and mosquito behavioural surveys, and using the model described in the methods section, allowed estimation of the biting rates experienced by residents at each hour of the night (Figure 5). This approach also allowed dissection of these mosquito-human interactions into distinct domestic compartments (Figure 6) where specific interventions may or may not reduce exposure. For example, ITNs are expected only to provide personal protection while sleeping so their protective efficacy is limited to those times of the night when users sleep and cannot exceed the proportion of exposure which would otherwise occur while asleep (\(\pi_s\); equation 6). In contrast, interventions which prevent house entry, such as mosquito proofing [60, 61] or spatial repellents such as DDT [100], could prevent any indoor exposure regardless of whether occupants are awake or in bed (\(\pi_i\); equation 8). It should be noted that the simpler form of this approach applied previously [85] did not allow estimation of exposure indoors while awake so it is not possible to compare Dar es Salaam with this rural precedent in terms of the relative contributions of exposure indoors and outdoors while awake. Nevertheless, it is possible to compare the proportion of exposure which an ITN might be expected to prevent (\(\pi_c\); equation 6).
Figure 5. Exposure to biting of *An. gambiae* s.s. for ITN users and non-users. The shadings represent the proportion of time spend in each compartment (outdoor; $1 - \pi_i$; equation 8, indoor awake; $\pi_a$; equation 7, indoor asleep; $\pi_s$; equation 6). Exposure to biting is shown overall as well as for different house qualities: Screened (Glass windows, screening with no or small holes), unscreened (no screening or badly torn/incomplete screens), ceiling (complete ceiling or partly ceiling), no ceiling (no ceiling board).
Figure 6. Proportion of people present in each compartment and their estimated exposure if not using a bednet (outdoor; \(1-\pi_i\); equation 8, indoor awake; \(\pi_a\); equation 7, indoor asleep; \(\pi_s\); equation 6), presented as an overall mean and for categories of different house qualities: Screened (Glass windows, screening with no or small holes), unscreened (no screening or badly torn), ceiling (complete ceiling or partly ceiling), no ceiling (no ceiling board).

Even though *An. gambiae s.s.* were exophagic in urban Dar es Salaam, a high quality ITN was expected to confer 59% protection against exposure to this mosquito for a typical resident in a typical house. Although such protection against exposure is clearly incomplete, it is almost as high as the 70% protection afforded against highly endophagic *An. gambiae* in rural Kilombero [85] which is known to provide effective protection against clinical disease even in this highly endemic rural setting [102, 103]. This slightly lower level of protection against exposure is because the number of bites which normally occur indoors and during sleeping
hours were lower in the city (79% and 74%, respectively) than in the rural area (90% and 80%, respectively). The less abundant *An. arabiensis* was not only exophagic in Dar es Salaam but also most active just before 10pm (Figure 3C) so the personal protection by an ITN against exposure to this species is estimated to be only 38%.

**Interdependence of protection measures and mosquito densities**

Members of the *An. gambiae* *s.l.* complex dominated malaria transmission in Dar es Salaam and, of these, only *An. gambiae s.s.* was present in sufficient numbers to undertake the following analysis in a meaningful way. The following results only describe those for *An. gambiae s.s.*, as confirmed by PCR, and assume it is responsible for essentially all transmission in the study area. In well-screened (glass windows, screening with no or small holes) and houses with complete ceiling boards (complete and partly ceiling board) ITNs conferred slightly less protection against *An. gambiae s.s.* because the proportion (Figures 5 and 6) and total (Figure 7) levels of exposure in such houses that occurred indoors were lower. It should be noted that much of the reduction of proportional and total exposure achieved with screening and ceilings resulted from adaptive changes in human behaviour with occupants spending more of their waking hours in the safer confines of the house (Figures 6 and 7).

Exploratory pair-wise correlation analysis showed that complete ceilings were associated with use of other protection methods ($r^2 = 0.323$, $P<0.01$) and good house screening ($r^2 = 0.267$, $P<0.01$), which was in turn associated with high outdoor densities of *Culex* sp ($r^2 = 0.136$, $P<0.05$). Interestingly, use of ITNs was associated with high indoor densities of *Culex* sp ($r^2 = 0.137$, $P<0.05$) and use of any bednet was negatively correlated with complete ceilings ($r^2 = -0.194$, $P<0.01$) and other protection methods ($r^2 = -0.209$, $P<0.01$). This suggests that
installation and maintenance of ceilings and screening, is motivated by local densities of nuisance mosquitoes whereas use of bednets may be a response to the failure or inability to apply these for socioeconomic reasons. The overall biting densities of *An. gambiae* showed only a negative association with complete ceilings ($r^2 = -0.160, P<0.05$) and good screening ($r^2 = -0.136, P<0.05$), suggesting that this vector species contributes little to motivating their utilization. Also, consistent with their known preference for eave entry and the results presented in figures 6 and 7, ceilings do confer protection against exposure to malaria transmission as does, to a lesser extent, good screening.

**Figure 7.** Mean number of bites received by a person in each of the three domestic and peri-domestic compartments (outdoor; $1-\pi_i$; equation 8, indoor awake; $\pi_a$; equation 7, indoor asleep; $\pi_s$; equation 6).

Principal component analysis of the relationship between vector densities and the various protection measures surveyed revealed three important factors (Table 4), suggesting that the uptake and use of these interventions is driven by a number of motivations and constraints in a complex manner (Figure 8). Interestingly, Factor 2 shows clear increase in use of all protective measures associated with increased density of *Culex* sp. but not *An. gambiae s.l.*, probably reflecting the motivation for uptake of all interventions at high densities of nuisance biting. Factors 1 and 3 seem to reflect quite different underlying motivations or limitations.
that determine intervention utilization at household level and interact to a greater or lesser extent with mosquito density. Factor 1 shows a clear association of mosquito proofed houses with low usage rates of treated or untreated bednets and with high usage rates of other protective measures. This maybe reflects the influence of socioeconomic status on the choices of interventions used by households with mosquito-proofing and other measures probably being associated with better households while bednets may be utilized to a greater extent in houses which cannot afford these. Factor 3 appears to be almost completely independent of bednet use, but exhibits a clear association of the use of other interventions with high densities of *An. gambiae s.l.* and poor or absent window screening. It is suggested that factor three reflects the response of residents to indoor exposure to *An. gambiae*, perhaps as a proxy for malaria transmission, when window screening is not present. However these suggestions have to be looked at with caution as they remain speculative until such surveys of practice are conducted on larger population scales and complemented with direct evaluations of socioeconomic and educational status, as well as associated knowledge and attitudes.

**Table 4** Protective measures and malaria and nuisance mosquito densities and their scores in three different factors and the percent of the variance these factors account for derived through principal component analysis.

<table>
<thead>
<tr>
<th></th>
<th>Factor 1</th>
<th>Factor 2</th>
<th>Factor 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>% of Variance</strong></td>
<td>24.55</td>
<td>21.41</td>
<td>15.67</td>
</tr>
<tr>
<td><strong>Scores</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete ceiling</td>
<td>- 0.477</td>
<td>0.646</td>
<td>0.039</td>
</tr>
<tr>
<td>Good screening</td>
<td>- 0.155</td>
<td>0.629</td>
<td>- 0.362</td>
</tr>
<tr>
<td>Other personal protection</td>
<td>- 0.528</td>
<td>0.392</td>
<td>0.374</td>
</tr>
<tr>
<td>Bednet use</td>
<td>0.773</td>
<td>0.236</td>
<td>- 0.223</td>
</tr>
<tr>
<td>ITN use</td>
<td>0.628</td>
<td>0.508</td>
<td>- 0.076</td>
</tr>
<tr>
<td>Mean log (<em>An. gambiae s.l.</em>)</td>
<td>0.334</td>
<td>- 0.068</td>
<td>0.764</td>
</tr>
<tr>
<td>Mean log Culex</td>
<td>0.289</td>
<td>0.463</td>
<td>0.430</td>
</tr>
</tbody>
</table>

*a* Complete and partly complete ceiling board

*b* Screening without holes, with small holes or glass windows

*c* Coils, spray and / or repellent
Figure 8. Three factors derived through principal component analysis and their association with different protective measures as well as mosquito densities.
3.5 Conclusions

Although the hourly biting pattern of *An. gambiae s.s.* remains essentially consistent with classical reports, *An. arabiensis* appears to have a much earlier peak biting time at 10 pm when a large proportion of people are still outdoors. ITNs confer little protection against exposure to this species, which is fortunately relatively rare in urban Dar es Salaam. *Anopheles arabiensis* only account for 16% of the *An. gambiae* complex in Dar es Salaam, so ITNs still provide useful individual protection. However, the observations from Dar es Salaam can have greater implications for malaria control in Africa where *An. arabiensis* is a very common and an important vector [5, 88, 104]. It cannot be determined whether the early biting of *An. arabiensis* in Dar es Salaam was induced by ITN use and/or improved housing quality. In this context, it seems relevant to note that this *An. arabiensis* is more tolerant to desiccation than *An. gambiae* [88, 105, 106] and may, therefore, be able to adapt more readily to earlier feeding despite the relatively low humidity that occurs in the early evening. The surprisingly exophagic behavior of *An. gambiae* in Dar es Salaam may also arise from increased bednet coverage as well as housing quality. This is consistent with another recently reported urban context [80] and an increasing number of sites in rural Africa [107-111].

Despite the clear exophagy of malaria vectors in Dar es Salaam, like elsewhere in Africa, ITNs confer useful but incomplete personal protection [59, 112]. Much bigger reductions of transmission can be attained at community level where high population coverage is achieved [44, 95, 113, 114]. Although additional vector control measures are desirable to cope with the remaining quarter of human exposure which occurs outdoors, ITNs should remain a high priority in urban settings. ITNs appear to be a second preference intervention in Dar es Salaam, with mosquito-proofing of houses being the most commonly implemented measure.
and probably the first choice of residents. It may, therefore, be feasible to develop programmes which promote and subsidize such efforts by vulnerable households to tackle their local malaria problems. Additional important options to prevent outdoor transmission include larviciding [115, 116] and environmental management [117-119], all of which merit further development as components of integrated programmes [1] in the tropical belt of Africa, where malaria transmission is at its most intense [5].

**Competing interests**

Part of the Urban Malaria Control Programme is financed by Valent Biosciences Corporation, a manufacturer of microbial larvicides. A substantial portion of the current salary and research support for the investigators depends on the achievement of documented suppression of malaria transmission and infection risk by this programme through systematic larviciding.

**Authors' contributions**

YG designed and implemented the study, analysed the data and drafted the manuscript. PC, BE, NJG, RS, VM, DM, HM, UF, SWL and KK were involved in designing and implementation the study. MCdC, MT and GFK participated in the study design, data analysis and drafting of the manuscript. All authors read and approved the final manuscript.

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Article 1: Domestic malaria prevention measures and mosquito-human interactions


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Article 1: Domestic malaria prevention measures and mosquito-human interactions


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Article 1: Domestic malaria prevention measures and mosquito-human interactions
4. A tool box for operational mosquito larval control: preliminary results and early lessons from the Urban Malaria Control Programme in Dar es Salaam, Tanzania

Ulrike Fillinger1*, Khadija Kannady2, George William2, Michael J. Vanek3, Stefan Dongus3,4, Dickson Nyika5,6, Yvonne Geissbühler2,3,5, Prosper P. Chaki1,2,5, Nico J. Govella1,2,5 Evan M. Mathenge7, Burton H. Singer8, Hassan Mshinda5, Steven W. Lindsay1, Marcel Tanner3, Deo Mtasiwa2, Marcia C. de Castro9 and Gerry F. Killeen1,3,5

1Durham University, School of Biological and Biomedical Sciences, South Road, Durham DH13LE, UK
2Dar es Salaam City Council, Ministry of Regional Administration and Local Government, United Republic of Tanzania;
3Swiss Tropical Institute, Department of Public Health and Epidemiology, P.O. Box, 4002 Basel, Switzerland;
4University of Freiburg, Department of Physical Geography, Freiburg, Germany;
5Ifakara Health Research and Development Centre, Coordination Office, PO Box 78373, Kiko Avenue, Mikoche, Dar es Salaam, United Republic of Tanzania;
6Ministry of Agriculture and Food Security, Dar es Salaam, United Republic of Tanzania;
7Kenya Medical Research Institute, PO Box 54840, Nairobi, Kenya;
8Office of Population Research, Princeton University, Princeton, NJ08544, USA;
9Harvard School of Public Health, Department of Population and International Health, 665 Huntington Avenue, Boston, MA 02115, USA

* Corresponding Author:
Ulrike Fillinger, 1Durham University, School of Biological and Biomedical Sciences, Durham DH13LE, United Kingdom; E-mail: ulrike.fillinger@durham.ac.uk

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4.1 Abstract

**Background:** As the population of Africa rapidly urbanizes, large populations could be protected from malaria by controlling aquatic stages of mosquitoes if cost-effective and scalable implementation systems can be designed.

**Methods:** A recently initiated Urban Malaria Control Programme in Dar es Salaam delegates responsibility for routine mosquito control and surveillance to modestly-paid community members, known as Community-Owned Resource Persons (CORPs). New vector surveillance, larviciding and management systems were designed and evaluated in 15 city wards to allow timely collection, interpretation and reaction to entomologic monitoring data using practical procedures that rely on minimal technology. After one year of baseline data collection, operational larviciding with *Bacillus thuringiensis var. israelensis* commenced in March 2006 in three selected wards.

**Results:** The procedures and staff management systems described greatly improved standards of larval surveillance relative to that reported at the outset of this programme. In the first year of the programme, over 65,000 potential *Anopheles* habitats were surveyed by 90 CORPs on a weekly basis. Reaction times to vector surveillance at observations were one day, week and month at ward, municipal and city levels, respectively. One year of community-based larviciding reduced transmission by the primary malaria vector, *Anopheles gambiae s.l.*, by 31% (95% C.I.=21.6-37.6%; p=0.04).

**Conclusion:** This novel management, monitoring and evaluation system for implementing routine larviciding of malaria vectors in African cities has shown considerable potential for sustained, rapidly responsive, data-driven and affordable application. Nevertheless, the true programmatic value of larviciding in urban Africa can only be established through longer-
term programmes which are stably financed and allow the operational teams and management infrastructures to mature by learning from experience.
4.2 Background

With the prospect of more than half of the African population living in urban areas by the year 2030, it is anticipated that the challenge and opportunity for tackling malaria burden in urban areas will also grow [1-3]. Compared to rural settings, malaria in urban Africa is generally characterized by lower intensities and more focal distribution of transmission, resulting in weaker immunity in the afflicted population and distribution of disease burden across older age groups [2, 3]. Compared to rural settings, urban areas usually offer more malaria control options because relatively good transport, communication, educational and health infrastructure is available to large populations in small geographic areas. Since there is relatively easy access to most urban area breeding sites, control interventions such as environmental control and larvicide application may be cost-effective [2, 3], but remain to be rigorously evaluated in the modern African context [4-6]. Although locally targeted approaches [7-9] are desirable, and this may be realizable in the future [10-13], all documented successes of larval control against African malaria vectors have depended on rigorous and comprehensive surveillance for aquatic stage mosquitoes [14] to enable wholesale suppression [15] and even elimination [16, 17]. To be sustainable in the context of African cities today, integrated vector management needs to be implemented through community-based systems using simple tools that are appropriately tailored to the enormous reservoir of affordable labour that is available in situ [18-20].

Although most malaria research has generally focused on rural settings [1-3, 21], Dar es Salaam in Tanzania is one of the few African cities in which the distinctive characteristics of urban malaria ecology and epidemiology have been examined in depth with useful records dating back almost a century [22-25]. The main vectors of malaria in the area of Dar es
Salaam are *Anopheles gambiae sensu stricto*, *Anopheles arabiensis*, *Anopheles funestus* and *Anopheles merus* [26]. *Plasmodium falciparum* is the most common malaria parasite, accounting for 90% of all cases [22]. Interestingly, malaria vectors in the city appear to have adapted to high coverage with bed nets and improved housing by predominantly feeding outdoors [26]. Thus, insecticide-treated nets confer slightly less protection than in rural areas so additional measures directed at aquatic stages of vector mosquitoes may have a useful role in this and similar urban settings [26].

This publication describes the principles and practices of a novel management system for implementing, monitoring and optimizing routine larviciding in African cities that was developed at the City Council of Dar es Salaam in Tanzania. It aims to provide an array of tools which can be adapted to different ecological settings for programmes aiming to integrate anti-larval interventions in ongoing malaria control programmes. Furthermore, preliminary results obtained in the first year of operation are described and the potential of these systems are discussed.

4.3 Material and Methods

Study site

The study was conducted in Dar es Salaam, Tanzania’s biggest and economically most important city with 2.7 million inhabitants and a total area of 1400 km² [22, 27]. The city is divided into three municipalities, namely Ilala, Kinondoni and Temeke. Each of these municipalities is further divided into wards and then neighbourhoods known as *mitaa* (singular *mtaa*) in Kiswahili, literally meaning street [28].
A recently-initiated Urban Malaria Control Programme (UMCP) in Dar es Salaam delegates responsibility for routine mosquito control and surveillance to modestly paid community members, known as Community-Owned Resource Persons (CORPs) in a decentralized manner [29]. However, baseline evaluation revealed that at the early stage of the UMCP the levels of coverage achieved by the CORPs were insufficient to enable effective suppression of malaria transmission through larval control, and that training, support and supervision of the CORPs was poor [24]. The authors concluded that novel surveillance systems were required to enable community-based integrated vector management [24].

Early experience also indicated that control of culicine species, responsible for the bulk of biting nuisance [30-32], would be essential to achieve community acceptance and support for the programme. It was therefore decided to prioritize intensive control of malaria vector species in habitats which are open to sunlight (referred to as “open habitats”) but to also implement less intensive control of sanitation structures, such as pit latrines, soakage pits, and container type habitats which are closed to the sun (referred to as “closed habitats”) and produce huge numbers of *Culex* and *Aedes*, but no *Anopheles* [33, 34]. Thus, the bulk of the programme description below prioritizes and focuses on the system for controlling open habitats suitable for *Anopheles*, with a brief section describing mosquito control in closed habitats, for which no detailed routine larval surveillance was undertaken.

**A strategic overview of the Dar es Salaam Urban Malaria Control Programme (UMCP)**

Fifteen wards were included in the Dar es Salaam UMCP (Figure 1) encompassing as wide a variety of malarialogical situations as possible. In total an area of 55 km² is covered with wards ranging from 0.96 to 15 km² in size. In 2002, 611,871 people, representing 23% of the urban population, lived within this area [27] which covers 4% of the surface area of urban
Dar es Salaam. By April 2007 all 15 wards had been mapped in detail as a precursor to systematic larviciding [28]. Acronyms and other specific terminology are defined and explained in Table 1. The Dar es Salaam UMCP was conceptualized and developed according to the key principles listed in Table 2 which were formulated on the basis of direct practical experience [23, 24, 29, 35-38] and an extensive literature review [5, 6, 12, 29]. The reporting structure of the UMCP consists of a matrix of activities which are hierarchically layered over a range of spatial and administrative scales (Figure 2). At each spatial and administrative scale, the programme reports to relevant stakeholders but remains essentially autonomous in terms of day-to-day activities. Importantly, lines of reporting are carefully designed with respect to the guiding principles of Table 2 so that competing interests of staff are minimized with respect to their implementation, support and supervision duties. For example, CORPs responsible for larval surveillance, and those responsible for the application of larvicides, report separately to their ward supervisors. Furthermore, adult mosquito surveillance is implemented by a separate team which primarily reports to the city mosquito control coordinator and secondarily to the three municipal coordinators so that this data reporting line is collected and reported independently of staff responsible for maintaining low vector densities. The implementation of each activity, as well as their integration into a coordinated management system is described in detail below. All data sheets and standard operating procedures were translated in Kiswahili to ease the work of community-based staff.
Figure 1. Wards included in the study area of the Dar es Salaam Urban Malaria Control Programme (UMCP), specifying those targeted for larviciding from March 2006 onwards (intervention), those considered to be the most comparable control (non-intervention wards) and those remaining.
**Table 1. Definitions and abbreviations**

| Closed habitat | Any stagnant or slow-flowing water body which is not exposed to the sun and therefore unlikely to produce *Anopheles* malaria vectors but may produce culicines, notably abundant *Culex quinquefasciatus* [33, 34]. |
| CORP | Community-Owned Resource Person. The responsibility for routine mosquito surveillance and application of larvicide is delegated to CORPs, who are individual community members appointed and managed through neighbourhood health committees [29]. |
| GIS | Geographical Information System. GIS is a set of tools for capturing, storing, retrieving, transforming and displaying spatial data. |
| GPS | Global Positioning System. An operational system that allows receiving and converting signals from satellites to a specific position on Earth. |
| Municipality | The Dar es Salaam City Region is subdivided into three municipalities (the equivalent term for districts in urban Tanzania), namely Ilala, Temeke and Kinondoni. |
| Neighbourhood | The 73 wards of the Dar es Salaam City Region are administratively subdivided into 368 neighbourhoods. The 15 wards covered by UMCP comprise 67 neighbourhoods. The local Kiswahili term for neighbourhood is *mtaa* (plural *mitaa*) which literally means “street”. |
| Open habitat | Any stagnant or slow-flowing water body which is openly exposed to sunlight, even if only partially and for a portion of the day. These constitute potential habitats for malaria vector *Anopheles* mosquitoes [61, 70], as well as a variety of culicines. |
| Plot | All TCUs within the wards covered by the UMCP are subdivided into plots. A plot is defined here as a specific physical area with an identifiable owner, occupant or user and with clearly defined boundaries within one specific TCU. The plot boundaries are defined by UMCP staff. Therefore, the plots do not always correspond to actual cadastral information such as land ownership. |
| Region | The United Republic of Tanzania is divided into 26 administrative regions, of which Dar es Salaam city and its associated hinterland is one. |
| TCU | Ten-Cell-Unit. The 368 neighbourhoods (mtaa) of the Dar es Salaam City Region are subdivided into several thousand ten-cell-units (TCUs). These are the smallest units of local government, headed by a locally elected chairperson. In principle, TCUs should comprise ten houses each but are typically larger in practice and sometimes exceed one hundred houses. |
| UMCP | Urban Malaria Control Programme of the Dar es Salaam City Medical Office of Health, developed in co-operation with national and international research and funding organizations. |
| Ward | The three municipalities of the Dar es Salaam City Region are subdivided into 73 administrative sub-units known as wards. Currently, 15 of these wards are covered by the UMCP. |
Table 2. Conceptual principles underlying development of the Dar es Salaam Urban Malaria Control Programme on the basis of direct practical experience [23, 24, 29, 35-38] and an extensive literature review [5, 6, 12, 29]

<table>
<thead>
<tr>
<th>Principle</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rapid response</strong></td>
<td>An. gambiae sibling species readily develop from egg to adult within a week in habitats that often occur transiently and unpredictably [61, 70] so surveillance and larvicide application must be implemented in cycles of a week or less, with consequent responses to observed failures executed within 24 hours [14, 17, 36].</td>
</tr>
<tr>
<td><strong>Community-based implementation</strong></td>
<td>Sustainable programmes in Africa will be predominantly staffed by community-based personnel with minimal educational qualifications [29, 71-73] so simple protocols and readily-verifiable targets that can be managed with minimal technology are essential to achieve effectiveness [12].</td>
</tr>
<tr>
<td><strong>Decentralization</strong></td>
<td>Given these resource limitations and the sheer abundance of mosquito aquatic habitats in tropical Africa, responsibility for surveillance and response to operational monitoring observations must therefore be devolved to staff assigned to geographic sub-units small enough to be traversed daily on foot.</td>
</tr>
<tr>
<td><strong>Comprehensive coverage</strong></td>
<td>Until reliable, generalizable and practical procedures are developed which allow targeting of the most productive malaria vector habitats [10, 11] under such programmatic circumstances, high coverage of all potential sources [4, 5, 14-17, 74] is necessary to achieve satisfactory reductions of malaria transmission and burden in African settings [12, 75].</td>
</tr>
<tr>
<td><strong>Rigorous vertical management</strong></td>
<td>To achieve sufficient coverage, such decentralized, community-based approaches will require new tools for hierarchical, centralized management that individualize responsibility for all program activities [5, 17] and allow rigorous monitoring, evaluation and adaptive tuning [24]. Each level of management from the CORPs up to the City Mosquito Control Coordinator is responsible for identifying and addressing all programmatic shortcomings under their purview before they are detected by the next highest level within the program or external evaluators such as donors or research partners.</td>
</tr>
<tr>
<td><strong>Adult mosquito densities as a priority performance indicator</strong></td>
<td>Larval surveillance alone is inadequate to monitor or evaluate larviciding programs because it only reflects observations in habitats successfully covered by surveillance activities. Weekly monitoring of adult mosquitoes is necessary to allow rigorous monitoring, evaluation and management. While clinical or parasitological indicators are essential for rigorous evaluation of program impact, these are usually collected and reported on timescales too slow to enable day-to-day management for optimal performance.</td>
</tr>
<tr>
<td><strong>Separation of surveillance and treatment responsibilities</strong></td>
<td>Larvicidal treatment, monitoring and evaluation activities should each be implemented by distinct groups of personnel so that competing interests in data collection and interpretation are minimized [5, 14, 17]</td>
</tr>
<tr>
<td><strong>Integration with existing infrastructure and governance mechanisms</strong></td>
<td>Larval control programs must be integrated with pre-existing local government structures and public health systems to minimize costs, maximize effectiveness and ensure sustained acceptance by communities, public services and governments [29, 71-73].</td>
</tr>
<tr>
<td><strong>Full time staff</strong></td>
<td>Larval control program staff must be allocated to the program full time. New responsibilities can not be taken over by established and often overburdened public health staff. Larval control staff will be recruited and managed through existing infrastructure and governance mechanisms as described above.</td>
</tr>
<tr>
<td><strong>Satisfactory evidence must precede scale up.</strong></td>
<td>Although some encouraging evidence does exist [14-17, 36, 74], strategies targeting aquatic stage mosquitoes, including systematic larviciding remain underdeveloped and have yet to be evaluated on scales that are meaningful for scale-up as priority malaria prevention measures in Africa.</td>
</tr>
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</table>
Participatory mapping

Although the use of remote sensing techniques for the detection of mosquito breeding habitats have proven useful [39], a large number of An. gambiae larval habitats are temporary and appear and disappear frequently in space and time especially in the urban context, which requires constant supervision. Maps of habitats need to be developed and updated on a weekly basis to keep up with the rapidly changing field situation. In this scenario, the use of remotely
sensed imagery to accurately monitor habitats demands the analysis of images at multiple times, which is likely to face financial and technical (e.g. cloud coverage) constraints.

Before any surveillance or control activities can be successfully implemented, the boundaries of all targeted areas must be mapped thoroughly in a way that is useful to both the highest levels of city management and the community-based staff responsible for executing most of the programme’s activities. A simple community-based mapping procedure that requires no electronic devices in the field was, therefore, developed [28], which formalizes ground-based sketch maps using laminated aerial photographs in the field and then digitizes them using Geographical Information Systems (Figure 3). Initial estimates from the first three wards mapped indicated that over 30% of the study area had not been included in the first round of sketch mapping by larval surveillance CORPs, mostly because they were non-residential or industrial areas that do not exist on local government residential lists [28]. This procedure, described in detail elsewhere [28], allows rapid identification and inclusion of these key areas for sketch mapping and routine mosquito control, as well as more equal distribution of work to field staff.

A key feature of this mapping procedure is that it allows every square meter of the study area to be assigned to a specific geographic unit known as a Ten Cell Unit (TCU) and a specific subunit within that TCU referred to as a plot [28]. This in turn allows each of the constituent TCUs in each ward and neighbourhood to be assigned to specific individual CORPs for weekly larval surveillance and larvicide application. Crucially, plots are small enough to allow unambiguous description of individual habitats by CORPs and subsequent identification by supervisory staff in the field. This can be achieved by using a larval habitat surveillance form in conjunction with the corresponding TCU sketch map and plot description form [see
Additional file 1]. This mapping procedure provides an essential frame of reference for weekly routine mosquito surveillance and insecticide application, as well as the supervision of these activities by management staff.

Figure 3. Example of a sketch map, aerial picture and field map.

A. Sketch map of TCU no. 40 in Kurasini ward, Shimo la Udongo neighbourhood, as drawn by the responsible CORP. Features comprise plots with continuous numbering, streets, drains, agricultural areas and ponds. B. The same area on an aerial picture. The yellow lines connect identical features on the sketch maps and the aerial picture. C. The same area on the laminated map used in the field. The features to be mapped (TCU boundaries and numbers) were marked with non-permanent red marker pens. D. Project management team discussing over the field map of a whole ward, and deciding on necessary follow-up actions. Reproduced from Dongus et al. 2007 [28].
Surveillance of potential *Anopheles* habitats

All essential standard operating procedures, posters and forms for adapting and reproducing the larval surveillance systems described below are available as an online supplement [see Additional files 2-6]. Approximately 90 larval surveillance CORPs were employed at any given time during the study and these were each assigned defined areas based initially on local knowledge of habitat abundance, difficulty of terrain and geographic scale of their own neighbourhoods. This workload was subsequently redistributed following detailed participatory mapping [28]. In general, CORPs were recruited through local administrative leaders known as street chairmen and received minimal emoluments (Tanzanian Shillings (TShs) 3,000/day or US$ 2.45/day) as volunteer workers through a system developed by the municipal councils of Dar es Salaam for sundry small-scale maintenance tasks such as road cleaning [24, 29]. All CORPs are assigned to a single neighbourhood or subset of TCUs from that neighbourhood [28] under the oversight of a single supervisor for the entire ward. CORPs follow predefined schedules of TCUs which they are expected to survey each day of the week, collecting forms from their ward supervisor at the Ward Executive Office each morning and returning them each afternoon. The return of forms each afternoon is normally used to discuss the day’s observations so that the supervisor can follow these up in a timely manner. The schedule of TCUs visited by surveillance CORPs follows one day after the application of microbial insecticides so that indicators of operational shortcoming, such as the presence of late-stage (3rd or 4th instar) mosquito larvae, can be reacted to in sufficient time to prevent unwanted emergence of adult mosquitoes.

Every potential *Anopheles* habitat found in each plot is described by using a standardized form [see Additional file 5] and classified as one of the following habitat types: 1: Puddles & tyre tracks, 2: Swampy areas, 3: Mangrove swamps / Saltwater marshes 4: Drains/Ditches, 5:
Construction pits/foundations/man-made holes, 6: Water storage containers, 7: Rice paddies, 8: Ridge and furrow agriculture known as Matuta, 9: Habitats associated with other agriculture, 10: Streams/river beds, 11: Ponds, 12: Others [see Additional files 2-6]. It is important to note that once a habitat is identified and assigned a habitat identification number, that number is retained for all subsequent rounds of surveillance so that a) the identity of those habitats can be unambiguously allocated and followed up in the field and b) the dynamics of larval populations in habitats of different types and characteristics can be assessed. Thus, when habitats contain no water, they are still recorded but described as being dry. The presence of mosquito larvae and pupae are determined by dipping potential breeding sites [40]. Up to 10 dips are taken with a white 350ml dipper. Anopheline and culicine larvae are differentiated macroscopically in the dipper according to whether they float parallel with the water surface (anophelines) or hang down from the surface (culicines) [41]. No further differentiation to species level is attempted. Records on presence or absence are taken for both genera separately. If larvae are present the sizes of the larvae are observed and classified as early (1st and 2nd instars) or/and late (3rd and 4th instars) stages. Morphological differentiation of pupae from different genera is very difficult and impracticable under field conditions in an operational malaria control programme implemented by staff with basic training [23, 37]. Pupae are, therefore, not differentiated between Anopheles and other genera. The approximate size, depth and associated vegetation for each habitat are also recorded [see Additional file 5].

The characteristics of the CORPs forms are also captured in the corresponding forms used by Municipal Mosquito Control Inspectors (MMCIs) who assure quality control of CORPs work independently of their ward supervisors (Figure 4). All MMCIs conduct weekly spot checks of six randomly assigned TCUs in their municipality, assessing the accuracy of the data collected by the CORP through direct on-the-spot observation.
**Figure 4.** Examples of spot-checking forms [see Additional file 5] for Municipal Mosquito Control Inspectors. **A.** A typical example signed on the bottom left by a City Mosquito Surveillance Officer to show it has been checked for consistency and signs of problems requiring corrective action by management at city, municipal and ward level. **B.** An example of where an inspector has found poor coverage of potential habitats for *Anopheles* larvae by a CORP but failed to highlight it or record any corrective action. Note the query of the City Mosquito Surveillance Officer at the bottom.
Spot checking of six TCUs takes approximately two days per week allowing enough time for the implementation of other duties e.g. supervision of data collection and training activities nevertheless ensuring that each larval survey CORP is visited at least once every two months. Additional larval habitats identified by the MMCI that had not been detected by the CORPs are recorded and additional clear discrepancies between the records of the CORPs and the observations of the inspector documented. It should be noted that although the observations of the inspectors are shared with the respective ward supervisors, they are primarily reported to the Municipal Mosquito Control Coordinator who takes responsibility for managing the Ward Supervisors.

**Larvicide application and stock management**

After one year of baseline data collection on habitat and larval seasonality and adult abundance the UMCP staff reviewed the performance of larval surveillance CORPs and Ward Supervisors for all 15 wards in order to select one ward from each municipality for larval control interventions in the following year. The research team based the decision of which wards will receive larviciding and which wards will be compared with the intervention wards mainly on the proven ability of the ward supervisors and ward-based CORPs to implement the required task. Specifically, their ability to collect, understand, use and submit high quality data during the baseline data collection period was the primary criterion for choosing these high priority wards. Since the success of larval control interventions largely depends on good management skills and supervision, the UMCP team selected the best performing wards for the evaluation of the first year’s intervention, whilst also striving to improve the performance of the remaining wards. One ward from each municipality, namely Buguruni, Mikocheni and Kurasini, were chosen for larviciding. In an attempt to facilitate representative comparison and analysis, one non-intervention ward from each municipality, namely Vingunguti,
Mwananyamala and Keko, were selected *a priori* on the same basis as the intervention wards. Along with the intervention wards, these non-intervention wards were targeted for particularly rigorous maintenance of larval surveillance standards so that valid evaluations of larvicide impact upon larval populations could be made. This choice of a limited number of controls (non-intervention wards) was considered essential to ensure that the laboriously-collected larval data from both, intervention and non-intervention areas, were similar in terms of their extent and intensity for the first year’s evaluation. In parallel, all remaining wards were subsequently evaluated and targeted for re-training activities or staff replacement, so that by the end of March 2007 all wards showed comparable performance.

