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

INAUGURALDISSERTATION

zur

Erlangung der Würde eines Doktors der Philosophie

vorgelegt der

Philosophisch-Naturwissenschaftlichen Fakultät

der Universität Basel

von

Yvonne Geissbühler

aus Lauperswil, BE

Basel, 2008

Table of contents

Acknowledgments

I had the great opportunity to carry out this PhD thesis in the frame of the Urban Malaria Control Program (UMCP) in Dar es Salaam, Tanzania which is a collaboration between the Dar es Salaam City Council, Swiss Tropical Institute (STI) and University of Durham. Many people were involved and contributed to this work and their help is gratefully acknowledged.

My sincerest thanks are addressed to my supervisor at STI, Prof. Marcel Tanner (Director STI) who helped designing this work and always supported and motivated me. Furthermore he was always very understanding during personally difficult situations. My special thanks go to my local supervisor, Dr. Gerry Killeen (Ifakara Health Research and Development Centre, Public Health Entomology Unit Leader) who was strongly involved in the rationale and design of the study and accompanied me during the last three years through the scientific writing and thinking. Without his enthusiasm, motivation and support during field work as well as his teaching skills of mathematical models and statistics, this work would have not been possible. I also wish to express my gratitude to some collaborators of the UMCP, Prof. Steve Lindsay, Dr. Ulrike Fillinger and Dr. Marcia Caldas de Castro who were also involved in the design of the study and who were always open for fruitful discussions.

In Dar es Salaam, I wish to express my gratitude to the late Mr. Michael Kiama, the former coordinator of the UMCP. Furthermore I would like to thank Dr. Deo Mtasiwa (former City Medical Officer of Health) for his support. Many thanks are addressed to Khadija Kannady (City Mosquito Control Coordinator) who was always supportive and always had an open ear for all my questions. I'm grateful for her support during difficult times and for her friendship. I would also like to thank George Williams and Basiliana Emidi with whom I started setting up the Adult Mosquito Monitoring System. Many nights with Basiliana Emidi in the field,

conducting human landing catch (HLC), also gave me a deeper insight into Tanzanian culture. Many thanks are addressed to my two counterparts, Prosper Chaki and Nicodemus Govella, for the excellent collaboration in the field, the interesting time and especially for their friendship.

I thank John Mpangile for taking over the Adult Mosquito Monitoring system. Furthermore I thank Athumani Mtandanguo and Valeliana Mayagaya for their laboratory work. I especially would like to thank the team who conducted HLC for their commitment and hard work. My thanks also go to the entire team of the UMCP who participated in the larval surveys and household surveys as well as the data entry team. Furthermore I would like to thank the residents of Dar es Salaam for their cooperation and facilitation during our regular visits.

Many thanks also to Dr. Hassan Mshinda (Director of Ifakara Health Research and Development Centre) and all my colleagues at the Ifakara Centre.

At the STI I would like to sincerely thank Christine Walliser, Eliane Ghilardi and Margrit Slaoui for their institutional support throughout the study. Thanks are also addressed to Prof. Mitchell Weiss (Head Department of Public Health and Epidemiology) and to PD Tom Smith for some statistical discussions. Special thanks also go to the library staff. Many thanks to my friends from STI who also worked in Tanzania and with whom I had a great time: Manuel Hetzel, Stefan Dongus, Michael Vanek, Andrea Kümmerle and Constanze Pfeiffer. I would also like to thank all my other colleagues at STI, especially some of my fellow students: Stefanie Granado, Barbara Matthys, Claudia Sauerborn, Bianca Plüss, Joshua Yukich, Laura Gosoniu, Ricarda Merkle, Andri Christen, Karin Gross, Peter Steinmann, Stefanie Knopp and all other fellow students as well as all the remaining STI staff.

Acknowledgments

I am indebted to my dear friends and flat mates in Tanzania: Natalie Groos, Michelle Gill and Sarah Furrer. Thanks go also to Matthew Cogan without his internet modem it would have been impossible to finish my PhD. Many thanks go to Lekundo Tingitana who always supported me. I would also like to thank all my other friends in Tanzania who made my stay there even more wonderful. And last but not least I would like to thank all my friends at home for their support, especially the ones who came to visit me.

I acknowledge the financial support from the Swiss Tropical Institute, the Bill and Melinda Gates Foundation, Valent Biosciences and USAID through its Environmental Health Programme, the Tanzanian Mission at Dar es Salaam and the President's Malaria Initiative. Gerry Killeen is supported by the Wellcome Trust through Research Career Development Fellowship number 076806. I also like to thank the dissertation fond of the University of Basel who partially financed the printing of this thesis.

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 km^2 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

Malaria stellt nach wie vor einen grossen Teil der weltweiten Krankheitsbelastung dar. Ungefähr 70% der klinischen Malariafälle treten im sub-saharischen Afrika auf. Das sub-saharische Afrika trägt das grösste Risiko, weil dort ideale klimatische Bedingungen für die Übertragung und gleichzeitig die effizientesten Malariaüberträger, *Anopheles gambiae* s.s., *Anopheles arabiensis* und *Anopheles funestus,* vorkommen. Obwohl geschätzt wird, dass im Jahr 2030 mehr als 50% der afrikanischen Bevölkerung in Klein- oder Grossstädten leben wird, ist über städtische Malariaepidemiologie, Larvenökologie und das Verhalten von adulten Mücken relativ wenig bekannt. Integrierte Malariakontrollprogramme, welche Umweltmanagement und Larvizidanwendung beinhalteten und erfolgreich waren bevor 1955 die globale Ausrottungskampagne begann, wurden nach der Erfindung von DDT vernachlässigt. Erst seit wenigen Jahren ist das Interesse an solchen Kontrollmethoden wieder geweckt, aber es bleibt zu untersuchen, ob diese im städtischen Afrika praktikabel und kosteneffektiv sind.

Ziel dieser Arbeit war es, das momentane Verständnis von städtischer Malariaepidemiologie- und ökologie zu verbessern und die Effektivität des Larvizids *Bacillus thuringiensis* (*Bti*) im Rahmen des städtischen Malariakontrollprogramms (UMCP) in Dar es Salaam, Tanzania, detailliert zu analysieren. Unsere Ergebnisse basieren auf Daten, die in den ersten drei Jahren des UMCP erhoben wurden. Die Datenerhebung erstreckte sich über den Zeitraum von März 2004 bis März 2007. Das sich über 55km^2 erstreckende Projektgebiet beinhaltet fünf Stadtteile in jedem der drei Stadtbezirke von Dar es Salaam und besteht aus 67 Untereinheiten, den sogenannten *mitaa*. In diesem Gebiet lebten zum Zeitpunkt der Volkszählung im Jahr 2002 611'871 Menschen. Das Erreichen der Ziele des UMCP hängt von drei Komponenten ab: 1) Dem Kartieren und Beobachten von möglichen *Anopheles* Brutstätten, 2) dem Monitoring von

adulten Mückendichten, und 3) einer Haushaltsstudie mit Fragebögen und Blutausstrichen zum Testen der Malariaparasiteninfektion. Im März 2006 begann in 3 von 15 Stadtteilen des UMCP die routinemässige Applikation des mikrobiellen Larvizids *Bti* in offenen bzw. *Bacillus sphaericus* (*Bs*) in geschlossenen Habitaten. Dies stellt einen Zusatz zu den schon existierenden Interventionen wie Mückennetzen, Raumdecken, Mückengittern an Fenstern, Insektenschutzmitteln, Insektizidsprays und Mückenspiralen dar. Zur gleichen Zeit wurde eine detaillierte Studie über Mückenstechverhalten, menschliches Verhalten und häusliche Schutzmassnahmen durchgeführt. Dies geschah in den 12 „Ten Cell Units“ (TCUs) mit den höchsten *An. gambiae* s.l. Dichten, welche während der Anfangsperiode des UMCP Überwachungssystems gemessen wurden. TCUs sind die kleinsten Untereinheiten der lokalen tanzanischen Verwaltung,. „Human landing catch“ (HLC) wurde in 216 Häusern durchgeführt, wobei in jeder Stunde von 18 Uhr bis 7 Uhr Mücken inner- und ausserhalb des Hauses gefangen wurden. Die Bewohner wurden zu ihrem Schlafverhalten befragt, wo sie sich am Abend aufhalten und welche Art von Schutzmassnahmen gegen Malaria sie verwenden. Der persönliche Schutz, den jemand durch den Gebrauch eines insektizidbehandelten Mückennetzes (ITN) erhält, wurde mit Hilfe eines erweiterten, kürzlich entwickelten mathematischen Modells evaluiert.