Larviciding is implemented exclusively with microbial insecticides, specifically *Bacillus thuringiensis* var. *israelensis* strain AM65-52 (*Bti*; VectoBac® Valent BioSciences Corporation, VBC, USA) and *Bacillus sphaericus* strain 2362 (*Bs*; VectoLex®, VBC, USA) because they are 1) highly efficacious against African malaria vectors, 2) selective in action, 3) environmentally safe to non-target organisms, 4) unlikely to result in resistance when used in combination or when only *Bti* is used, 5) safe for human handling and consumption, 6) easy to handle by staff with minimal training and protective measures, and 7) their impact can be easily monitored [35, 36, 41-44]. *Bti* is efficacious in all types of habitats but is less potent in high concentrations of organic matter, such as open sewers, and closed habitats, such as pit latrines and septic tanks. *Bti* needs to be applied weekly, but is relatively cheap compared with *Bs* [36]. Nevertheless, *Bs* has the advantage of being efficacious in very polluted water and even recycling by propagating itself in the cadavers of the mosquito larvae it kills [45-51]. Although *Bs* can have a residual effect and may not require weekly application, its efficacy in open habitats is difficult to predict. Furthermore, the habitat monitoring requirements to enable timely re-application and the decision making process necessary to decide when and
where to apply a larvicide with residual effect might be a source for errors. Therefore, the
application of *Bs* was not considered appropriate for the start of a programme. Moreover, *Bs*
formulations are about three times more expensive than *Bti* formulations [36] and need to be
applied in higher dosages to produce a persistent residual effect [35] which is likely to be less
cost-effective than labour intensive treatment with *Bti* [52]. In closed habitats which are not
exposed to solar radiation and support densities of culicine mosquitoes that are high enough to
enable sustained recycling, a single treatment with a sufficient dosage of *Bs* can be reliably
expected to suppress emergence for several weeks [51, 53-55].

Two formulations of larvicides are used in the programme: water-dispersible granules (WDG)
are applied as aqueous suspensions using Solo® 475 knapsack sprayers, whereas corn
granules (CG) are applied by hand. CG was preferred for the vast majority of habitats that are
open to the sun. Although hand application of CG treats large areas less rapidly and less
evenly than WDG, it is broadly applicable under different environmental conditions.
Moreover, it can be readily applied by community-based personnel with minimum training.
Granules can penetrate vegetation to reach targeted water surfaces and can be distributed
further than liquid aerosols, thereby allowing treatment of less accessible sites. CG was also
preferred for treating closed habitats because it is easy to apply to such domestic mosquito
sources by CORPs and even the house owners. Liquid application of WDG with knapsack
sprayers was preferred for extensive areas of stagnant water with little emergent or floating
vegetation that might prevent the sprayed aerosol from reaching the water surface.

Based on evaluations of *Bti* and *Bs* in western Kenya [35, 36], the formulations-dosage
combinations described in Table 3 were recommended for larval control, although in practice
these dosages were often accidentally exceeded especially by inexperienced staff and in very
small habitats. Training materials and detailed guidelines for insecticide application, based on locally implemented calibration exercises, were prepared in a participatory manner and refined through early field piloting [see Additional files 7 and 8]. While open habitats with the potential to produce *Anopheles* are treated weekly by Mosquito Control CORPs assigned to neighbourhoods or portions of neighbourhoods, closed habitats are treated every three months by small teams of additional CORPs working through entire wards on a quarterly cycle.

The specificity of these microbial insecticides makes stock control substantially easier because they do not have any uses, other than mosquito control, which avoids financial incentive for theft, misuse or misappropriation. Nevertheless, insecticide stocks are carefully managed at a central storage site and distributed to locked cabinets in each ward office on a weekly basis. Insecticide stocks are distributed on a ‘first-in, first-out’ basis and decentralized stocks at the ward level are replenished weekly on the basis of consumption and projected need. Simple, but readily verifiable records of the daily use of insecticide by each individual CORP allows decentralized detection and correction of inappropriate use rates by Ward Supervisors and other management personnel [see Additional file 7] in a manner similar to programmes for indoor residual spraying of chemical insecticides in southern Africa [56]. Consumption rates at the ward level can also be reconciled with city level records at the central storage and delivery facility. These central stock management procedures also allow timely ordering of new stock which is currently sourced from the USA and therefore entails a delay of at least two months between ordering and delivery by surface freight.
Table 3. Formulation-dosage combinations recommended to UMCP staff to achieve 100% control of mosquito larvae within 24 hours.

<table>
<thead>
<tr>
<th>Producta</th>
<th>Active Ingredientb</th>
<th>Dosage Kg/hectare</th>
<th>Application Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Open habitats</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VectoLex® WDG (650 ITU/mg)</td>
<td>Bs</td>
<td>2.0</td>
<td>0.20</td>
</tr>
<tr>
<td>VectoBac® WDG (3000 ITU/mg)</td>
<td>Bti</td>
<td>0.4</td>
<td>0.04</td>
</tr>
<tr>
<td>VectoLex® CG (50 ITU/mg)</td>
<td>Bs</td>
<td>30</td>
<td>3</td>
</tr>
<tr>
<td>VectoBac® CG (200 ITU/mg)</td>
<td>Bti</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td><strong>Closed habitats</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VectoLex® CG (50 ITU/mg)</td>
<td>Bs</td>
<td>10</td>
<td>1</td>
</tr>
</tbody>
</table>

ITU = International Toxic Units, describes the potency of larvicide, the higher the number, the more toxic is 1mg the less is needed to kill 100% of larvae within 24hrs.
bBti; Bacillus thuringiensis var israelensis, Bs; Bacillus sphaericus.
cSee box 1 for definitions.

Adult mosquito surveillance

It was originally planned that the CORPs would also report densities of adult mosquitoes at sentinel sites distributed throughout the study area using Mbita-design bed net traps [57-59]. However, 181 full night samples with these traps executed over two months yielded over 4,000 Culex, Mansonia and Aedes of various species, but only one An. gambiae sensu lato caught in one of the traps placed outdoors. While the very low sensitivity of Mbita traps is consistent with other reports [60], additional observations suggest a broader limitation to existing trapping methods for malaria vectors in Dar es Salaam. Further investigation showed that CDC light traps beside occupied bed nets, indoor pyrethrum spray catch and Mbita bed net traps all failed to catch significant numbers of Anopheles indoors in Dar es Salaam, while three nights of outdoor human landing catch at one location yielded 136 An. gambiae s.l., 30 other Anopheles and 806 culicines, two nearby Mbita traps (one placed indoor and another outdoor) caught only 176 culicines and no Anopheles on the same nights. Two nearby CDC-light traps placed beside occupied untreated bed nets (one indoors and one outdoors), which is normally a reliable trapping method for malaria vectors in sub-Saharan Africa [58], captured 423 culicines, but only three An. gambiae s.l. and 14 other Anopheles. Notably, all An. gambiae s.l. caught in light traps were found in traps placed outdoors and it has since been
shown, through detailed behavioural studies, that *An. gambiae* and *An. arabiensis* are both predominantly exophagic in this highly urbanized environment [26]. The inability of CDC light traps and pyrethrum spray knockdown to capture *An. gambiae s.l.* in modern Dar es Salaam contrasts with previous programmes up to 1996, suggesting that this behavioural shift is a relatively recent adaptation, possibly resulting from increased bed net use and house screening.

This unexpected difficulty in monitoring adult mosquitoes was overcome by conducting human landing catches as an interim monitoring and evaluation measure while alternative trapping technologies were developed to replace it. Detailed protocols and training materials for the adult mosquito surveillance procedures are not provided for adaptation elsewhere because this cannot be considered a routine procedure for wide-scale programmatic use. The potential health risks associated with human landing catches necessitate careful consideration, justification and ethical review. The human landing catches executed in these early stages of the Dar es Salaam UMCP are undertaken as an interim research tool only. Practical, safe and effective new surveillance procedures have since been developed to prototype stage and will be reported elsewhere after full evaluation in terms of efficacy and effectiveness (NJ Govella, personal communication).

The procedures applied to monitor and evaluate mosquito densities [26] are described briefly as follows. One resident was recruited from each of the 67 neighbourhoods in the study area and employed as an Adult Mosquito Surveillance CORP to conduct one full night of human landing catch each week. All human landing catches are done outdoors. Each CORP is assigned four sampling sites which are distributed approximately evenly across his neighbourhood. For safety reasons, these are typically within walled compounds but are
nevertheless chosen on the basis of not only the location, but also the co-operation of the residents and accessibility of the site to city-level supervisors for unannounced spot checks. Once every four weeks at each location, human landing catch are conducted from 6 pm to 6 am for 45 minutes of each hour, allowing 15 minute breaks for rest. Each afternoon a city level team led by two Adult Mosquito Control Supervisors distributes a kit to each CORP scheduled to work that night. The kit consists of netting-covered cups for each hour’s catch, an aspirator and a simple form upon which each hour’s catch can be recorded so that, upon random inspection at any hour of the night, the recordings and content of the cup can be reconciled. Each morning the kits, with all caught mosquitoes in their respective cups, are collected and returned to a central laboratory. All collected mosquitoes are identified morphologically to genus and, in the case of *Anopheles*, to species complex level [61]. Members of the *An. gambiae* species complex are further resolved to sibling species level by polymerase chain reaction [62]. The sporozoite infection status of each mosquito gets determined by enzyme-linked immuno-absorbent assay [63].

**Integration and coordination**

Larval surveillance data are primarily summarized and interpreted at the level of the Ward Supervisors to enable the rapid response of larvicide application to observed operational failures. This is accomplished in a practical, affordable and scaleable manner using weekly summary forms [see Additional file 9], which are filled out each afternoon by the supervisor when the Larval Surveillance CORPs under his/her oversight return the filled forms from their work that morning. The total number of habitats and the subset of those which contain water and mosquito larvae of various stages are totalled from each form (and the TCU it represents) provided by the CORPs by simply counting the number of ticks in each column (see Figure 4 which closely resembles the equivalent form for CORPs). These totals are then entered in the
supervisor’s weekly summary sheet, inspected immediately for signs of poor larvicide application, and totalled for each neighbourhood when all its TCUs have been completed (Figure 5). Supervisors are expected to note any such indicators of programme failure and consequent follow-up action on these forms, signing and dating all such notes, as well as the confirmation that they have read and checked the form before filing. This approach formalizes the obligation to read and respond to all larval surveillance data within 24 hours, and allows unambiguous assessment of performance and responsibility by municipal and city-level management. Furthermore, it simplifies, accelerates and decentralizes an otherwise vast data aggregation burden without using any computing technology beyond that provided by a pocket calculator.

Figure 5. Example of a completed weekly ward summary form [see Additional file 9] filled out by the Ward Supervisor and totalled along the bottom with a pocket calculator to enable rapid entry into monthly report templates at the municipal level.
All the Larval Surveillance CORPs’ forms are collated in order of their TCU numbers in pre-labelled folders with the ward supervisor’s summary sheet on top of the cluster of TCUs it summarizes. These folders are provided to the Municipal Mosquito Control Coordinator (MMCC) each week. The MMCC or the MMCIs directly under his/her supervision then checks that all forms have been filled out and submitted correctly, recording the results of this quality control exercise in a checklist designed for that purpose [see Additional file 9]. The totals for each neighbourhood in this checklist, at the bottom of each ward supervisor’s summary form (Figure 5), are then entered into a password protected excel spreadsheet template, tailored to each municipality. This template automatically generates summary statistics, tables and charts [see Figures 6 and 7] that form the backbone of the MMCCs monthly report to the City Mosquito Control Coordinator (CMCC). More importantly, the MMCC is responsible for identifying and reacting to signs of programme failure in the content of these forms within a week of their occurrence, documenting any actions taken in writing on those forms. These standard, automatically generated tables and charts are supplemented with written narratives summarizing successes, failures and responses to these monthly observations, as well as plans and requests for support to implement further action. A crucial part of the MMCCs duties is to coordinate, assess and execute corrective action in relation to the observations of his/her inspectors when conducting random spot checks to assure the quality of data reported by larval surveillance CORPs (Figure 4). The results of these quality control assessments by the MMCIs are also entered into the municipal monthly report template for examination by the CMCC and his/her two City Mosquito Surveillance Officers (CMSOs). The MMCC also receives a summary of the adult mosquito surveillance data for that week directly from the city-level Adult Mosquito Surveillance Supervisors so that this independent and more direct assessment of programme impact can be used to rigorously triangulate and interpret the larval surveillance data.
### Municipal larval survey: Monthly summary report

<table>
<thead>
<tr>
<th>Ward/Location</th>
<th>Folder</th>
<th>Month</th>
<th>Year</th>
<th>Total habitats</th>
<th>Wet habitats</th>
<th>Habitats with early instar Anopheles</th>
<th>Habitats with late instar Anopheles</th>
<th>Habitats with late instar Culicines</th>
<th>Habitats with early pupae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ilala</td>
<td></td>
<td>12</td>
<td>2005</td>
<td>100</td>
<td>300</td>
<td>700</td>
<td>150</td>
<td>300</td>
<td>150</td>
</tr>
<tr>
<td>Mtakuja</td>
<td>1</td>
<td></td>
<td></td>
<td>100</td>
<td>300</td>
<td>700</td>
<td>150</td>
<td>300</td>
<td>150</td>
</tr>
<tr>
<td>Mission Kota</td>
<td>2</td>
<td></td>
<td></td>
<td>100</td>
<td>300</td>
<td>700</td>
<td>150</td>
<td>300</td>
<td>150</td>
</tr>
<tr>
<td>Kasulu</td>
<td>3</td>
<td></td>
<td></td>
<td>100</td>
<td>300</td>
<td>700</td>
<td>150</td>
<td>300</td>
<td>150</td>
</tr>
<tr>
<td>Kisiwani</td>
<td>4</td>
<td></td>
<td></td>
<td>100</td>
<td>300</td>
<td>700</td>
<td>150</td>
<td>300</td>
<td>150</td>
</tr>
</tbody>
</table>

**Figure 6.** Example of a mosquito larval surveillance component in a municipal monthly report template [see Additional files 10 and 11]. A. The overall data entry table in which each row corresponds to one, or occasionally two (see bottom row for example of a very large neighbourhood) folders, each containing 4 or 5 sequential weekly ward summary forms and respective sets of CORPs larval surveillance forms. Note that weeks overlapping two months are assigned to specific calendar months in advance so that each operational month has a predefined start and end date, spanning exactly 4 or 5 weeks. B. A typical automatically generated chart summarizing the observed distribution of larval habitat abundance and mosquito occupancy in one ward.
### ILALA WARD

**Figure 7.** Example of a mosquito adult surveillance component in a municipal monthly report template. A. The overall data entry table (empty fields indicate missing data) B. A typical automatically generated chart summarizing the observed distribution of adult mosquitoes.

This data are also included in the monthly municipal report with a preformatted component of the spreadsheet which automatically generates summary statistics and charts.
The City Mosquito Control Coordinator (CMCC) expects to receive the previous month’s municipal reports in the first week of each month and is expected to provide verbal feedback, as well as annotated comments, on these reports in a meeting with the CMSOs, MMCCs and MMCIs to be held on or before the end of the second week of the month. The CMCC collates these data and adds them to existing records to generate a series of trend graphs and summary statistics that quantify and illustrate the progress of the programme in terms of impact on larval (Figure 8 and 9) and adult-stage mosquitoes (Figure 10). By the start of 2007, the CMCC had begun presenting these reports at bimonthly coordination meetings with the partners of the primary donor for the programme at that time (US President’s Malaria Initiative of the United States Agency for International Development).

**Figure 8.** Monthly average of aquatic habitats surveyed in the three municipalities Kinondoni, Ilala and Temeke from February 2005 to March 2007 in relation to rainfall.
Figure 9. Impact of seasonal rainfall variation and larvicide application on aquatic-stage mosquito populations between April 2005 and June 2007. Larvicide application started in the intervention sites in March 2006 week number 1. A: Proportion of aquatic habitats containing late instar culicine larvae at weekly surveys. B: Proportion of aquatic habitats containing late instar anopheline larvae at weekly surveys.
Figure 10. Impact of seasonal rainfall variation and larvicide application on weekly adult mosquito densities between April 2005 and June 2007. A. Rainfall and densities of adult *Culex* species, B. Rainfall and densities of adult *Anopheles gambiae s.l.*, C. The ratio of densities of *An. gambiae s.l.* in intervention wards relative to non-intervention wards. The line representing the x-axis in panel C represents equivalence of densities in intervention and *a priori* selected non-intervention wards while the vertical black line represents the initiation of larviciding activities. The thick, broken horizontal line in panel C represents the ratio of exposure estimated to be provided by an insecticide-treated net in urban Dar es Salaam [26].
Analyses

To describe changes in mosquito densities associated with larviciding the percentage reduction in mosquito densities in larviciding areas was calculated using an established formula [35, 42, 64] which takes into account that natural changes (for instance through predation or changes in climatic conditions) in the mosquito populations are taking place over time at the same level and rate in both treated (intervention) and untreated (non-intervention) sites. Therefore, the percentage reduction is defined as follows:

\[
\% \text{ reduction} = 100 - \left( \frac{C_1}{T_1} \times \frac{T_2}{C_2} \right) \times 100
\]

where \( C_1 \) and \( C_2 \) describe the average density of mosquitoes in untreated (non-intervention) sites during baseline and intervention periods, and \( T_1 \) and \( T_2 \) describe the average density of mosquitoes in intervention sites during baseline (when no larviciding took place yet) and intervention periods (when larvicides were applied weekly) [64]. All figures presented as “percentage reduction” throughout the paper have been calculated using this formula.

All measured adult mosquito biting densities were multiplied by 1/0.75 to get biting rates for a full hour [26]. Generalized estimating equations (GEE) were run with SPSS 15.0 to calculate differences in mosquito biting rates and EIR between intervention and non-intervention areas with ten-cell units as a subject unit, log linked mosquito densities and intervention and non-intervention areas as the factor (Table 4). In order to adjust for total exposure indoors and outdoors, outdoor mosquito densities were multiplied by the ratio of the total true human exposure (the sum of the hourly mean of the indoor and outdoor biting rates, weighted according the proportion of time human beings typically spend in these two compartments) divided by the total outdoor biting rate as estimated previously [26]. These ratios were derived from an in depth mosquito survey which was conducted during the main rainy season in 2006 (\( \text{An. gambiae} \): 0.67, \( \text{An. funestus} \): 0.725, \( \text{Anopheles coustani} \): 0.448 and \( \text{Culex} \): 0.94) [26].
Ethics

All participants provided informed consent. No persons in high risk groups, namely people under 18 years or women of reproductive age, were recruited to conduct human landing catches. Furthermore, the catchers are screened every week for malaria by microscopic examination of thick smear peripheral blood samples and treated with artesinin-based combination therapy when diagnosis was positive. Research clearance was obtained from the Medical Research Coordination Committee of the National Institute of Medical Research in Tanzania (NIMR/HQ/R.8a/Vol. IX/279) the Tanzanian Commission of Science and Technology (No. 2004-69-MFS-2004-24) and Durham University’s Ethics Advisory Committee (No. 03 EAC R131).

Table 4. Comparison of mean human biting rates (HBR) of An. gambiae s.l. and Culex sp. and entomological inoculation rate (EIR) for An. gambiae s.l. in the intervention and non-intervention wards during baseline and first year of intervention. 95% confidence intervals in parenthesis.

<table>
<thead>
<tr>
<th></th>
<th>Pre-Intervention a</th>
<th>First intervention year b</th>
<th>Percentage Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-Intervention Wards</td>
<td>Intervention Wards</td>
<td>$p$ c</td>
</tr>
<tr>
<td><strong>Annual mean</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily HBR</td>
<td>0.93 (0.60-1.46)</td>
<td>0.72 (0.51-1.02)</td>
<td>0.367</td>
</tr>
<tr>
<td><em>An. gambiae</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Annual EIR</td>
<td>1.05 (0.68-1.65)</td>
<td>0.81 (0.58-1.15)</td>
<td></td>
</tr>
<tr>
<td>Daily HBR</td>
<td>173.9 (140.7-214.9)</td>
<td>86.8 (72.7-103.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><em>Culex sp.</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Dry season mean (July-August-September)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily HBR</td>
<td>0.59 (0.32-1.11)</td>
<td>0.46 (0.29-0.72)</td>
<td>0.505</td>
</tr>
<tr>
<td><em>An. gambiae</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EIR</td>
<td>0.67 (0.36-1.26)</td>
<td>0.52 (0.33-0.81)</td>
<td></td>
</tr>
<tr>
<td><em>An. gambiae</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily HBR</td>
<td>196.3 (157.9-244.0)</td>
<td>98.4 (82.2-117.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><em>Culex sp.</em></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a April 2005 – March 2006; March 2006 has been included in the calculation for the baseline year since reductions of adult mosquitoes due to larviciding cannot be expected earlier than 3-4 weeks into the intervention [36].


c Generalized estimating equations (GEE) were used to analyse pre-intervention data and data from the first year of intervention, respectively. In each analyses mean densities are compared between non-intervention and intervention sites. Ten-cell units were used as a subject unit, log linked mosquito densities and intervention and non-intervention areas as the factor.
4.4 Results

Overall, the vector surveillance and management systems developed in Dar es Salaam allowed timely collection, interpretation and reaction to field-collected entomologic data with reaction times at ward, municipal and city levels of one day, week and month, respectively. In fact, the vector density patterns as presented in Figure 9 and 10 were drafted into manuscript format figures within three weeks of their collection through these standard low-technology procedures, therefore serving as an instant monitoring and teaching tool. In contrast, more complex, research driven analyses (Table 4), which require elaborate data entry procedures, can only be achieved with several months delay.

The implementation of the programme through local community-based staff led to high community acceptance and support. The procedures and staff management systems described, greatly improved standards of larval surveillance relative to that reported at the outset of this programme [24]. Vanek and others [24] reported that only 42% of potential Anopheles habitats were detected by CORPs prior to the introduction of the programme management systems described here. By the end of 2005, the independent spot checks of the Municipal Mosquito Control Inspectors revealed that all three municipalities had larval surveillance coverage levels exceeding 75% (Figure 11). Based on this result the decision was taken to implement larviciding in three selected wards since substantial reductions of malaria exposure and burden for resident populations [10-12] were expected if such coverage levels could be approached with actual larvicidal control.

Larviciding began in three wards in the first week of March 2006 (Figure 1). By that time more than 65,000 potential Anopheles habitats spread out over a 55 km² area occupied by
more than 612,000 people were surveyed on a weekly basis. At any sampling date, between 10 and 50% of all habitats contained water (Figure 8).

Figure 11. Proportion of habitats successfully detected (sensitivity) and correctly identified (specificity) by larval surveillance CORPs in November 2005, as determined from the random on-site spot checks of the Municipal Mosquito Control Inspectors using methodology essentially identical to earlier evaluations of larval surveillance [24].

The first year of larviciding successfully reduced the number of larval habitats (Figure 9). In the three non-intervention wards the proportion of habitats that contained late instar anopheline and culicine larvae increased from March 2006 onwards by an average of 53% and 37%, respectively, as compared to the baseline year. This is probably associated with more rainfall in 2006 (1526 mm) compared to 2005 (979 mm) leading to an increase in fresh water and suitable habitats (Figure 8). In marked contrast, the number of habitats with anophelines and culicines both fell in average by over 90% in the three intervention wards as compared to the baseline year. Overall percentage reduction in Anopheles larval habitat abundance was 96.5% assuming that without larviciding larval populations would have risen by the same rate as in non-intervention wards [64].
The vast majority of 245,927 adult mosquitoes collected in the year before intervention were culicines represented by *Culex* sp. (97.7%), *Mansonina* sp. (0.9%) and *Aedes* sp. (0.4%). Only 1% (2,468) of these were anophelines. *An. gambiae s.l.* represented 76.6% (1,864) of the anophelines and was by far the most common vector. Only a small number of *An. funestus* (85; 3.5%) were identified through the adult surveillance system. Laboratory analyses confirmed transmission by both *An. gambiae s.l.* and *An. funestus* with sporozoite rates of 0.31% and 1.25% [65], respectively. A sub-sample of 1,099 members of the *An. gambiae* species complex were identified as 75.6% *An. gambiae s.s.*, 21.3% *An. arabiensis* and 3.1% *An. merus*.

Culicine mosquitoes were abundant in all study wards and showed little seasonality throughout the year (Figure 10 A). During the baseline data collection the average culicine human biting rate was nearly twice as high in the wards chosen *a priori* as controls for the intervention and this proportion did not change during the intervention (Table 4) indicating that routine larvicide application did not suppress the nuisance biting rate.

Adult densities of the primary vector, *An. gambiae s.l.*, were highly seasonal (Figure 10 B). Although the mean *An. gambiae s.l.* human biting rate and annual EIR was higher in the control wards than the intervention wards during the baseline year, this difference was not significant (Table 4). In contrast, in the first 12 months of intervention, the mean human biting rate and annual EIR remained approximately the same in the non-intervention wards (Table 4) but decreased by one third in wards where larval control was implemented following the general trend observed in the larval surveys. The difference in transmission intensity between non-intervention and intervention wards was significant (p=0.04) in the first year of larval control (Table 4) even though an overall percentage reduction of 31.3% might appear modest
compared to the impact shown on larval habitat abundance. Notably, the dry season larviciding in July-August-September 2006 led to a percentage reduction in transmission by 87% when compared with the same time period pre-intervention and non-intervention sites. In marked contrast to the pre-intervention year, weekly mean adult mosquito densities in intervention areas were constantly lower than those in non-intervention areas for six consecutive months from May to October 2006, and for five consecutive months from mid January to mid June 2007 (Figure 10 C). However, little to no effect was achieved during the primary (March-June) and secondary (October-December) rainy seasons in 2006 (Figure 10 B). Larviciding was only begun with the onset of the main rains of 2006 and it took several weeks for programme staff to refine their performance based on hands-on experience. Although the proportion of habitats containing late instar larvae decreased from the start of larviciding, it is important to note that the actual numbers of habitats available increased substantially in March 2006 (Figure 8), resulting in significant larval development and possibly emergence. Thus, although adult *An. gambiae s.l.* densities in intervention wards steadily dropped till the end of September 2006 (Figure 10), the introduction of the intervention came too late to prevent the bulk of transmission resulting from the main rains from March to May 2006.

An additional challenge confronted the programme staff during the short rains at the end of 2006. Simultaneous rains and municipal maintenance of waste water settlement ponds in each of the intervention wards generated substantial areas of inaccessible larval habitats ideal for *An. gambiae s.l.* on the surface of freshly drained mud flats (Figure 12).
Figure 12. Examples of inaccessible but productive *Anopheles* aquatic habitats in the wards of Buguruni (A), Mikocheni (B) and Kurasini (C) during the period October to December 2006. Note that all the open soil surfaces depicted are in fact very soft mud which is impossible to walk across. Although these ponds had been freshly drained for maintenance, their low porosity, and the rainfall which immediately followed their exposure, resulted in abundant and stable surface water in multiple inaccessible depressions on the surface for two months. These areas closely resemble similarly challenging sites in flooding river valleys of West Africa which can be rigorously controlled with powered granule-blowing equipment [42].
Crucially, these three water treatment facilities were located within 100 meters of at least one adult mosquito surveillance site each so their influence upon recorded *An. gambiae s.l.* densities was substantial. Once these ponds had been fully renovated and these areas either dried out or were filled up, malaria vector densities were once again successfully controlled. Nevertheless, because of programme limitations during both seasonal rainfall peaks in 2006, the overall impact on malaria transmission for the first intervention year was very modest. Preliminary results from the main rains (April-June) in 2007 (Figure 10) though indicate an improvement in the operational procedures which led to a percentage reduction of transmission by 71% as compared to the same time period at baseline and by 62% as compared to the start of the intervention in 2006.

### 4.5 Discussion

After only one year of operational larviciding in Dar es Salaam a clear impact of the intervention on malaria vectors was demonstrated. Overall anopheline larval abundance was reduced by 96% in the intervention wards compared to historical and contemporary controls which consequently resulted in a significant reduction of 31% of malaria transmission by *An. gambiae s.l.*. Furthermore, preliminary analyses of parasitological surveys (Y. Geissbühler and M.C. De Castro, personal communication) showed that the larviciding was associated with an overall reduction of 40% (p<0.001) of *P. falciparum* infection prevalence in the study population and that highest impact was achieved during the dry season of 2006. Interestingly, the majority of infected mosquitoes in Dar es Salaam were found during the dry seasons which also coincided with maximum larval control success (Y. Geissbühler, personal communication).
The control of nuisance mosquitoes remained unsatisfactory. Similar to observations in other urban centres in East Africa, where anti-larval measures for malaria control were implemented [66], the overall culicine densities remained high in the intervention wards which might be explained by the large number of closed habitats like pit latrines, soakage pits, septic tanks and water storage tanks, which were not included in the weekly larvicide applications. The three-month cycle for interventions targeted at closed habitats is probably too long to suppress larval development in these often highly polluted breeding sites. Furthermore, no rigorous system existed for monitoring coverage of these habitats, to which access is often difficult or not possible at all. While targeting the interventions at *Anopheles* breeding sites makes economic sense, it may not be practicable. Culicine mosquitoes are responsible for over 100 bites per exposed person per night in Dar es Salaam. Targeting *Anopheles* habitats only would most likely lead to the withdrawal of the communities’ support as has been shown in the past [30-32]. Nevertheless, *Culex* control appears not worth doing unless the numbers can be reduced sufficiently to convince inhabitants that larval control, in general, is a good idea. Therefore, new strategies including the implementation of environmental modifications need to be urgently developed to address the nuisance biting problem in Dar es Salaam.

The UMCP’s unique feature is the surveillance and management system described here which proved to be practical and affordable [52] and allowed operational response times to changing ecological and programmatic conditions that were previously unthinkable at this scale. The strong involvement of community-based staff, local capacity building, direct governmental participation and commitment in all phases of the programme, data-driven decision making and hands-on technical and programmatic support from national and international partners constitute a strong basis for future sustainability of control activities and have been pointed out to be important factors for success in malaria control programmes [18-20].
Despite the overall encouraging impact on malaria transmission, the wet season results in 2006 were clearly unsatisfactory. Nevertheless, it needs to be cautioned that adult mosquito sampling was most likely somewhat biased towards overestimating the contribution of the settlement ponds illustrated in Figure 12. Furthermore, detailed spatial analyses of the data need to be carried out to investigate the possibility of immigration of adult mosquitoes from non-treated areas outside the relatively small intervention wards. This might have contributed to the overall modest difference in adult densities between control and intervention wards which stands in sharp contrast to the observations of larval abundance. It is noteworthy, however, that the levels of suppression achieved before and after the short rains in late 2006 comfortably exceeded recent estimates [26] for the personal protection against exposure provided by an insecticide-treated net in this urban setting (Figure 10 C).

To achieve effective control, larviciding programmes must clearly suppress transmission not only in the dry season when mosquitoes are most vulnerable but also when their numbers peak during and after the wet season. Both wet seasons in 2006 provided useful lessons and highlight the importance of long-term commitment for successful malaria control with larvicides in urban Africa. The first and most important wet season of 2006 illustrates the importance of being prepared for major transmission surges and the value of hands-on experience. Consistent with our observations of improving staff skills and performance, the impact of larviciding steadily increased following initiation, but the intervention was started too late for improving effectiveness to have a major impact on the intense peak of transmission in 2006.

Much of this can be attributed to the slow financing mechanisms for the programme at that time. All of the financial support for this programme was only secured in mid 2006, with
limited interim pre-financing and insecticide donations provided in advance by the research partners at their own risk. These cash flow restrictions meant that equipment, supplies, personnel costs and training could not be assembled and coordinated before this key transmission season, so a vital opportunity to reduce malaria exposure was missed. For most of the programme’s existence it has been necessarily pre-financed on an *ad hoc* (and therefore intermittent and unreliable) basis by its primary research partners, without which none of the data or methodologies presented would have been realized. The lack of sustainable funding has been identified as one of the major obstacles in the planning and implementation of mosquito control interventions in general [18, 19, 67] and a recent evaluation of malaria control programmes in Eritrea, Brazil, India and Vietnam [18] showed that sufficient and flexible financing, decentralized control of resources and local prioritisation of spending was key to success. As of March 2007, one of the research partners of the UMCP has instituted a risk-assessed pre-financing mechanism specifically to support smooth distribution of cash, equipment and supplies to the programme during the slow process of grant allocation and administration from donors. Such credit support from intermediary institutions is, however, likely to be the exception rather than the rule and stable funding mechanisms must be developed if larviciding programmes which rely on continuous weekly application cycles are to be stably implemented and supplied based on long-term development plans.

The unforeseen creation of major, inaccessible larval habitats during the short rainy season at the end of 2006 underlines the importance of experience and long-term commitment to programmes which rely so much on locally-specific tactical adaptation. While the need for powered granule blowers for occasionally difficult habitats [42] is now obvious, this was not the case at the outset of this endeavour. With the scheduled scale-up of the interventions to nine wards from June 2007 and 15 wards from June 2008 further surprises are anticipated.
Solutions to such challenges are likely to be found, however, the maturation of the programme’s capacity to tackle the full range of such operational challenges will require at least additional 1-2 years of practical implementation experience.

It is necessary to point out that the UMCP is currently a combination of an operational programme, a research project and a training platform to provide the evidence and capacity needed for future programmes. Therefore, the activities implemented to date are very comprehensive and intensive. As the programme matures there should be opportunity to scale down some of these activities. For example, the mapping and recording of every plot could be simplified since for a solely operational programme not each individual water body needs to be characterised by an individual ID number. Furthermore, while weekly application of larvicides to all aquatic habitats remains necessary, the weekly larval surveillance (follow up) of every single habitat could be reduced to spot checks of a representative number of randomly selected habitats every week for monitoring and evaluation purposes. Nevertheless, it needs to be emphasized that such strategies should only be developed and fine-tuned over time as the program staff gains more experience. To monitor the disease impact of a vector control programme household and malaria surveys [68] need to be implemented. Nevertheless, these need not to be necessarily part of the vector control programme but should be implemented through national disease monitoring and evaluation procedures, preferably integrated in health information systems for core health and poverty indicators that serve local, national and global needs [69].
4.6 Conclusions

A novel management system for implementing systematic larviciding of malaria vectors in African cities, that includes an intensive monitoring and evaluation component, has exhibited considerable potential for sustained, rapidly responsive, data-driven and affordable application. Despite operational and financial limitations in the first year of intervention it could be demonstrated that large-scale larviciding programmes can reduce malaria transmission in urban Africa. The true programmatic value of larviciding though can only be established through longer-term programmes which are stably financed and allow the capacities of operational teams and infrastructures to mature through direct experience of locally relevant ecological, epidemiological and institutional challenges.

Competing interests

The programme evaluated in this manuscript is partially supported by Valent BioSciences Corporation, a commercial manufacturer of microbial larvicides. Nevertheless, none of the funders of this work had any role neither in the analysis or interpretation of the results nor in the drafting of the manuscript.

Authors' contributions

UF, KK, MCC, GW and GFK developed all standard operating procedures concerning larval surveillance and control in a participatory manner with field staff at city, municipal and ward level. YG, PPC, NJG were involved in the development of adult mosquito sampling protocols, field data collection and analyses. KK oversaw all activities implemented by the UMCP. UF,
KK and GFK planned and oversaw the larval control intervention. MCC and KK created all databases. SD and DN developed and oversaw the participatory mapping. MJV and EMM helped with protocol refinement based on evaluation of CORPs’ performance. DM, MT, SWL, HM and BHS were involved in the overall design of the UMCP and regular review of progress. UF and GFK were involved in the analyses of the data and drafted the manuscript. All authors read and approved the final manuscript.

4.7 Acknowledgements

The late Gabriel Michael Kiama planned and managed the predecessor of the UMCP and initiated its development into the form described here. We are greatly indebted to him for his enormous commitment and contribution towards this programme. We would like to thank the people of Dar es Salaam and their district and ward authorities for their excellent cooperation. We are grateful to Abdulla Hemedi, Bryson Shoo, Ali Adinani, Johnson Ndaro, James Msami, Ally Babu, Jaffary Lyimo, Winnie Ernest, Nelly Richard, Muller Shabani, Musa Saidi, Oswald Temba, Martin Kuoku, Martin Kalongolela, Abraham Mwambona, Mercy Kinenekejo, Fanuel Kipesha, Deo Mtalikika, Pascal Kashindye, Mashauri Malimi, Joan Joshua, Luiza Mhando, Juma Malipula, Thomas Mshana, Daudi Sylvester, Shabani Omary, Patric Mshana and all the larval surveillance, larval control and adult monitoring CORPs for their tireless work in the field and laboratory. We thank Dr. Alex Mwita, Dr Renate Madnike and Dr Azma Simba from the National Malaria Control Programme for their invaluable support of the programme. We are grateful to Peter DeChant, Dr. Steven Krause and Valent BioSciences Corporation for technical support and donation of microbial larvicides. This work was supported financially by the Swiss Tropical Institute, the United States Agency for International Development (Environmental Health Project, Dar es Salaam Mission and the
U.S. President’s Malaria Initiative), the Bill & Melinda Gates Foundation, Valent BioSciences Corporation and the Wellcome Trust (Research Career Development Fellowship number 076806 awarded to GFK). All persons shown in the photographs have consented to publication. This manuscript has been published with kind permission of Dr Andrew Kitua, the Director of the National Institute for Medical Research of the United Republic of Tanzania.
4.8 References


5. **REDUCTION IN MALARIA PREVALENCE IN DAR ES SALAAM, TANZANIA AFTER CONTROL WITH LARVICIDES**

Marcia C. de Castro¹, Khadija Kannady², Burton H. Singer³, Deo Mtasiwa², Hassan Mshinda⁴, Marcel Tanner⁵, Yvonne Geissbühler²,⁴,⁵, Steve W. Lindsay⁶, Ulrike Fillinger⁶, Gerry F. Killeen⁴,⁵,⁶

¹Harvard School of Public Health, Department of Population and International Health, 665 Huntington Avenue, Bldg. I, Room 1113, Boston, MA 02115, USA
²City Medical Office of Health, Dar es Salaam City Council, P.O. Box 63320, Dar es Salaam, United Republic of Tanzania
³Princeton University, Office of Population Research, Wallace Hall, Princeton, NJ 08544, USA
⁴Ifakara Health Research and Development Centre, Coordination Office, P.O. Box 78373, Kiko Avenue, Mikocheni B, Dar es Salaam, United Republic of Tanzania
⁵Swiss Tropical Institute, Department of Public Health and Epidemiology, Socinstrasse 57, 4002 Basel, Switzerland
⁶Durham University, Institute of Ecosystems Science, School of Biological and Biomedical Sciences, South Road, Durham, DH1 3LE, UK

Paper in preparation
5.1 Abstract

Background

Although antilarval mosquito control measures have been successfully implemented in the past, this strategy is largely neglected in contemporary malaria control programs in sub-Saharan Africa. Recent studies have shown that microbial larvicides significantly reduced Anopheles mosquito density and malaria transmission intensity in Africa but impact upon Plasmodium falciparum infection in sub-Saharan Africa remains to be proven. Here we evaluate whether large-scale use of microbial larvicides could reduce the prevalence of malaria in the city of Dar es Salaam in the United Republic of Tanzania.

Methods

In March 2004 an Urban Malaria Control Program (UMCP) was launched in 15 wards (614,000 inhabitants) to train local personnel, develop new implementation protocols and collect baseline information on larval and adult mosquito density, prevalence of malaria infection, and socio-economic, ecological and behavioral characteristics. In March 2006, routine application of microbial larvicides – Bacillus thuringiensis var. israelensis and B. sphaericus, was initiated in 3 wards (128,000 inhabitants).

Findings

After only one year of larval control, wards treated with microbial larvicides had a 63% (95% CI 53-71%; p<0.0001) decline in the odds of infection with P. falciparum during the intervention period, when compared with the pre-intervention one. This compared with only a 32% (95% CI 29-39%) decline in the non-intervention wards. When one considers only the intervention period, there was a 59% (95% CI 29-95%) greater chance of infection in non-treated wards than treated ones. This represents a major contrast compared with the pre-
intervention period, when there was no statistically significant difference in the chance of infection between treated and non-treated wards.

**Conclusions**

Our findings suggest that large scale application of microbial larvicides can contribute substantially to reducing *P. falciparum* infection prevalence under operational conditions. Microbial larvicides therefore represent an important option for reducing malaria burden in urban areas and may be incorporated into an integrated package of malaria control interventions.
5.2 Introduction

Malaria control in urban Africa has a history dating back more than a century [1] and is receiving increasing attention in response to the rapid urban expansion in the continent [2-4]. The urban population of Africa is likely to double between 2000 and 2030 [5], and it is estimated that more than half of Africans will live in urban areas by 2030 [6]. Therefore, attempts to understand and to control urban malaria in Africa not only serve as a response to anticipated problems, but also to mitigate potential future malaria transmission in these settings.

The influence of urbanization on malaria transmission can be three-fold. First, it might contribute to a reduction in the number of places that could potentially act as *Anopheles* breeding sites given the large extent of built-up areas and drainage [7]. Second, the initial process of urban expansion in the periphery of the city is most often characterized by fast developing unplanned settlements, lacking basic infrastructure and therefore accompanied by increases in *Anopheles* larval habitats [8]. Finally, high levels of population density, such as currently observed in urban agglomerations, ultimately contribute to reduce the intensity of malaria transmission [2,4,9].

Dar es Salaam, Tanzania, is typical of urban areas in Africa experiencing rapid growth. The city started as a trading center established during colonial times, and is currently the most densely populated area in Tanzania (1,793 people/km²). The urban population in the country increased from 5% in 1967 to 23% in 2002 mostly due to rural-urban migration [10]. It is estimated that in Dar es Salaam 70% of the population lives in unplanned settlements [11]. In addition, urban agriculture is a common practice in locations with a high water table. Raised planting beds leaving pooled water in ridges (called as *tuta* in Kiswahili, and *matuta* being the plural form of the word), irrigated rice fields, garden wells, and irrigation channels favor the
development of mosquito breeding sites [12-14]. Another important characteristic in the city is the network of anti-malaria drains, some dating back to the early 1900s [15-17]. Although intended to reduce the potential breeding sites, these drains lack proper and regular maintenance resulting in waste accumulation, water stagnation, and the proliferation of breeding sites for malaria vectors.

It is in this scenario that an Urban Malaria Control Program (UMCP) was launched in March 2004 [18-20]. The UMCP covers 15 of the 73 wards of Dar es Salaam, 5 in each municipality (Figure 1), encompassing a total area of 56 km² and more than 610,000 residents. These 15 wards are classified as urban by the Tanzania National Bureau of Statistics (NBS). Although the NBS criterion for defining an urban area is not precise, urban wards typically have a nuclear center, and provide basic infrastructure and social services, while rural wards are mostly dominated by agriculture. The NBS also defines mixed areas as having both urban and rural characteristics (Figure 1).

Prior to February 2006, UMCP activities were concentrated on developing new operational procedures [18,19] collecting baseline information and training of local personnel. Baseline data was obtained through 4 different surveys focused on: (i) density and species diversity of adult mosquitoes, (ii) mapping of breeding sites and larval surveillance, (iii) individual and household characteristics, and (iv) parasitological assessment. All surveys use the ten-cell unit (TCU) – a cluster of 10 -20 houses - as the basic spatial unit. In addition, an assessment was conducted between June 2005-March 2007 in order to produce an inventory of drains and their current condition. The objective of this survey is to enable environmental management (EM) of Anopheles breeding sites, focusing on cleaning anti-malaria drains, and promoting community sensitization on environmental and hygienic issues related to malaria transmission and control [21].
Figure 1. Administrative units of Dar es Salaam, UMCP targeted area, and intervention wards. Administratively, Dar es Salaam comprises three municipalities – Ilala, Kinondoni and Tembeke – and is divided into 73 wards (22 in Ilala, 27 in Kinondoni, and 24 in Tembeke), classified by the Tanzania National Bureau of Statistics (NBS) as urban, rural or mixed. The wards are further divided into smaller areal units called mitaa (a Kiswahili word for street, written in the singular form as mtaa). Each mtaa is subdivided into ten-cell units (TCU), or clusters of approximately 10-20 houses, although some TCUs aggregate a much higher number of houses (the figure shows one example of mtaa and TCU boundaries for Mikocheni ward). The UMCP targets 15 of the 73 wards in Dar es Salaam. Use of microbial larvicides started in 3 wards in March 2006, and was expanded to 6 additional wards in June 2007.
Since March 2006, microbial larvicides \textit{–Bacillus thuringiensis var. israelensis} (VectoBac\textsuperscript{®}) or \textit{B. sphaericus} (VectoLex\textsuperscript{®}) – were applied weekly to all potential mosquito breeding sites as a control strategy in 3 wards, one in each municipality (Figure 1) \cite{18}. Details of the methodology are presented elsewhere \cite{18,19}. Antilarial mosquito control measures, although successfully implemented in the past \cite{22-28}, have been largely neglected in contemporary malaria control programs in sub-Saharan Africa. Recent studies suggest that larvicides have the potential to be an effective control strategy \cite{29-33}. Indeed, data from Kenya indicate that areas treated with larvicides experienced a reduction of 95\% in \textit{Anopheles} larval density and a 92\% decline in human exposure to mosquito bites \cite{31}. Although these results are highly encouraging, no assessment of the impact of larvicide use on the prevalence of infection with malaria parasites has been demonstrated in a contemporary African setting. Here we present initial evidence that the use of microbial larvicides can significantly reduce the prevalence of \textit{Plasmodium falciparum} infection after only one year of intervention.

5.3 Methods

Study site

Dar es Salaam is the commercial capital of Tanzania, located in Eastern Africa (Figure 1). Administratively, the city comprises three municipalities – Ilala, Kinondoni and Temeke – and is divided into 73 wards (22 in Ilala, 27 in Kinondoni, and 24 in Temeke). Wards are further divided into smaller neighborhood units called \textit{mitaa} (a Kiswahili word for street, written in the singular form as \textit{mtaa}) \cite{19}. Each \textit{mtaa} is subdivided into ten-cell units (TCU), or clusters of approximately 10-20 houses, although some TCUs contain a much larger number of houses \cite{19}. Dar es Salaam has a hot and humid tropical climate with two rainy seasons: an intense one observed during the months of March, April, and May, and a milder
one occurring in November and December. The area is endemic for malaria and transmission is perennial [34].

**Household and parasitological surveys**

As part of the activities carried out by the UMCP, household and parasitological surveys started in Dar es Salaam in May 2004. All data collected were georeferenced. Community involvement is a strong component of the surveys: interviewers and nurses are members of the community, and in preparation for each wave of household and parasitological data collection, meetings were conducted with TCU leaders in order to promote sensitization.

**Sample frame.** The sampling unit was the TCU. A list of TCUs by ward was assembled in March 2004, and it was regularly updated [19]. For each one of the 15 UMCP wards, 10 TCUs were randomly sampled at each survey wave. All houses located in the sampled TCUs were visited and individuals invited to participate in the survey. Four waves of data collection were conducted between May 2004 and May 2007 (Table 1). Each wave had 5 stages, and in each stage the survey was conducted in 3 out of the 15 UMCP wards (one in each municipality). The duration of each wave, as well as the interval between them, varied due to unforeseen events e.g. presidential elections impacting people’s perception about the apolitical nature of the survey, replacement of personnel and other reasons. After the 1st wave of data collection, two approaches were adopted: (i) a follow-up survey of subjects interviewed in the 1st wave, and (ii) a cross-sectional survey of new subjects in randomly selected TCUs. The goal of the former was to serve as sentinel areas routinely appraised.
Table 1 Waves/Phases of household and parasitological surveys. Four waves of data collection have been concluded between May/2004-March/2007. Each wave has 5 stages, and 3 wards of each municipality are included in each stage. Month overlaps reflect slightly different duration of data collection in each ward. Municipalities are coded as: Ilala=I, Kinondoni=K, Temeke=T. The wards included in each stage are: Stage I – Vingunguti (I), Mwananyamala (K), and Azimio (T); Stage II – Buguruni (I), Mzimuni (K), and Keko (T); Stage III – Mchikichini (I), Mikocheni (K), and Miburani (T); Stage IV – Ilala (I), Ndogumbi (K), and Mtoni (T); and Stage V – Kipawa (I), Magomeni (K), and Kurasini (T). Use of microbial larvicides started in March 2006 in Buguruni (I), Mikocheni (K), and Kurasini (T).

<table>
<thead>
<tr>
<th>Wave</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>2004</td>
<td>2005</td>
<td>2006</td>
<td>2007</td>
</tr>
<tr>
<td>Months</td>
<td>M J</td>
<td>J A</td>
<td>S O</td>
<td>N D</td>
</tr>
</tbody>
</table>

Survey instruments. The questionnaire utilized in the household survey was divided into 6 parts: (i) locational information, (ii) characteristics and structural conditions of the house, (iii) information about the head of the household, (iv) socio-economic and agricultural characteristics of the household, (v) measures for protection against malaria, and (vi) individual, demographic, behavioral and health related information. The contents were chosen in order to ascertain which members of the household have had at least one diagnosed or perceived episode of malaria in the previous two weeks; to appraise their utilization of public, private, and informal health systems; and to collect information on likely confounders. The 1st wave of household data collection in the UMCP wards started in May 2004.

Community nurses accompanied the interviewer in each house and were responsible for measuring body temperature and collecting finger prick blood samples from each study subject. Malaria parasites were identified by species using thin smears while parasites count (number of parasites per 200 white blood cells) was determined using thick smears [35], and results recorded in a separate questionnaire. The results were forwarded to the community nurses within 24 hours after screening, and individuals who tested positive for the presence of
malaria parasites were initially treated with Fansidar until August 2006, when it was replaced by a combination therapy – Maladar (each tablet contains 50 mg of Artesunate and 135 mg of Amodiaquine). In the case of side effects, individuals were advised to report to a health facility to receive alternative treatment. In the case of severe malaria or any other disease, patients were immediately referred to the nearest health facility.

**Statistical analyses**

Weekly rainfall data were used to categorize periods as dry or wet, based on the classification of rain intensity adopted by the Tanzania Meteorological Agency (http://www.meteo.go.tz): "very light" – scattered drops that do not completely wet a surface; "light" – rainfall greater than a trace and up to 0.10 inch an hour; "moderate" – rate of fall is between 0.11 to 0.30 inch per hour; and "heavy" – over 0.30 inch per hour. Assuming that daily rainfall events last on average 3 hours, and occurred during at least 30% of the time period considered (week), we established cutoff rainfall amounts for very light, light, moderate and heavy precipitation. These were further aggregated into 2 categories: (i) dry, combining dry and light; and (ii) wet, combining moderate and heavy.

Starting in March 2006 (slightly after the onset of the main rainy season; Figure 2) biological larvicides were applied weekly to all open sunlit water bodies which might produce malaria vectors. Considering that the 3rd wave of household and parasitological data collection ended in May 2006 (Table 1), and the biological time lag between reducing larval survival and reducing malaria transmission from human to human, two time periods were defined for the purposes of assessing the impact that the use of biological larvicides had on the prevalence of *P. falciparum* infection: (i) pre-intervention period, consisting of the 3rd wave of data collection – September/2005-May/2006, and (ii) intervention period, 4th wave of data collection – July/2006 – March/2007. Prevalence of *P. falciparum* infection was calculated
based on the microscopy results, and the analyses here presented include both sexes and all age groups combined.

![Rainfall Graph](image)

**Figure 2.** Monthly rainfall in Dar es Salaam, 2004-7. Rainfall measurements observed at the Dar es Salaam JK Nyerere airport station, and provided by the Tanzania Meteorological Agency, Ministry of Infrastructure and Development - [http://www.meteo.go.tz](http://www.meteo.go.tz). Dar es Salaam is characterized by two rainy seasons: an intense one observed during the months of March, April, and May, and a mild one occurring in November and December.

Odds ratios for the prevalence of infection in treated and non-treated areas were computed comparing pre-intervention and intervention periods, in order to assess if significant declines in the odds of infection were indeed observed following control with microbial larvicides. In addition, odds ratios for the prevalence of infection were also calculated for the intervention period comparing non-intervention wards (contemporary controls) with intervention ones, in
order to assess if areas treated with larviciding do have significantly lower odds of infection. Confidence intervals for prevalence of infection and odds ratios were also obtained. Appraisal of prevalence rates and odds ratios was detailed by rain intensity (wet and dry, as detailed above), facilitating the evaluation of the intervention during distinct seasonal patterns of precipitation. Data cleaning and calculation of prevalence rates, odds ratios and confidence intervals were performed in STATA® software, version 9.2 [36]. Databases were created in Epi Info™ version 3, and a double entry routine set up for the purposes of quality control.

Ethical clearance

The Medical Research Coordination Committee of the National Institute of Medical Research in Tanzania (NIMR/HQ/R.8a/Vol. IX/279), Tanzanian Commission of Science and Technology (No. 2004-69-MFS-2004-24) and Durham University Ethics Advisory Committee provided ethical clearance for all UMCP activities. All the laboratory work follows protocols developed by the World Health Organization [35]. The survey was not restricted to specific subjects, including all age ranges and sex groups. Individual human subjects invited to participate in the survey, upon agreement, signed informed consent forms. In the case of children (aged 15 or younger) consent was granted and documented through signature by a parent or designated guardian. In the event that individual subjects are illiterate, a finger print replaced the signature.

5.4 Results

Accurate levels of prevalence of infection were not available a priori at the onset of the survey. A range of 2-10% had been reported for the urban area of Dar es Salaam [12], and the overall prevalence of infection for the 1st wave of the household survey was 16.4% (95% CI = 15.5-17.2%). Using a range of 10-16%, the required sample size to detect a 50% change in
prevalence with significance error of 5% and 80% power was 283-474. Nevertheless, very small prevalence rates (1-5%) were expected in a few locations due to seasonal patterns and spatial heterogeneity. A targeted sample size of 400-450 was therefore chosen as a compromise. An average of 404 people per wave/municipality/ward have been interviewed since May 2004.

In an urban context such as Dar es Salaam, loss due to follow-up results mostly from migration (inside or outside the city) and temporary traveling. During the 2nd wave, refusal to participate in the follow-up survey, mainly observed among adults, reached a maximum of 28%, and was a consequence of several factors. Common reasons included complaints that the finger prick was painful, and misconceptions about malaria transmission, such as: “everybody has malaria and therefore repeated tests are useless”, and that parasite counts provided in the 1st wave were “impossible” numbers based on blood slide results usually provided by private health facilities (interviewees often suggested that blood tests made at private facilities were frequently positive and reported a parasite count of 1 or 2). All these issues were properly addressed in sensitization efforts conducted by interviewers and nurse practitioners with the support of TCU leaders, resulting in higher participation in subsequent waves. During the 4th wave the refusal rate was, on average, 17%. Multiple attempts (up to 3) to enroll subjects were made to achieve full coverage of each house. Starting on the 3rd wave, the list of subjects to be followed-up were randomly drawn from the population of individuals interviewed in the 2nd wave (new subjects) in order to account for the losses due to follow-up, and guarantee the minimum required sample size.

Rainfall (Figure 2) was greater and more extended in 2006 (1448 mm) than in 2004 (1095 mm) or 2005 (901 mm). In 2006, the heavy and protracted rains resulted in significant
flooding in several areas of the city, unlike in the preceding years where there was little flooding. Regarding malaria, the prevalence of *P. falciparum* infection declined during the peak of precipitation and rose afterwards (Figure 3).

![Figure 3](image-url)

**Figure 3.** Prevalence of infection and rainfall by month. Error bars represent 95% confidence intervals for the prevalence of *P. falciparum* infection observed in all 15 UMCP wards. No data was collected in June 2006. Periods of heavy rains are usually associated to low prevalence of infection, while the opposite is observed during drier periods.

At an aggregate level, intervention and non-intervention wards do not reveal significant differences regarding basic demographics, use of bednets, house crowding, ownership of house, and practice of agriculture (Table 2).

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1 The rainfall data reflect measurements observed at only one meteorological station, located at the Dar es Salaam JK Nyerere airport. Therefore, there is an underlying assumption that the airport station suffices to represent pluviometric patterns of all 15 wards under study. While this assumption imposes no constraints for a global analysis, the pattern shown in Figure 3 is likely to hide local precipitation variability.
Table 2 Basic characteristics of the population in intervention and non-intervention wards. Aggregated descriptive statistics for intervention wards (treated with microbial larvicides: Buguruni, Mikocheni, and Kurasini) and non-intervention wards, observed during the 3rd (Sep/2005-May/2006) and 4th (Jul/2006-March/2007) waves data collection.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Intervention wards</th>
<th>Non-intervention wards</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average age (years)</td>
<td>22.3</td>
<td>22.2</td>
</tr>
<tr>
<td>Sex distribution (%)</td>
<td>63.5 Fem 36.5 Male</td>
<td>65.2 Fem 34.8 Male</td>
</tr>
<tr>
<td>Average % of people that slept under a net the night before the interview</td>
<td>83.5</td>
<td>86.6</td>
</tr>
<tr>
<td>Average % of people that slept under a treated net the night before the interview</td>
<td>22.1</td>
<td>26.5</td>
</tr>
<tr>
<td>Average number of people per house</td>
<td>11.3</td>
<td>10.7</td>
</tr>
<tr>
<td>Average number of households per house</td>
<td>1.7</td>
<td>1.5</td>
</tr>
<tr>
<td>Average % of households that own a house</td>
<td>75.0</td>
<td>74.7</td>
</tr>
<tr>
<td>Average % of households that cultivate a crop</td>
<td>10.7</td>
<td>9.6</td>
</tr>
</tbody>
</table>

The overall prevalence of *P. falciparum* infection for the 15 UMCP wards during the pre-intervention period was 10.4% (95% confidence interval (CI) = 9.8-10.9%). In the intervention period the overall prevalence in these wards dropped to 6.6% (95% CI = 6.2-7.0%). Large variability in the prevalence of infection was observed at different scales (Table 3). Indeed, in an urban context such as Dar es Salaam, the prevalence is expected to be spatially autocorrelated. Previous research revealed that transmission follows a gradient, with low rates in the city center and higher rates as one moves away from the center to the periphery [12]. Although all 15 UMCP wards are classified as urban by the NBS (Figure 1), they have rather different patterns of urban morphology, mixing upper scale housing, unplanned settlements lacking basic infrastructure, and newly expanded areas in the periphery. Although a universal definition of urban does not exist [37-39], in a future study we will propose an alternative characterization of urban morphology for the UMCP targeted area, which will facilitate the evaluation of the existence of gradients of malaria transmission.
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Table 3 Range of the prevalence of *P. falciparum* infection observed at the ward, *mtaa* and TCU levels. Maximum and minimum values of prevalence of *P. falciparum* infection observed at different levels of spatial scale in each municipality. Intervention wards are Buguruni, Mikocheni, and Kurasini. Pre-intervention period is represented by the 3rd wave of data collection (Sep/2005-May/2006). Intervention period is represented by the 4th wave of data collection (Jul/2006-March/2007). Although larval control commenced in March 2006, initial months faced challenges due to heavy rainfall and adaptation of staff members to the new activity. Therefore, using the wave as a reference, instead of the month per se, accounts for these problems.

<table>
<thead>
<tr>
<th>Period and area</th>
<th>Prevalence of <em>P. falciparum</em> infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ilala</td>
</tr>
<tr>
<td></td>
<td>Kinondoni</td>
</tr>
<tr>
<td></td>
<td>Temeke</td>
</tr>
<tr>
<td>Ward</td>
<td>Mtaa</td>
</tr>
<tr>
<td></td>
<td>TCU</td>
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<tr>
<td></td>
<td>Ward</td>
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<tr>
<td></td>
<td>Mtaa</td>
</tr>
<tr>
<td></td>
<td>TCU</td>
</tr>
<tr>
<td></td>
<td>Ward</td>
</tr>
<tr>
<td></td>
<td>Mtaa</td>
</tr>
<tr>
<td></td>
<td>TCU</td>
</tr>
<tr>
<td>Pre-intervention</td>
<td></td>
</tr>
<tr>
<td>wards</td>
<td>4.5-10.9 3.1-13.9</td>
</tr>
<tr>
<td></td>
<td>0-22.2 7.7-12.1 4.4-25.5</td>
</tr>
<tr>
<td></td>
<td>0-40.0 9.1-14.2 6.3-22.6 0-47.1</td>
</tr>
<tr>
<td>Intervention wards</td>
<td>11.9 5.6-15.0</td>
</tr>
<tr>
<td></td>
<td>0-29.4 9.4 7.1-20.7</td>
</tr>
<tr>
<td></td>
<td>0-30.0 12.7 9.8-17.0 4.8-33.3</td>
</tr>
<tr>
<td>Intervention period</td>
<td></td>
</tr>
<tr>
<td>Non-intervention</td>
<td>5.3-11.0 3.3-13.0</td>
</tr>
<tr>
<td>wards</td>
<td>0-26.7 4.9-9.6 3.8-13.4</td>
</tr>
<tr>
<td></td>
<td>0-26.1 3.0-10.7 0.9-14.3 0-33.3</td>
</tr>
<tr>
<td>Intervention wards</td>
<td>4.6 2.9-7.7</td>
</tr>
<tr>
<td></td>
<td>0-25.0 4.7 0-6.5 0-12.2 4.4 0-7.8</td>
</tr>
<tr>
<td></td>
<td>0-27.3</td>
</tr>
</tbody>
</table>

The prevalence of infection declined in both intervention and non-intervention wards in 2006 (Figure 4). However, the largest decline was observed in the intervention wards where there was a 63% (95% CI 53-71%) decline in the odds of infection during the intervention period, when compared with the pre-intervention one. This compared with only a 32% (95% CI 29-39%) decline in the non-intervention wards. When one considers only the intervention period, there was a 59% (95% CI 29-95%) greater chance of infection in non-treated wards than treated ones. This represents a major contrast compared with the pre-intervention period, when there was no statistically significant difference in the chance of infection between treated and non-treated wards.
Figure 4. Prevalence of infection during pre-intervention and intervention periods. Error bars represent 95% confidence intervals for the prevalence of *P. falciparum* infection. Three wards were treated with microbial larvicides: Buguruni (I), Mikocheni (K), and Kurasini (T). Pre-intervention period is represented by the 3rd wave of data collection (Sep/2005-May/2006). Intervention period is represented by the 4th wave of data collection (Jul/2006-March/2007). Although larval control commenced in March 2006, initial months faced challenges due to heavy rainfall and adaptation of staff member to the new activity. Therefore, using the wave as a reference, instead of the month per se, accounts for these problems.

During the pre-intervention period the prevalence of infection was similar in intervention and non-intervention wards and for all seasons (Table 4). However, following mosquito control with larvicides, the prevalence of infection was significantly lower throughout the dry season. During the intervention period the reduction in the odds of infection seen in the intervention wards (62%, 95% CI 48-72%) declined much more than the non-intervention wards (24%, 95% CI 15-33%) during the dry periods than in the wet periods (64% in intervention wards,
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95% CI 48-75%, and 53% in non-intervention wards, 95% CI 42-62%). Therefore, the expected seasonal burden of malaria transmission after the rainy season (Figure 3) was mitigated in intervention wards following control with larvicides. This is consistent with historical reports that suggested the use of larviciding to be easier and more effective during the dry season [22,25].

Table 4 Prevalence of \textit{P.falciparum} infection by rainfall season observed in intervention and non-intervention wards during pre-intervention and intervention periods. Rainfall season categories are based on the classification of rain intensity adopted by the Tanzania Meteorological Agency ([http://www.meteo.go.tz](http://www.meteo.go.tz)). Wet season describe a period of heavy or moderate rainfall, while a dry season refer to absence of light rainfall. Average prevalence \textit{P.falciparum} infection presented with the binomial confidence interval. Intervention wards are Buguruni, Mikocheni, and Kurasini. Pre-intervention period is represented by the 3\textsuperscript{rd} wave of data collection (Sep/2005-May/2006). Intervention period is represented by the 4\textsuperscript{th} wave of data collection (Jul/2006-March/2007). Although larval control commenced in March 2006, initial months faced challenges due to heavy rainfall and adaptation of staff members to the new activity. Therefore, using the wave as a reference, instead of the month per se, accounts for these problems.

<table>
<thead>
<tr>
<th>Period and area</th>
<th>Prevalence of \textit{P.falciparum} infection by precipitation seasonal pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry</td>
</tr>
<tr>
<td>\textit{Pre-intervention} Non-intervention wards</td>
<td>10.1</td>
</tr>
<tr>
<td>Intervention wards</td>
<td>10.7</td>
</tr>
<tr>
<td>\textit{Intervention} Non-intervention wards</td>
<td>8.0</td>
</tr>
<tr>
<td>Intervention wards</td>
<td>5.1</td>
</tr>
</tbody>
</table>

5.5 Discussion

Recent studies in Africa have shown that the use of microbial larvicides reduced the \textit{Anopheles} larval density by 95% and malaria transmission intensity by 92% [31]. In this paper we offer new evidence that this control strategy also reduces the prevalence of \textit{P. falciparum} infection. Based on the assessment of an operational urban malaria control in Dar es Salaam, we show that areas systematically treated with larvicides experienced a 63% decline in the odds of infection after only one year of interventions, whilst non-treated areas had a 32%
Article 3: Reduction in malaria prevalence in Dar es Salaam, after control with larvicides

decline. Although a longer period of time is needed to ascertain long-term effectiveness and sustainability, the maturation of programs through experience and refinement are likely to further improve impact of this intervention. Our results indicate that using microbial larvicides as an antilarval mosquito control measure is an important option for reducing the burden of malaria in urban areas, and that may be incorporated in integrated packages of malaria control interventions [40].

In parallel to the UMCP activities, the National Malaria Control Program (NMCP) is currently promoting early diagnosis and proper treatment, bednet distribution, and community programs to promote sensitization. The impact of these interventions in Dar es Salaam (and therefore in the 15 UMCP wards) has not been evaluated. Although part of the decline observed during the intervention period may be a result of these and other confounding factors (e.g. fast urban growth), the use of microbial larvicides indicates a significant reduction in the prevalence of infection particularly during the dry season. This finding has 2 major implications: (i) the peak in malaria usually observed after the rains can be mitigated by the use of microbial larvicides, facilitating the reduction of the disease burden; and (ii) additional strategies and/or improved procedures and practice may be needed during the wet season in order to further reduce transmission (e.g. environmental management through sanitary engineering works).

Our results do not consider fine-grained differences in ecological settings and socioeconomic characteristics. This will be accomplished in a future study performed at multiple levels of temporal and spatial scales [41]. Results will shed further light on selection of additional control strategies (in combination with larvicides) that should comprise an integrated package for malaria control in urban settings [40,42].
In conclusion, malaria control programs designed for African cities are needed so that future problems linked with rapid urban expansion can be mitigated in a timely manner. Although a variety of control strategies other than use of microbial larvicides have been successfully implemented [43-46], we believe that the organizational structure and approach implemented by the UMCP has a unique feature. The strong community involvement in malaria control strategies, based on local capacity building, and the direct governmental participation and commitment in all phases of the program constitute a strong basis for future sustainability of control activities [18,20]. Our findings indicate that larval control with microbial larvicides can substantially reduce malaria infections in Dar es Salaam and similar programs should be encouraged in other African cities.

**Competing Interests**

The urban malaria control program evaluated in this paper is partially supported by Valent Biosciences Corporation, a commercial manufacturer of microbial larvicides.