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 Malariaprävelenz hat seit 2004 stetig abgenommen, während der Gebrauch von Mückengittern an Fenstern, Raumdecken und effektivere Medikamente wie Amodiaquine und auf Artemisinin basierende Medikamente zugenommen 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 ganzjährliche 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

UMCP entwickelt wurden und eine effektive gemeindebasierende Ausführung in einer dezentralisierten Art und Weise ermöglichten. Der Kontrollstandard hat sich seit Anfang des Programms sehr verbessert. Die Reaktionszeiten für Vektorkontrolle betragen einen Tag, eine Woche und einen Monat auf Stadtteil-, Stadtbezirk- und Stadtlevel.

Die Resultate vom wechselnden Stechverhalten des hauptsächlichen Malariavektors im städtischen Gebiet, welches zu einem geringeren, aber immer noch nützlichen persönlichen Schutz durch ein ITN führt, zeigen den Bedarf nach zusätzlichen, ergänzenden Vektorkontrollmethoden wie Umweltmanagement und den Gebrauch von Larviziden. Das UMCP mit seinem neuen, praktischen Management- und Kontrollsystem hat gezeigt, dass durch routinemässige Applikation von mikrobiellen Larviziden grosse Reduktionen der Malariaprävalenz erreicht werden können. Da dies die Anfangsresultate des Programms sind, werden in der Zukunft beträchtliche Verbesserungen durch mehr Investitionen und Zeit erwartet. Das UMCP zeigt zum ersten Mal seit der globalen Ausrottungskampagne eine Erfolgsgeschichte eines Malariakontrollprogramms, welches die Anwendung von Larvizid beinhaltet. Da dies eine kosten-effektive Option darstellt, könnte es ohne Umstände auch in anderen afrikanischen Städten adaptiert werden.

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 *Anopeheles 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 21th century (Hay et al. 2004) (Figure 1).

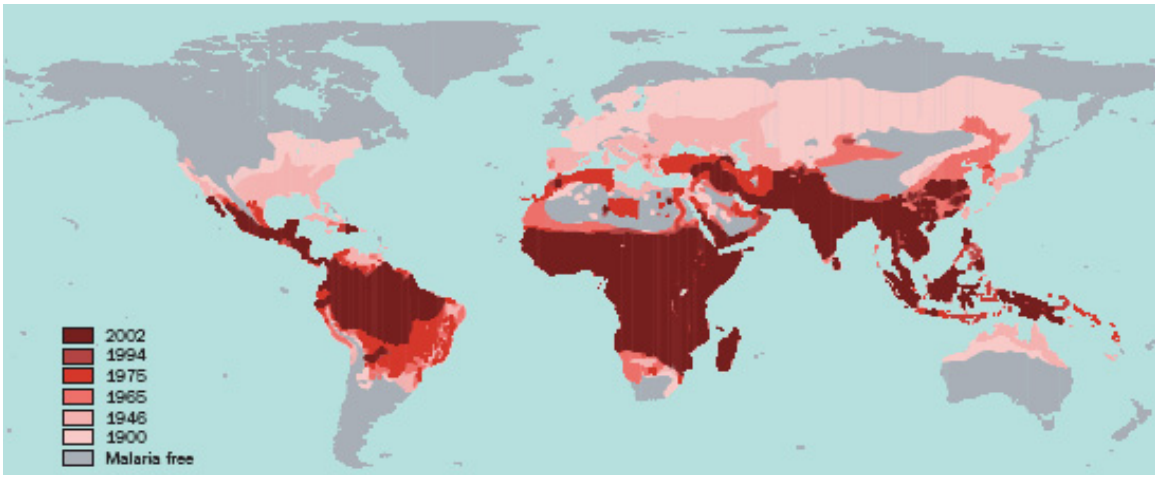


Figure 1 The global distribution of malaria since preintervention (1900-2002) (Hay et al. 2004).

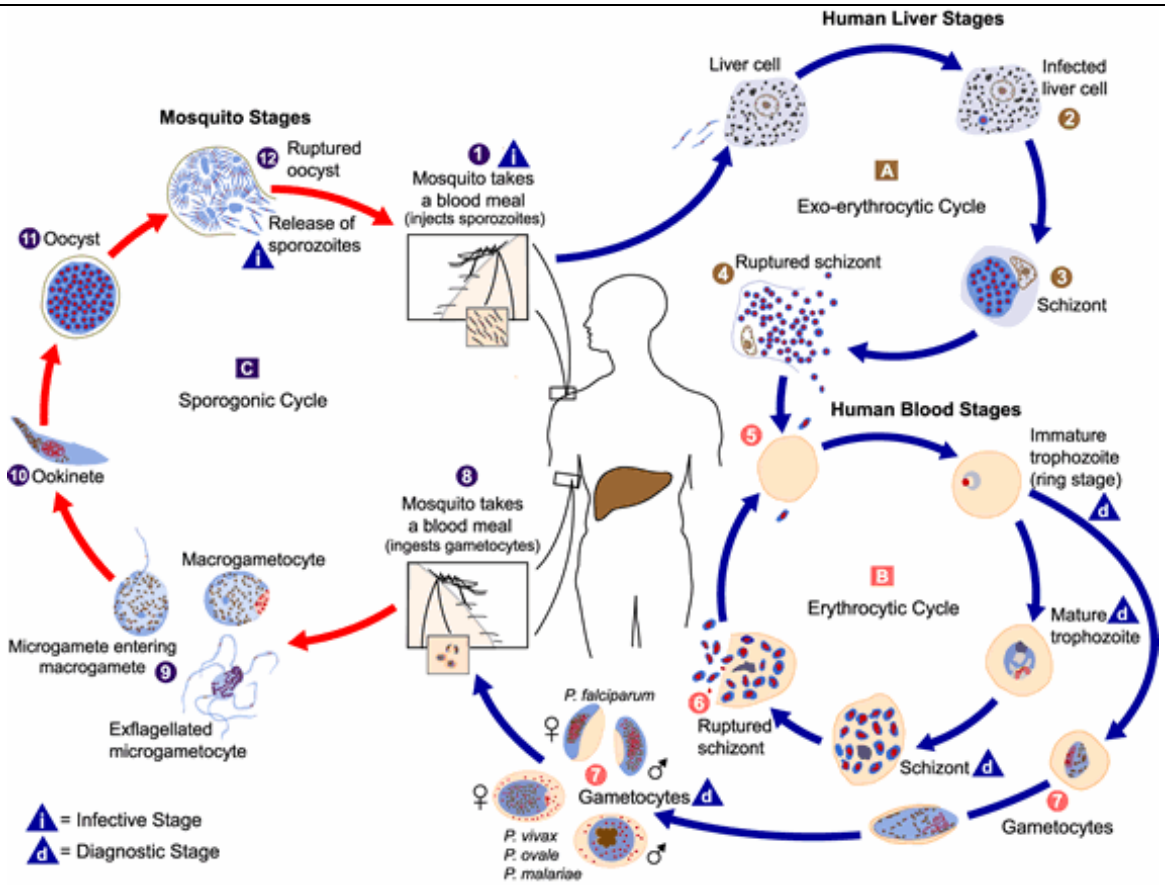
In Tanzania between 14 to 18 million malaria cases and 100,000 to125,000 deaths occur per year. Malaria accounts for 40 % of outpatient attendances (MOH 2002) and is caused mainly by *Plasmodium falciparum* (Clyde 1967; DHS, Tanzania 2005) which is also the most common malaria parasite worldwide (Roll 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).

Box 1. Life cycle of malaria parasite (*Plasmodium)*.

(Source: 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 ❶. Sporozoites infect liver cells ❷and mature into schizonts ❸, which rupture and release merozoites ❹. After this initial replication in the liver (exo-erythrocytic schizogony Ⓐ), the parasites undergo asexual multiplication in the red blood cells (erythrocytes) (erythrocytic schizogony Ⓑ). Merozoites infect red blood cells ❺. The ring stage trophozoites mature into schizonts, which rupture releasing merozoites ❻. Some parasites differentiate into sexual erythrocytic stages (gametocytes) ❼.The gametocytes, male (microgametocytes) and female (macrogametocytes), are ingested by an *Anopheles* mosquito during a blood meal ❽. The parasites' multiplication in the mosquito is known as the sporogonic cycle Ⓒ. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes ❾. The zygotes in turn become motile and elongated (ookinetes) ❿which invade the midgut wall of the mosquito where they develop into oocysts ⓫. The oocysts grow, rupture, and release sporozoites ⓬, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites ❶into 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

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. funests* 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. *An. gambiae* s.l. and *An. funestus* are considered to be endophagic and endophilic (Gillies and DeMeillon 1968; Gillies and Coetzee 1987). *An. gambiae* s.l. and *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 *An. gambiae* s.l. (either *An. gambiae s.s., An. arabiensis* or species not resolved) as well as *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 *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 *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 exito-repellent 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, behavioural, 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; 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.

1.4 References

Abose T, Ye-Ebiyo Y, Olana D, al. E (1998) Re-orientation and Definition of the Role of Malaria Vector Control in Ethiopia: The Epidemiology and Control of Malaria with Special Emphasis on the Distribution, Behaviour and Susceptibility of Insecticides of Anopheline Vectors and Chloroquine Resistance in Zwai, Central Ethiopia and Other Areas. (WHO/MAL/981085) WHO, Geneva: 1-30.

Adiamah JH, Koram KA, Thomson MC, Lindsay SW, Todd SJ et al. (1993) Entomological risk factors for severe malaria in a peri-urban area of The Gambia. Ann Trop Med Parasitol 87(5): 491-500.

Afolabi BM, Amajoh CN, Adewole TA, Salako LA (2006) Seasonal and temporal variations in the population and biting habit of mosquitoes on the Atlantic coast of Lagos, Nigeria. Med Princ Pract 15(3): 200-208.

Afrane YA, Klinkenberg E, Drechsel P, Owusu-Daaku K, Garms R et al. (2004) Does irrigated urban agriculture influence the transmission of malaria in the city of Kumasi, Ghana? Acta Trop 89(2): 125-134.

Baird JK (1998) Age-dependent characteristics of protection v. susceptibility to *Plasmodium falciparum*. Ann Trop Med Parasitol 92(4): 367-390.

Bang YH, Sabuni IB, Tonn RJ (1975) Integrated control of urban mosquitoes in Dar es Salaam using community sanitation supplemented by larviciding. East African Medical Journal 52(10): 578-588.

Bang YH, Mrope FM, Sabuni IB (1977) Changes in mosquito populations associated with urbanization in Tanzania. East African Medical Journal 54(7): 403-411.

Barat LM (2006) Four malaria success stories: how malaria burden was successfully reduced in Brazil, Eritrea, India, and Vietnam. Am J Trop Med Hyg 74(1): 12-16.

Barbazan P, Baldet T, Darriet F, Escaffre H, Djoda DH et al. (1998) Impact of treatments with *Bacillus sphaericus* on Anopheles populations and the transmission of malaria in Maroua, a large city in a savannah region of Cameroon. J Am Mosq Control Assoc 14(1): 33-39.

Bates I, Fenton C, Gruber J, Lalloo D, Medina Lara A et al. (2004) Vulnerability to malaria, tuberculosis and HIV/AIDS infection and disease. Part 1:determinants operating at individual and household level. Lancet Infect Dis 4: 267-277.

Bayoh MN, Lindsay SW (2003) Effect of temperature on the development of the aquatic stages of *Anopheles gambiae sensu stricto* (Diptera: Culicidae). Bull Entomol Res 93(5): 375-381.

Bayoh MN, Lindsay SW (2004) Temperature-related duration of aquatic stages of the Afrotropical malaria vector mosquito *Anopheles gambiae* in the laboratory. Med Vet Entomol 18(2): 174-179.

Beales PF, Gillies HM (2002) Rationale and technique of malaria control. In Essential Malariology. London: Arnold, a member of the Hodder Headline Group.

Beier JC (1998) Malaria parasite development in mosquitoes. Annu Rev Entomol 43: 519-543.

Beier JC, Killeen GF, Githure J (1999) Short report: Entomologic inoculation rates and *Plasmodium falciparum* malaria prevalence in Africa. Am J Trop Med and Hyg 61(1): 109-113.

Beier JC, Oster CN, Onyango FK, Bales JD, Sherwood JA et al. (1994) *Plasmodium falciparum* incidence relative to entomological inoculation rates at a site proposed for testing malaria vaccines in Western Kenya. Am J Trop Med Hyg 50(5): 529-536.

Bogh C, Pedersen EM, Mukoko DA, Ouma JH (1998) Permethrin-impregnated bed net effects on resting and feeding behaviour of lymphatic filariasis vector mosquitoes in Kenya. Med Vet Entomol 12: 52-59.

Bogh C, Clarke SE, Jawara M, Thomas CJ, Lindsay SW (2003) Localized breeding of the *Anopheles gambiae* complex (Diptera: Culicidae) along the River Gambia, West Africa. Bull Entomol Res 93(4): 279-287.

Bogh C, Lindsay SW, Clarke SE, Dean A, Jawara M et al. (2007) High spatial resolution mapping of malaria transmission risk in the Gambia, west Africa, using LANDSAT TM satellite imagery. Am J Trop Med Hyg 76(5): 875-881.

Boudin C, Robert V, Carnevale P, Ambroise-Thomas P (1992) Epidemiology of *Plasmodium falciparum* in a rice field and a savanna area in Burkina Faso. Comparative study on the acquired immunoprotection in native populations. Acta Trop 51(2): 103-111.

Bourgon R, Delorenzi M, Sargeant T, Hodder AN, Crabb BS et al. (2004) The serine repeat antigen (SERA) gene family phylogeny in Plasmodium: the impact of GC content and reconciliation of gene and species trees. Mol Biol Evol 21(11): 2161-2171.

Braimah N, Drakeley C, Kweka E, Mosha FW, Helinski M et al. (2005) Tests of bednet traps (Mbita traps) for monitoring mosquito populations and time of biting in Tanzania and possible impact of prolonged ITN use. Int J Trop Insect Sci 25(3): 208-213.

Bruce-Chwatt L (1984) Malaria: from eradication to control. New Scientist: 17-20.

Cano J, Descalzo MA, Moreno M, Chen Z, Nzambo S et al. (2006) Spatial variability in the density, distribution and vectorial capacity of anopheline species in a high transmission village (Equatorial Guinea). Malar J 5: 21.

Carnevale P, Robert V, Boudin C, Halna JM, Pazart L et al. (1988) La lutte contre le plaudisme par des moustiquaires impregnees de pyrethroides au Burkina Faso. Bull Soc Pathol Exot Filiales 81(5): 832-846.

Castro MC, Yamagata Y, Mtasiwa D, Tanner M, Utzinger J et al. (2004) Integrated urban malaria control: a case study in Dar es Salaam, Tanzania. Am J Trop Med Hyg 71 (Supplement 2): 103-117.

Charles JF, Nicolas L (1986) Recycling of *Bacillus sphaericus* 2362 in mosquito larvae: a laboratory study. Ann Inst Pasteur Microbiol 137B(1): 101-111.

Charlwood JD, Pinto J, Ferrara PR, Sousa CA, Ferreira C et al. (2003) Raised houses reduce mosquito bites. Malar J 2(1): 45.

Charlwood JD, Qassim M, Elnsur EI, Donnelly M, Petrarca V et al. (2001) The Impact of Indoor Residual Spraying with Malathion on Malaria in Refugee Camps Eastern Sudan. Acta Trop 80: 1-8.

Charlwood JD, Kihonda J, Sama S, Billingsley PF, Hadji H et al. (1995) The rise and fall of *Anopheles arabiensis* (Diptera: Culicidae) in a Tanzanian village. Bull Entomol Res 85: 37-44.