**Author Contributions**

MCC and KK designed and implemented the household and parasitological surveys, and created all databases. KK oversaw all operational activities, in consultation with HM, DM, SWL, BS and MT. UF, KK and GFK planned and oversaw the larval control intervention. YG and GFK set up the adult mosquito surveillance system. MCC cleaned the data, conducted the statistical analysis, and wrote the paper. All authors read and approved the final version of the paper.
5.6 Acknowledgments

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5.7 References


Article 3: Reduction in malaria prevalence in Dar es Salaam, after control with larvicides


6. Urban malaria epidemiology and the impact of microbial larvicides upon infection prevalence in Dar es Salaam, United Republic of Tanzania

Yvonne Geissbühler, Research Scientist\textsuperscript{1,2,3}, Khadija Kannady, City Malaria Control Officer\textsuperscript{2}, Prosper Chaki, Research Scientist\textsuperscript{2,3,4}, Basiliana Emidi, Research Assistant\textsuperscript{2,5}, Nicodemus J. Govella, Research Scientist\textsuperscript{2,3,4}, Valeliana Mayagaya, MSc candidate\textsuperscript{3,5}, Michael Kiama, City Malaria Control Officer\textsuperscript{2}, Deo Miasiwa, Chief Medical Officer\textsuperscript{6}, Hassan Mshinda, Director\textsuperscript{3}, Steven W. Lindsay, Professor\textsuperscript{4}, Marcel Tanner, Professor & Director\textsuperscript{1}, Ulrike Fillinger, Public Health Entomologist\textsuperscript{4}, Marcia Caldas de Castro, Assistant Professor\textsuperscript{7}, Gerry F. Killeen, Research Fellow\textsuperscript{3,4}

\textsuperscript{1}Swiss Tropical Institute, Department of Public Health and Epidemiology, Basel, Switzerland
\textsuperscript{2}Dar es Salaam City Council, Ministry of Regional Administration and Local Government, Dar es Salaam, United Republic of Tanzania
\textsuperscript{3}Ifakara Health Research and Development Centre, Coordination Office, Dar es Salaam, United Republic of Tanzania
\textsuperscript{4}Durham University, School of Biological and Biomedical Sciences, Durham, United Kingdom
\textsuperscript{5}Department of Zoology and Marine Biology, University of Dar es Salaam, Dar es Salaam, Tanzania
\textsuperscript{6}Ministry of Health and Social Welfare, Dar es Salaam, United Republic of Tanzania
\textsuperscript{7}Harvard School of Public Health, Department of Population and International Health, Boston, Massachusetts, USA

Correspondence to: Y Geissbühler, Y.Geissbuehler@unibas.ch

\* Sadly, Michael Kiama passed away before completing the present work.
6.1 Abstract

**Objective** Elucidate malaria epidemiology in urban Africa and evaluate the impact of microbial larvicide (*Bacillus thuringiensis var. israelensis (Bti)*) application upon malaria infection prevalence.

**Design** Routine entomological surveillance data from the Urban Malaria Control Program (UMCP) was combined with household surveys of malaria infection status, household characteristics, human behaviour, and use of various malaria control measures.

**Setting** Fifteen wards of Dar es Salaam in Tanzania with 612,000 residents where the UMCP monitors and controls malaria transmission on an ongoing basis.

**Participants** All age groups.

**Intervention** Application of microbial larvicide *Bti* to open larval breeding sites was initiated in March 2006 to complement existing personal and household protection measures.

**Main outcome measures** Prevalence of malaria infection, mosquito densities and entomological inoculation rate (EIR) of malaria.

**Results** From May 2004 to March 2007, use of window screening, complete ceilings, amodiaquine and artemisin-based therapies increased, presumably contributing to steadily decreasing malaria prevalence despite stable transmission intensity (EIR≈1.3 infectious bite per person per year) outside of larvicide-treated areas. Malaria infection prevalence was
highest and most responsive to exposure in children ≤5 years, despite this relatively low transmission intensity. Community-based larval control with *Bti* in the third year of the study reduced transmission intensity (OR= 0.708 [0.505 to 0.991], P = 0.044) and was the only significant determinant of malaria infection risk (OR=0.434 [0.263 to 0.714], P=0.001) other than time (P<0.001) and location (P=0.001) among young children. Separate analyses of prevalence by year suggest modest benefits of insecticide-treated nets (OR=0.805 [0.642 to 1.009], P=0.060; year 1) and houses with complete ceilings (OR=0.776 [0.620 to 0.970], P=0.026; year 2).

**Conclusion** These early benefits of larviciding can be substantially improved upon with time, investment, experience and creativity. After half a century of neglect, larval control now merits further development, investment and evaluation in urban Africa.
6.2 Introduction

Although awareness and support for controlling malaria has increased greatly in recent years, current financial commitments total only 20% of that required \(^1\) and malaria remains a major contributor to the global disease burden \(^2-^4\). Malaria research and control has traditionally focused on rural areas but it is increasingly recognized that malaria also poses a major problem in urban settings \(^5-^9\). Even though malaria transmission is generally lower in urban areas \(^5\) \(^9\) \(^11\) \(^12\), improved understanding and evidence–based strategies for controlling urban malaria are urgently needed because more than 50% of the African population will live in towns or cities by 2030 \(^13\). Recent advances in analytical modelling \(^14-^16\) illustrate how the lower exposure levels typically occurring in urban Africa lead to lower immunity in the urban population, and higher prevalence of infection, morbidity, mortality and infectiousness in older age groups \(^5\) \(^9\) \(^17\).

Malaria epidemiology is complex and malaria prevalence is not only influenced by the entomological inoculation rate (EIR) \(^18\). It is also affected by the socioeconomic status (SES) of the household, the education level of the head of household or travelling to rural areas with higher transmission levels \(^19-^25\). While poorer and less educated people, as well as people travelling to rural areas, are typically at higher risk of contracting malaria, SES and education also influence what kind of protective, diagnostic and curative measures against malaria inhabitants can afford and use \(^26-^31\).

In Dar es Salaam, the largest city of the United Republic of Tanzania, inhabitants use different protective measures like ceiling boards, window screening, sprays, coils, repellents and insecticide treated nets (ITNs) depending on what they can afford and also depending on their
knowledge and perception of risk\textsuperscript{32}. Tanzania has emphasized widespread use of ITNs as a priority malaria vector control strategy\textsuperscript{33} but recent observations indicate that malaria vectors tend to bite outdoors in Dar es Salaam so ITNs confer less protection than in rural areas\textsuperscript{32}. Alternative strategies which reduce larval abundance and hence adult vector populations may be of great utility in other urban areas, particularly those with similarly exophagic vectors. Successes of larval control and integrated vector control programs including environmental management have been clearly recorded in the past, although it should be noted that none of these historical examples have since been sustained consistently\textsuperscript{12,34-36}. The Urban Malaria Control Program (UMCP) in Dar es Salaam has been initiated by the Dar es Salaam City Council as a pilot program to develop sustainable and affordable systems for larval control as part of routine municipal services. Specifically, the UMCP implements the regular application of microbial larvicides (\textit{Bacillus thuringiensis} var. \textit{israelensis} (\textit{Bti}) and \textit{B. sphaericus} (\textit{Bs})) through community-based but vertically managed delivery systems\textsuperscript{37,38}.

Here we have taken an in-depth look at malaria epidemiology and seasonal patterns in urban Dar es Salaam and evaluate the impact of a carefully managed larviciding system\textsuperscript{38} upon malaria transmission and infection prevalence in the presence of existing malaria control measures such as ITNs, ceiling boards, window screening and therapeutic drugs.

\subsection{6.3 Methods}

\textit{Study site}

This study was conducted in Dar es Salaam, the biggest and economically most important city in Tanzania, which is situated on the shores of the Indian Ocean\textsuperscript{12}. It has around 2.5 million inhabitants and covers a total area of 1400 km\textsuperscript{2} (Ref. 39). Dar es Salaam is divided into 3
municipalities: Temeke, Ilala and Kinondoni, which together comprise 73 wards. The wards are further subdivided into neighbourhood-sized administrative subunits known as *mtaa* (singular *mtaa*), the Kiswahili word for street, which normally compromises between 20 and 100 *mashina* (singular *shina*) or Ten Cell Unit (TCU). The TCU is the smallest subunit and normally includes 20 – 30 houses but some even exceed 100 (Figure 1).

**Figure 1.** Wards included in the study area of the Dar es Salaam Urban Malaria Control Program (UMCP), specifying those targeted for larviciding from March 2006 onwards (intervention) and those which did receive any larviciding over the course of the study (non-intervention wards).
The findings presented here are based on data derived from the first 3 years of the UMCP, where household surveys including malaria infection status were initiated in May 2004. The project area includes 5 wards in each of the three municipalities, comprising a total of 67 mitaa. This study site covers a surface area of 55 km² in which 611,871 people resided in 2002. The new management and delivery systems developed which underpin this program are described in detail elsewhere. The surveillance activities of the UMCP are briefly described below and rely on 3 crucial components: 1) Mapping and surveillance of potential Anopheles breeding sites, 2) Monitoring of adult mosquito densities, and 3) Household surveys of parasite infection status and potential determinants thereof (Castro et al. unpublished). In the third year of the UMCP, beginning in March 2006, the routine application of the microbial larvicide Bti to open habitats and Bs to closed habitats was initiated in 3 of the 15 wards in the study area, adding to existing interventions such as bednets, house screening, ceiling boards, repellents, coils and spray. Buguruni, Mikocheni and Kurasini wards in Ilala, Kindondoni and Temeka Municipalities, respectively, are home to a total of approximately 128,000 residents and were chosen for intervention with larvicides because comprehensive and detailed maps had been completed for these wards. The study is divided into years of programmatic activity as follows: Year 1: April 2004 till March 2005 was the first year, during which household surveys were initiated and systems for mapping and monitoring larval habitats were developed. Year 2 spans the period April 2005 to March 2006 and was also defined as a pre-intervention year because no larviciding was implemented. In year 2 household surveys were complemented with entomological baseline data (larval and adult surveys) allowing subsequent rational implementation and evaluation of larviciding. Year 3 is the subsequent intervention year during which systematic larviciding was introduced to the three selected wards and spanned the period of April 2006 to March 2007. Although the first larviciding began in March 2006 these activities took some weeks to
scale up to the full three targeted wards so for analytical purposes we consider March 2006 to be the last month of pre-intervention year 2. Apart from the programmatic rationale for this assumption, biologically-determined time lags in the processes affected suggest that substantial impact upon either adult mosquito density or, even more so, upon malaria infection prevalence cannot be expected any earlier. The underlying epidemiology of malaria in this urban setting and the impact of various interventions on the prevalence of malaria infection were examined using appropriate statistical models and qualitative analyses as described below.

1. Data collection

LARVAL HABITAT SURVEILLANCE

Before surveillance or control activities started, all active or potential breeding sites in each TCU were sketch mapped by community own resource persons (CORPs) \(^{40}\). Approximately 90 larval surveillance CORPs survey all water bodies in their assigned area on a weekly basis for the presence of *Anopheles* and Culicine mosquitoes and report their observations using standardized forms. Quality control and decentralized *in-situ* reaction to field observations is ensured through a carefully designed management system described elsewhere \(^{38,40}\).

ADULT MOSQUITO SURVEILLANCE

In each of the 67 *mitaa*, one resident was recruited as an Adult Mosquito Surveillance CORP in order to conduct human landing catch (HLC) \(^{41}\). In each *mtaa*, four different sampling locations were chosen. HLC was conducted once every four weeks at each location outdoors from 6pm to 6am for 45 minutes of each hour, allowing 15 minutes break for rest. Measured biting densities were therefore divided by 0.75 to obtain
biting rates for a full hour. In order to estimate the total true exposure experienced both indoors and outdoors by residents, these directly measured outdoor mosquito densities were multiplied by the coefficient of the estimated total true human exposure divided by the estimated total outdoor biting rate obtained from detailed studies of mosquito-human interactions. These coefficients (Anopheles gambiae: 0.670, An. funestus: 0.725, An. coustani: 0.448 and Culex: 0.94) were derived from an in-depth mosquito survey which was conducted during the main rainy season of April to June 2006. All mosquitoes were identified morphologically to genus and, in the case of Anopheles, to species complex level. Members of the An. gambiae complex were further identified to sibling species level by polymerase chain reaction (PCR). The sporozoite infection status of each mosquito was determined by enzyme-linked immunoabsorbent assay as previously described.

HOUSEHOLD SURVEY

Four rounds of household surveys were conducted, the first of which took place from May until September 2004. The second started in November 2004 and ended in July 2005. Round 3 went from September 2005 till May 2006 and round 4 from July 2006 till March 2007. During each round, 10 TCUs were randomly sampled in each of the 15 UMCP wards. From the second round onwards, the cohort of TCUs sampled on the first round was followed-up for the duration of the study. The household surveys utilized a questionnaire that recorded the following information about the household: (i) geographical identification of the area, (ii) house structure with an emphasis on features that prevent mosquito entry, (iii) information about education, occupation and knowledge about malaria of the head of the household, (iv) assets, expenditures and income sources, (v) anti-malarial measures in use, and (vi) individual, demographic, behavioural and health
related information like sleeping behaviour, travelling habits and treatment seeking behaviour. All consenting participants also provided finger-pricked blood samples for Giemsa-stained thick and thin smear microscopic examination. The accuracy of these blood smear diagnoses was quality controlled internally as previously described 23. Individuals who were found to be infected with malaria parasites were then treated with appropriate front-line anti-malarial drugs (until August 2006 it was sulphadoxine-pyrimethamine (Fansidar®) which was subsequently replaced by artesunate-amodiaquine (Maladar®)), retested a week later and, if necessary, referred to hospital for treatment of recrudescent infections (Castro et al. unpublished).

2. Implementation of larval control

Larviciding started in March 2006 in one ward of each municipality, namely Buguruni, Mikocheni and Kurasini. These intervention wards were chosen based on the ability of the ward supervisors and the ward-based CORPs to collect, understand, use and submit high quality data during the baseline data collection period 38. The microbial insecticides applied were Bacillus thuringiensis var. israelensis (VectoBac®) for open (light-exposed) habitats and Bacillus sphaericus (VectoLex®) for closed (covered, often highly polluted) habitats. Open habitats, which have the potential to produce Anopheles larvae, were treated weekly by the Mosquito Control CORPs each of whom assigned to a specific mtaa or portions of an mtaa. Closed habitats which mainly produce Culex quinquefaciatus were treated every three months by an additional team of CORPs 38.
Analytical methods

All statistical analyses were executed using SPSS 15.0. In order to calculate a wealth index as a proxy for the SES, we applied principal component analysis (PCA) to the recorded assets of each household. All protective measures such as mosquito nets, window screenings and ceiling boards were excluded as this would have compromised the value of such an index as an independent determinant of malaria risk. All livestock ownership variables were also excluded because only a few people owned animals while ownership of beds and mattresses were excluded because almost all households had them. Factor 1, which was concluded to best reflect the asset index, accounted for 28.6% of the variance (Appendix Table A1).

Generalized estimating equations (GEE) were used to estimate impact on mosquito densities and EIR by treating active larviciding in that time and place as a categorical independent factor in the model. TCU was treated as the unit of geographic location and year as the indicator of time, with vector densities and EIRs estimated as means for each TCU over either the full year or the duration of the July-September dry season when control appeared most effective. TCU identity was treated as a subject variable and mosquito density or total EIR as the dependent variable, using a logarithmic link function and normal distribution, weighted according to the number of catcher nights for each location. The repetition of measurements within the same TCU experimental units was accounted for by treating year as a source of first order autoregressive within-subject variance. Note that in this analysis all 12 non-intervention wards were used for comparison with the 3 intervention wards which differs from an earlier report limited to 3 non-intervention wards for which larval habitat data of sufficient quality was available.
Determinants of malaria infection prevalence were estimated using a similar GEE approach but treating infection status as the dependent variable with a binary distribution and logit link function. Individual human participants were considered the experimental units of measurement, treating date as a source of first order autoregressive within-subject variance. Records of infection status in subjects treated for malaria taken a week after therapy were not included in this analysis so the only repeated measures in this data set are for those subjects in the cohort of TCUs followed up twice a year over the course of the study. Mtaa rather than TCU was treated as the unit of geographic location because only TCUs included in the cohort were surveyed more than once so most of these fine-scale sampling units occur in only one survey round. Survey round was treated as the unit of temporal variation and the model fit was optimized by backward stepwise selection (exclusion criterion; $P>0.10$) of all potential determinants of malaria risk, such as socioeconomic status and protective measures like coils, sprays and repellents.

In order to enable detailed, critical examination of trends in vector density, malaria transmission and infection prevalence, these data are also presented in the appendix (Tables A2 and A3) stratified by year and whether the ward was selected for intervention. Data presented in this manner were also analysed on a year-by-year basis using similar models but using intervention/non-intervention ward status to make comparisons and pooling prevalence data from all relevant rounds in a given program year. Specific details of each analysis which are relevant to interpretation are detailed in the text and or footnotes of the tables.
6.4 Results

Mosquito densities, malaria prevalence and seasonality

Between May 2004 and March 2007 the crude prevalence of malaria infection across all age groups averaged 11.7% (4969/42,447) but steadily declined from 17.6% in year 1 (2189/12,431) to 11.9% (1614/13,563) in year 2 and 7.1% (1166/16,453) in year 3. A total of 3,868 An. gambiae sensu lato, 160 An. funestus, 936 An. coustani and 444,156 Culex were collected between April 2005 and March 2007 over a total of 5463 catcher nights. In the pre-intervention year 2, 1,864 An. gambiae s.l., 85 An. funestus, 485 An. coustani and 240,295 Culex were collected over 2,468 catcher nights. In the intervention year 2,995 catcher nights yielded 2,004 An. gambiae s.l., 75 An. funestus, 451 An. coustani and 203,861 Culex.

Mosquito abundance and malaria prevalence followed seasonal patterns in Dar es Salaam (Figure 2 and 3). Peak An. gambiae s.l. densities occurred shortly after the peak of the main rains in April-May (Figure 2B and 3B), whilst An. funestus had a much longer time lag as densities peaked around July and August (Figure 2C and 3C). In Dar es Salaam all 3 species of Anopheles, namely An. gambiae s.l., An. funestus and An. coustani, were identified as malaria vectors. Although An. funestus densities were low the limited sporozoite infection data suggest this species may nevertheless be an important malaria vector because they had a much higher sporozoite prevalence (1.25% (2/160)) than either An. gambiae (0.41% (16/3868), \(\chi^2=2.42, P<0.5\)) or An. coustani (0.53% (5/936), \(\chi^2=1.10, P>0.5\)). The crude mean entomological inoculation rates (EIRs) in these two years was calculated as 1.00, 0.13 and 0.20 infectious bites per person per year for An. gambiae, An. funestus and An. coustani, respectively, although it should be noted that intense spatial heterogeneity exists over scales as fine as hundreds of meters \(^{32}\) (Castro et al. unpublished).
Figure 2. Monthly variations in rainfall, temperature (A), mosquito biting densities (B – E) and malaria prevalence (F) in the intervention and non-intervention areas over the first three years of the urban malaria control program (UMCP). Climatic and prevalence data was available from May 2004 till March 2007 whereas mosquito data was only collected from April 2005 till March 2007. Meteorological data was derived from meteorological station at Nyerere International Airport and assumed representative of both intervention and non-intervention areas.
An. funestus and An. coustani together were responsible for one quarter of the transmission in Dar es Salaam, which occurs at a crude rate of 1.33 infectious bites per person per year for the average resident. An. coustani densities were highest in January shortly after the short rainy season, following which they almost disappear, reappearing and persisting immediately after the main rains. Note, however, that An. coustani densities in the intervention areas are generally very low, only appearing in June and July (Figure 2D and 3D). Culex sp. densities were also highest during and shortly after the main rainy season (Figure 2E).

Figure 3. Seasonal patterns of rainfall and temperature (A), seasonal distribution of mosquito biting densities (B – D) and sporozoite-infected mosquitoes in the non-intervention areas (G), as well as relative biting rates in the pre-intervention and the intervention year (E, F). Relative biting densities were aggregated over pre-intervention year 2 (E: April 2004 till March 2005) and intervention year 3 (F: April 2005 till March 2006)) while direct observations of transmission in the non-intervention areas (G) were summed over both years to consolidate the limited numbers of observations in a qualitatively useful manner.
Interestingly, malaria prevalence peaked at different times each year (Figure 2F). In 2004, prevalence reached extremely high levels in November, appearing to reflect an active epidemic. Epidemic-prone conditions may have resulted from the low prevalence and immunity levels experienced during the exceptionally dry periods in 2003 and early 2004. In both 2005 and 2006 there was a clear peak in or around May (Figure 2F). This corresponds to the abundance of sporozoite infected mosquitoes over these two years with three seasonal peaks: in April-May, July-September and November-January (Figure 3G).

**Malaria prevalence as a function of age and exposure**

Initial attempts to examine determinants of malaria prevalence, without considering year-to-year variations over the course of the study, proved difficult to interpret. Original attempts to fit logistic regression models produced counter-intuitive outcomes such as higher social economic status (SES) associated with higher malaria prevalence and high An. gambiae densities associated with lower malaria prevalence. We therefore took an in-depth look at possible confounders such as the age-distribution of prevalence (Figure 4), using data from all 15 wards from the year immediately before intervention. Although Dar es Salaam is an urban area with mostly rather low EIR values, the distribution of prevalence across various age groups was consistent with rural areas where prevalence declines when people get older. Overall malaria prevalence was only very weakly related to locally measured EIR, being only slightly and non-significantly higher in TCUs with EIR values greater than 0.3 infectious bites per year (Figure 4A and 4E). When prevalence was stratified by age, only the infection status of young children (0 – 5 years old) showed any association with EIR (Figure 4B and 4E). For children over the age of 5, no relationship between prevalence and EIR was observed (Figure 4C and 4E).
Figure 4. Association between malaria prevalence and entomological inoculation rate (EIR) as a function of age. The proportion of residents patently infected in each Ten Cell Unit (TCU) where EIR was also determined is presented as open circles in panels A-D for all ages (A), young children (B), older children and young adults (C) and older adults (D) with best-fit logistic models of prevalence as a function of EIR plotted as continuous lines. These trends are summarized in panel E where three strata of transmission intensity (n=1063, 497 and 845 for log (EIR + 1) = 0 – 0.1, 0.1001 – 0.3, and > 0.3, respectively) were fitted accordingly by a logistic model treating age-group as a determinant of prevalence. Prevalence data presented is derived from people living in the areas of the adult mosquito monitoring system in the year before larviciding started (April 2005 – March 2006).
Although not significantly, prevalence amongst adults does appear to decreased slightly with increasing EIR, presumably due to higher exposure in childhood and therefore elevated levels of acquired immunity (Figure 4D and 4E). Overall, this modest but clear peak of prevalence in young children reflects early exposure to infection and development of immunity amongst residents of Dar es Salaam. While such early acquisition of infection and immunity are consistent with reports from rural areas with similarly low transmission levels\textsuperscript{48,52-55}, the overall prevalence in Dar es Salaam is much lower. This might be explained by faster parasite clearance rates, presumably due to high availability and utilization of curative drugs\textsuperscript{56-58} in this urban setting with relatively well developed health services\textsuperscript{59-61}, possibly augmented by immunity acquired to higher levels of exposure occurring in years preceding this study.

**Impact of larvicides upon mosquito densities and malaria transmission**

Larviciding suppressed densities of both secondary vectors in Dar es Salaam, namely *An. funestus* and *An. coustani*, as well as *Culex* sp. (Table 1). Although no significant suppression of the primary vector *An. gambiae* was observed, total EIR calculated from the combined annual mean densities and sporozoite prevalence of all three malaria vectors, revealed that larviciding reduced human exposure to malaria by 29.2\% (Table 1).

While the failure to detect a significant impact of larviciding upon annual mean densities of *An. gambiae* (Figure 2 B, Table 1) contrasts somewhat with analyses restricted to 6 of the study wards, this is not surprising as *An. gambiae* was controlled more effectively during drier periods and there were two major relapses of control during the two wet periods of this first year of intervention\textsuperscript{38}. The first one occurred due to cash flow and therefore procurement restrictions so larviciding didn’t begin early enough to prevent the bulk of *An. gambiae*
proliferation during the main rainy season. The second relapse occurred due to newly generated, inaccessible larval habitats in waste water settlement ponds 38.

The observation that infection prevalence and responsiveness to exposure was concentrated in young children prompted us to restrict our analysis of determinants of malaria risk upon children of age five years or less. Mosquito densities were not included as a determinant of risk because this is an intermediate outcome of, and therefore covariant with vector control interventions such as larviciding. It was therefore possible to include all ≤5 children in the analysis, rather than just those living in TCUs for which adult mosquito surveillance data were available, thus greatly increasing the sample size. In order to clearly resolve spatial and temporal variation in malaria risk from the impact of larviciding which was delivered to specific geographic areas at specific times, infection status and questionnaire data for all three years were analysed treating survey round and neighbourhood as units of temporal and spatial variation, respectively.

While the model presented in table 1 was achieved through backward stepwise selection, survey round, neighbourhood, and larviciding were consistently the three most important independent sources determinants of variance in all iterations (P≤0.001). Interestingly, individuals surveyed for the first time as “fresh” recruits to the study had a higher risk of infection. This suggest that the parasite-clearing effect of treatment with the front-line drug six months before the subject was followed-up had a lasting effect consistent with the limited exposure and re-infection rates implied by the entomological data (P<0.1). Larviciding clearly reduced malaria risk by approximately half (Table 1). Although neither ITNs nor any other personal or household protection measure appeared to reduce malaria risk when data from all three years were analysed in this manner (Table 1), separate analyses of prevalence data from
each year (Appendix Table A3) suggest modest benefits of ITN use (OR=0.805 [0.642 to 1.009], P=0.060; year 1) and living in a house with a complete ceiling (OR=0.776 [0.620 to 0.970], P=0.026; year 2).

Examining time trends for the use of protective measures and drugs over these 3 years, overall ITN usage remained consistently low but window screening and ceiling boards became increasingly common (Figure 5). Also for under 5 years old ITN usage was consistently low, only increasing from 26.3% to 28.0%. Interestingly, the use of both amodiaquine and artemisin-based drugs increased while the use of quinine and sulphadoxine-pyrimethamine decreased significantly over the three years. Although usage of artemisinin-based therapies increased slightly over the three years of the study, this treatment option remained a remarkably infrequent choice. We attribute poor uptake of this high priority intervention to lack of affordable, subsidized drugs at public facilities until early 2007 and the predominant reliance upon private sector outlets amongst Dar es Salaam residents. Indeed the phasing out of sulphadoxine-pyrimethamine seems to have resulted in higher use of amodiaquine rather than artemisinin-based therapies. These modest increases in the use of effective drugs, perhaps combined with increasing use of screening and complete ceilings, may well have played a role in the overall reduction of malaria prevalence over these three years. There were also differences in usage of different control measures in the intervention versus non-intervention areas but none of these differences are consistent with, or of a sufficient magnitude to plausibly explain, the massive reduction of malaria risk in the intervention wards during year 3 (Appendix Table A4).
Table 1 Impact of larviciding on malaria prevalence in children up to five years, transmission intensity and mosquito density over the course of the study.

<table>
<thead>
<tr>
<th>Model details</th>
<th>Malaria Prevalence</th>
<th>Annual mean mosquito densities (bites per night) and malaria transmission intensity (infectious bites per person per year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>7173 human subjects over years 1 to 3 Mtaa</td>
<td>5228 catcher nights over years 2 and 3 TCU</td>
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<thead>
<tr>
<th>An. gambiae s.l.</th>
<th>An. funestus</th>
<th>An. constant</th>
<th>Total EIR</th>
<th>Culex</th>
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</thead>
<tbody>
<tr>
<td>Parameters excluded</td>
<td>P-value</td>
<td>Parameter</td>
<td>P-value</td>
<td>Parameter</td>
</tr>
<tr>
<td>Ceiling board</td>
<td>0.966</td>
<td>Year</td>
<td>0.635</td>
<td>Larviciding</td>
</tr>
<tr>
<td>ITN</td>
<td>0.902</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Repellent</td>
<td>0.676</td>
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<tr>
<td>Sleep elsewhere</td>
<td>0.357</td>
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<td></td>
</tr>
<tr>
<td>Untreated bednets</td>
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<td></td>
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<tr>
<td>Window screening</td>
<td>0.301</td>
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<td></td>
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<tr>
<td>Coil</td>
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<tr>
<td>Spray</td>
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<tr>
<td>Parameters included</td>
<td>Estimate [95% CI]</td>
<td>P-value</td>
<td>Estimate [95% CI]</td>
<td>P-value</td>
</tr>
<tr>
<td>Constant</td>
<td>0.388 [0.213, 0.706]</td>
<td>0.002</td>
<td>0.028 [0.016, 0.049]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fresh recruit</td>
<td>OR [95% CI]</td>
<td>0.090</td>
<td>OR [95% CI]</td>
<td>OR [95% CI]</td>
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<tr>
<td>Location</td>
<td>NP</td>
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<td>Survey Round</td>
<td>NP</td>
<td>&lt;0.001</td>
<td></td>
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</tr>
<tr>
<td>Larviciding</td>
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<td>0.001</td>
<td>0.320 [0.103, 0.992]</td>
<td>0.048</td>
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<tr>
<td>NP not presented</td>
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Figure 5. Time trends of protective measures and drug use in the survey areas of the Urban Malaria Control Program. The overall trends over time were calculated using a logistic regression model with the protection measures and drugs as an outcome. Except for ITN usage \((P = 0.507)\), usage of other protective measures and drugs all significantly increased or decreased \((P < 0.001)\).

**Interactions between transmission seasonality and intervention impact**

Such dramatic impacts of larviciding on malaria prevalence might be surprising given that no obvious reductions in the mean annual biting rates of *An. gambiae*, the major vector in Dar es Salaam, were observed (Table 1, Appendix Table A2, Figure 2B, C, D and reference 38). Qualitative examination of seasonal patterns of transmission and control effectiveness suggests a rational and interesting potential explanation for this surprising level of impact on human malaria burden. Crucially, control of *An. gambiae* varied seasonally and previous analyses have shown that reduction of *An. gambiae* densities 38 was greatest during the dry season following the main rains (Figure 3E and 3F). Interestingly, we observed that almost half of all directly observed transmission events in non-intervention wards occurred between July and September (Figure 3G) when control of all three confirmed vectors, including *An. gambiae*, was most effective (Figure 3E and 3F): 45% (9/20) of all sporozoite-infected mosquitoes caught in the 12 non-intervention wards occurred in this three month period.
The ratio of *An. gambiae* biting densities for intervention versus non-intervention areas was particularly reduced by larviciding in July and August of year 3 compared to the same period of the pre-intervention year 2 (Figure 3E and 3F). Furthermore, the density ratio of both *An. funestus* and *An. coustani*, which are responsible for about a quarter of all transmission, were greatly reduced throughout the whole intervention year (Table 1, Appendix Table A2, Figure 3E and 3F). Consistent with previous analyses restricted to 6 of the study wards, analyses of mosquito densities over the July to September period reveal more impressive reductions of *An. gambiae* (OR= 0.278 [0.145 to 0.531], P<0.001) densities. It is therefore likely that more suppression of transmission was actually achieved than is reflected in table 1, which is based on annual mean sporozoite prevalence, because the greatest suppression of *An. gambiae* occurred when this vector population was most infectious to humans. In summary, substantial suppression of malaria prevalence in young children by routine application of *Bti* in Dar es Salaam can be explained by fortuitous temporal targeting of effective control of the primary malaria vector, combined with successful all-year-round abatement of the secondary vectors.

### 6.5 Discussion

Seasonal surges in mosquito numbers often lag behind rainfall in Kenya and rural Tanzania. Substantial delays have been observed between rainfall and peak malaria prevalence and in Dar es Salaam malaria prevalence appears to peak during the three periods of the year when most sporozoite-infected mosquitoes are caught in the act of feeding upon humans. Although a few sporozoite-infected *An. gambiae* were caught when their abundance peaks in April and May, most were caught at the end of the cold season, when temperatures rose again, allowing faster parasite development and higher mosquito survival.
It is well established that during peaks of mosquito abundance, the vast majority of the population are young and therefore not infectious but that when densities decline, the proportion of sporozoite-positive mosquitoes increase. Malaria prevalence is heavily influenced by EIR but also by a number of interrelated non-entomological factors, including urbanization that are difficult to dissect analytically. Personal protection measures like coils, spray and repellents were infrequently used and so had no obvious impact on overall prevalence even though they are known to give personal protection from mosquito bites. There was some evidence from year-by-year analyses (Appendix Table A3) that better established protective measures like ITNs and well-protected houses, which not only have an individual but also community effects, modestly reduced malaria prevalence. Although window screening is also known to offer individual protection, this was not detected in our study, possibly due to the known preferences of afro tropical vectors to enter through the eaves. Indeed, detailed entomological studies in this context have shown that sealed ceilings reduce house entry more than intact screening. It is particularly interesting that increasing levels of mosquito-proofing of houses had achieved almost 3 times greater coverage than ITNs even though the latter is actively promoted and subsidized as a priority intervention by the National Malaria Control Program of Tanzania. Given that house-screening and ceiling boards are much more expensive than ITNs, this observation confirms that mosquito-proofing homes is a highly acceptable and desirable intervention for residents that could be promoted and developed further as a component of a national strategy for integrated vector management.

By comparison, application of Bti in the third year of this study halved malaria prevalence and was clearly the malaria control measure with by far the highest impact. It has been proven...
before that *Bti* effectively kills malaria vector mosquito larvae under laboratory and field conditions \(^{87-90}\). It is also known that microbial larvicides can reduce adult mosquito densities and therefore malaria transmission in selected African settings, including Dar es Salaam \(^{38}\) \(^{87}\). The impact upon malaria disease burden of microbial larvicides and other forms of larval control against African malaria vectors has been demonstrated in qualitative terms \(^{34}\) \(^{91-102}\) and predicted with simulation models \(^{103-105}\). Here we demonstrate, for the first time, the effectiveness of a large scale operational malaria control program using *Bti* in sub-Saharan Africa in terms of reduced infection prevalence. Community-based larval control with *Bti*, delivered using the novel management and delivery systems developed by the UMCP \(^{38}\) \(^{40}\) had a major impact on malaria prevalence in this setting and such approaches may have great potential in towns and cities all across Africa. At an annual cost of approximately US$0.94 per person protected \(^{106}\), the routine application of larvicides in Dar es Salaam, compares well with the US$1.48 to US$2.64 estimated per year of protection from a long lasting ITN \(^{107}\) although it should be remembered that the latter often protects more than one person.

Although our analyses do not capture the communal effects of ITNs, which can be just as important as personal protection \(^{84-86}\), these results suggest that larviciding may be at least as cost-effective as ITNs in cities and merits consideration for broader development, implementation and evaluation in urban Africa.

We anticipate that even greater impacts can be achieved as the proficiency of operational teams matures through direct experience and innovation in response to locally-specific operational challenges, as well as improved institutional and financing mechanisms \(^{38}\). Tactically, we emphasize the specific need to tackle malaria vector populations in Dar es Salaam more effectively during the long rains while building upon successes during drier times of the year when much transmission occurs but larval habitats are both less abundant...
and easier to access\textsuperscript{108,109}. Strategically, we conclude that larviciding has a true potential for sustainable malaria control in African cities but emphasize that the encouraging results presented here merely represent an early demonstration which can be substantially improved upon with time, investment, experience and creativity.