Chinery WA (1984) Effects of ecological changes on the malaria vectors *Anopheles funestus* and *Anopheles gambiae* complex mosquitoes in Accra, Ghana. J Trop Meg Hyg 87: 75-81.

Clarke SE, Bogh C, Brown RC, Pinder M, Walraven GEL et al. (2001) Untreated nets protect against malaria infection. Trans R Soc Trop Med Hyg 95: 457-462.

Clyde DF (1967) Malaria in Tanzania. London: Oxford University Press. 167 p.

Coene J (1993) Malaria in urban and rural Kinshasa: the entomological input. Med Vet Entomol 7(2): 127-137.

Cohuet A, Simard F, Wondji CS, Antonio-Nkondjio C, Awono-Ambene P et al. (2004) High malaria transmission intensity due to *Anopheles funestus* (Diptera: Culicidae) in a village of savannah-forest transition area in Cameroon. J Med Entomol 41(5): 901-905.

Collins WE, Jeffery GM (1999) A retrospective examination of secondary sporozoite- and trophozoite- induced infections with *Plasmodium falciparum*: development of parasitologic and clinical immunity following secondary infection. Am J Trop Med Hyg 61(1 Suppl): 20-35.

Coluzzi M, Corbellini G (1995) [The places of Mal'aria and the causes of malaria]. Med Secoli 7(3): 575-598.

Corbel V, N'Guessan R, Brengues C, Chandre F, Djogbenou L et al. (2007) Multiple insecticide resistance mechanisms in *Anopheles gambiae* and *Culex quinquefasciatus* from Benin, West Africa. Acta Trop 101(3): 207-216.

Craig MH, Snow RW, le Sueur D (1999) A climate-based distribution model of malaria transmssion in sub-Saharan Africa. Parasitol Today 15(3): 105-111.

Curtis C, Maxwell C, Lemnge M, Kilama WL, Steketee RW et al. (2003) Scaling-up coverage with insecticide-treated nets against malaria in Africa: who should pay? Lancet Infect Dis 3(5): 304-307.

Curtis CF, Maxwell CA, Finch RJ, Njunwa KJ (1998) A comparison of use of a pyrethroid either for house spraying or for bednet treatment against malaria vectors. Trop Med Int Health 3(8): 619-631.

Cuzin-Ouattara N, Van den Broek AHA, Habluetzel A (1999) Wide-scale installation of insecticide-treated curtains confers high levels of protection against malaria transmission in a hyperendemic area of Burkina Faso. Trans R Soc Trop Med Hyg 93: 473-479.

DHS, Tanzania (2005) Tanzania Demographic and Health Survey 2004-2005. National Bureau of Statistics, Dar es Salaam, Tanzania: 163-175.

Diuk-Wasser MA, Toure MB, Dolo G, Bagayoko M, Sogoba N et al. (2005) Vector abundance and malaria transmission in rice-growing villages in Mali. Am J Trop Med Hyg 72(6): 725-731.

Doannio JM, Konan YL, Amalaman K, Attiah J (2004) [Knowledge, attitudes and practices of populations towards mosquitoes in urban and rural area (Cote d'Ivoire--West Africa)]. Bull Soc Pathol Exot 97(4): 295-301.

Dolo G, Briet OJ, Dao A, Traore SF, Bouare M et al. (2004) Malaria transmission in relation to rice cultivation in the irrigated Sahel of Mali. Acta Trop 89(2): 147-159.

Donnelly MJ, McCall PJ, Lengeler C, Bates I, D'Alessandro U et al. (2005) Malaria and urbanization in sub-Saharan Africa. Malar J 4(1): 12.

Dossou-Yovo J, Diarrassouba S, Doannio J, Darriet F, Carnevale P (1999) [The aggressive cycle of *Anopheles gambiae s.s.* inside houses and malaria transmission in the Bouake region (Cote d'Ivoire). Value of using impregnated mosquito nets]. Bull Soc Pathol Exot 92(3): 198-200.

Dukeen MYH, Omer SM (1986) Ecology of the malaria vector *Anopheles arabiensis* Patton (Diptera: Culicidae) by the Nile in northern Sudan. Bull Entomol Res 76: 451-467.

Edillo FE, Tripet F, Toure YT, Lanzaro GC, Dolo G et al. (2006) Water quality and immatures of the M and S forms of *Anopheles gambiae s.s.* and *An. arabiensis* in a Malian village. Malar J 5: 35.

Eisele TP, Keating J, Swalm C, Mbogo CM, Githeko AK et al. (2003) Linking field-based ecological data with remotely sensed data using a geographic information system in two malaria endemic urban areas of Kenya. Malar J 2(1): 44.

El Sayed BB, Arnot DE, Mukhtar MM, Baraka OZ, Dafalla AA et al. (2000) A study of the urban malaria transmission problem in Khartoum. Acta Trop 75(2): 163-171.

Elliott R (1972) The influence of vector behavior on malaria transmission. Am J Trop Med Hyg 21(5): 755-763.

Evans PJ (1994) Community knowledge, attitudes and practices-urban mosquitoes and sustainable mosquito control [Degree of Doctor of Philosophy in Geography]: University of Exeter. 308 p.

Faye O, Fontenille D, Herve JP, Diack PA, Diallo S et al. (1993) [Malaria in the Saharan region of Senegal. 1. Entomological transmission findings]. Ann Soc Belg Med Trop 73(1): 21-30.

Faye O, Konate L, Gaye O, Fontenille D, Sy N et al. (1998) Impact of the use of permethrin pre-impregnated mosquito nets on malaria transmission in a hyperendemic village of Senegal. Med Trop (Mars) 58(4): 355-360.

Fillinger U, Lindsay SW (2006) Suppression of exposure to malaria vectors by an order of magnitude using microbial larvicides in rural Kenya. Trop Med Int Health 11(11): 1629-1642.

Fillinger U, Knols BG, Becker N (2003) Efficacy and efficiency of new *Bacillus thuringiensis* var *israelensis* and *Bacillus sphaericus* formulations against Afrotropical anophelines in Western Kenya. Trop Med Int Health 8(1): 37-47.

Fillinger U, Sonye G, Killeen GF, Knols BGJ, Becker N (2004) The practical importance of permanent and semi-permanent habitats for controlling aquatic stages of *Anopheles gambiae sensu lato* mosquitoes: operational observations from a rural town in Western Kenya. Trop Med Int Health 9: 1274-1289.

Fletcher M, Teklehaimanot A, Yemane G (1992) Control of mosquito larvae in the port city of Assab by an indigenous larvivorous fish, *Aphanius dispar*. Acta Trop 52: 155-166.

Fried M, Nosten F, Brockman A, Brabin BJ, Duffy PE (1998) Maternal antibodies block malaria. Nature 395: 851-852.

Gillies MT (1988) Anopheline mosquitos: vector behaviour and bionomics. Edinburgh: Churchill Livingstone. 453-485 p.

Gillies MT, DeMeillon B (1968) The Anophelinae of Africa South of the Sahara (Ethiopian zoogeographical region). Johannesburg: South African Institute for Medical Research.

Gillies MT, Coetzee M (1987) A supplement to the Anophelinae of Africa South of the Sahara (Afrotropical region). Johannesburg: South African Medical Research Institute.

Gilroy AB, Bruce-Chwatt LJ (1945) Mosquito-control by swamp drainage in the coastal belt of Nigeria. Croydon: HR Grubb.

Gimnig JE, Ombok M, Kamau L, Hawley WA (2001) Characteristics of larval anopheline (Diptera: Culicidae) habitats in Western Kenya. J Med Entomol 38(2): 282-288.

Gimnig JE, Ombok M, Otieno S, Kaufman MG, Vulule JM et al. (2002) Density-dependent development of *Anopheles gambiae* (Diptera: Culicidae) larvae in artificial habitats. J Med Entomol 39(1): 162-172.

Gopaul R (1995) Surveillance entemologique a Maurice. Santé 5: 401-405.

Govere J, Durrheim D, la Grange K, Mabuza A, Booman M (2000) Community knowledge and perceptions about malaria and practices influencing malaria control in Mpumalanga Province, South Africa. S Afr Med J 90(6): 611-616.

Gu W, Novak RJ (2005) Habitat-based modeling of impacts of mosquito larval interventions on entomological inoculation rates, incidence, and prevalence of malaria. Am J Trop Med Hyg 73(3): 546-552.

Gu W, Regens JL, Beier JC, Novak RJ (2006) Source reduction of mosquito larval habitats has unexpected consequences on malaria transmission. Proc Natl Acad Sci U S A 103(46): 17560-17563.

Haddow AJ (1942) The mosquito fauna and climate of native huts at Kisumu, Kenya. Bull Entomol Res 33: 91-142.

Haddow AJ (1943) Measurement of temperature and light in artificial pools with reference to the larval habitat of *Anopheles (Myzomia) gambiae* Giles and *A. (M.) funestus* Giles. Bull Entomol Res 34: 89.

Haddow AJ, Gillet JD, Highton RB (1947) The mosquitoes of Bwamba country, Uganda. V. The vertical distribution and biting cycle of mosquitoes in rain forest, with further observations on microclimate. Bull Entomol Res 37: 307-330.

Hawley WA, Phillips-Howard PA, ter Kuile FO, Terlouw DJ, Vulule JM et al. (2003) Community-wide effects of permethrin-treated bednets on child mortality and malaria morbidity in western Kenya. Am J Trop Med Hyg 68 (Supplement 4): 121-127.

Hay SI, Guerra CA, Tatem TA, Noor AM, Snow RW (2004) The global distribution and population at risk of malaria: past, present and future. Lancet Infect Dis 4: 327-336.

Hay SI, Guerra CA, Tatem AJ, Atkinson PM, Snow RW (2005) Urbanization, malaria transmission and disease burden in Africa. Nat Rev Microbiol 3: 81-90.

Hoffman SL, Oster CN, Plowe CV, Woollett GR, Beier JC et al. (1987) Naturally acquired antibodies to sporozoites do not prevent malaria: vaccine development implications. Science 237(4815): 639-642.

Holstein MH (1954) Biology of *Anopheles gambiae*. Geneva: World Health Organization. 173 p.

Hoshen MB, Morse AP (2004) A weather-driven model of malaria transmission. Malar J 3: 32.

Hougard JM (1990) Formulation and persistence of *Bacillus sphaericus* in *Culex quinquefasciatus* larval sites in tropical Africa.; de Barjac H, Gutherland DJ, editors. New Brunswick: Rutgers University Press. 295-306 p.

Ijumba J, Lindsay S (2001) Impact of irrigation on malaria in Africa: paddies paradox. Med Vet Entomol 15: 1-11.

Ilboudo-Sanogo E, Cuzin-Ouattara N, Diallo DA, Cousens SN, Esposito F et al. (2001) Insecticide-treated materials, mosquito adaptation and mass effect: entomological observations after five years of vector control in Burkina Faso. Trans R Soc Trop Med Hyg 95(4): 353-360.

Impoinvil DE, Ahmad S, Troyo A, Keating J, Githeko AK et al. (2007) Comparison of mosquito control programs in seven urban sites in Africa, the Middle East, and the Americas. Health Policy 83(2-3): 196-212.

Jacob BG, Arheart KL, Griffith DA, Mbogo CM, Githeko AK et al. (2005) Evaluation of environmental data for identification of Anopheles (Diptera: Culicidae) aquatic larval habitats in Kisumu and Malindi, Kenya. J Med Entomol 42(5): 751-755.

Jaenson TG, Gomes MJ, Barreto dos Santos RC, Petrarca V, Fortini D et al. (1994) Control of endophagic Anopheles mosquitoes and human malaria in Guinea Bissau, West Africa by permethrin-treated bed nets. Trans R Soc Trop Med Hyg 88(6): 620-624.

Julvez J (1995) Historique du paludime insulaire dans l'ocean Indien (sud-Ouest). Une approche eco-epidemiologique. Santé 5: 353-357.

Karch S, Monteny N, Jullien JL, Sinegre G, Coz J (1990) Control of *Culex pipiens* by *Bacillus sphaericus* and role of nontarget arthropods in its recycling. J Am Mosq Control Assoc 6(1): 47-54.

Karch S, Garin B, Asidi N, Manzambi Z, Salaun JJ et al. (1993) [Mosquito nets impregnated against malaria in Zaire]. Ann Soc Belg Med Trop 73(1): 37-53.

Keating J, MacIntyre K, Mbogo C, Githeko A, Regens JL et al. (2003) A geographic sampling strategy for studying relationships between human activity and malaria vectors in urban Africa. Am J Trop Med Hyg 68(3): 357-365.

Keiser J, Singer BH, Utzinger J (2005) Reducing the burden of malaria in different eco-epidemiological settings with environmental management: a systematic review. Lancet Infect Dis 5(11): 695-708.

Keiser J, Utzinger J, Castro MC, Smith TA, Tanner M et al. (2004) Urbanization in sub-Saharan Africa and implication for malaria control. Am J Trop Med Hyg 71(2 Suppl): 118-127.

Khaemba BM, Mutani A, Bett MK (1994) Studies of anopheline mosquitoes transmitting malaria in a newly developed highland urban area: a case study of Moi University and its environs. East Afr Med J 71(3): 159-164.

Kilama WL (1991) Control of arthropods of public health importance. In: Mwaluko GMP, Kilama WL, Mandara PM, Murru M, MacPherson CNL, editors. Health and Disease in Tanzania. London: Harper Collins Academic.

Kilama WL (1994) Malaria in Tanzania: past and present. Proceedings of the 11th Annual Joint Scientific Conference with a Seminar on Malaria Control Research. Arusha, Tanzania: National Institute for Medical Research.

Killeen GF, Smith TA (2007) Exploring the contributions of bed nets, cattle, insecticides and excitorepellency to malaria control: a deterministic model of mosquito host-seeking behaviour and mortality. Trans R Soc Trop Med Hyg 101(9): 867-880.

Killeen GF, Fillinger U, Knols BGJ (2002) Advantages of larval control for African malaria vectors: Low mobility and behavioural responsiveness of immature mosquito stages allow high effective coverage. Malar J 1: 8.

Killeen GF, Knols BG, Gu W (2003) Taking malaria transmission out of the bottle: implications of mosquito dispersal for vector-control interventions. Lancet Infect Dis 3(5): 297-303.

Killeen GF, Seyoum A, Knols BGJ (2004) Rationalizing historical successes of malaria control in Africa in terms of mosquito resource availability management. Am J Trop Med Hyg 71 (Supplement 2): 87-93.

Killeen GF, McKenzie FE, Foy BD, Bogh C, Beier JC (2001) The availability of potential hosts as a determinant of feeding behaviours and malaria transmission by mosquito populations. Trans R Soc Trop Med Hyg 95: 469-476.

Killeen GF, Fillinger U, Kiche I, Gouagna LC, Knols BGJ (2002) Eradication of *Anopheles gambiae* from Brazil: lessons for malaria control in Africa? Lancet Infect Dis 2: 618-627.

Killeen GF, McKenzie FE, Foy BD, Schieffelin C, Billingsley PF et al. (2000) The potential impacts of integrated malaria transmission control on entomologic inoculation rate in highly endemic areas. Am J Trop Med Hyg 62(5): 545-551.

Killeen GF, McKenzie FE, Foy BD, Schieffelin C, Billingsley PF et al. (2000) A simplified model for predicting malaria entomologic inoculation rates based on entomologic and parasitologic parameters relevant to control. Am J Trop Med Hyg 62(5): 535-544.

Killeen GF, Tanner M, Mukabana WR, Kalongolela MS, Kannady K et al. (2006) Habitat targeting for controlling aquatic stages of malaria vectors in Africa. Am J Trop Med Hyg 74(4): 517-518; author reply 519-520.

Killeen GF, Kihonda J, Lyimo E, Oketch FR, Kotas ME et al. (2006) Quantifying behavioural interactions between humans and mosquitoes: Evaluating the protective efficacy of insecticidal nets against malaria transmission in rural Tanzania. BMC Infect Dis 6(1): 161.