### 6.6 Conclusions

Routine larviciding constituted only one component of a suite of interventions actively applied in Dar es Salaam. Although no other single intervention had a comparably dramatic attributable benefit, malaria prevalence steadily decreased over the three years of the UMCP, even before application of larvicides. Here we show for the first time that community-based larval control with \textit{Bti} on a large scale operational level (128,000 residents protected) has a dramatic impact on malaria prevalence. As the last successes of larval control rapidly fade from living memory\textsuperscript{34-36,91,110-113}, perhaps it is time to re-examine the theoretical considerations\textsuperscript{109,114} which led to half a century of exclusive emphasis upon adult mosquito control for malaria prevention in Africa and beyond\textsuperscript{34,36}. We suggest that larval control should be re-integrated into the priorities of national malaria control programs and evaluated in further rigor over the long term, particularly in urban areas where feasibility and cost-benefit ratio may be highest.

\textit{What is already known about this topic}

Integrated malaria control programs incorporating larviciding, conducted before the Malaria Eradication Campaign started, successfully reduced or even eliminated malaria.

\textit{Bacillus thuringiensis} var. \textit{israelensis} (\textit{Bti}) effectively reduces larval as well as adult mosquito abundance in the laboratory and in small-scale field trials of efficacy.
What this study adds

Community-based larval control with Bti on a large operational scale in Dar es Salaam, a major African city, reduced malaria infection prevalence by half, providing more measurable protection than any other intervention and was at least as cost-effective as an insecticide-treated net.

Larval control strategies should be integrated into the priorities of national malaria control programs and evaluated in further rigor over the long term, particularly in urban areas where feasibility and costs-benefit ratio is likely to be highest.

6.7 Acknowledgments

Michael Kiama planned and managed the program upon which the UMCP was based and we are greatly indebted to him for his enormous commitment and contribution towards this program. We thank the entire team who participated in these surveys but especially those who conducted human landing catch studies for their perseverance and commitment to this challenging undertaking. Furthermore we would like to thank the residents of Dar es Salaam and their municipal and ward authorities for their cooperation and facilitation. Thanks go to S. Dongus for drafting the map of the study area. We would also like to thank P. McElroy, S. Mkude, A. Simba and A. Mwita for their helpful comments and support for the program. We thank T. Smith for statistical advice and P. DeChant, S. Krause, E. Dankwa, E. Brantly and J. O’Sullivan for logistical, technical and financial support. This paper is published with kind permission of Dr. Andrew Kitua, Director of the National Institute for Medical Research, United Republic of Tanzania.
Contributors: YG designed and implemented the adult mosquito monitoring system in consultation with the other authors, analysed the data and drafted the manuscript. PC, BE, NJG, VM all participated in the design and implementation of various aspects of the adult mosquito surveillance and corresponding laboratory analysis. MCC designed the household survey in consultation with UK, KK, GFK, DM, HM, SWL and MT and developed all data management systems for the program. UF, KK and GFK designed and implemented the larviciding system in consultation with DM, HM, SWL and MT. GFK supervised all aspects of the study design, implementation, data analyses and drafting of the manuscript. All authors read and approved the final manuscript.

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Competing interests: A substantial portion of the current salary and research support for the investigators depends on the achievement of documented suppression of malaria transmission
and infection risk by this program through systematic larviciding. The Urban Malaria Control Program was partially supported by Valent Biosciences Corporation, a manufacturer of microbial larvicides. None of the funders had any role in the evaluation design, data collection, analysis, interpretation, drafting of the manuscript or decision to publish. Furthermore all authors declare that the answer to the questions on your competing interest form are all “No” and therefore have nothing to declare.

**Ethical considerations:** All activities of the UMCP, including these field surveys were approved by the Medical Research Coordination Committee of the National Institute for Medical Research, Ministry of Health, Government of Tanzania (Reference numbers NIMR/HQ/R.8a/Vol. IX/279 and 324) and Durham University’s Ethics Advisory Committee. No persons in high risk groups, namely people under 18 years or women of reproductive age, were recruited to conduct human landing catch. Furthermore, all human landing catchers were screened weekly for malaria by microscopic examination of thick smear peripheral blood samples and, when found infected, treated with artemisin-based combination therapy. Participants of the household survey signed an informed consent form after receiving information about the goals of the survey. For children under 18 years, parents or designated guardians granted consent. Individual information was kept in strict confidence by storing in locked rooms and cabinets and password-protected computers.
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7. **Discussion and conclusions: Opportunities for improved malaria control through integrated vector management in urban Africa**

7.1 **Abstract**

Most entomological and epidemiological malaria research to date has focused upon rural areas of Africa. Urban areas have been neglected although more than 50% of the African population will live in towns or cities by 2030. In order to control urban malaria successfully, it will be necessary to better understand larval ecology, behavioural interactions between mosquitoes and humans, urban malaria epidemiology and the seasonal population biology of vectors and parasites. When the Urban Malaria Control Program (UMCP) in Dar es Salaam, Tanzania started, relatively little was known about urban malaria, but it was increasingly recognized that malaria poses a major problem in urban areas. More recently, several studies have been conducted in urban settings in African countries, confirming that *Anopheles gambiae* s.l. has adapted to urban settings by ovipositing and developing in atypical larval habitats such as domestic containers and polluted water bodies. Furthermore it is now recognized that urban agriculture poses a major problem by increasing the availability of suitable larval habitats for malaria vectors. The importance of heterogeneity in urban malaria transmission was also recognized. Exophagic biting behaviour of *Anopheles* species has been reported from two cities, Dar es Salaam, Tanzania and Lagos, Nigeria. This has major implications for personal protection gained by usage of insecticide treated nets (ITNs) or mosquito proofed houses. While vector control priorities at national level focus primarily on ITN usage and indoor residual spraying (IRS), here we have shown urban inhabitants of Dar es Salaam tend to prefer personal protection like mosquito proofed housing if their socioeconomic status allows it. While all of these protective measures have been shown to reduce malaria prevalence in
Discussion and conclusions

different urban settings, here we have shown that a community-based larval control system readily can be at least as effective in Dar es Salaam, Tanzania. While the Dar es Salaam example represents a model that other cities could adopt, a number of challenges remain as local vector ecology, distribution of larval habitats and seasonality differ from country to country and city to city. There is still a substantial gap in the scientific literature, not only in larval ecology of African vectors but also on their control and the heterogeneity of transmission in densely populated urban areas. The latter is especially important in order to further evaluate control measures, as such interactions are complex and have a major influence upon both the cost and effectiveness of vector control. Further research should also focus upon human factors in cities as behaviour, the perception of community members, social structure, health care seeking and the equity of malaria risks differ substantially from rural areas. To our knowledge, the UMCP is the first integrated vector control program using *Bacillus thuringiensis* on a large scale programmatic level. Further large scale field trials with well-defined monitoring and evaluation tools are needed to evaluate the sustainability and effectiveness of this approach in the longer term. Essential steps towards the sustainability of integrated malaria control programs include the institutionalization and enhancement of training capacity and securing long-term financing for programmatic implementation.

Larviciding appears to be a cost-effective option with the annual costs of larviciding per person protected being similar to the costs estimated for long lasting ITNs and similar impacts on malaria prevalence reduction.

We therefore conclude that larvicides should be prioritized at national policy and donor levels along with subsidized effective drugs and existing protective measures such as ITNs and ceiling boards. In order to maximize cost-effectiveness in such programs, local larval and adult mosquito ecology should be evaluated prior to implementation so that the intervention
can be targeted specifically in time and space. Such exploratory evaluations of local vector ecology also constitute an essential pre-requisite step, enabling the development of appropriate monitoring and evaluation systems that will allow sustained, effective and successful day-to-day management of decentralized community-based larval control programs.

7.2 Larval ecology and mosquito biting behavior in urban areas and its implications for vector control

In rural areas, *An. gambiae* s.l. prefers to breed mainly in sunlit habitats like rice fields, borrow pits and stagnant waters such as pools, puddles and hoof prints. In contrast, *An. funestus* is typically found in more or less permanent water bodies shaded by vegetation such as marshes, river edges or rice fields. Both species generally prefer clean and unpolluted waters and are absent from habitats contaminated with faeces or containing rotting plants (Gillies and DeMeillon 1968; Service 2000). It is therefore generally considered that urbanization reduces natural larval habitat abundance by polluting, draining or covering surface water bodies (Keating et al. 2003; Keating et al. 2004). On the other hand, new larval habitats are also created by human activity so modest increases in human population density can sometimes increase overall habitat availability (Chinery 1984; Jacob et al. 2003; Keating et al. 2003; Castro et al. 2004). In recent years it was confirmed that *An. gambiae* s.l. adapted to urban settings by ovipositing and developing in sewage ponds and organically polluted water habitats in Dar es Salaam, Tanzania (Sattler et al. 2005) and in Man, Côte d’Ivoire, waste did not affect the presence and density of *Anopheles* larvae (Matthys et al. 2006). In Accra, Ghana *An. gambiae* was found in pit latrines (Chinery 1969, 1984) and in Kisumu and Malindi, Kenya, pollution was even a predictor for the presence of *An. arabiensis* (Jacob et al.
A multiplicity of *Anopheles* larvae were also found in urban agricultural sites like matuta (a type of agriculture where plants are grown on top of small ridges), rice fields, irrigated vegetable fields and irrigation wells (Afrane et al. 2004; Sattler et al. 2005; Matthys et al. 2006; Vanek et al. 2006) which was reflected in higher malaria incidence and prevalence in their proximity (Afrane et al. 2004; Klinkenberg et al. 2005; Matthys et al. 2006). Urban agriculture therefore poses a major hazard in terms of increasing the exposure to transmission in surrounding areas. These studies, and others from Dakar, Senegal and Maputo, Mozambique, further emphasized that urban malaria transmission is highly heterogeneous, with malaria incidence and prevalence declining rapidly with distance from breeding sites (Trape et al. 1992; Thompson et al. 1997; Staedke et al. 2003). The highly localized and patchy nature of malaria transmission in urban areas generally occurs over remarkably fine spatial scales (Castro et al. 2004; Keiser et al. 2004) because mosquito dispersal is restricted by the ready available human blood meal hosts (Service 1997; Killeen et al. 2003).

In rural Africa, a wealth of qualitative reports have shown that the bulk of human exposure to transmission occurs indoors during the middle of the night (Holstein 1954; Gillies and DeMeillon 1968; Gillies and Coetzee 1987) although explicit quantitative analysis has occurred only recently (Killeen et al. 2006). By contrast, very little research has been conducted into mosquito biting behaviour in urban areas. More outdoor than indoor biting with closed doors and windows has been observed in Dakar, Senegal (Trape et al. 1992) and both, Dar es Salaam, Tanzania and Lagos, Nigeria have reported exophagic behaviour of *Anopheles* species (Oyewole and Awolola 2006; Geissbühler et al. 2007). These reports collectively suggest a trend towards exophagic behaviour in large cities. In Dar es Salaam this behaviour could have been induced by a high coverage of bednets, ceiling boards and window screenings. Interestingly, in Dar es Salaam *An. arabiensis* was mainly biting before 10pm.
similar to reports from Lagos for *An. gambiae* s.s., whereas *An. gambiae* s.s. in Dar es Salaam had a main biting peak around midnight and a second one between 4 and 5am. Both cities have a tropical climate although mean minimum temperature, average annual rainfall and humidity are lower in Dar es Salaam. It was observed before that the tolerance to desiccation of *An. arabiensis* (Gillies and Coetzee 1987; Lindsay et al. 1998; Gray and Bradley 2005) enabled it to feed in the early evening despite low humidity. Similar biting behaviour of *An. arabiensis* was also observed in rural Eritrea where it was found to be exophagic, exophilic and mainly biting before 10pm (Shililu et al. 2004).

Such differences in mosquito behaviour have major implications for personal protection gained by usage of insecticide treated bednets (ITNs) or mosquito proofed houses. We estimated that, in Dar es Salaam, ITNs confer 59% personal protection against *An. gambiae* s.s. and only 38% against *An. arabiensis* which was fortunately less abundant (Geissbühler et al. 2007). Therefore ITNs confer limited but still useful personal protection. It has to be emphasized that here only personal protection was considered but that ITNs have an important community-level effect at high levels of population-wide coverage (Maxwell et al. 2002; Hawley et al. 2003; Killeen and Smith 2007; Le Menach et al. 2007). Importantly, not only mosquito behaviour changes in the city but also human behaviour. Urban dwellers tend to go to bed later and if they have a good quality house they tend to spend more time indoors in the evening (Geissbühler et al. 2007). Of further importance is that although bednet coverage levels are high in Dar es Salaam, treatment levels of these nets were consistently low over the past three years (Geissbühler et al. 2007; Geissbühler et al. 2008). Therefore the introduction of long lasting insecticide treated nets (LLIN) will definitely contribute to increase ITN coverage although the magnitude of this effect will depend on the type of net (Graham et al. 2005; Nafo Traore 2005; Roll Back Malaria Partnership 2005; Yates et al. 2005; Maxwell et
al. 2006). Scaling-up and sustaining ITN coverage could also be achieved through a catch-up (large scale distribution of free ITNs) and keep up (routinely providing ITNs to pregnant women and children through public health clinics or commercial outlets) strategies (Grabowsky et al. 2007; Lengeler et al. 2007). Although on the national level ITNs and indoor residual spraying (IRS) are the priorities in vector control in most African countries, urban inhabitants seem to prefer mosquito-proofed housing if their socioeconomic status allows it. In Accra and Kumasi, Ghana residents preferred window and door screening (Klinkenberg et al. 2006) to ITNs and in Dar es Salaam, Tanzania inhabitants similarly preferred window screening and ceiling boards to ITNs (Geissbühler et al. 2007). Therefore it might be feasible to develop programs which promote and subsidize the efforts of vulnerable residents to effectively mosquito-proof their houses. Furthermore, to prevent outdoor transmission, larviciding (Killeen et al. 2002; Fillinger and Lindsay 2006) and environmental management (Utzinger et al. 2001; Utzinger et al. 2002; Keiser et al. 2005) should be integrated into existing vector control programs especially in cities where breeding sites are less abundant and easier to tackle (Killeen et al. 2002).

### 7.3 Surveillance and management systems for effective vector control

In order to implement larviciding and environmental management successfully, cost-effective and scalable implementation systems with good monitoring and evaluation systems have to be designed and put in place. There are several important lessons from the era before the Global Eradication Campaign started in 1955, when vector control programs using larvicides and/or environmental management were successfully implemented in Brazil, Zambia and Egypt (Utzinger et al. 2001; Killeen et al. 2002). The need for rigorous and comprehensive surveillance is one of them (Watson 1953). In Brazil a centralized larval surveillance system,
as well as an adult mosquito monitoring system, was used to ensure the quality of work done by the larval inspectors. Another important feature of the program was a separate reporting system at district level for anti-larval and anti-adult control teams (Soper and Wilson 1943; Killeen et al. 2002). In Zambia vector densities and malaria incidence rates were used to survey and appropriately tune environmental management strategies (Utzinger et al. 2001). More recent vector control programs, even though not using larvicides or environmental management, strongly emphasized the need for good surveillance systems (Sharp et al. 2007) which work in a vertically, decentralized manner (Barat 2006). Other programs identified the lack of mosquito surveillance as a shortcoming (Impoinvil et al. 2007). In Dar es Salaam such a decentralized, community-based approach with a hierarchical, centralized management in order to systematically apply larvicide was put in place (Fillinger et al. 2008). The origins of this decentralized, grassroots level approach lay in the initial pilot program of the Ilala Municipality (Mukabana et al. 2006; Fillinger et al. 2008). The need for better surveillance systems was recognized at the beginning of the UMCP when larval surveillance CORPs (Community-Owned Resource Persons) reported less than half of the potential Anopheles habitats (Vanek et al. 2006). This improved tremendously after independent spot checks by Municipal Mosquito Control Inspectors were implemented. Larval surveillance coverage rose, and now typically exceeds 75% (Fillinger et al. 2008). The strength of this program lays in the surveillance systems in place at different administrative levels. Each level of management is responsible for identifying and addressing programmatic shortcomings. Also the use of insecticide by individual CORPs is recorded to avoid inappropriate use rates which is done in a similar way as IRS programs in South Africa and Mozambique (Booman et al. 2003; Fillinger et al. 2008). In order to rigorously survey all potential larval breeding sites on a weekly basis they were sketch mapped at the beginning of the program and mapping was later improved by using aerial photographs and basic GIS (Dongus et al. 2007). Another important
feature is the separation of reporting systems for the larval surveillance CORPs and the ones responsible for larvicidal treatment, as this minimizes competing interests in data collection and interpretation (Fillinger et al. 2008). In order to assure high coverage of larviciding, larval surveillance CORPs visit all potential larval habitats one day after Bti application. Also adult mosquito surveillance is implemented by a separate team which primarily reports to the city program manager and secondarily to the three municipal coordinators. Adult mosquito monitoring is also of major importance for rigorous and timely monitoring and managing of larval habitat surveillance activities (Fillinger et al. 2008). To ensure quality of the adult mosquito monitoring unannounced nightly spot checks are conducted by the Adult Mosquito Control Supervisor.

A very short reaction time is achieved at the level of ward supervisor by identifying shortcomings in larvicide application within 24 hours. As mosquito development takes place within a week (Haddow 1943; Holstein 1954; Gillies and DeMeillon 1968) and some larval habitats occur transiently and can be easily overlooked, ability to respond to gaps is absolutely essential (Soper and Wilson 1943; Watson 1953; Fillinger and Lindsay 2006). Rigorous mapping, weekly surveillance of potential breeding sites and application of Bti also reduced larval and adult mosquito densities in a rural area in Eritrea (Shililu et al. 2007). In Dar es Salaam at the municipality level, reaction time is one week as ward supervisor’s hand in weekly summary sheets to the Municipal Mosquito Control Coordinator. This data is then entered into spreadsheets which generate summary statistics, tables and charts which form the backbone of the monthly report to the City Mosquito Control Coordinator. Municipal Mosquito Control Coordinators also receive weekly adult mosquito reports which are fed into the same system and help them to independently and more directly assess the program impact. With this vector surveillance and management system, larviciding led to a 92% decrease of
habitats containing anophelines and culicines (Fillinger et al. 2008). This system maturated over time and could be easily adopted in other African cities.

7.4 Protective measures and malaria risk factors in urban settings

Prevalence is heavily dependent upon the entomological inoculation rate (EIR) (Beier et al. 1999) but is also influenced by a number of non-entomological factors such as socio-economic status, education, usage of personal protective measures, travel to rural areas, age and urbanization (Ng'andu et al. 1989; Koram et al. 1995; Stephens et al. 1995; MacIntyre et al. 2002; Doannio et al. 2004; Mensah and Kumaranayake 2004; Klinkenberg et al. 2006; Ronald et al. 2006; Wang et al. 2006; Wang et al. 2006) as well as frequency and longevity of infection and disease outcome (Smith et al. 2005) which are interrelated and therefore difficult to dissect analytically (Bates et al. 2004). In Dar es Salaam, existing personal protective measures like ITNs, ceiling boards and window screening have now been complemented by regular application of the microbial larvicide *Bacillus thuringiensis* var. *israelensis* (*Bti*) through the vertically-managed delivery system of the UMCP (Fillinger et al. 2008). Therefore we were able to explore how these protective measures, as well as *Bti* application, influence malaria prevalence in this urban context. As observed in several cities in East and West Africa (van der Kolk et al. 2003; Matthys et al. 2006; Wang et al. 2006; Geissbühler et al. 2008), malaria prevalence followed a classical distribution of prevalence across age groups typical of highly endemic rural areas with infection risk peaking in young children. Protective measures like ITNs and ceiling boards reduced malaria prevalence each by about one fifth in Dar es Salaam but the highest impact on prevalence was achieved by the application of *Bti* (Geissbühler et al. 2008). In other African cities without application of *Bti*, main risk factors were proximity to potential breeding sites, travel to rural areas and low socioeconomic status,
whereas having window screening reduced the risk of malaria episodes (Afrane et al. 2004; Klinkenberg et al. 2005; Klinkenberg et al. 2006; Matthys et al. 2006; Ronald et al. 2006).

Surprisingly, An. coustani, although generally believed to be of minor importance as it is mainly zoophagic (Gillies and DeMeillon 1968), was found to be a secondary vector in Dar es Salaam (Geissbühler et al. 2008). Recently its potential as a secondary vector was also shown in several sites in Cameroon (Antonio-Nkondjio et al. 2006), though its importance as secondary vector in low transmission areas has been discussed in East Africa previously (Gillies 1964). The malaria prevalence reduction accomplished with Bti in Dar es Salaam was achieved through all-year-round reduction of the secondary malaria vectors An. funestus and An. coustani, as well as fortuitous temporal targeting of An. gambiae at the time of the highest transmission (Geissbühler et al. 2008).

### 7.5 Integrated vector control: The way forward

The majority of documented applications of integrated vector control occurred before the advent of DDT and the start of the Global Eradication Campaign (1955-1969) (Killeen et al. 2002; Keiser et al. 2005). Nevertheless, only a few programs were implemented in Africa during the pre-DDT era using different kinds of environmental management and larviciding (Ross 1907; Gilroy and Bruce-Chwatt 1945; Shousha 1948; Kitron 1987; Utzinger et al. 2001; Utzinger et al. 2002). In the more recent post-eradication era, then the overwhelming focus of vector control in Africa has been on pyrethroid treated nets and IRS (Roll Back Malaria Partnership 2005). With increasing insecticide resistance of malaria vectors against pyrethroids (Sina and Aultman 2001; Hemingway et al. 2002; N'Guessan et al. 2007), complementary options such as larviciding and environmental management are receiving
Discussion and conclusions

renewed consideration (Utzinger et al. 2001; Killeen et al. 2002; Utzinger et al. 2002; Killeen 2003; Keiser et al. 2005). The UMCP in Dar es Salaam proved that application of the biological larvicide *Bti* immensely contributed to the reduction of malaria prevalence in the city (Geissbühler et al. 2008) (Castro et al. unpublished). To our knowledge this is the first integrated vector control program using *Bti* on a large scale programmatic level and it is furthermore the first vector control program applying larvicides since the advent of DDT. Developed over the course of three years, it could be used and adopted now in other cities and other countries. Lessons learned during the development of the program underline the importance of exhaustive coverage with larval control strategies, based on mapping and remapping of all potential larval habitats, giving individual responsibility to each larval surveillance CORP, a strategy which was very successful half a century ago. The more authoritarian approach of the Brazilian campaign was replaced by a community based, decentralized, well-organized and judicious vertically applied management system which allows detection of short-comings in a timely manner (Soper and Wilson 1943; Shousha 1948; Killeen et al. 2002; Killeen et al. 2006; Mukabana et al. 2006; Fillinger et al. 2008).

During the short rainy season of the intervention year, program limitations due to inaccessible larval habitats in waste water settlement ponds led to a resurgence in adult mosquito densities. Slow financial mechanisms, also resulted in the delayed start of larviciding during the main rainy season, which was too late to prevent the bulk of transmission (Fillinger et al. 2008; Geissbühler et al. 2008). This emphasizes the need for sustainable and stable financing for programs, an issue which has historical precedents dating back to the era of malaria eradication with indoor residual spraying (Kouznetsov 1977).
Large scale vector control programs complemented by larviciding seem to be feasible first of all because mosquito larvae are easier to target as they can not avoid interventions like adult mosquitoes (Killeen et al. 2002) and in urban areas access to breeding sites is relatively easy and can therefore be cost-effective (Robert et al. 2003; Keiser et al. 2004). In fact in Dar es Salaam larviciding appeared to be highly cost-effective with an annual cost of approximately US$0.94 per person protected per year by larviciding (Worrall 2007), which compares well with the US$1.48 to US$2.64 estimated per year of protection from a long lasting ITN (Yukich et al. 2007) even though the latter often protect more than one person. Larviciding has been proven to be highly effective in Dar es Salaam by reducing malaria prevalence by 50% (Geissbühler et al. 2008) over the three years whereas ITNs only reduced malaria prevalence by 20% compared to control groups without nets in year 1 in a stable malaria setting although in a more unstable setting (EIR < 1), which is the case in many areas of Dar es Salaam, a 42% reduction was observed (Lengeler 2004). Therefore in this kind of an urban setting with low transmission both interventions are likely to be cost-effective. In order to further evaluate the cost-effectiveness of Bti more large scale operational programs will have to be implemented.

Some authors have pointed out that in order to achieve effective integrated vector control substantial locally-relevant information about vector ecology, distribution of larval habitats and environmental conditions is necessary (Walker and Lynch 2007). Improvements in the human resources devoted to control, by building up a cadre of technical, managerial and operational staff is needed and it also requires an improved policy framework (Killeen et al. 2002; Killeen et al. 2003; Killeen et al. 2004; Townson et al. 2005; Mukabana et al. 2006). Here we demonstrated that, given stable long-term financing and enhanced in-country training
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capacities, larviciding in urban areas can be integrated effectively into national vector control programs by adapting the monitoring and evaluation tools described here.

7.6 Conclusion

In an urban setting like Dar es Salaam with predominantly exophagic malaria vectors, additional vector control measures like larviciding and environmental management are highly recommended. Although personal protection by ITNs and ceiling boards reduced malaria prevalence, the application of the microbial, environmentally safe larvicide had an even stronger impact on malaria prevalence reduction and should be considered in designing integrated programs.

Nevertheless it should be emphasized that ITNs do confer personal protection and presumably also community-level suppression of transmission.(Howard et al. 2000; Maxwell et al. 2002; Hawley et al. 2003; Killeen and Smith 2007) and should therefore remain a priority regardless of the availability of new options. Amongst those new options are both improved housing and larviciding. Mosquito proofed housing reduced malaria cases drastically in Italy at the end of the 19th century (Celli 1901, 1901) and it is believed to have contributed significantly to the eradication of malaria in the USA (Byrd 1914; Boyd 1926; Kiker 1941). Unfortunately, as with larviciding and environmental management, this intervention was abandoned when the Global Eradication Campaign began (Lindsay et al. 2002). The work described here supports the view that improved housing is a grossly under-utilized control measure that should be given greater priority by national programs. In fact, we specifically recommend that strategies for promoting and subsidizing improved mosquito-proofing for vulnerable households may merit active consideration as this is the intervention of choice for residents.
Most importantly, we conclude that all these vector control measures should be complemented by the use of larvicides. Therefore larviciding should be prioritized at national policy and donor levels alongside ITNs, IRS and effective drugs in niches, such as cities, where it may be appropriate. Further large-scale field trials with well-defined monitoring and evaluation tools are needed to evaluate the sustainability and effectiveness of this approach in the longer term and different urban settings. In particular, larviciding needs to be evaluated on even larger programmatic scales and impact upon incidence of clinical disease and mortality needs to be documented rigorously. In order to achieve sustainable success at programmatic level, local larval and adult mosquito ecology have to be evaluated through appropriate surveillance systems prior to implementation so that maximum targeting efficiency in time and space is attained in each particular setting (Gu and Novak 2005; Killeen et al. 2006; Smith et al. 2007).

Since the Global Eradication Campaign started half a century ago and larval control was abandoned, the UMCP represents the first large scale integrated vector program in Africa implementing larval control through new surveillance and management systems. First results are encouraging but substantial improvement with time and investment are expected. This could be the beginning of a new era of integrated vector control programs with successfully implemented larval control.
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Annex 1

Additional files of Article 2

Additional file 1: Participatory mapping guidelines and TCU mapping and description forms
Additional file 2: Larval surveillance guidelines and standard operating procedure for open habitats
Additional file 3: Posters describing categories for open habitats
Additional file 4: Posters describing categories for closed habitats
Additional file 5: Larval surveillance forms for open and closed habitats
Additional file 6: Training presentation for larval surveillance
Additional file 7: Guidelines for larvicide application.
Additional file 8: Training presentation for larvicide application
Additional file 9: Ward-level weekly summary form for larval surveillance data and form checklist for collation in pre-labelled folders and evaluation by municipal management.
Additional file 1:

Guidelines for 10-cell unit mapping to be carried out by the community owned resource persons and the wards malaria vector control supervisors

I. Introduction

To find all mosquito breeding habitats, you have first to know each and every square metre in your Mtaa. Each Mtaa is composed of several 10-cell units, which now need to be divided into plots, typically numbering between 10 and 20 per 10-cell unit. The only sure way to do this is to know who owns, occupies or uses which plot of land regardless of whether it is surveyed or unsurveyed. For the purposes of our programme, a plot is defined as a specific physical area with an identifiable owner, occupant, or user and with clearly defined boundaries within one specific 10-cell unit. A plot is our basic access unit for surveying larval habitats. In the built up areas, a plot is that area covered by, and surrounding a house that is owned or occupied by a named and identifiable person. In the seemingly no-man’s land, a plot is that unit that a specific person owns, claims to own, or he/she regularly uses. Thus, when we refer to an “owner” of a “plot”, this goes beyond just those surveyed plots with legal owners to include river valleys, open fields, swamps, cultivated areas etc. Knowledge of who owns, occupies or uses a certain plot is very important if you are to gain unlimited and regular access in future as this is the person who has the power to say yes or no! Consequently, to find, name and define the plots within a 10-cell unit, you must be accompanied by the 10-cell unit leader or their representative from that 10-cell unit and those from the adjoining 10-cell units. The purpose of conducting a mapping exercise is to lay a platform that will guide the larval habitat survey. It is only after every metre square within a 10-cell unit has been assigned to a specific plot that you can start a larval habitat survey. However, even before you can start walking around finding out who owns which plot of land, it is important that the community members are made aware of who you are, where you are from, what you are doing, why you are doing it, of what benefit is it to them, and how they can be part of it. These questions are addressed though proper and continuous community sensitisation.
II. Step-by-step guide for plot mapping

1. First, obtain the 10-cell mapping forms (Annex 1) from your supervisor at the ward level.
2. Go to the specific 10-cell unit that you intend to map and get in touch with the 10-cell leader. Explain clearly to him what you are doing and request him to take you on a detailed guided tour of his 10-cell unit. In this tour, let him take you from plot to plot and to all plots within his 10-cell unit. Explain to the 10-cell unit leader that exhaustive mapping is important for conducting a thorough larval search and eventual larval control. In defining the 10-cell unit boundaries, it is important to involve the 10-cell unit leaders of the adjoining 10-cell units. Explain to the 10-cell unit leaders that unless the boundaries are correctly and mutually agreed upon, mosquitoes will breed in these boundary areas and fly into the 10-cell units.
3. On the 10-cell unit mapping form, fill in the date, the name of the Municipality, the Ward, the Mtaa, the 10-cell unit number and the name of the 10-cell unit leader.
4. Once on a specific plot, assign an identification number (Plot ID) to it and fill in this number in the column named “Plot ID” in the 10-cell unit mapping form. If it is within a surveyed/built-up area, also include the house number in the column named “House Number” in the 10-cell unit mapping form. For each and every 10-cell unit, assigning of plot ID numbers should be independent of the plot numbers of the other 10-cell units.
5. Then, ask who owns, occupies or regularly uses the plot and write down his/her name in the column named “Owner’s Name” in the 10-cell unit mapping form.
6. With the help of the owner, occupant or regular user, clearly define the boundaries making a rough sketch of the plot on a piece of paper. This will assist you in constructing a map for all plots in that 10-cell unit (see step 9). Since two or more 10-cell units may share some of the open areas, it is important to involve all the 10-Cell Unit Leaders from the adjoining 10-cell units to define boundaries for plots as well as those for the 10-cell units. Great care should be taken when defining boundaries so that no part of the boundary is left unassigned to a plot. Therefore, the only way to define a boundary is to know what is on the other side of the boundary i.e. another plot in a different 10-cell unit, or in a different ward. This will ensure complete and full coverage of each and every square metre of a 10-cell unit. For areas covered by common facilities and infrastructure like roads, rail, drains etc, assign them to one plot with a specified plot ID number (look at how the drain in annex 2 B has been allocated to plots).
7. Describe in details the location of the plot such that even a stranger to the 10-cell unit can locate it using your description. Fill in this description in the column named “Plot location description (where is it in the 10-cell unit) and its basic characteristics” in the 10-cell unit mapping form.
8. Explore the plot and describe its basic characteristics (for example, is it flat, flooded, what is growing there, rocky, hilly, cultivated, construction ongoing, well or poorly drained etc.) in the column named “Plot location description (where is it in the 10-cell unit) and its basic characteristics” in the 10-cell unit mapping form. However, if there is no unique feature or characteristics in the plot, then describing its location in step 7 above will be enough.
9. After you have defined all the plots in a 10-cell unit, have completed steps 5-10 above for each and every plot in that 10-cell unit and have agreed on the 10-cell unit boundary with the leaders of the adjoining 10-cell units, on a separate page named “10-cell unit plots map” (Annex 1B), draw a map of the 10-cell unit to include all the plots you have described in it. Remember to include the Plot ID number for each plot on the map. Also fill in the date, the municipality, the ward, the 10-cell unit number and the name of the 10-cell leader at the top part of this 10-cell unit plots map form.
10. After the map is completed move to the next 10-cell unit and repeat the above procedure.
11. Later, when checking the quality of your 10-cell unit mapping, either the ward supervisor, or
the municipal malaria control inspector for vector control, will assist you fill in the GPS
readings in the column named “GPS” in the 10-cell unit mapping form.
12. Attached (Annex 2) is a hypothetical example on how to go about the 10-cell unit mapping
exercise. Study in carefully as this will help you develop an idea on how to carry out this
exercise.
13. After the 10-cell unit plots map forms are filled in and the maps drawn, they should be taken to
the ward office. From here the supervisor will take them for photocopying at the Municipal
Malaria Control Coordinator’s office. He (the supervisor) will receive copies of the filled in
forms and maps to take them back to the Community Owned Resource Persons for their day-to-
day reference.

NB: Remember that you will use the filled-in 10-cell unit mapping forms to guide you in your
larval survey exercise and therefore you should fill them in carefully and accurately!!

Always fill in the forms using black or black ball pens.
Annex 1A: 10-cell unit plots map form

<table>
<thead>
<tr>
<th>Plot E</th>
<th>House Number</th>
<th>Owner’s name</th>
<th>Plot location description (where is it in the 10-cell unit) and its basic characteristics</th>
<th>GPS (UTMK/UTM)</th>
<th>Nothing</th>
<th>Existing</th>
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Signatures: Sponsor: _______________________
CORP: ___________________________
Fomu ya ramani ya shina

Tarehe:__/__/______

Manispaa__________________Kata_______________Mtaa_________________
Namba ya shina______________Jina la Mjube_________________
Annex 2: a hypothetical example on how to go about the 10-cell unit mapping exercise. Below are two diagrams (A & B). Study them carefully and then read the notes that follow.