Kirby MJ, Lindsay SW (2004) Responses of adult mosquitoes of two sibling species, *Anopheles arabiensis* and *A. gambiae s.s.* (Diptera: Culicidae), to high temperatures. Bull Entomol Res 94(5): 441-448.

Kiszewski A, Mellinger A, Spielman A, Malaney P, Sachs SE et al. (2004) A global index representing the stability of malaria transmission. Am J Trop Med Hyg 70(5): 486-498.

Kitron U (1987) Malaria, agriculture, and development: lessons from past campaigns. International Journal of Health Services 17(2): 295-326.

Kitron U, Spielman A (1989) Suppression of transmission of malaria through source reduction: antianopheline measures applied in Israel, the United States, and Italy. Review of Infectious Diseases 11(3): 391-406.

Kleinschmidt I, Torrez M, Schwabe C, Benavente L, Seocharan I et al. (2007) Factors influencing the effectiveness of malaria control in Bioko Island, equatorial Guinea. Am J Trop Med Hyg 76(6): 1027-1032.

Klinkenberg E, Takken W, Huibers F, Toure YT (2003) The phenology of malaria mosquitoes in irrigated rice fields in Mali. Acta Trop 85(1): 71-82.

Klinkenberg E, McCall PJ, Hastings IM, Wilson MD, Amerasinghe FP et al. (2005) Malaria and irrigated crops, Accra, Ghana. Emerg Infect Dis 11(8): 1290-1293.

Klinkenberg E, McCall PJ, Wilson MD, Akoto AO, Amerasinghe FP et al. (2006) Urban malaria and anaemia in children: a cross-sectional survey in two cities of Ghana. Trop Med Int Health 11(5): 578-588.

Knols BGJ, Takken W (1998) The widescale use of impregnated bednets for malaria control in Africa: impact on mosquitoes. Proc Exp Appl Entomol 8: 15-20.

Koram KA, Bennett S, Adiamah JH, Greenwood BM (1995) Socio-economic risk factors for malaria in a peri-urban area of The Gambia. Trans R Soc Trop Med Hyg 89(2): 146-150.

Kouznetsov RL (1977) Malaria control by application of indoor spraying of residual insecticides in tropical Africa and its impact on community health. Tropical Doctor 7: 81-93.

Kulkarni MA, Kweka E, Nyale E, Lyatuu E, Mosha FW et al. (2006) Entomological evaluation of malaria vectors at different altitudes in Hai district, northeastern Tanzania. J Med Entomol 43(3): 580-588.

Lacey LA (2007) *Bacillus thuringiensis* serovariety *israelensis* and *Bacillus sphaericus* for mosquito control. J Am Mosq Control Assoc 23(2 Suppl): 133-163.

Laganier R, Randimby FM, Rajaonarivelo V, Robert V (2003) Is the Mbita trap a reliable tool for evaluating the density of anopheline vectors in the highlands of Madagascar? Malar J 2(1): 42.

Le Menach A, McKenzie FE, Flahault A, Smith DL (2005) The unexpected importance of mosquito oviposition behaviour for malaria: non-productive larval habitats can be sources for malaria transmission. Malar J 4(1): 23.

Le Menach A, Takala S, McKenzie FE, Perisse A, Harris A et al. (2007) An elaborated feeding cycle model for reductions in vectorial capacity of night-biting mosquitoes by insecticide-treated nets. Malar J 6: 10.

Lengeler C (2004) Insecticide-treated nets for malaria control: real gains. Bull World Health Organ 82(2): 84.

Lengeler C (2004) Insecticide-treated bed nets and curtains for preventing malaria. Cochrane Database Syst Rev(2): CD000363.

Lengeler C, Grabowsky M, McGuire D, DeSavigny D (2007) Quick Wins Versus Sustainability: Options for the Upscaling of Insecticide-Treated Nets. Am J Trop Med Hyg 77(6 Suppl): 222-6

Lindblade KA, Walker ED, Onapa AW, Katungu J, Wilson ML (2000) Land use change alters malaria transmission parameters by modifying temperature in a highland area of Uganda. Trop Med Int Health 5(4): 263-274.

Lindsay SW, Snow RW (1988) The trouble with eaves: house entry by vectors of malaria. Trans R Soc Trop Med Hyg 82(645-646).

Lindsay SW, Emerson PM, Charlwood JD (2002) Reducing malaria transmission by mosquito-proofing homes. Trends Parasitol 18(11): 510-514.

Lindsay SW, Adiamah JH, Miller JE, Armstrong JRM (1991) Pyrethroid-treated bednet effects on mosquitoes of the *Anopheles gambiae* complex. Med Vet Entomol 5: 477-483.

Lindsay SW, Campbell H, Adiamah JH, Greenwood AM, Bangali JE et al. (1990) Malaria in a periurban area of The Gambia. Ann Trop Med Parasitol 84: 553-562.

Lindsay SW, Wilkins HA, Zieler HA, Daly RJ, Petrarca V et al. (1991) Ability of *Anopheles gambiae* mosquitoes to transmit malaria during the dry and wet seasons in an area of irrigated rice cultivation in The Gambia. J Trop Med Hyg 94(5): 313-324.

Lindsay SW, Armstrong Schellenberg JRM, Zeiler HA, Daly RJ, Salum FM et al. (1995) Exposure of Gambian children to *Anopheles gambiae* vectors in an irrigated rice production area. Med Vet Entomol 9: 50-58.

Lindsay SW, Jawara M, Paine K, Pinder M, Walraven GE et al. (2003) Changes in house design reduce exposure to malaria mosquitoes. Trop Med Int Health 8(6): 512-517.

Lindsay SW, Alonso PL, Armstrong Schellenberg JRM, Hemingway J, Adiamah JH et al. (1993) A malaria control trial using insecticide-treated bed nets and targeted chemoprophylaxis in a rural area of The Gambia, West Africa. 7. Impact of permethrin-impregnated bed nets on malaria vectors. Trans Roy Soc Trop Med Hyg 87 (Supplement 2): 45-51.

Lines J, Harpham T, Leake C, Schofield C (1994) Trends, priorities and policy directions in the control of vector-borne diseases in urban environments. Health Policy and Plan 9(2): 113-129.

Lines J, Lengeler C, Cham K, de Savigny D, Chimumbwa J et al. (2003) Scaling-up and sustaining insecticide-treated net coverage. Lancet Infect Dis 3(8): 465-468.

Lines JD, Myamba J, Curtis CF (1987) Experimental hut trials of permethrin-impregnated mosquito nets and eave curtains against malaria vectors in Tanzania. Med Vet Entomol 1: 37-51.

Louis JP, Albert JP (1988) Malaria in the Republic of Djibouti. Strategy for control using a biological antilarval campaign: indigenous larvivorous fishes (*Aphanius dispar*) and bacterial toxins. Medicine Tropicale 48(2): 127-131.

Mabaso ML, Sharp B, Lengeler C (2004) Historical review of malarial control in southern African with emphasis on the use of indoor residual house-spraying. Trop Med Int Health 9(8): 846-856.

Mabaso ML, Craig M, Ross A, Smith T (2007) Environmental predictors of the seasonality of malaria transmission in Africa: the challenge. Am J Trop Med Hyg 76(1): 33-38.

MacDonald G (1957) The epidemiology and control of malaria. London: Oxford University Press.

MacIntyre K, Keating J, Sosler S, Kibe L, Mbogo CM et al. (2002) Examining the determinants of mosquito avoidance practices in two Kenyan cities. Malaria J 1: 14.

Magesa SM, Wilkes TJ, Mnzava AEP, Njunwa KJ, Myamba J et al. (1991) Trial of pyrethroid impregnated bednets in an area of Tanzania holoendemic for malaria. Part 2 Effects on the malaria vector population. Acta Trop 49: 97-108.

Maire N, Smith T, Ross A, Owusu-Agyei S, Dietz K et al. (2006) A model for natural immunity to asexual blood stages of *Plasmodium falciparum* malaria in endemic areas. Am J Trop Med Hyg 75(2 Suppl): 19-31.

Majambere S, Lindsay SW, Green C, Kandeh B, Fillinger U (2007) Microbial larvicides for malaria control in The Gambia. Malar J 6: 76.

Manda H, Gouagna LC, Foster WA, Jackson RR, Beier JC et al. (2007) Effect of discriminative plant-sugar feeding on the survival and fecundity of *Anopheles gambiae*. Malar J 6(1): 113.

Marsh K (1992) Malaria--a neglected disease? Parasitology 104 Suppl: S53-69.

Marsh K, Kinyanjui S (2006) Immune effector mechanisms in malaria. Parasite Immunol 28(1-2): 51-60.

Marsh K, Forster D, Waruiru C, Mwangi I, Winstanley M et al. (1995) Indicators of life-threatening malaria in African children. N Engl J Med 332(21): 1399-1404.

Matanmi BA, Federici BA, Mulla MS (1990) Fate and persistence of *Bacillus sphaericus* used as a mosquito larvicide in dairy wastewater lagoons. J Am Mosq Control Assoc 6(3): 384-389.

Matthys B, N'Goran EK, Kone M, Koudou BG, Vounatsou P et al. (2006) Urban agricultural land use and characterization of mosquito larval habitats in a medium-sized town of Cote d'Ivoire. J Vector Ecol 31(2): 319-333.

Matthys B, Vounatsou P, Raso G, Tschannen AB, Becket EG et al. (2006) Urban farming and malaria risk factors in a medium-sized town in Cote d'Ivoire. Am J Trop Med Hyg 75(6): 1223-1231.

Maxwell CA, Wakibara J, Tho S, Curtis CF (1998) Malaria-infective biting at different hours of the night. Med Vet Entomol 12(3): 325-327.

Maxwell CA, Myamba J, Njunwa KJ, Greenwood BM, Curtis CF (1999) Comparison of bednets impregnated with different pyrethroids for their impact on mosquitoes and on re-infection with malaria after clearance of pre-existing infections with chlorproguanil-dapsone. Trans R Soc Trop Med Hyg 93(1): 4-11.

Maxwell CA, Msuya E, Sudi M, Njunwa KJ, Carneiro IA et al. (2002) Effect of community-wide use of insecticide-treated nets for 3-4 years on malarial morbidity in Tanzania. Trop Med Int Health 7(12): 1003-1008.

Maxwell CA, Chambo W, Mwaimu M, Magogo F, Carneiro IA et al. (2003) Variation of malaria transmission and morbidity with altitude in Tanzania and with introduction of alphacypermethrin treated nets. Malar J 2(1): 28.

Mbogo CNM, Baya NM, Ofulla AVO, Githure JI, Snow RW (1996) The impact of permethrin-impregnated bednets on malaria vectors of the Kenyan coast. Med Vet Entomol 10: 251-259.

Mensah OA, Kumaranayake L (2004) Malaria incidence in rural Benin: does economics matter in endemic area? Health Policy 68(1): 93-102.

Miller JE, Lindsay SW, Armstrong JRM (1991) Experimental hut trials of bednet impregnated with synthetic pyrethroid and organophosphate insecticides for mosquito control in The Gambia. Med Vet Entomol 5: 465-476.

Minakawa N, Seda P, Yan G (2002) Influence of host and larval habitat distribution on the abundance of African malaria vectors in Western Kenya. Am J Trop Med Hyg 67(1): 32-38.

Minakawa N, Githure JI, Beier JC, Yan G (2001) Anopheline mosquito survival strategies during the dry period in western Kenya. J Med Entomol 38(3): 388-392.

Minakawa N, Sonye G, Mogi M, Yan G (2004) Habitat characteristics of *Anopheles gambiae s.s.* larvae in a Kenyan highland. Med Vet Entomol 18(3): 301-305.

Minakawa N, Mutero CM, Githure JI, Beier JC, Yan G (1999) Spatial distribution and habitat characterization of Anopheline mosquito larvae in Western Kenya. Am J Trop Med Hyg 61(6): 1010-1016.

Minakawa N, Sonye G, Mogi M, Githeko A, Yan G (2002) The effects of climatic factors on the distribution and abundance of malaria vectors in Kenya. J Med Entomol 39(6): 833-841.

Minakawa N, Omukunda E, Zhou G, Githeko A, Yan G (2006) Malaria vector productivity in relation to the highland environment in Kenya. Am J Trop Med Hyg 75(3): 448-453.

Minakawa N, Munga S, Atieli F, Mushinzimana E, Zhou G et al. (2005) Spatial distribution of anopheline larval habitats in Western Kenyan highlands: effects of land cover types and topography. Am J Trop Med Hyg 73(1): 157-165.

Modiano D, Sirima BS, Sawadogo A, Sanou I, Pare J et al. (1998) Severe malaria in Burkina Faso: influence of age and transmission level on clinical presentation. Am J Trop Med Hyg 59(4): 539-542.

Moffett A, Shackelford N, Sarkar S (2007) Malaria in Africa: vector species' niche models and relative risk maps. PLoS ONE 2(9): e824.

MOH (2002) National malaria medium term strategic plan, 2002-2007. Dar es Salaam: Ministry of Health, United Republic of Tanzania & World Health Organization. 55 p.

Molineaux L, Muir DA, Spencer HC, Werndorfer WH (1988) The epidemiology of malaria and its measurment. Edinburgh: Churchill Livingstone. 999-1090 p.

Molineaux L, Trauble M, Collins WE, Jeffery GM, Dietz K (2002) Malaria therapy reinoculation data suggest individual variation of an innate immune response and independent acquisition of antiparasitic and antitoxic immunities. Trans R Soc Trop Med Hyg 96(2): 205-209.

Muirhead-Thomson RC (1951) Mosquito behaviour in relation to malaria transmission and control in the tropics. London: Edward Arnold & Co. 219 p.

Muirhead-Thomson RC (1960a) The significance of irritability, behaviouristic avoidance and allied phenomena in malaria eradication. Bull World Health Organ 22: 721-734.

Muirhead-Thomson RC (1960b) The winter activities of *An. gambiae* at high altitudes in Southern Rhodesia. unpublished WHO working document.

Munga S, Minakawa N, Zhou G, Mushinzimana E, Barrack OO et al. (2006) Association between land cover and habitat productivity of malaria vectors in western Kenyan highlands. Am J Trop Med Hyg 74(1): 69-75.

N'Guessan R, Corbel V, Akogbeto M, Rowland M (2007) Reduced Efficacy of Insecticide-treated Nets and Indoor Residual Spraying for Malaria Control in Pyrethroid Resistance Area, Benin. Emerging Infectious Diseases 13(2): 199-206.

Ng'andu N, Watts TE, Wray JR, Chela C, Zulu B (1989) Some risk factors for transmission of malaria in a population where control measures were applied in Zambia. East Afr Med J 66(11): 728-737.

Njau RJA, Mosha FW, Nguma JFM (1993) Field trials of pyrethroid impregnated bednets in northern Tanzania. 1.Effects in malaria transmission. Insect Sci Appl 5: 575-584.

Nyarango PM, Gebremeskel T, Mebrahtu G, Mufunda J, Abdulmumini U et al. (2006) A steep decline of malaria morbidity and mortality trends in Eritrea between 2000 and 2004: the effect of combination of control methods. Malar J 5: 33.

O'Meara WP, Collins WE, McKenzie FE (2007) Parasite prevalence: a static measure of dynamic infections. Am J Trop Med Hyg 77(2): 246-249.

Oesterholt MJ, Bousema JT, Mwerinde OK, Harris C, Lushino P et al. (2006) Spatial and temporal variation in malaria transmission in a low endemicity area in northern Tanzania. Malar J 5: 98.

Omumbo JA, Guerra CA, Hay SI, Snow RW (2005) The influence of urbanisation on measures of *Plasmodium falciparum* infection prevalence in East Africa. Acta Trop 93(1): 11-21.

Oyewole IO, Awolola TS (2006) Impact of urbanization on bionomics and distribution of malaria vectors in Lagos, southwestern Nigeria. J Vector Borne Dis 43: 173-178.

Pantuwatana S, Maneeroj R, Upatham ES (1989) Long residual activity of *Bacillus sphaericus* 1593 against *Culex quinquefasciatus* larvae in artificial pools. Southeast Asian J Trop Med Public Health 20(3): 421-427.

Pates H, Curtis C (2005) Mosquito behavior and vector control. Annu Rev Entomol 50: 53-70.