**Notes on the Diagrams**

- Diagram A represents how a part of Dar es Salaam City would look like to any other person who is not interested in 10-cell units mapping whereas diagram B represents what we would like to achieve in our 10-cell unit mapping exercise. Note that the two diagrams represent the same and one area.
- Diagram A represents how things appear to us (on the ground & in our minds) before carrying out the 10-cell units mapping exercise whereas diagram B represent how things will be on paper and in our minds after carrying out the 10-cell units mapping.
The dark houses represent the 10-cell units leaders’ houses in this particular locality. Therefore there are 4 10-cell units represented in this diagram, 3 (Numbers 11, 12 & 17) are in the same Mtaa while 1 (number 45) is from another Mtaa.

Now, assume that today, you want to carry out a plot mapping exercise in 10-cell unit number 12 located in a Mtaa called Mtambani in Vingunguti Ward of Ilala Municipality.

1. The first step would be to collect the 10-cell unit mapping form from your ward supervisor (the vector control supervisor for Vingunguti Ward).

2. Then you would move to the 10-cell unit number 12 and contact its leader (TCU Leader 12). After explaining the purpose of your visit to the TCU Leader 12, ask him to take you on a detailed guided tour of his 10-cell unit. In this tour, let him take you from plot to plot and to all plots (11 in this case). Then follow steps the follow steps 3-12 as explained in the Step-by-step guide for plot mapping.

After the exercise, you should have a completed 10-cell unit mapping form and a map for that 10-cell unit (See below).

Note that the 4 10-cell units leader should be involved in defining the boundaries of TCU 12

### Completed 10-cell unit mapping form for 10-cell unit number 12 shown in diagrams A & B above
Completed 10-cell unit plot map for 10-cell unit number 12 shown in diagrams A & B

10-cell unit plots map form

Municipality: Ilala  Ward: Vingunguti  Mtaa: Mtambani  10-cell Unit: 12

Date: 24/6/04  10-cell unit leader: Abdalla Mwasiba
GUIDELINES TO SEARCHING FOR MOSQUITO BREEDING HABITATS (STAGNANT WATER) AND CONDUCTING LARVAL SURVEY

Revised March 2005

Background:
Although all mosquitoes breed in water the available type of breeding habitat is likely to change at different times of the year. *Anopheles* larvae prefer water that is exposed to the sun, whilst *Culex* larvae can be found everywhere. *Anopheles* larvae and especially pupae are usually concentrated in certain parts of large breeding sites, which make larval collection difficult. Edges of sites and patches of vegetation are often places where larvae can be found; sun exposure and wind also determine where mosquito larvae occur. Since mosquitoes breed in almost any kind of water body it is important to check all water bodies during a larval survey.

This larval survey is designed to inform us of the distribution of the aquatic stages of disease transmitting mosquitoes over space and time. After collecting baseline data on mosquito habitats and larvae for one year we plan to begin larval control operations in selected areas. The larval surveys will help us target future control activities.

Our Goal: To survey all potential mosquito breeding habitats (all stagnant water bodies) in 15 wards of urban Dar es Salaam in order to plan efficiently the interventions for larval control from 2006 onwards.

Why do we need to collect data on mosquitoes in a malaria control programme?

To control the mosquitoes that transmit malaria, we have to know them well! We need to have the basic but accurate information that is essential for proper planning of our control measures. We need to know **WHAT** kind of mosquitoes we are going to target, **WHEN** are we going to target them, **WHERE** we going to target them and **HOW** are we going to target them.

Therefore, we need to identify:
- If malaria mosquitoes are present in the area, and if present, which ones?
- Which other mosquitoes are around that are not malaria mosquitoes.
- Where are the different mosquitoes breeding?
- Are the breeding places available throughout the year?
- How do the breeding places look like, how do they differ?
- How can we prevent mosquitoes from breeding in the various sites?

All this information is necessary to design a powerful mosquito larval control operation and to assess, in the following years, whether we have been successful in our control operations. We should be able to compare the mosquito densities before and after larvicide treatments or environmental management and see a remarkable reduction in the number of mosquitoes. Furthermore, The Demographic Survey Teams of the programme are
collecting data on malaria cases in the preparation phase as well as during the larval control activities to show if we have an impact on malaria in the community with our control operation. A good baseline data collection period is most important if a control operation is to be successful in future! Therefore all field staff involved ought to have great interest, motivation, responsibility and enthusiasm to make the programme work! If we succeed during this pilot phase, the programme can be continued, expanded and improved for the benefit of all inhabitants of Dar Es Salaam City.

**Mosquito Larval Survey**

**Why do we carry out larval survey?**

We carry out larval survey in order to:

- Identify potential mosquito breeding habitats (stagnant water bodies) and ascertain the presence or absence of mosquito larvae in them.
- Determine the availability of mosquito breeding habitats around the year (during dry and rainy seasons).
- Determine the preferred larval habitats for mosquitoes.
- Describe changes in mosquito larvae densities over time.
- Assess the impact of mosquito control activities on larval abundance.

**Where do mosquitoes breed?**

- Mosquitoes breed only in water! The larvae cannot survive anywhere else, they DO NOT breed in grass or bushes.
- Mosquitoes can breed in any kind of water and therefore ALL kinds of water bodies have to be checked for mosquito larvae in a larval survey.
- Mosquitoes do not breed in fast running water of rivers, but can breed at the edges where the water is not moving fast, in cattle hoof prints along a river and in slow flowing drains.

To identify ALL mosquito larval habitats it is essential to be exhaustive and check all possible breeding places (any stagnant water body), even those that are hard to reach, this enables determination of the types of habitats most likely to harbour the larvae of mosquitoes

**Mosquito habitat types to be distinguished in larval surveys**

In this larval survey, we try to characterise the breeding habitats (water bodies) we find, to investigate which habitats are the most common habitats and which of them are most attractive and productive for mosquito larvae. In our programme, we will survey **Open Habitats** weekly and **Closed Habitats** every 3 months.

**What are Open Habitats?**

Open habitats are defined as water bodies that are exposed to the open air and light. This means that light can reach the water surface, also plants can grow inside. In most of these sites the water can be readily reached with the dipper.

**What are Closed Habitats?**

Closed Habitats are in contrast to the open habitats defined as water that can be found in closed and dark environments. Often it will be more complicated to reach the water surface with the dipper since openings to access have to be identified and opened in many cases.

We want to characterise the open and closed mosquito larval habitats of Dar es Salaam following closely the definitions below.
Open Habitats: Habitat Codes (see data sheet) and Habitat definition

1: Puddles and Tyre Tracks

Puddles are small to medium sized stagnant water areas. Most puddles are less than 10 m in perimeter (<10 m). Some of them might though reach between 10 and 100 m in perimeter (10-100 m). The source of the water is rain water and water run off. The water is shallow, less than 0.5 m deep (<0.5 m). Tyre tracks are just as special type of a puddle. Vehicles often leave tracks in the ground especially if the ground is wet. These tracks/depressions hold water longer than the surrounding areas and thus serve as potential mosquito breeding grounds.

2: Swampy Areas

The habitat code Swampy Areas summarises a number of different looking water bodies. They all have in common that because of a very high ground water table there is water standing on the ground for quite some time during the year or even continuously. The source of the water is ground water, but can additionally be fed by rainwater.

Swampy areas are for example areas that border a large water body like a river or creek where water is permanent throughout the year. Often can the water here inside the swamp be deep (>0.5 m). The vegetation is often characterised by tall reeds (left photo) and/or floating plants.

Other swampy areas might be characterised by short grassy vegetation (right photo) where water stands due to high water table or due to a spring/seepage that brings water from the ground to the surface.

3: Mangrove swamp

These are areas near the sea only, they can not be found far away from the sea. They have mangrove trees growing with water underneath. The water is tidal because it comes from the sea but some small pools might remain throughout. The water is salty.
4: Drains and Ditches

Drains and ditches are man-made and constructed for the purpose of getting rid of water or to irrigate an area. Drains specifically are constructed for water to flow and therefore to drain water from or irrigate the area. However, most of them get blocked with litter thus holding water for longer duration. Ditches are also man-made but do not necessarily support water to flow, they support stagnant water bodies. Drains and ditches can be cement lined but also can just be dug in the ground. It is important to notice that they are man-made and made for a specific purpose to channel the water. They can also be small for example to channel water from a tap to the garden, as long as that channel is man-made. It would be very desirable if you could describe what type of a drain or ditch you are recording in the comment area of your data sheet.

5: Construction pits, foundations and man-made holes

These are small to medium sized man-made habitats that can collect water, for example unfinished constructions of pit latrines, holes in the ground for rubbish collection, holes for water collection or storage, holes for ground water collection for irrigation (wells), foundations of houses that will be built, any man-made pit structure that holds water and is open (also pits holding water from the bathroom). These habitats are usually in the ground and are therefore not moveable.

6: Water storage or other Man-made containers:

Any container that holds water that could serve mosquitoes to breed in for example open water storage tanks, barrels, tyres, buckets, clay pots, livestock feeding trays. Most of these habitats are therefore on top of the ground and can be moved from one place to another (except big open cemented water tanks etc.)

7: Rice paddy (Rice field)

These are plots where rice grows. Those plots can be flooded for longer periods of time. Larvae can mainly be found on the edges of the fields. You need to pay close attention to fields that are drying up because the water is collected in small pools all over the field. The mosquito larvae can then be concentrated in very small water collections that might not easily be found.

8: Matuta

These are raised ridges on agricultural plots. The furrows created hold water for longer duration. The water in the furrows is not evenly distributed and therefore keen observation for larvae in very small depressions particularly on the fringes is important.
9: Other Agriculture

Besides Rice and Matuta other agricultural fields might provide stagnant water bodies for mosquito larvae. The water might be supplied by irrigation or by a high water table, or even rainfall.

10: Stream and River beds

Streams and Rivers are usually fast flowing water bodies that are not good for mosquito larvae to develop in. But with these streams and rivers there are often fringe area associated where the water only moves very slowly or is stagnant in areas where water pools along the river and stream edges. These fringe areas can provide good breeding habitats for mosquito larvae. Also rivers and streams that are drying up leave stagnant, pooling water behind that can serve as larval habitats.

11: Ponds

Ponds are medium to large in size. Ponds are permanent water bodies or are at least present for several months in the year. They might decrease in size with the dry season. Ponds are at least during the rainy season more than 0.5 m deep (>0.5 m, in the middle of habitat). Ponds can contain tall vegetation and floating plants, mosquito larvae are usually associated with the shallow edges of ponds.

12: Others (please describe them)

Under this category you can record any other stagnant water bodies that could be mosquito larval habitats that do not fit under any of the above-described habitats. Before you decide to record a habitat under category 12, please make sure you have checked the definitions of habitat categories 1 to 11 to make sure this habitat type is not considered there. Please, describe the habitat recorded under category 12 in the comment section of the data sheet. Be in your description as detailed as possible.

Closed Habitats: Habitat Codes (see data sheet) and Habitat definition

There are fewer types of closed habitats than the open ones. We want to distinguish between the following:

**Pit latrines:** These are dug on the ground and often contain water in closed and dark environments. They are good breeding habitats for culicine mosquitoes.

**Soakage pits:** These are closed pits connected to the latrines and often contain water. They serve as breeding grounds for culicine mosquitoes.

**Septic tanks:** These are constructed as underground (closed) waste storage containers. They are normally sealed but if they have a small opening, and contain water, mosquitoes do breed in them.

**Others:** Here you can record any other closed habitat that you encounter that does not fall under the definitions above. Please describe the habitat in the comment section.
Sampling mosquito larvae

The most common and easiest technique to investigate the presence or absence mosquito larvae in a habitat is dipping.

**ALWAYS** have:
- A dipper. A dipper can vary in shape and size, including small pans, soup ladles etc. A dipper should be light in colour inside to see the larvae easily.
- A Pen/pencil, a notebook, and the standard data recording forms/sheets

**Sometimes** (If need be)
- A pipette,
- Vials to collect specimen (sometimes if the samples are needed for identification).
- Ethanol to kill specimen and preserve them immediately (when the samples are needed for further processing)
- Bigger bottles or suitable containers to transport larvae alive (If live specimens are needed)

**Where to do a mosquito larval search in the habitat (stagnant water body)**

Note that preferred (but not restricted) sites where Anopheles larvae can be found, are:
- sunlit water bodies or the sun-exposed area of a water body,
- edges of water bodies,
- around low vegetation e.g. grass tufts, round swimming debris and leaves,
- in-between floating vegetation
- except in very small sites, Anopheles larvae are usually NOT evenly distributed over the entire surface area.

**The dipping technique**

- While dipping, you should take care so that your shadow is cast away from the habitat as larvae are very sensitive and will dive to the bottom once your shadow is cast on the water
- Lower the dipper gently in an angle of 45° just below the surface so that water flows in together with any larvae that might be present. The important point to note here is that we sample by displacement suction and not by scooping. The diagram below how dipping should be done.
- Take care not to disturb the water too much as this will make larvae dive downwards. If the water is disturbed, wait for three minutes before continuing dipping.

- When lifting the water, take care not to spill the water containing the larvae and pupae.
- Hold dipper steadily until larvae and pupae rise to the water surface in the dipper (this can take several minutes, especially for older instars).
- Take at least 10 dips per habitat in different locations where mosquito larvae can be expected (edges of habitats, around vegetation, shallow areas etc.). In the case of water channels and drains or large swamps or mangrove swamps, walk along/around the habitat and take up to 60 dips/habitat to investigate for the presence of mosquito larvae.
- If specimen are needed for further studies in the laboratory collect larvae and pupae by means of a pipette and transfer them to a bottle or vials, label the vials (date, name of sampling habitat), throw the water on the ground.
- REMEMBER that Anopheles mosquito densities are often quite low compared with other genera, and therefore, you have to extend your time and efforts to detect them! Furthermore, sampling pupae is extremely difficult because they are very sensitive and fast, the slightest disturbance and they disappear (dive down), additionally they are even more clustered at one spot than larvae, and therefore you should thoroughly search the habitats for pupae.

Where there is dense, floating vegetation:
- Disturb the water thus causing larvae and pupae to sink below the surface
- Clear away vegetation with the dipper and wait a few minutes for larvae and pupae to return to surface
- In clumps of vegetation e.g. grass, press dipper into it so that water flows in.

For extremely small habitats like hoof prints, you can sample larvae or pupae either with a very small sieve, with a spoon, with a pipette, or make direct observation: It can be helpful to stir the water with a stick to make it muddy and wait for the larvae and pupae to rise because they are now easily seen against the muddy background.

**Step-by-step guide to searching for mosquito larval habitats, characterization of the habitats and filling in the data forms**

**Introduction**

All the data on mosquito larval habitats is recorded in the forms provided. It is therefore important that the forms are filled in correctly so that they reflect the true picture on the ground. Therefore, it is important that we know what to fill in the forms and how to fill in them. We have two types of forms 1) *Open Habitats Forms* and 2) *Closed Habitats Forms*. Please follow the guidelines step by step as given below:

**Open Habitats (these are visited once every week)**

1. First, obtain the open habitats larval survey forms from your supervisor at the ward level. The Basic Operational Unit for our programme is the 10-cell unit and thus the forms are designed accordingly. Also carry with you the 10-cell unit maps and their description forms that you had made during the mapping exercise
2. Go to the specific 10-cell unit that you had previously mapped into plots. Once on a 10-cell unit, fill in the date, the name of the Municipality, the Ward, and the Mtaa on the top part of the form. Also fill in the 10-cell unit number and the name of the 10-cell unit leader
3. Then, with the help of your 10-cell unit map, move from plot to plot as shown on your map.
4. Once on a specific plot within that 10-cell unit, fill in its Plot ID number as it appears on your map. If the plot has a house, fill in the house number.
5. Then walk exhaustively and keenly on the plot to searching for mosquito breeding habitats. Sometimes, you may not find a habitat within a plot, then write ‘No Habitat’ (but once you had found a certain habitat on the compound that might now be dry, record its number from previous mappings and record dry)
6. Once a habitat is located, assign it a number (Habitat ID), and then fill its type (Habitat type) on the form using the habitat codes provided on the top part of the form for open habitats. If you are not sure of the habitat type, refer to the notes and pictures on different habitat types.
7. Give a brief but accurate description and location of the habitat on the column labelled ‘Habitat description’. This description will help you remember each and every habitat they way you have arranged them in your form. Always describe habitats in a way that you can easily remember which they are and where they are.

8. Since each and every habitat will be visited every week, the habitat type might change with time e.g. from a matuta to a rice paddy. If the habitat is the same as it was the last time you visited it, then fill in 1 in the column with the question ‘Same habitat type from first visit?’ If the habitat type has changed, then fill in 2 and then fill in the code for the habitat type it has changed to in the column labelled ‘New habitat type’.

9. After filling in the above information on a habitat, the actual data collection begins. This is what should be filled in each column. Tick (√) where appropriate.

**Wet?** Here you observe if the habitat contains water or it is dry and tick (√) the appropriate box. The recording of when the habitat is wet or dry help us in judging how stable the habitat is over time. A stable habitat will always pose the danger of continuously producing mosquitoes.

**Habitat perimeter:** Here many people get confused. Perimeter means the distance all round the habitat. You get the perimeter by walking round the habitat. Approximately, each step that you walk is a meter. For example, a drain can be half a step wide but 45 steps long as shown below. If you walk around it, then you will walk 91 steps. The perimeter of the drain will be 91 meters and for the habitat perimeter the column ‘10-100’ in the form should be ticked (√).

```
0.5 m

Drain
```

Important here is that you measure the perimeter of the water body not necessarily of the whole habitat that could contain water. So the drain might be very long but might have only water in a short area, measure the short area of water only. If a habitat is dry, meaning it does not contain any water then you can not measure any thing so the column remains blank.

The importance of estimating the habitat perimeter is to enable us calculate the amount of larvicide to be used during the larval control operations. Larvicide dosage is always calculated in terms of hectares of water surface to be covered. Therefore, the perimeter of a habitat is a good estimate of the area covered by that habitat.

**Plants:** The presence or absence of plants in a habitat determines the kind of larvicide to be used and the method of application. Observe the habitat for the presence or absence of plants and tick (√) where appropriate. We want to distinguish between short vegetation (not higher than your knee) and tall vegetation (much higher than your knee), floating vegetation that can be found on the water surface or no vegetation at all. Multiple ticks are possible.

**Water depth:** The depth of water determines the stability of a habitat as well as the type of mosquito breeding in it. Use the handle of your dipper to estimate the depth and tick (√) where appropriate. Remember, when the habitat is dry, you can not measure any water depths and therefore this column will be left blank.

**Larval stage:** To fill this column, you must dip the habitat. Take at least 10 dips per habitat in different locations where mosquito larvae can be expected (edges of habitats, around vegetation, shallow areas etc.). In the case of water channels and drains or large swamps or mangrove swamps, walk along/around the habitat and take up to 60 dips/habitat to investigate for the presence of mosquito larvae.

Record by ticking (√) all the larval stages (Early, Late, or Both) that you see. Recording of the stage or stages of the larvae in a given habitat is important as the larvicide only kill the larvae at a
certain stage. Therefore, we need to know what stage the larvae are before we can treat the habitat with the larvicide. Late instars larvae are indicators of poor or no larvicide treatment in the recent days. Remember that it is possible to have different larval stages in the same habitat at the same time.

**Pupa:** It is important to check and record the presence or absence of pupae this is the final stage of the mosquito in water. Their presence or absence helps us in judging whether a larval control operation has been successful or not. Record the presence or absence of pupae by ticking (✓) the appropriate box.

**Comments:** Note down anything that you think is important in your larval survey exercise on the ‘Comments’ column of the form. Make good use of the comment section.

10. After a careful and exhaustive searching, and after dipping for larvae in all the habitats move to the next plot.
11. After you have completed all the plots in a 10-cell unit, move to the next 10-cell unit and repeat the above procedure.

**Closed Habitats**

For a survey of the closed habitats, you will be looking for Closed Habitats like Pit Latrines, Septic Tanks, Soakage Pits and Other types of closed habitats like Covered Waste Water Storage Tanks in build up areas (Inside compounds/Houses). Therefore, the focus is on houses within the 10-cell units.

1. First, obtain the closed habitats larval survey forms from your supervisor at the ward level. The Basic Operational Unit for our programme is the 10-cell unit and thus the forms are designed accordingly. Also carry with you the 10-cell unit maps and their description forms that you had made during the mapping exercise.
2. Go to the specific 10-cell unit that you had previously mapped into plots. Once on a 10-cell unit, fill in the date, the name of the Municipality, the Ward, the Mtaa, the 10-cell unit number and the name of the 10-cell unit leader on the top part of the form.
3. Then, with the help of your 10-cell unit map, move from plot to plot as shown on your map.
4. Once on a specific plot within that 10-cell unit, fill in its Plot ID number as it appears on your map. Then fill in the house number.
5. Then ask to be shown the toilets, the soak pits, the septic tanks, and any other structure associated with human waste and wastewater disposal.
6. For each and every type of the above listed habitats that you find in that compound/house, assign it an Identification Number (Habitat ID). For example, if you enter a house/compound and the first type of habitat you find is a Soakage pit, assign it 1 on the ‘Habitat ID’ column in the form. If the second that you find is an underground wastewater storage tank, then assign it 2 on the ‘Habitat ID’ column in the form.
7. Once a habitat has been identified, use the Habitat codes provided at the top of the form to assign it a number for its type on the column labelled ‘Habitat Type’ in the form. For example, if it is a soakage pit, its habitat type is coded 3, and therefore you fill in 3 in the column labelled ‘Habitat Type’ in the form.
8. Then give a brief description of the habitat in a way that will assist you always remember it.
9. After filling in the above information on a habitat, **the actual data collection begins**. This is what should be filled in each column. Tick (√) where appropriate.

   **Wet:** Check whether the habitat is dry or it contains water and tick (√) where applicable. Habitats that contain water are always potential in producing mosquitoes.

   **Condition of the toilet:** For pit latrines examine whether their conditions are **good**, **bad** or **full** and tick (√) accordingly in the column named ‘**Condition of the latrine**’.

   **Habitat perimeter:** Approximate the perimeter of the habitat by walking around it and tick (√) in the column corresponding to its size in the ‘**Habitat perimeter**’ section of the form.

   **Water depth:** Approximate the depth of the habitat using the handle of your dipper or a longer stick and tick (√) where appropriate in the ‘**Water depth**’ section of the form.

   **Larval stage:** To fill this column, you **must dip** the habitat. Use the dipper with a long handle to sample. Record by ticking (√) all the larval stages (Early, Late, or Both) that you see.

   **Pupae:** Record the presence or absence of pupae by ticking (√) the appropriate box.

   **Comments:** Note down anything that you think is important in your larval survey exercise in the ‘**Comments**’ column of the form. For example, a pit latrine cannot be sampled because the hole is very narrow, or it is very deep, note this down in the ‘**Comments**’ column of the form.

10. After a careful and exhaustive searching, and after dipping for larvae in all the habitats move to the next plot.

11. After you have completed all the plots in a 10-cell unit, move to the next 10-cell unit and repeat the above procedure.
### Open Habitats (12 habitats codes)

<table>
<thead>
<tr>
<th>1: Puddles and Tyre Tracks</th>
<th>6: Water storage or other Man-made containers:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Puddles</strong></td>
<td>• any container that holds water for more than one week</td>
</tr>
<tr>
<td>• small to medium sized</td>
<td>• examples: open water storage tanks, barrels, tyres,</td>
</tr>
<tr>
<td>• stagnant water</td>
<td>buckets, clay pots, livestock feeding trays</td>
</tr>
<tr>
<td>• water source = rain water and water run off</td>
<td>• can be moved from one place to another (except</td>
</tr>
<tr>
<td>• shallow water = less than 0.5 m deep (&lt;0.5 m)</td>
<td>big open cemented water tanks etc.)</td>
</tr>
<tr>
<td><strong>Tyre tracks</strong></td>
<td></td>
</tr>
<tr>
<td>• made by wheels of vehicles</td>
<td></td>
</tr>
<tr>
<td>• these tracks/depressions hold water longer than the surrounding areas, these are potential mosquito breeding grounds</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2: Swampy Areas</th>
<th>7: Rice paddy (Rice field)</th>
</tr>
</thead>
<tbody>
<tr>
<td>• very high ground water table, but can be fed by rainwater</td>
<td>• plots where rice grows</td>
</tr>
<tr>
<td>• can border a large water body like a river or creek</td>
<td>• when fields are drying up the mosquito larvae can then be concentrated in very small water pools</td>
</tr>
<tr>
<td>• tall reeds and/or floating plants (left photo)</td>
<td></td>
</tr>
<tr>
<td>• short grassy vegetation with water seepage (right photo)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>3: Mangrove swamp</th>
<th>8: Matuta</th>
</tr>
</thead>
<tbody>
<tr>
<td>• near the sea only</td>
<td>• raised ridges on agricultural plots</td>
</tr>
<tr>
<td>• salty water</td>
<td>• furrows created to hold water for longer duration</td>
</tr>
<tr>
<td>• water is tidal</td>
<td></td>
</tr>
<tr>
<td>• small pools</td>
<td></td>
</tr>
<tr>
<td>• mangrove trees growing with water underneath</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>4: Drains and Ditches</th>
<th>9: Other Agriculture</th>
</tr>
</thead>
<tbody>
<tr>
<td>• man-made for getting rid of water or to irrigate an area</td>
<td>• other agricultural area that might provide stagnant water bodies for mosquito larvae</td>
</tr>
<tr>
<td>• the water should flow, but if blocked with litter, the drains and ditches can become stagnant water bodies</td>
<td>• water source = irrigation or high water table or rainfall</td>
</tr>
<tr>
<td>• cement lined or just dug in the ground</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>5: Construction pits, foundations and man-made holes:</th>
<th>10: Streams and River beds</th>
</tr>
</thead>
<tbody>
<tr>
<td>• small to medium sized</td>
<td>• flowing water bodies</td>
</tr>
<tr>
<td>• man-made habitats to collect water</td>
<td>the edge of the stream or river, where the water is slow</td>
</tr>
<tr>
<td>• open stagnant water</td>
<td>moving or stagnant and when streams and rivers are</td>
</tr>
<tr>
<td>• in the ground and not moveable</td>
<td>drying up leave stagnant pooling water that can serve as larval habitats</td>
</tr>
<tr>
<td>• examples: unfinished constructions of pit latrines, holes in the ground for rubbish collection, holes for water collection or storage, holes for ground water collection for irrigation (wells), foundations of houses</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>6: Water storage or other Man-made containers:</th>
<th>11: Ponds</th>
</tr>
</thead>
<tbody>
<tr>
<td>• any container that holds water for more than one week</td>
<td>• medium to large in size</td>
</tr>
<tr>
<td>• examples: open water storage tanks, barrels, tyres, buckets, clay pots, livestock feeding trays</td>
<td>• open water</td>
</tr>
<tr>
<td>• can be moved from one place to another (except big open cemented water tanks etc.)</td>
<td>• permanent water or present for several months in the year</td>
</tr>
<tr>
<td></td>
<td>• tall vegetation and floating plants</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>7: Rice paddy (Rice field)</th>
<th>12: Others (please describe them)</th>
</tr>
</thead>
<tbody>
<tr>
<td>• plots where rice grows</td>
<td>• any other stagnant water bodies that could be mosquito larval habitats and do not fit under any of the habitat categories 1 to 11</td>
</tr>
<tr>
<td>• when fields are drying up the mosquito larvae can then be concentrated in very small water pools</td>
<td>• In the comment section of the data sheet, the description should be as detailed as possible</td>
</tr>
</tbody>
</table>
Closed Habitats (4 habitats codes)

1: Pit latrines: dug in the ground and often contain water

2: Septic tanks:
   - underground (closed) waste storage containers
   - normally sealed but may have a small opening

3: Soakage pits: closed pits connected to the latrines and often contain water

4: Others:
   - any other closed habitat that does not fall under the definitions above
   - please describe the habitat in the comment section
# Larval surveillance forms

## Ward level mosquito larval habitat survey - Open habitats

<table>
<thead>
<tr>
<th>Plot ID</th>
<th>Habitat ID</th>
<th>Habitat type</th>
<th>Same habitat type from last visit? 1=Yes 2=No 3=First visit</th>
<th>Previous habitat type</th>
<th>Wet?</th>
<th>Habitat perimeter</th>
<th>Plants</th>
<th>Water depth</th>
<th>Larval stage</th>
<th>Anoph.</th>
<th>Culex</th>
<th>Pupae</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>

**Habitat codes:**

1: Puddles & tire tracks
2: Swampy areas
3: Mangrove Swamp / Saltwater marsh
4: Drain/Ditch
5: Construction pits-foundations/man-made holes
6: Water storage container
7: Rice paddy
8: Matuta
9: Other agriculture
10: Stream/river bed
11: Pond
12: Other (describe below)

**Serial number of this form**

**Serial number on the map form**

**Ten cell unit identifier**


CORPS Signature: ___________________ Date: ________ / ________ / ________ Supervisors Signature: ___________________ Date of check: ________ / ________ / ________

Page 1
## Ward level mosquito larval habitat survey - Closed habitats

<table>
<thead>
<tr>
<th>PLOT ID</th>
<th>HOUSE NUMBER</th>
<th>HABITAT ID</th>
<th>HABITAT TYPE</th>
<th>HABITAT DESCRIPTION</th>
<th>WEATHER</th>
<th>CONDITION OF LATRINE</th>
<th>HABITAT PERIMETER</th>
<th>WATER DEPTH</th>
<th>LARVAL STAGE</th>
<th>PUPAE</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>dry</td>
<td>contains water</td>
<td>&lt; 10 m</td>
<td>10-100 m</td>
<td>&gt; 100 m</td>
<td>None</td>
<td>Early</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Good</td>
<td></td>
<td>Full</td>
<td>&lt; 0.5 m</td>
<td>&gt; 0.5 m</td>
<td>Present</td>
<td>Late</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Bad</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>None</td>
<td>Absent</td>
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<td></td>
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</tbody>
</table>

**Habitat codes:** 1 - Pit Latrine  2 - Soakage Pit  3 - Septic Tank  4 - Other

**CORPS signature:** ___________________  Date: ______/______/_______  **Supervisors signature:** ___________________  Date of check: ______/______/_______
<table>
<thead>
<tr>
<th>Aina ya mazalio:</th>
<th>Namba ya utambulisho wa ploti</th>
<th>Namba ya zalio la awali</th>
<th>Maji?</th>
<th>Ukumbwa wa zalio</th>
<th>Mamea</th>
<th>Kiina cha maji</th>
<th>Hatua za viluwiluwi</th>
<th>Mabuu</th>
<th>Maoni</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: Dimbwi na kashata za matairi ya magari</td>
<td>1=Ndio 2=Hapana</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2: Mboojmbijiwi la matope</td>
<td>1=Ndio 2=Hapana</td>
<td></td>
<td></td>
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<td></td>
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<td>3: Bwawa maji chumvi/Mikoko</td>
<td>1=Ndio 2=Hapana</td>
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<td>4: Milereji/Kijilo</td>
<td>1=Ndio 2=Hapana</td>
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<td>5: Shimo/Ujenzi/Mchanga</td>
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<td>6: Chembo cha kuhifahia maji</td>
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<td>9: Aina nyingine ya kilimo</td>
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<td>12: Aina Nyingine (Elezea)</td>
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Namba ya uchunguzi wa mazalio ya wazi ya mbu ngazi ya kata

Taarifa ya uchunguzi wa mazalio ya wazi ya mbu ngazi ya kata

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Taarifa ya uchunguzi wa mazalio ya wazi ya mbu ngazi ya kata
### Fomu ya mazalio ya mbu kwenye vyoo na makaro Ngazi ya kata

**Serial namba ya fomu hii**

**Serial namba ilioke kwenye fomu ya ramani ya shina hili**

**Namba ya shina ya pekee**

<table>
<thead>
<tr>
<th>Manisipaa:</th>
<th>Kata:</th>
<th>Mtaa:</th>
<th>Namba ya shina:</th>
<th>Jina la Mjumbe:</th>
</tr>
</thead>
</table>

**Aina ya zalio:** 1 - Choo cha shimo  2 - mashimo ya maji machafu  3 - Mashimo ya maji taka  4 - Mengineyo

<table>
<thead>
<tr>
<th>Namba ya Utambulisho wa ploti</th>
<th>Namba ya nyumba</th>
<th>Aina ya zalio</th>
<th>Maji yapo?</th>
<th>Hali ya choo</th>
<th>Ukubwa wa zalio</th>
<th>Kina cha maji</th>
<th>Hatua za viluwiluwi</th>
<th>Mabuu</th>
<th>Maoni</th>
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<tr>
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<td>kina maji</td>
<td>&lt; 10 m</td>
<td>10-100 m</td>
<td>&gt; 100 m</td>
<td>&lt; 0.5 m</td>
<td>&gt; 0.5 m</td>
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<td>hakuna</td>
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</tr>
</tbody>
</table>

**Jina La CORP:** _____________ Tarehe: _____ / _____ / ______

**Jina La Supervisor:** _____________ Tarehe: _____ / _____ / ______
Municipal mosquito larval habitat spot check - Open habitats

Serial number of this form

Serial number on the map form

Ten cell unit identifier

Municipality:_____________________     Ward:________________________     MTAA:_______________________  10-cell unit:_____________________  10-cell leader:________________________

GPS(UTM/WGS84): Northing_________________________ Easting_______________________________

Date of CORP's data sheet:________/________/__________

Serial number on the map form___________________________

Yes          No
Is there a map? 1: Puddles/tire tracks 5: Construction pits/foundations/man-made holes 9: Other agriculture
Is the map accurate? 2: Swampy areas 6: Water storage & any other man-made container 10: Stream/river bed
Does map match city copy? 3: Mangrove Swamp 7: Rice paddy 11: Pond
4: Drain/Ditch 8: Matatu 12: Others (describe below)

<table>
<thead>
<tr>
<th>Plot ID</th>
<th>House No.</th>
<th>Habitat ID</th>
<th>Habitat type</th>
<th>Habitat type correct?</th>
<th>Correct habitat type found by the CORPs?</th>
<th>Habitat Description</th>
<th>Wet?</th>
<th>Habitat perimeter</th>
<th>Plants</th>
<th>Water depth</th>
<th>Larval stage Anoph.</th>
<th>Larval stage Culex</th>
<th>Larval stage Absent</th>
<th>Pupae</th>
<th>Comments</th>
</tr>
</thead>
</table>

Dry: contains water, 5 cm; contain water, 10-100 m; > 1 m

Plants:
- None
- Short vegetation
- Tall vegetation
- Floating plants

Water depth:
- < 0.5 m
- 0.5 m - 1 m
- > 1 m

Larval stage:
- Anoph.: Absent, Early, Late
- Culex: Absent, Early, Late

Inspectors signature:________________________________  Date of check :______/______/_______

Municipal Coordinators signature:_____________________________  Date of check :______/______/_______
# Municipal mosquito larval habitat spot check - Closed habitats

Serial number of this form: [ ]
Serial number on the map form: [ ]
Ten cell unit identifier: [ ]

**Municipality:** [ ]  **Ward:** [ ]  **MTAA:** [ ]  **10-cell unit:** [ ]  **10-cell leader:** [ ]

**GPS (UTM/WGS84):** Northing [ ]  Easting [ ]

<table>
<thead>
<tr>
<th>Is there a map?</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serial number on the map form</td>
<td>[ ]</td>
<td></td>
</tr>
</tbody>
</table>

| Does map match city copy? | [ ] |

**Habitat codes:**
- 1 - Pit Latrine
- 2 - Soakage Pit
- 3 - Septic Tank
- 4 - Other

<table>
<thead>
<tr>
<th>Plot ID</th>
<th>House No.</th>
<th>Habitat ID</th>
<th>Correct habitat type?</th>
<th>Habitat found by the CORPs? (1=Yes 2=No)</th>
<th>Habitat Description</th>
<th>Wet?</th>
<th>Condition of the latrine</th>
<th>Habitat perimeter</th>
<th>Water depth</th>
<th>Culicine stage</th>
<th>Pupae</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>dry</td>
<td>Good</td>
<td>10-100 m</td>
<td>&gt; 10 m</td>
<td>None</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>contains water</td>
<td>Bad</td>
<td>&lt; 10 m</td>
<td>&lt; 0.5 m</td>
<td>Early</td>
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<td></td>
<td></td>
<td>full</td>
<td>Full</td>
<td>&gt; 10 m</td>
<td>&gt; 0.5 m</td>
<td>Late</td>
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</table>

**Inspectors signature:** [ ]  **Date of check:** [ ]

**Municipal Coordinators signature:** [ ]  **Date of check:** [ ]
LARVAL SURVEYS FOR OPEN HABITATS

The Urban Malaria Control Program (UMCP), Dar es Salaam
WARD LEVEL mosquito larval habitat survey - Open habitats

WARD LEVEL mosquito larval habitat survey - Open habitats

Serial number of this form ________________________________
Serial number on the map form ____________________________
Date: __________________________

Municipality: __________________________ Ward: __________________________
MTAA: __________________________ 10-cell unit: __________________________
10-cell leader: __________________________

GPS(UTM/WGS84): Northing___________________ Easting_______________________

Habitat codes:
1: Puddles&tire tracks 5: Construction pits/ foundations/ man-made holes
2: Swampy areas 6: Water storage container
3: Mangrove Swamp 7: Rice paddy
4: Drain/Ditch 8: Matuta
9: Other agriculture
10: Stream/river bed
11: Pond
12: Other (describe below)

<table>
<thead>
<tr>
<th>Plot ID</th>
<th>Habitat ID</th>
<th>Habitat type</th>
<th>Same habitat type</th>
<th>Last visit?</th>
<th>Previous habitat type</th>
<th>Habitat description</th>
<th>House number</th>
<th>Wet?</th>
<th>Habitat perimeter</th>
<th>Plants</th>
<th>Water depth</th>
<th>Larval stage</th>
<th>Pupae</th>
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</table>

Comments

New

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How to fill in the data sheets

• Plot No.
• House No. All **unique** and **continuous**
• Habitat ID.

• example
2 habitat types in same plot

1: Puddle

4: Drain
### Ward level - data sheet

**WARD LEVEL mosquito larval habitat survey - Open habitats**

<table>
<thead>
<tr>
<th>Plot ID</th>
<th>Habitat ID</th>
<th>Habitat type</th>
<th>Same habitat type from last visit?</th>
<th>Previous habitat type</th>
<th>Habitat description</th>
<th>House number</th>
<th>Wet?</th>
<th>Habitat perimeter</th>
<th>Plants</th>
<th>Water depth</th>
<th>Larval stage</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>Large drain with flowing water</td>
<td>X</td>
<td>X</td>
<td>&lt; 10 m</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>Present</td>
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<tr>
<td>7</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>Small open shallow puddle</td>
<td>X</td>
<td>X</td>
<td>&lt; 10 m</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>Present</td>
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</tbody>
</table>

**Habitat type = 1 to 12 codes**

**Habitat ID = how many different habitats in one plot**

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Dry or No habitat ???

7: Rice field - dry habitat (with the potential of being a larval breeding site)

11: Pond

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Wet / Dry / no habitat

• **Both** wet and dry sites need description

4: Drain & Ditches

**Wet habitat**
1.4.05

4: Drain & Ditches

**Dry habitat**
7.4.05
### 2 weeks of ward level data sheets

**WARD LEVEL mosquito larval habitat survey - Open habitats**

<table>
<thead>
<tr>
<th>Plot ID</th>
<th>Habitat ID</th>
<th>Habitat type</th>
<th>Same habitat type</th>
<th>Last visit?</th>
<th>1st visit?</th>
<th>Previous habitat</th>
<th>Wet? Habitat perimeter</th>
<th>Plants</th>
<th>Water depth</th>
<th>Larval stage</th>
<th>Pupae</th>
<th>Comments</th>
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</tbody>
</table>

**Serial number of this form**: 0723

**Serial number on the map form**: 0333

**Date**: 7/4/05

**Municipality**: Kinondoni  
**Ward**: Ndgumbi  
**MTAA**: Vigaeni  
**10-cell unit**: 38  
**10-cell leader**: Omary Bauari

**Habitat codes**:
1: Puddles&tire tracks  
2: Swampy areas  
3: Mangrove Swamp  
4: Drain/Ditch  
5: Construction pits/foundations/man-made holes  
6: Water storage container  
7: Rice paddy  
8: Matata  
9: Other agriculture  
10: Stream/river bed  
12: Other (describe below)

**Same code = same site habitat & no more man-made construction**

**Full habitat description even if dry**

---

Comment: Water flowing = water tap on 22 X X X X X X X in between the houses
Habitat perimeter (m)

- walk around and count your steps
- one step = one meter (1m)
Plants Height

Tall Plants

Level = knee height

Short Plants
Floating Plants
Water depth

Water depth: less than knee high=shallow; more than knee high=deep

Put dipper into middle of water to find the water level
Mosquito types

• Mosquitoes breed in **all types of water**, it is important to **check all** water bodies during a larval survey.

• **Anopheles** larvae and **Culex** larvae physically distinguishable but the pupae are **not** physically distinguishable
Mosquito types

*Anopheles* larva has no obvious siphon and lies parallel to the water surface.

*Culex* larva hang down from the water surface at an angle.
**WARD LEVEL mosquito larval habitat survey - Open habitats**

Serial number of this form: ____________________________
Serial number on the map form: ____________________________

Date: __________ / __________ / __________
Municipality: ____________________________
Ward: ____________________________
MTAA: ____________________________

10-cell unit: ____________________________

GPS(UTM/WGS84): Northing ____________
Easting ____________

10-cell leader: ____________________________

<table>
<thead>
<tr>
<th>Plot ID</th>
<th>Habitat ID</th>
<th>Habitat type</th>
<th>Previous habitat type</th>
<th>Same habitat type from last visit?</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Habitat description</th>
<th>House number</th>
<th>Wet?</th>
<th>Habitat perimeter</th>
<th>Plants</th>
<th>Water depth</th>
<th>Larval stage</th>
<th>Pupae</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>dry</td>
<td>Contains water</td>
<td>&lt; 10 m</td>
<td>10-100 m</td>
<td>&gt; 100 m</td>
<td>none</td>
<td>floating pears</td>
<td>0.5 m</td>
<td>&gt; 0.5 m</td>
</tr>
</tbody>
</table>

**Comments**

**CORPS signature Date**

**Inspectors signature Date**

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Habitat type = 12 codes

1: Puddles and Tyre Tracks
2: Swampy Areas
3: Mangrove swamp
4: Drains and Ditch
5: Construction pits, foundations and man-made holes
6: Water storage or other Man-made containers:
7: Rice paddy (Rice field)
8: Matuta
9: Other Agriculture
10: Stream and River beds
11: Ponds
12: Others (please describe them)
Is the site natural or man-made?

**Natural**
- 2: Swampy Areas
- 3: Mangrove swamp
- 10: Stream and River beds
- 11: Ponds

**Man-made**
- 1: Puddles and Tyre Tracks
- 4: Drains and Ditches
- 5: Construction pits, foundations, man-made holes
- 6: Water storage or other man-made containers
- 7: Rice paddy (Rice field)
- 8: Matuta
- 9: Other Agriculture

Is it freshwater or salt water?

**Freshwater**
- 2: Swampy Areas
- 10: Stream and River
- 11: Ponds

**Saltwater**
- 3: Mangrove swamp

Is the water stagnant or flowing or should it be flowing?

**Stagnant**
- 2: Swampy Areas
- 11: Ponds

**Flowing**
- 10: Stream and River

Drain is straight and man-made and river meanders and is natural
1: Puddles and Tyre Tracks
1: Puddles and Tyre Tracks
2: Swampy Areas

- very high ground water table
- water present always or most of the year
- water source = ground water & rainwater
- often border a large water body e.g. river
- usually depth >0.5 m
- often tall reeds, short grass or / & floating plants
2: Swampy Areas

Long plants = bushes

Short plants = grass
2: Swampy Areas

Short plants = grass

Long plants = reeds
3: Mangrove swamp

- usually near the sea = salty water from the sea
- mangrove trees growing with water underneath
- mangrove trees roots exposed
- water is tidal, when tide out:
  - small pools
  - crab holes in mud
  - shells on mangrove tree barks
3: Mangrove swamp

Tree roots in water

Sea water
3: Mangrove swamp

- Crab holes
- Sea shells on the mangrove tree
4: Drains and Ditches

- man-made
- Usually getting rid of water or to irrigate
- flowing water
  or if blocked with litter = stagnant water
- can be cement lined or just be dug in the ground
4: Drains and Ditches

Short plants

Tall plants
4: Drains and Ditches

Dry Habitat
5: Construction pits, foundations and man-made holes

- small to medium sized
- man-made habitats
- stagnant water
- water source = rain or ground water (garden wells), or filled by people
- function to collect water
- habitats in the ground - **not** moveable
5: Construction pits, foundations and man-made holes
5: Construction pits, foundations and man-made holes

Sharp man-made edges
5: Construction pits, foundations and man-made holes
6: Water storage or other Man-made containers:

- **any** container that holds water that could serve mosquitoes to breed (which were left for **more than a week**)

- **open water** storage tanks, barrels, tyres, livestock feeding trays

- Do not record all small buckets, flower pots, watering cans etc, since the water will be used and their position changed
6: Water storage or other Man-made containers:

Rain water = potential breeding site
7: Rice paddy (Rice field)

- plots where rice grows
- drying up = small pools = concentrated mosquito larvae
7: Rice paddy (Rice field)
7: Rice paddy (Rice field)
8: Matuta

- **raised ridges** on agricultural plots
- **man-made** furrows = hold water for longer duration
- larvae in very small depressions
8: Matuta
8: Matuta
9: Other Agriculture

• **stagnant** water bodies

• water source = irrigation or rainfall or high water table
9: Other Agriculture
10: Stream and River beds

- **Fast or slow flowing** water, although it can be seasonal
- Natural, not man-made
- twisting course **not** straight as for ditches and drains
- mosquito larvae habitats usually at
  - edges very slow flow or stagnant
  - seasonal rivers and creeks dry up at certain times in year and leave stagnant pooling water
10: Stream and River beds

Flow = water current
10: Stream and River beds

10: River

2: Swampy Areas
11: Ponds

- medium to large size stagnant water

- water present for several months in the year

- rainy season (depth can be $>0.5$ m, in the middle of habitat)
11: Ponds
12: Others

• any other stagnant water bodies that could be mosquito larval habitats

• please make sure you have **checked** the definitions of habitat categories 1 to 11

• please **describe** the habitat recorded under category 12
First Intervention Phase March 2006 to March 2007: Intervention Ward Selection
Following preliminary data analyses and field visits 3 wards have been selected as intervention sites (1 from each municipality) for 2006 while the other 12 wards will remain untreated controls. Of these wards where no insecticides will be applied this year, 3 (1 from each municipality) have been selected to be compared with the intervention wards for final analyses. The selection of the sites was based on the following observations:

All study wards have shown to greatly differ during the baseline data collection period in their habitat numbers available, the proportion of available habitats colonised by *Anopheles* larvae, the density and seasonality of adults found in houses and the malaria prevalence. The research team based the decision of which wards will receive larviciding and which wards will be compared with the intervention wards mainly on the proven ability of the ward supervisors and ward-based CORPs to implement the required task. Specifically, their ability to collect, understand, use and submit high quality data during the baseline data collection period was the primary criterion for choosing these high priority wards.

Specific Objectives of the Mosquito Larval Control Pilot Studies in 2006
- To identify and characterise all potential aquatic habitats of culicine and vector anophelines in the study wards and to study their availability over time
- To study seasonal larval population dynamics of Culex and vector anophelines
- To establish the level of biting intensity by anopheline and culicine mosquitoes and determine human malaria exposure, measured as the entomological inoculation rate (EIR) during the dry and rainy seasons
- To determine the prevalence of malaria infections in the population
- To implement the microbial larval control intervention in 3 study communities (wards)
- To ensure community consent and cooperation

Study Hypothesis
Larval mosquito control in urban Dar es Salaam where malaria transmission is relatively low and focal will decrease densities of adult mosquitoes to such an extent that malaria transmission will also decline and reduce the level of malaria infection prevalence in local communities/wards where larviciding takes place.

Timeline
- Collection of baseline data from March 2005 to February 2006:
  - Availability of aquatic habitats (weekly)
  - Colonisation of habitats with Anopheles mosquitoes (weekly)
  - Adult mosquito densities in houses (weekly)
  - Malaria prevalence and incidence in population (twice per year in each ward)
- Training on application of microbial larvicides in February 2006
Implementation of weekly monitoring and larviciding in intervention sites from March 2006 to March 2007
  - Monitoring and Evaluation of intervention from March 2006 to March 2007 will use the same surveillance system described above for the baseline period

Pilot study design

Site Selections

Non-Intervention Sites
- Keko
- Vingunguti
- Mwananyamala

Intervention Sites
- Kurasini
- Buguruni
- Mikocheni

Bacillus formulations – Background

- Discovery of the mosquitocidal Bacteria strains of *Bacillus thuringiensis* var. *israelensis* (*Bti*) and *Bacillus sphaericus* (*Bs*) during the mid-1970s
- Advantages of microbial larvicides:
  - Highly effective (need very little to kill mosquito larvae)
  - Selective in action (kill only mosquito and blackfly larvae in recommended dosage)
  - Environmentally safe to non-target organisms (other organisms living in water like those that feed on mosquito larvae will not be killed)
  - Safe for human handling and consumption: Microbials are natural mosquito diseases that can in no way harm humans. In fact WHO recommends it for drinking water.
  - Easy and safe to handle
- Resistance: *Bs* can introduce resistance but this can be reversed by rotating with an alternative insecticide. Resistance to *Bti* has never been observed in over 30 years of use around the world.

Bacillus formulations - Mode of action

- *Bacillus* is a bacteria that forms spores when conditions become adverse.
- During formation of spores a special protein is produced
- This protein is toxic to mosquito larva but only when eaten by them.
- The mosquito-killing protein is activated by digestive enzymes and alkaline pH in midgut of the mosquito larvae
- These special proteins then attack the midgut causing the formation of pores (small holes) and destruction of the cells that line the midgut
- Midgut pH drops to neutral
- Larvae can no longer digest food and die
- Only mosquito and blackfly larvae provide conditions in gut to activate the mosquito-killing protein so the microbials do not affect any other living organism
- The toxins do not act on pupae because they do not feed anymore
- The younger the larvae the less toxin they need to digest to die, therefore they usually die quicker than late instars
Products
Commercially available products
Manufacturer: Valent BioSciences, Illinois, USA

We have to distinguish between two microbials and the two formulations of each microbial that might be used:

Microbials
- Bacillus thuringiensis var. israelensis (VectoBac®)
- Bacillus sphaericus (VectoLex®)

Formulations and application methods
- Water-dispersible Granule (WDG) applied as a liquid with knapsack sprayers
- Corn Granule (CG) applied by hand

Mode of application
water-dispersible granule (WDG) – diluted in water, applied as liquid with a knapsack sprayer
corn granule (CG) – applied as granular, undiluted finished product by hand

When to use what?

Liquid application with knapsack sprayer:
- Effective and easy to apply in sites that have little emergent or floating vegetation
- If there is large amount of emergent vegetation the spray may not penetrate the vegetation and get into the water

Granule application by hand:
- Slower to apply to large areas but broadly applicable, will reach the target in all circumstances
- Particularly effective in sites with emergent or floating vegetation that liquid applications cannot penetrate
- Granule penetrates vegetation and drops on water surface
- Granule can often be thrown a larger distance than liquid and can therefore be used to treat less accessible sites

Bti (VectoBac)
- In all habitats, less good in very polluted habitats (e.g. latrines)
- Needs to be applied weekly
- Cheap

Bs (VectoLex)
- In all habitats, also in very polluted water
- Can show an extended residual effect, application when late instar larvae occur, this needs weekly monitoring
- expensive

Application Dosages
Before the Bti and Bs. formulations can be used in the field, their actual potency and efficacy has to be evaluated against the different indigenous mosquito species. To assess the minimum effective dosage bioassays need to be carried out in the laboratory following World Health Organisation (WHO) guidelines. To assess the optimum effective dosages field trials either in natural or in artificial habitats need to be carried out. The outcome of these preliminary tests on larval control answer the following questions: What is the minimum and optimum effective dosage of the formulations against indigenous Anopheles and Culex mosquitoes? Is Bti/Bs suitable for the control of anopheline mosquitoes in the area? Which concentrations have to be used? In which intervals have re-treatments to take place? Which formulations are most powerful? Which are the best application methodologies?
Preparatory studies have been carried out at ICIPE, Mbita, western Kenya between 2002 and 2004. Following the results from these studies recommended formulations and dosages for open, potentially *Anopheles*-producing habitats are shown in the table below:

To achieve 100% control of mosquito larvae in any habitat in 24 hours, use:

<table>
<thead>
<tr>
<th>Larvicide</th>
<th>Dosage (mg/m²)</th>
<th>Dosage (kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VectoLex WDG (650 ITU/mg)</td>
<td>0.20</td>
<td>2.0</td>
</tr>
<tr>
<td>VectoBac WDG (3000 ITU/mg)</td>
<td>0.04</td>
<td>0.4</td>
</tr>
<tr>
<td>VectoLex CG (50 ITU/mg)</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>VectoBac CG (200 ITU/mg)</td>
<td>1</td>
<td>10</td>
</tr>
</tbody>
</table>

ITU = International Toxic Units, describes the potency of larvicide, the higher the number, the more toxic is 1mg the less is needed to kill 100% of larvae within 24hrs.

Always note Lot number & ITU of product used in the field, ITU and Lot number are indicated on the product.

**Any Bti product ( VectoBac) NEEDS to be applied in WEEKLY intervals. Bti products do not have any longer residual effect.**

Selection of Larvicide for Dar es Salaam in 2006

We will take two approaches to two different categories of habitats. Open habitats which are exposed to sunlight and hence potential sources of *Anopheles* will be treated directly by the program Mosquito Control CORPs with Bti (VectoBac) only. For closed habitats in domestic settings which are not exposed to sunlight and produce no *Anopheles* but lots of nuisance culicine mosquitoes, small amounts of Bs (VectoLex) will be provided to households by programme staff.

Since we deal with highly polluted habitats in the urban area we will double the optimum dosage as identified above for routine use in Dar es Salaam.

**For open habitats we will apply:**

VectoBac (Bti) CG at 1 gram per square meter (10 kg per hectare)

**OR**

VectoBac (Bti) WDG at 0.04 gram per square meter (0.4 kg per hectare)

For our first year larviciding we have decided to use only Bti (VectoBac) for open larval habitats. Bti will be applied as corn granule (CG) formulations for hand application and water dispersible granule (WDG) for application as a liquid with knapsack sprayers where this formulation is appropriate. We will use Bti only for open habitats and this product must be applied weekly because it has no residual activity but is the cheapest option and does not require any additional monitoring and decisions on re-application dates.

**Application Equipment and Procedures**

**Liquid application:** Solo 475 knapsack sprayers with a capacity of 14 L will be used to apply water dispersible granular (WDG) formulations. They are an effective method of application in sites that have little emergent vegetation. If there is a large amount of emergent vegetation the spray may not penetrate and get into the water. The selected knapsack sprayers are relatively light and simple to use. They use compressed air above the spray mixture to push the mixture out of the tank through a hose and nozzle. The output of the sprayer is dependent on the pressure used, the nozzle type and the speed of walking during the application. Calibration of the knapsack sprayers can be practiced easily following standard operating procedures. The WDG formulations are easy to use since they dissolve in water easily. Therefore, it can be directly mixed in the knapsack sprayer by adding the larvicide and filling the sprayer to its maximum mark. The sprayer needs to be shaken well before pressure is added to the spray mix. To fill a full tank of the Solo 475 sprayer, 400 grams of WDG powder can be dispersed in water by mixing with agitation in approximately half a tank of water (7L), adding the remainder of the water to achieve a total
volume of 14L, and then mixing vigorously for 2 minutes. To prepare a half a tank, mix 200g of powder with 3 to 4 liters of water and make up to 7 liters or the halfway mark in a similar fashion. Only when the powder is fully dispersed into liquid form can pressure be applied and application begin: An application pressure of approximately 3 bar is achieved and maintained by pumping a Solo 475 sprayer with a number 2 disk and no core to pressure setting number 3. Calibration in Dar es Salaam indicates a typical mosquito control CORP achieves a swath width of 10 m, a flow rate of 0.74 litres per minute, and a walking rate of 54 m. With this dilution, flow rate, walking speed and swath width, a full tank is expected to cover one full hectare but no more. This is equivalent to 10 x 100 meter swaths across a perfectly square area of one hectare (100m x 100m) or 1000 meters of continuous swath. The spray wand should be moved quickly and continuously across a 180° arc using a full swing while walking the length of the swaths.

*Calibrated application specifications for liquid application:*
- Dilution: 400 g of WDG for a full tank (14L) or 200g for a half tank (7L)
- Backpack configuration: Number 2 disk with no core.
- Pressure: Backpack setting number 3 (approximately 3 bar)
- Walking speed: Approximately 50 meters per minute
- Swath Width: 10 meters
- Expected usage rate: 1 full tank should treat one hectare or 1000 meters of swath length (eg 10 swaths across a perfectly square 1 hectare area: 100m x 100m). This means that each litre should last for approximately 70 meters of swath length.

**Hand application:** Granular formulations (CG) may be applied by hand, similar to scattering seeds. However, it takes practice to obtain an even application or maintain the recommended application rate. It is very important for the field staff to practice this exercise well to gain experience in achieving even coverage as per the recent calibration workshop. For hand application from granular formulation buckets are used on a carrying strap to be hung around the shoulders allowing it to rest on the belly. The carrying strap can be adjusted for individual comfort and effectiveness. As determined during the recent calibration workshop, our objective is to achieve a coverage rate of 1 gram of VectoBac CG per square meter (m²), equivalent to 10 Kg per hectare. For medium to large areas (>9m² or 3m x 3m) with multiple habitats, this is best achieved by treating 3m-wide swaths with one handful spread over 10m of swath length. For smaller, distinct habitats, the area of the habitat should be measured and appropriate fractions of a handful (One handful = 25g) or a teaspoon (one teaspoon=2g) should be applied. For example, for a small habitat of approximately one meter squared, half a teaspoonful should be spread evenly by hand throughout the habitat. For a larger habitat of, for example 12 m² (3m x4m), half a handful should be spread evenly across the habitat. For long, narrow (<1m) habitats such as remnants of foundation trenches running alongside walls, simply scatter granules in the target area as you walk the length of the habitat, aiming to cover 20-30m of habitat per handful of granules. For all these habitat types you can practice on surfaces where granules area readily seen, aiming to achieve even coverage with approximately 4 granules per 10 cm x 10 cm area. We summarize these application specifications for easy reference as follows:

*Calibrated application specifications for liquid application:*
- Coverage: Approximately 4 granules per 100 cm² or 10 cm x 10 cm area.
- Application rate for small to medium habitats: 1 teaspoon full per 2 m²
- Swath width for habitats > 9 m² in size: 3 meters
- Application rate for swaths across habitats > 9 m² in size: 1 handful per 10 meters of swath length walked
- Application rate for long narrow habitats: 1 handful per 20 to 30 m of habitat length

**Evaluation of Larval Control Success**
In our study we hypothesize that in comparison to the non-intervention year and the non-intervention sites controlling the larval stages of mosquitoes in the 3 intervention wards will result in:
- Smaller proportion of habitats colonised by early instar mosquito stages.
• Late-instar larvae and especially pupae should be rare and extremely difficult to find.
• Much fewer (80% less than otherwise) adult *Anopheles* biting humans.
• Reduced malaria infection and illness in children.

**Success depends on:**
• Identification of all available aquatic habitats within the study area
• Treatment of all aquatic habitats in required dosages (e.g. treatment of drains for the full lengths) Proper performance of the larvicides
• Treatment at regular weekly intervals so that no late instar larvae are recorded in the sites
• No pupation and emergence takes place in any sites.

**IMPLEMENTATION PROCEDURES & DATA RECORDING**

**Community sensitization**
It is mandatory to inform and gain consent from the administration, community leaders and the community members before any larviciding can take place in the intervention areas. Community members are usually very concerned about any pesticide applied by research teams. There is usually the fear that pesticides applied on water could affect human beings or live stock.

**District administration officials (and others) need to be visited and informed about the planned activities, their appearance at community sensitization meetings might be helpful.** Community leaders need to be informed and with their help community meetings need to be held. Any questions and concerns of the community need to be answered to the best of your knowledge. Questions that can not be answered immediately need to be discussed with the scientists and information brought back to the community. **Families that farm in the intervention areas should be especially addressed to ensure that the information reaches them well, since those will be much concerned with the weekly larviciding and might fear for their crops or animals.** A community information leaflet and a frequently asked questions fact sheet will be distributed during the sensitization meetings.

Community sensitization will be done using various methods, these are:

1. Meetings with well known community members/leaders including Ten Cell leaders.
2. Public addressing using megaphone by passing with a car through all the mitaa just before the intervention
3. Public meeting with the community by using traditional ngomas
4. Distribution of leaflet and frequently asked questions at all meetings.
5. Availability of larvicides for Household Control of closed habitats (packaging of VectoLex CG)
   Leaflet and announcements to households from intervention areas to ward office/meeting point to pick up larvicides for mosquito control in pit latrines and other closed habitats

**Field Staff – Mosquito Control CORPs and Larval Mosquito Surveillance CORPs**
During the intervention year the weekly larval surveys will be implemented by the *Larval Mosquito Surveillance* CORPs following the same standard procedures as during the baseline data collection. Additionally, in the intervention wards a team of *Mosquito Control CORPs* has been recruited so that surveillance and control of all the habitats in the targeted wards are conducted separately. Larval surveillance and application of larvicides will be implemented independently (these two teams of CORPS do not cover the area together! Instead, the surveillance team follows, using the same lists of ten cell units two days later).

Mosquito Control CORPs for the 3 intervention wards for 2006 were recruited in January 2006 and have followed the Mosquito Larval Surveillance CORPs for a one month to familiarise themselves with the area of operations. Larviciding will start 1\(^{st}\) March 2006. A special timetable has been developed for larval survey CORPs and spraymen specifying days of the week and TCUs to be
visited at these days. Spraymen will visit the TCUs first and apply larvicides to all aquatic sites. The CORP will survey the same TCUs one day later for larvae.

**Larval Survey Data Recording – Mosquito larval surveillance CORPs**
Larval habitat and density data will be recorded weekly in intervention and non-intervention wards following the same procedures and data sheets as for the baseline data collection. All available aquatic habitats will be recorded and larval presence noted. In the intervention wards the larval survey CORP monitors the activity of the sprayman in his/her respective area of responsibility. If the CORP identifies sites with late instar larvae, he needs to highlight them in the data sheet and report this observation back to the supervisor the same day when he/she brings the data sheets back to the ward office. All larval survey CORPs need to return their data sheets to the ward office after finishing the day’s work and inform the supervisor verbally at the same day about any TCUs and sites where old larvae have been found and where larvicide application still needs to be done. The supervisor needs to discuss this with the sprayman responsible for the area.

**Larviciding Data Recording – Mosquito Control COPRs**
In his area of responsibility (mtaa or part of an mtaa) the Mosquito Control CORPs will have to treat ALL available sites that contain water at the moment of the visit. This must happen weekly and irrespective of the presence or absence of larvae. Therefore, the Mosquito Control CORPs will not carry a dipper and will not record every single habitat that has been treated. The Mosquito Control CORPs searches every TCU that he or she is supposed to visit on this date (following the timetable prepared by supervisors and CMSOs) for any site that contains water (open habitats) using also the experiences gained from following the larval survey CORP during the first 4 weeks of training. BUT it is important that the Mosquito Control CORP does not only visit the sites he has learned to have water during his training but finds and treats all potential sites.

Note: The Mosquito Control CORPs are trained during the dry season! He will experience several times more habitats during the rains. Supervisors and Mosquito Control CORPs need to be trained to this effect and CMSOs need to remind them regularly.

The Mosquito Control CORP has to record the following information:
Week and date of application, TCU visited for larviciding, the total number of TCUs visited, the amount of larvicide received per day (as weight and indicated in data sheet by supervisor), amount of larvicide left after day’s work (as weight and indicated in data sheet by supervisor) and the calculated amount of larvicide used per day (calculated and recorded in data sheet by supervisor).

A mosquito control CORP will have 1 data sheet for every day in the week (Mon, Tue, Wed, Thu, Fri), see example below:

**Ward level larviciding - Open habitats**

| MUNICIPALITY: ___Temeke___ WARD: ___Kurasini___ MTAA: ___Kurasini___ |
| Round (filled at City level): ________________________________ |

<table>
<thead>
<tr>
<th>Day</th>
<th>Date</th>
<th>TCU number</th>
<th>Wet habitats present?</th>
<th>Larvicide applied?</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td>MON</td>
<td>01.01.06</td>
<td>001</td>
<td>x</td>
<td>x</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>002</td>
<td>x</td>
<td>x</td>
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<td></td>
<td></td>
<td>005</td>
<td>x</td>
<td>x</td>
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<td></td>
<td></td>
<td>007</td>
<td>x</td>
<td>x</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>008</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>009</td>
<td>x</td>
<td>x</td>
<td>no application because access was denied by residents</td>
</tr>
</tbody>
</table>

Total number of TCUs where larvicide application took place today: __________
Amount of Larvicide received today (in kg): __________
Amount of Larvicide left (in kg): __________
Amount of Larvicide used today (in kg): __________

Mosquito Control CORP signature: ____________________ Supervisor’s Signature: _________________
Records on Larvicide use and areas treated – Ward Supervisor

The ward supervisors (and the assistant supervisor in Mikocheni) need to keep daily records of the material released and returned per day and need to prepare a weekly summary of used material per Mosquito Control CORP:

- The larvicide will be stored at the ward offices in the intervention wards.
- The ward supervisors will hand out larvicides to the Mosquito Control CORP every morning between 7.00 and 8.00am.
- The released material has to be recorded per Mosquito Control CORP. Both, supervisor and Mosquito Control CORPs have to sign.
- A separate material recording sheet will be used for each Mosquito Control CORP and therefore for each area/Mtaa.
- The supervisor weighs the material and indicates the amount released in his own larvicide release data sheet and in the ward level larviciding data sheet of the Mosquito Control CORP.
- The Mosquito Control CORP returns in the afternoon after finishing the day’s work to the ward office.
- The supervisor weighs the remaining amount of larvicides and indicates this in his own and in the Larval Control CORP’s data sheet and calculates the amount of larvicide used.
- The larviciding data sheet of this day remains then in the ward office.
- These datasheets need to be checked immediately when they are submitted and if there is no problem identified need to be filed in a separate file for larvicide application (1 file per Mtaa or subzone of Mtaa=1 Mosquito Control CORP).
- In case any problem can be identified from the data sheet the ward supervisor must discuss with the larval control COPRs and investigate further. The supervisor needs to discuss the problem with the inspector, plan and implement appropriate action promptly. In case problems arise that can not be addressed by the ward supervisor he/she should consult the inspector and, if necessary municipal coordinator immediately. If the problem still cannot be resolved promptly, help should be sought from the City Office immediately.

<table>
<thead>
<tr>
<th>Larvicide Release Records</th>
</tr>
</thead>
<tbody>
<tr>
<td>MUNICIPALITY:</td>
</tr>
<tr>
<td>Supervisor's name:</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Week:</th>
<th>Day:</th>
<th>Date:</th>
<th>Amount of Granule received (in kg):</th>
<th>Signature Sprayman</th>
<th>Amount of granule returned (in kg):</th>
<th>Amount of granule used</th>
<th>Total number of TCUs treated (as per Mosquito Control CORP data sheet):</th>
<th>Signature Supervisor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Tue</td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>1</td>
<td>Wed</td>
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<tr>
<td>1</td>
<td>Thu</td>
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<tr>
<td>1</td>
<td>Fri</td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>Weekly Total:</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Mon</td>
<td></td>
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<tr>
<td>2</td>
<td>Tue</td>
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</tr>
</tbody>
</table>

Record on the daily release of larvicides will be taken on 1 data sheet per Mosquito Control CORPs per months. In this data sheet the supervisor also indicates how many TCU’s have been treated according to the Mosquito Control CORP every day. At the end of the week the supervisor calculates the weekly total. This data sheet can then be sent back to the City with the weekly summary records from the larval surveys.

Culex Control in Closed Habitats

Closed habitats can not be managed by the spaymen of the program and they will focus on the open habitats only. Given that a large number of closed habitats (latrines, soakage pits, water tanks etc) produce a substantial number of nuisance Culex mosquitoes the community of the intervention wards might be disappointed because they might not feel a big reduction in nuisance biting. To increase community support we will offer larvicides for treatment of closed habitats for households free of charge. Small bags of granule will be made available at the mtaa office at
certain dates for households in the intervention wards. Members of those households can come to pick up the larvicide and a leaflet with directions for use and can treat the closed habitats themselves. We will use *Bacillus sphaericus* (Bs) granules (CG) for treatment of closed habitats. Bs is very effective in highly polluted water and has a long residual effect in closed habitats. Treatment of closed habitats has to take place every 2-3 months. One small bag of larvicide will contain 10 grams of granule which is sufficient to treat up to 10m$^2$ of water surface.

Organisation: Closed habitat treatment campaigns will be implemented every 3 months in all the intervention wards. The distribution of larvicides for householders will take place on Mtaa level at specific dates. Community sensitisation will take place a few days before the distribution to inform the community on which date and where they can come to collect larvicides for their closed habitats on household level. The Mtaa chairman will be involved in the release of larvicides to ensure provision only to eligible households members. The householders name, address, type of closed habitats and number of larvicide bags will be recorded per Mtaa.

**Storage and Distribution of Larvicides**

The larvicides will be shipped to the City Office and will be stored at a central store (Kisutu Office). The keys for the store will be handled by City Council staff ONLY. Once a week, the necessary amount of larvicides will be delivered to the ward offices under supervision of the CMSOs. Records will be kept at the central store and at ward level, (account book for in and out need to be available). Ward supervisors have to sign for the weekly amount of larvicides they receive. The weekly supply will be delivered on Fridays. All ward offices will keep their larvicide stock in a dry and secure place that will be locked and can only be accessed by the ward supervisor. All four sites have been provided with locked cabinets for secure storage of larvicides.

**Supervision and Support System for Intervention wards**

**Inspectors:**

To support the intervention wards in the first year of larviciding one of the municipal inspectors has been assigned to the priority intervention ward and the non-intervention ward assigned for comparison in each municipality. The inspector will help the ward supervisors with all his/her duties, assists in problem solving, communication with City Office and will implement independent spot check to ensure good quality mosquito control in the intervention wards and data quality in non-intervention wards. Twelve randomly selected spot checks need to be implemented per week: 6 in the high priority (intervention plus comparison ward) and 6 in the lower priority (remaining three) wards; the visit of TCUs in the intervention ward need to be implemented 24-48 hrs after scheduled larvicide application by the sprayman (therefore the inspector has to check timetable of spraymen and plan day of spot check).

### Municipal mosquito larval habitat spot check - Open habitats

<table>
<thead>
<tr>
<th>Plot ID</th>
<th>House No.</th>
<th>Habitat ID</th>
<th>Habitat type</th>
<th>WNs Habitat Code</th>
<th>Wet?</th>
<th>Plants</th>
<th>Larval stage</th>
<th>Water</th>
<th>Water Perimeter Depth</th>
<th>Water depth</th>
<th>Last application of larvicide as per sprayman?</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>

Additional targeted spot checks in areas of known larvae production or identified problem areas should be implemented by the inspector and ward supervisor throughout the week.
The second municipal inspector will be responsible for the remaining 3 lower priority wards in the municipality and will implement his/her routine duties. The routine TCU spot check data sheets remain the same as during the baseline data collection period except for 1 additional column where the latest larvicide application date (as per timetable of Mosquito Control CORPs) needs to be indicated in the intervention wards.

The results of the additional targeted spot checks in the intervention ward, identified problems and the action taken need to be included in the inspector’s reports.

City Malaria Surveillance Officers:
CMSOs also need to implement independent spot checks in the 3 intervention wards weekly. Special attention needs to be given to areas where larval habitats are abundant. Spot checks should preferably take place 24-48 hrs after scheduled application. CMSOs should record the TCUs and habitats (Plot & Habitat ID) visited and the presence or absence of larval stages & pupae. The CMSOs should also enquire whether the spaymen has been seen by the community and record whether any sign of biocide granule (CG) can be seen. A special intervention spot check data sheet (see below) will help to record the observations. This data sheet can be used by CMSOs, inspectors and municipal coordinators. When ever late instar larvae or pupae can be observed in checked habitats or complains from the community are received immediate action has to be taken (contact ward supervisor, inspector and spraymen, identify source of and help solving problem).

<table>
<thead>
<tr>
<th>Intervention Spot Checks</th>
</tr>
</thead>
<tbody>
<tr>
<td>MUNICIPALITY: INTERVENTION WARD:</td>
</tr>
<tr>
<td>checked by (name and position):</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date</th>
<th>Mtaa</th>
<th>TCU number</th>
<th>Plot ID</th>
<th>Habitat ID</th>
<th>Larval stage</th>
<th>Pupae seen?</th>
<th>Signs of CG?</th>
<th>Sprayman Signs of CG?</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Anoph.</td>
<td>Culex</td>
<td>Absent</td>
<td>Late</td>
<td>Absent</td>
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</table>

Research Permit
Bti and Bs products are not registered in Tanzania for individual or commercial use. Therefore, we applied for a research permit from the Tropical Pesticide Research Institute to use these products in the UMCP. Photocopies of the permit should be with all the ward supervisors.
Calibration For Application of Microbial Mosquito Larvicides

Peter DeChant
Valent BioSciences Corporation
Libertyville, IL
Objective

Provide practical training in calibration for application of microbial mosquito larvicides for control of malaria vectors.
Agenda

- The global malaria problem
- Current strategies in malaria control
- Mosquito life cycle and control strategies
- Microbial mosquito larvicides
- Microbial mosquito larvicide formulations
- Application Equipment
- Calibration methods
A Global Problem

Image courtesy of The World Health Organization

Image courtesy of Roll Back Malaria, World Health Organization

Dar UMCP
Dar es Salaam
January 2006
Malaria in Sub-Saharan Africa

Annual global burden of malaria (2002 estimates):

- 1.1 million deaths (mostly children)
- 300-500 million cases
- 44 million disability adjusted life years (DALYs)
- Reduction of GNP of more than half in Malaria endemic countries

Over 90% of the disease burden is in sub-Saharan Africa, and almost all deaths (due to Plasmodium falciparum) occur in Africa.

The Special Programme for Research and Training in Tropical Diseases (TDR)
Malaria Control Challenges

- Vaccine not yet developed
- Multiple drug resistance
- Insecticide resistance
- Poverty (cause & effect)
- Infrastructure
- Local capacity

Image courtesy of The World Health Organization

Copyright (c) 1998-2004 by A. Richard Palmer & Ron Koss.
Agenda

- The global malaria problem
- Current strategies in malaria control
- Mosquito life cycle and control strategies
- Microbial mosquito larvicides
- Microbial mosquito larvicide formulations
- Application Equipment
- Calibration methods
Malaria Control Strategies

- Treating the ill and preventing transmission
  - Drug chemotherapies
  - Insecticide treated nets (ITN’s)
  - Larviciding

PHOTO COURTESY OF C.F. CURTIS - U of M Website
Mosquito Life Cycle

- Adult Stage
- Pupae Stage
- Larval Stage
- Egg Stage
Methods of Mosquito Control

- Source Reduction
- Larviciding
- Adulticiding

American Mosquito Control Association’s Pesticide Environmental Stewardship Program Strategy Document

PHOTO COURTESY OF C.F. CURTIS - U of M Website
Source Reduction
(Environmental Management)

- Removal or reduction of mosquito larval habitats
  - Drainage
  - Sanitation or hygiene
  - Community Participation
Larviciding

- Application of substances to kill mosquito larvae or pupae in water
  - Liquid spray, granular application, direct application
  - No loosers
Adulticiding

- Application of chemicals to kill adult mosquitoes
- Residual spray & ITN.

PHOTO COURTESY OF C.F. CURTIS - U of M Website
Larviciding Philosophy (CIA)

- Mosquito Larvae are generally:
  - Concentrated
  - Immobile
  - Accessible

- Adult mosquitoes spread out over a much larger area.

- CIA = efficiency of larval control.
New Look at Environmental Management and Larval Control

Adult Anopheles Abundance pre-, during and post-intervention (12 sentinel houses, PSC method)

- **Pre-intervention**
  - 2001: 1139 mm
  - Average No. of Anopheles bites per person per sampling date: 0.7

- **Post-intervention**
  - 2004: 2300 mm
  - Average No. of Anopheles bites per person per sampling date: 1.05

- **92% decrease**

- **94% increase**

- **Intervention**
  - 2002: 1534 mm
  - Average No. of Anopheles bites per person per sampling date: 0.06

**Average No. of Anopheles bites per person per sampling date**
- **2001**: 0.7
- **2002**: 0.06
- **2003**: 1.05
- **2004**: 1.05

**Comment:**
- **Start in Feb**
- **Courtesy of U Fillinger**

**Rainfall (mm)**
- **Pre-intervention**
  - RAW TEXT (numbers and months)
- **Post-intervention**
  - RAW TEXT (numbers and months)
Agenda

✓ The global malaria problem
✓ Current strategies in malaria control
✓ Mosquito life cycle and control strategies
☐ Microbial mosquito larvicides
☐ Microbial mosquito larvicide formulations
☐ Application Equipment
☐ Calibration methods
Mosquito Larvicides

- **Chemicals**
  - OP’s (temephos)

- **Surface Agents**
  - oils, monomolecular films

- **Microbials**
  - *Bacillus thuringiensis israelensis (Bti)*
  - *Bacillus sphaericus (Bs)*
**B. thuringiensis subsp. israelensis** (Bti)

**VectoBac = Bti**

Bacteria that produces 5 toxins (ICP)
ICP = Insecticidal Crystal Protein
Protein is not toxic until digested by larvae
**Bacillus sphaericus (Bs)**

**VectoLex = Bs**

Bacteria that produces 2 toxins (ICP)

ICP = Insecticidal Crystal Protein

Protein is not toxic until digested by larvae

Figure courtesy of Jean-François Charles
Mode of Action

- Enlarged Section of Midgut
- Perforation of gut wall through toxin action
- Spore = crystal =
Insecticidal Crystal Protein

The larvae’s Last Meal

Figure courtesy of Stephen L. Doggett
OUR GOAL
“Give all the larvae a good FINAL meal.”

- ICP’s are not contact poisons
- Effective dose must be eaten by all larvae
- ICP’s are not water soluble
  - Will not move laterally (diffusion)
- Total area needs to be evenly treated
- Must penetrate vegetation
OUR GOAL
“Give all the larvae a good FINAL meal.”

- KEYS TO OUR GOAL:
  - FORMULATION
    - Delivers ICP to the feeding zone
  - APPLICATION
    - Proper dose and even coverage
Agenda

- The global malaria problem
- Current strategies in malaria control
- Mosquito life cycle and control strategies
- Microbial mosquito larvicides
- Microbial mosquito larvicide formulations
- Application Equipment
- Calibration methods
MICROBIAL LARVICIDE FORMULATIONS

- Granules (on corncob) - CG
- Water dispersible granules - WDG
- Tablets - DT
- Water soluble pouches – WSP
- Aqueous suspensions - AS
- Technical powders - TP
VectoBac® and VectoLex® Formulations
CG & WDG
VectoLex® and VectoBac® CG

Granular formulations for dry application
Why Choose CG Formulation?

- Stable formulations
- No mixing required
- Penetrates vegetation
- Can be hand applied to small areas easily by community members
Examples of Equipment for CG Application

Hand

Manual

Power
Agenda

✓ The global malaria problem
✓ Current strategies in malaria control
✓ Mosquito life cycle and control strategies
✓ Microbial mosquito larvicides
✓ Microbial mosquito larvicide formulations
✓ Application Equipment
☐ Calibration methods
What is Calibration?
Why Calibrate?
Why Calibrate?

REMEMBER OUR GOAL

“Give all the larvae a good FINAL meal.”

- Accurate dose and even coverage of the larval habitat.
- Saves material, time and money.

VectoBac CG dose is 10 kg/ha

We aim to achieve this dose.
VectoBac® CG Calibration Method

How do we apply the right amount?

Rate is 10 KG per hectare.

Think of this as granules per square meter.
Granules Are Applied By Hand

- Your hands and feet are the application tool.
- You must learn the weight of granules in your handful or measure with teaspoon.
- You must learn the distance of your step.
- Knowing these, you can develop the skill to make an even application at the correct dose.
Two Methods

Small Areas
(<3 meters x 3 meters)

Large Areas
(>3 meters x 3 meters)
Hand Application of Granules
For Small Areas (< 3 meters x 3 meters)

- Rate = 1 gram per square meter (1/2 teaspoon)
- Know the size of the area
- Spread small amounts at a time to make application even.
- Was there enough to finish? (Was it too much?)
- Check if application “looks OK”
$2 \times 3 = 6$ square meters

Needs 6 grams
# Hand Application Rates

<table>
<thead>
<tr>
<th>KG/HA</th>
<th>VectoBac CG</th>
<th># PER M2</th>
<th>10 cm x 10 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>410 granules/gram</td>
<td>205</td>
<td>2</td>
</tr>
<tr>
<td><strong>10</strong></td>
<td><strong>410</strong></td>
<td><strong>410</strong></td>
<td><strong>4</strong></td>
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<tr>
<td>15</td>
<td>615</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>20</td>
<td>820</td>
<td></td>
<td>8</td>
</tr>
</tbody>
</table>
Good Hand Application Rate
10 Kilogram/Hectare (AT LEAST FOUR)
HAND APPLICATION RATES of CG
To Large Areas (> 3 meters x 3 meters)

- Your Walking STEP (meters per step)
- SWATH (3 meters wide)
- Weight of your HANDFUL (of granules)

- How many steps do we take per handful?
Calibration Steps for CG Hand Application

- Measure your STEP
- Know your SWATH (3 meters)
- Know your HANDFUL WEIGHT
- Determine how many steps for each HANDFUL
Hand Calibration for CG

\[ \text{RATE} = \frac{\text{HANDFUL}}{\text{STEPS PER HANDFUL} \times \text{SWATH} \times \text{STEP}} \]

\[ \text{STEPS PER HANDFUL} = \frac{\text{HANDFUL}}{\text{STEP} \times \text{SWATH}} \]

\[ \text{RATE} = \text{GRAMS PER SQUARE METER} = 1 \text{ GRAM PER METER SQUARE} \]
\[ \text{STEP} = \text{METERS PER STEP} \]
\[ \text{SWATH} = 3 \text{ METERS} \]
\[ \text{HANDFUL} = \text{GRAMS PER HANDFUL} \]
Example for VectoLex CG

RATE = GRAMS PER SQUARE METER = 1 GRAM PER METER SQUARE
STEP = METERS PER STEP = 1 METER
SWATH = 3 METERS
HANDFUL = GRAMS PER HANDFUL = 15

STEPS PER HANDFUL = 5
TOTAL HANDFULS = 12 HANDFULS

20 METERS
STEP = 1 METER PER STEP
3 meter SWATH
3 meter SWATH
Verification of Application

- Does actual use match expected use.
  - Size of each area treated
  - Rates intended
  - Overall inventory vs use accounting
  - End of day match?
- Do the applications “look OK”
Spraying Strategies

• Responsibility for product & equipment

• Safety

• Team Work

• Material Transport – Backpack

• Start on edge
  (Better to spray some land than miss water)

• Reporting material use
Take Pride in your work. Every mosquito you kill could mean one less person getting malaria!
It will take teamwork roll back malaria.
Let’s all push!
Calibration For Application of VectoBac WDG

Peter DeChant
Valent BioSciences Corporation
Libertyville, IL
Objective

Provide practical training in calibration for application of VectoBac WDG for control of malaria vectors.
Agenda

- Microbial mosquito larvicide formulations
- VectoBac WDG
- Application Equipment
- Calibration methods
- Verification
MICROBIAL LARVICIDE FORMULATIONS

- Granules (on corncob) - CG
- Water dispersible granules - WDG
- Tablets - DT
- Water soluble pouches – WSP
- Aqueous suspensions - AS
- Technical powders - TP
VectoBac® and VectoLex® Formulations
CG & WDG
VectoBac® WDG

The stability of a granule with the application flexibility of a liquid.
When Choose WDG Formulation?

- **Large areas with open water**
  - Breeding sites larger than 2000 sq meters (45m x 45m)
  - *Larger than 15 swaths x 15 swaths (granules)?*
  - Breeding sites requiring more than one pack (2 kg) of granules to treat
  - Inform Ward Supervisor and Inspector

- **Why?**
  - Less product to carry into the field
  - More area covered before returning
  - More economical
  - Backpack spray = wide swath
VectoBac WDG
Ideal particle size and suspension characteristics in water

5 microns
Insecticidal Crystal Protein - The larvae’s Last Meal

Small particles suspend in feeding zone

Figure courtesy of Stephen L. Doggett
Agenda

✓ Microbial mosquito larvicide formulations
✓ VectoBac WDG
☐ Application Equipment
☐ Calibration methods
☐ Verification
Examples of Spray Equipment for WDG Application
Why Calibrate?

REMEMBER OUR GOAL

“Give all the larvae a good FINAL meal.”

- Accurate dose and even coverage of the larval habitat.
- Saves material, time and money.

**VectoBac WDG dose is 400 gm/ha**

We aim to achieve this dose.
Factors That Determine Application Rate

- **SPEED** of travel (meters per minute)
- width of **SWATH** (meters wide)
- **FLOW** rate of sprayer (liters per minute)
- **DILUTION** rate of product (grams per liter)
Calibration Steps for WDG

- Measure working SPEED
- Measure sprayer SWATH
- Measure sprayer FLOW
- Calculate DILUTION

SPEED = METERS/MINUTE

SWATH

SWATH
Calibration requirements

- 1 container of WDG
- 1 sprayer with D2 nozzle
- 1 Tape measure
- 1 Stop watch
- 1 Calculator
- Data forms
- Boots
Measure the distance
Measure the walking speed to determine the required dilution
Measure the flow rate
Backpack Spray Calibration for WDG

**APPLICATION RATE** = **SPRAY RATE** x **DILUTION**

(GRAMS PRODUCT PER HECTARE) = (LITERS SPRAYED PER HECTARE) x (GRAMS PRODUCT PER LITER)

**DILUTION** = **APPLICATION RATE** / **SPRAY RATE**

**SPRAY RATE** = (FLOW x 10,000)/ SPEED x SWATH

**APPLICATION RATE** = GRAMS PRODUCT APPLIED PER HECTARE
SPEED = METERS PER MINUTE
SWATH = METERS
FLOW = LITERS PER MINUTE
SPRAY RATE = LITERS OF SPRAY MIX APPLIED PER HECTARE
DILUTION = GRAMS PRODUCT PER LITER OF SPRAY MIX
Measuring Swath Width

- Find a flat, clean surface such as a parking lot.
- Measure “Full Swath”
  - “Full swath” will be equal to two times the projection distance using a 180 degree sweep to distribute the material.
- Apply product with appropriate sweep while stationary and measure width covered
- Subtract 50% for overlap
Results of Swath Tests - Meters

10 METER SWATH

(10 STEPS)
Measuring Flow Rates For WDG Sprays

- Flow rate of liquids measured with a graduated cylinder or other liquid measuring device.
- The spray pressure is maintained at a standard level, and spray is discharged into the cylinder for one minute.
- The flow rate per minute is determined by the volume of liquid in the cylinder.
Results of Flow Tests – Liters per Minute

AVERAGE = 0.74 LITERS PER MINUTE

[Bar chart showing the average flow rates for different samples, with the mean value of 0.74 L/min highlighted.]
Measuring Your Working Speed

- Measure and mark 50 meters in typical habitat.
- Time how long it takes to walk 50 meters at a comfortable working pace while carrying equipment and pretending to spray.
- Repeat the measurement three times.
- Make an average of your times.
- 50 divided by average time = meters per minute.
Results of Speed Tests – Meters per Minute

**AVERAGE =**

<table>
<thead>
<tr>
<th>Name</th>
<th>REP1</th>
<th>REP2</th>
<th>REP3</th>
<th>Mean</th>
<th>AVERAGE</th>
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<td>45</td>
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<tr>
<td>R. Kipanga</td>
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<tr>
<td>Ali S</td>
<td>56</td>
<td>54</td>
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<tr>
<td>Mean</td>
<td>54</td>
<td>54</td>
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<td>54</td>
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</tbody>
</table>
Calibration for VectoBac WDG

Solo Backpack with #2 disk (no core); On pressure setting #3 (approx 3 bar)

APPLICATION RATE = 400 GRAMS PER HECTARE
SPEED = 54 METERS PER MINUTE
SWATH = 10 METERS
FLOW = 0.74 LITERS PER MINUTE
SPRAY RATE = \( \frac{0.74 \times 10000}{54 \times 10} = 14 \) LITERS PER HECTARE
DILUTION = 400 GRAMS / 14 LITERS or 200 GRAMS / 7 LITERS

One Tank (14 l) = One Hectare
Mixing instructions

1) Add half of the water
2) Add pre-measured WDG slowly while shaking/stirring as you add
3) Add the rest of the water
4) Shake vigorously for 2 minutes
Standardizing Calibrations

- Calibrate each sprayer
- Repeat calibration during season.
- When sweeping the spray wand, make a full swing.
- Make a fast enough sweep for even coverage.
- Standardize against expected use rates.
- Practice, Practice, Practice…
Mix carefully and thoroughly:
Add and mix small amounts at a time
Apply evenly and consistently
Treat the entire surface area with 10 meter-wide swaths
Verification of Application

- Does actual use match expected use.
  - Size of each area treated
  - Rates intended
  - Overall inventory vs use accounting
  - End of day match?
### Weekly habitat summary data sheet

**Signature Supervisor**: 
**Date**: 
**Municipality**: 
**Ward**: 
**Signature Inspector**: 
**Date**: 
**Mtaa**: 
**Signature Co-ordinator**: 
**Date**: 

<table>
<thead>
<tr>
<th>Year</th>
<th>Month</th>
<th>Week</th>
<th>10-cell unit</th>
<th>No. of habitats</th>
<th>No. of habitats with water</th>
<th>No. of habitats with Anopheles early</th>
<th>No. of habitats with Anopheles late</th>
<th>No. of habitats with Culex early</th>
<th>No. of habitats with Culex late</th>
<th>No. of habitats with pupae</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td></td>
<td></td>
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<th>Year</th>
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<th>No. of habitats</th>
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<th>No. of habitats with Culex early</th>
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<th>No. of habitats with pupae</th>
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**Signature:**
Annex 2

APPENDIX of Article 4

Urban malaria epidemiology and the impact of microbial larvicides upon infection prevalence in Dar es Salaam, United Republic of Tanzania

Yvonne Geissbühler, Research Scientist¹,²,³ Khadija Kannady, City Malaria Control Officer², Prosper Chaki, Research Scientist²,³,⁴ Basiliana Emidi, Research Assistant²,⁵ Nicodemus J. Govella, Research Scientist²,³,⁴ Valeliana Mayagaya, MSc candidate³,⁵ Michael Kiama²*, City Malaria Control Officer, Deo Masiwa, Chief Medical Officer⁶, Hassan Mshinda, Director³, Steven W. Lindsay, Professor⁴, Marcel Tanner, Professor & Director¹, Ulrike Fillinger, Public Health Entomologist⁴, Marcia Caldas de Castro, Assistant Professor⁷, Gerry F. Killeen, Research Fellow³,⁴

¹Swiss Tropical Institute, Department of Public Health and Epidemiology, Basel, Switzerland, ²Dar es Salaam City Council, Ministry of Regional Administration and Local Government, Dar es Salaam, United Republic of Tanzania, ³Ifakara Health Research and Development Centre, Coordination Office, Dar es Salaam, United Republic of Tanzania, ⁴Durham University, School of Biological and Biomedical Sciences, Durham, United Kingdom, ⁵Department of Zoology and Marine Biology, University of Dar es Salaam, Dar es Salaam, Tanzania, ⁶Ministry of Health and Social Welfare, Dar es Salaam, United Republic of Tanzania, ⁷Harvard School of Public Health, Department of Population and International Health, Boston, Massachusetts, USA

Correspondence to: Y Geissbühler, Y.Geissbuehler@unibas.ch

* Sadly, Michael Kiama passed away before completing the present work.
| Table A1 Asset ownership for households in each socioeconomic status quintile |
|---------------------------------|---------------------|-----------------|-----------------|-----------------|-----------------|
|                                 | SES quintiles       | Poorest         | Very poor       | Poor            | Less poor       | Least poor      |
| Clothing cupboard (%)           | 15                  | 20              | 93              | 97              | 99              |
| Sofa set (%)                    | 31                  | 96              | 99              | 100             | 100             |
| Watch/ clock (%)                | 33                  | 91              | 97              | 98              | 100             |
| Iron (%)                        | 27                  | 84              | 98              | 99              | 100             |
| Radio (%)                       | 69                  | 99              | 100             | 100             | 100             |
| Bicycle (%)                     | 3                   | 5               | 7               | 10              | 18              |
| Motorcycle (%)                  | 0                   | 0               | 0               | 1               | 8               |
| Car / tractor (%)               | 0                   | 0               | 0               | 1               | 16              |
| TV (%)                          | 2                   | 10              | 25              | 72              | 87              |
| Satellite dish (%)              | 0                   | 0               | 0               | 0               | 8               |
| Fan (%)                         | 4                   | 11              | 24              | 75              | 93              |
| Sewing machine (%)              | 1                   | 2               | 4               | 11              | 31              |
| Video (%)                       | 0                   | 3               | 10              | 40              | 84              |
| CD player (%)                   | 0                   | 2               | 6               | 61              | 96              |
| Camera (%)                      | 0                   | 0               | 0               | 1               | 36              |
| Telephone (%)                   | 0                   | 0               | 0               | 1               | 26              |
| Refrigerator (%)                | 5                   | 9               | 28              | 66              | 74              |
### Table A2: Comparison of mosquito densities, combined crude indirect EIR of *An. gambiae*, *An. funestus* and *An. coustani* in the intervention and non-intervention area in the two years of the entomological survey. Mosquito survey started in year 2 (April 2005 – March 2006) and the intervention with larvicide (*Bti*) started in year 3 (April 2006 – March 2007).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Year 2</th>
<th>Year 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-intervention area</td>
<td>Intervention area</td>
</tr>
<tr>
<td></td>
<td>Mean [95% CI]</td>
<td>Mean [95% CI]</td>
</tr>
<tr>
<td><em>An. gambiae</em> (bites per night)</td>
<td>0.664 [0.538 – 0.819]</td>
<td>0.719 [0.507 – 1.019]</td>
</tr>
<tr>
<td><em>An. funestus</em> (bites per night)</td>
<td>0.036 [0.021 – 0.061]</td>
<td>0.021 [0.007 – 0.065]</td>
</tr>
<tr>
<td><em>An. coustani</em> (bites per night)</td>
<td>0.143 [0.077 – 0.267]</td>
<td>0.003 [0.001 – 0.010]</td>
</tr>
<tr>
<td>Total EIR (infectious bites per year)</td>
<td>1.435 [1.137 – 1.813]</td>
<td>1.178 [0.804 – 1.725]</td>
</tr>
<tr>
<td><em>Culex</em> (bites per night)</td>
<td>130 [115 – 146]</td>
<td>87 [73 – 104]</td>
</tr>
</tbody>
</table>

Generalized estimating equations (GEE) was used with TCU as a subject unit, log linked mosquito densities which were weighted by number of catcher nights as a dependent and intervention and non-intervention as a factor.
Table A3  Summary of the logistic regression model, for children under 5 years, estimating the impact of protection measures and vector control upon malaria prevalence in areas surveyed in year 1 of the household survey (May 2004 – March 2005), year 2, before intervention (larviciding) (April 2005 – March 2006) and year 3, the year of the intervention (April 2006 – March 2007).

<table>
<thead>
<tr>
<th>Overall prevalence</th>
<th>Year 1 23.1 % (534/2310)</th>
<th>Year 2 16.2 % (392/2418)</th>
<th>Year 3 10.2 % (242/2371)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Explanatory variables</td>
<td>n  % OR [95% CI]</td>
<td>P-value</td>
<td>n  % OR [95% CI]</td>
</tr>
<tr>
<td>Constant</td>
<td>0.347 [0.296, 0.408]</td>
<td>&lt; 0.001</td>
<td>0.202 [0.162, 0.253]</td>
</tr>
<tr>
<td>Round</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>1948 84.3 1.000</td>
<td></td>
<td>1492 61.7 1.000</td>
</tr>
<tr>
<td>Follow up</td>
<td>362 15.7 1.356 [1.051, 1.749]</td>
<td>0.019</td>
<td>926 38.3 0.957[0.764, 1.198]</td>
</tr>
<tr>
<td>Net usage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No net\textsuperscript{a,f}</td>
<td>1704 73.8 1.000</td>
<td></td>
<td>1771 73.2 1.000</td>
</tr>
<tr>
<td>ITN</td>
<td>606 26.2 0.805 [0.642, 1.009]</td>
<td>0.060</td>
<td>647 26.8 0.814[0.629, 1.053]</td>
</tr>
<tr>
<td>Window screening</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No\textsuperscript{a,f}</td>
<td>1108 48.0 1.000</td>
<td></td>
<td>942 39.0 1.000</td>
</tr>
<tr>
<td>Complete\textsuperscript{e}</td>
<td>1202 52.0 0.900 [0.727, 1.115]</td>
<td>0.335</td>
<td>1476 61.0 1.122[0.893, 1.409]</td>
</tr>
<tr>
<td>Ceiling board</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No\textsuperscript{c}</td>
<td>1518 65.7 1.000</td>
<td></td>
<td>1353 56.0 1.000</td>
</tr>
<tr>
<td>Complete\textsuperscript{d}</td>
<td>792 34.3 0.879 [0.699, 1.104]</td>
<td>0.268</td>
<td>1065 44.0 0.776 [0.620, 0.970]</td>
</tr>
<tr>
<td>Larviciding area</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No\textsuperscript{f}</td>
<td>1896 82.1 1.000</td>
<td></td>
<td>1969 81.4 1.000</td>
</tr>
<tr>
<td>Yes</td>
<td>414 17.9 0.771 [0.590, 1.009]</td>
<td>0.058</td>
<td>449 18.6 1.350 [1.036, 1.759]</td>
</tr>
</tbody>
</table>

Generalized estimating equations (GEE) was used with individual as a subject unit, date as a within subject unit, log linked prevalence data and Fresh or Follow up, net usage, window screening, ceiling board and larvicide area as factors.

\textsuperscript{a} no or untreated net
\textsuperscript{b} Complete screening, screening with small holes, glass windows
\textsuperscript{c} No screening or badly damaged screening
\textsuperscript{d} Complete and partly complete ceiling board
\textsuperscript{e} No ceiling board
\textsuperscript{f} Reference category
Table A4. Comparison of protective measures and drug use in intervention and non-intervention areas of the Urban Malaria Control Program. Usage of different protection measures and drugs in intervention and non-intervention area was compared by Chi Square for each year. The application of larvicide (Bti) started beginning of year 3.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-intervention area</td>
<td>Intervention area</td>
<td>Non-intervention area</td>
</tr>
<tr>
<td></td>
<td>n/N</td>
<td>%</td>
<td>n/N</td>
</tr>
<tr>
<td></td>
<td>χ²</td>
<td>P</td>
<td>χ²</td>
</tr>
<tr>
<td>Personal protection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ITN</td>
<td>2481/10507 23.6</td>
<td>479/2058 23.3</td>
<td>1.0</td>
</tr>
<tr>
<td>Window screening</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete a</td>
<td>758/1252 60.5</td>
<td>163/270 60.4</td>
<td>1.0</td>
</tr>
<tr>
<td>Complete b</td>
<td>526/1252 42.0</td>
<td>101/271 37.3</td>
<td>0.8</td>
</tr>
<tr>
<td>Drug use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloroquine</td>
<td>30/721 4.2</td>
<td>1/119 0.8</td>
<td>0.2</td>
</tr>
<tr>
<td>SP</td>
<td>461/721 63.9</td>
<td>93/119 78.2</td>
<td>2.0</td>
</tr>
<tr>
<td>Amodiaquine</td>
<td>86/721 11.9</td>
<td>8/119 6.7</td>
<td>0.5</td>
</tr>
<tr>
<td>Quinine</td>
<td>151/721 20.9</td>
<td>21/119 17.6</td>
<td>0.8</td>
</tr>
<tr>
<td>Artemisin</td>
<td>11/721 1.5</td>
<td>0/119 0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Traditional</td>
<td>1/721 0.1</td>
<td>0/119 0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

*a Complete screening, screening with small holes, glass windows compared to no screening or badly damaged screening

*b Complete and partly complete ceiling board compared to no ceiling board
Curriculum vitae

Yvonne Geissbühler

Nationality: Swiss

Date of Birth: 25.04.1977

Education:

1984 – 1990 Primary and Secondary School, Langenthal, Switzerland
1990 – 1997 Pregymnasium and Gynasium, Freies Gymnasium Bern, Bern, Switzerland
1997 Matura, Freies Gymnasium, Bern, Switzerland
1998 – 1999 Botanics, Physics, Chemistry, University of Basel, Switzerland
1999 – 2000 Zoology, Biochemistry, Statistics, University of Basel, Switzerland
2001 Semester of Microbiology, Epidemiology and Parasitology, Universidade federal de Pelotas, Brazil
2001 – 2002 Plant ecology, Neurobiology, Microbiology, Vertebrates, University of Basel, Switzerland
2003 Master of Science, Swiss Tropical Institute, University of Basel, Switzerland
   “Mosquito control by polystyrene beads in Dar es Salaam, Tanzania”
2004 - 2007 PhD, Swiss Tropical Institute, University of Basel, Switzerland
   “Ecology and epidemiology of integrated malaria vector management in Dar es Salaam, Tanzania”
   Supervision by Prof. Dr. Marcel Tanner and Dr. Gerry F. Killeen

Language courses:

1994 French course, Les Paccots, Switzerland
1995 English course, Riverside, California, United States
1996 English course, Edinburgh, Scotland
1998 Spanish course, Malaga, Spain
2001 Portuguese course, Pelotas, Brazil
2004 Kiswahili course, Stone town, Zanzibar, Tanzania

Certifications:

2007 Award of excellence for the 3th best poster presentation at the scientific conference of NIMR in Arusha