Qari SH, Shi YP, Pieniazek NJ, Collins WE, Lal AA (1996) Phylogenetic relationship among the malaria parasites based on small subunit rRNA gene sequences: monophyletic nature of the human malaria parasite, *Plasmodium falciparum*. Mol Phylogenet Evol 6(1): 157-165.

Ragavoodoo C (1995) Situation du paludimse a Maurice. Santé 5: 371-375.

Ribeiro JMC, Seulu F, Abose T, Kidane G, Teklehaimanot A (1996) Temporal and spatial distribution of anopheline mosquitoes in an Ethiopian village: implications for malaria control strategies. Bull World Health Organ 74(3): 299-305.

Rishikesh N (1966) Observations on Anopheline of Malaria in an Upland Valley in Ethiopia. (Unpublished document, WHO/MAL/66554) WHO, Geneva.

Robert V, Carnevale P (1991) Influence of deltamethrin treatment of bed nets on malaria transmission in the Kou valley, Burkina Faso. Bull World Health Organ 69(6): 735-740.

Robert V, Awono-Ambene HP, Thiolouse J (1998) Ecology of larval mosquitoes, with special reference to *Anopheles arabiensis* (Diptera: Culicidae), in market gardener wells in urban Dakar, Senegal. J Med Entomol 35: 948-955.

Robert V, MacIntyre K, Keating J, Trape JF, Duchemin JB et al. (2003) Malaria transmission in urban sub-Saharan Africa. Am J Trop Med Hyg 68(2): 169-176.

Roberts DR, Alecrim WD, Hshieh P, Grieco JP, Bangs M et al. (2000) A probability model of vector behavior: effects of DDT repellency, irritancy, and toxicity in malaria control. J Vector Ecol 25(1): 48-61.

Rogier C, Trape JF (1995) [Study of premunition development in holo- and meso-endemic malaria areas in Dielmo and Ndiop (Senegal): preliminary results, 1990-1994.Med Trop (Mars) 55(4 Suppl): 71-76.

Roll Back Malaria http//:wwwrbmwhoint.

Roll Back Malaria Partnership a (2005) World Malaria Report. Geneva: World Health Organization.

Roll Back Malaria Partnership b (2005) Scaling up insecticide treated netting programmes in Africa: a strategic framework for coordinated national action. Geneva: World Health Organization.

Ronald LA, Kenny SL, Klinkenberg E, Akoto AO, Boakye I et al. (2006) Malaria and anaemia among children in two communities of Kumasi, Ghana: a cross-sectional survey. Malar J 5: 105.

Ross A, Killeen G, Smith T (2006) Relationships between host infectivity to mosquitoes and asexual parasite density in *Plasmodium falciparum*. Am J Trop Med Hyg 75(2 Suppl): 32-37.

Ross A, Maire N, Molineaux L, Smith T (2006) An epidemiologic model of severe morbidity and mortality caused by *Plasmodium falciparum*. Am J Trop Med Hyg 75(2 Suppl): 63-73.

Ross R (1907) An address on the prevention of malaria in British possessions, Egypt, and parts of America. Lancet 2: 879-887.

Ross R (1911) The Prevention of Malaria. London: Murray.

Rowland M, Freeman T, Downey G, Hadi A, Saeed M (2004) DEET mosquito repellent sold through social marketing provides personal protection against malaria in an area of all-night mosquito biting and partial coverage of insecticide-treated nets: a case-control study of effectiveness. Trop Med Int Health 9(3): 343-350.

Rozendaal JA (1997) Vector Control. Methods for use by individuals and communities. Geneva: WHO.

Sabatinelli G, Blanchy S, Majori G, Papakay M (1991) Impact of the use of the larvivorous fish, *Poecilia reticulata* in the transmission of malaria in the Federal Islamic Republic of Comoros. Annales Parasitologique Humaine et Comparativ 66(2): 84-88.

Sachs J, Malaney P (2002) The economic and social burden of malaria. Nature 415: 680-685.

Sattler MA, Mtasiwa D, Kiama M, Premji Z, Tanner M et al. (2005) Habitat characterization and spatial distribution of Anopheles sp. mosquito larvae in Dar es Salaam (Tanzania) during an extended dry period. Malar J 4(1): 4.

Service MW (1971) Studies on sampling larval populations of the *Anopheles gambiae* complex. Bull World Health Organ 45(2): 169-180.

Service MW (1997) Mosquito (Diptera: Culicidae) dispersal--the long and short of it. J Med Entomol 34(6): 579-588.

Service MW (2000) Medical entomology for students. Liverpool: Cambridge University Press.

Service MW, Townson H (2002) The Anopheles vector in Essential Malariology. London: Arnold, a member of the Hodder Headline Group.

Sharp BL, Ridl FC, Govender D, Kuklinski J, Kleinschmidt I (2007) Malaria vector control by indoor residual insecticide spraying on the tropical island of Bioko, Equatorial Guinea. Malar J 6: 52.

Sharp BL, Kleinschmidt I, Streat E, Maharaj R, Barnes KI et al. (2007) Seven years of regional malaria control collaboration--Mozambique, South Africa, and Swaziland. Am J Trop Med Hyg 76(1): 42-47.

Shiff CJ, Minjas JN, Hall T, Hunt RH, Lyimo S et al. (1995) Malaria infection potential of anopheline mosquitoes sampled by light trapping indoors in coastal Tanzanian villages. Med Vet Entomol 9: 256-262.

Shililu J, Mbogo C, Ghebremeskel T, Githure J, Novak R (2007) Mosquito larval habitats in a semiarid ecosystem in Eritrea: impact of larval habitat management on *Anopheles arabiensis* population. Am J Trop Med Hyg 76(1): 103-110.

Shililu J, Ghebremeskel T, Seulu F, Mengistu S, Fekadu H et al. (2004) Seasonal abundance, vector behavior, and malaria parasite transmission in Eritrea. J Am Mosq Control Assoc 20(2): 155-164.

Shililu JI, Tewolde GM, Brantly E, Githure JI, Mbogo CM et al. (2003) Efficacy of *Bacillus thuringiensis israelensis*, *Bacillus sphaericus* and temephos for managing Anopheles larvae in Eritrea. J Am Mosq Control Assoc 19(3): 251-258.

Shousha AT (1948) Species-eradication. the eradication of *Anopheles gambiae* from Upper Egypt, 1942-1945. Bull World Health Org 1: 309-353.

Skovmand O, Bauduin S (1997) Efficacy of a granular formulation of *Bacillus sphaericus* against *Culex quinquefasciatus* and *Anopheles gambiae* in West African countries. J Vector Ecol 22(1): 43-51.

Smith DL, McKenzie FE (2004) Statics and dynamics of malaria infection in Anopheles mosquitoes. Malar J 3(1): 13.

Smith DL, Dushoff J, McKenzie FE (2004) The risk of a mosquito-borne infection in a heterogeneous environment. PLoS Biol 2(11): e368.

Smith T, Maire N, Dietz K, Killeen GF, Vounatsou P et al. (2006) Relationship between the entomologic inoculation rate and the force of infection for *Plasmodium falciparum* malaria. Am J Trop Med Hyg 75(2 Suppl): 11-18.

Smith T, Charlwood JD, Kihonda J, Mwankusye S, Billingsley P et al. (1993) Absence of seasonal variation in malaria parasitemia in an area of intense seasonal transmission. Acta Trop 54: 55-72.

Snow RW, Gilles HM (2002) The epidemiology of malaria in Essential Malariology. London: Arnold, a member of the Hodder Headline Group.

Snow RW, Marsh K (2002) The consequences of reducing transmission of *Plasmodium falciparum* in Africa. Adv Parasitol 52: 235-264.

Snow RW, Guerra CA, Noor AM, Myint HY, Hay SI (2005) The global distribution of clinical episodes of *Plasmodium falciparum* malaria. Nature 434(7030): 214-217.

Snow RW, Peshu N, Forster D, Bomu G, Mitsanze E et al. (1998) Environmental and entomological risk factors for the development of clinical malaria among children on the Kenyan coast. Trans R Soc Trop Med Hyg 92: 381-385.

Snow RW, Omumbo JA, Lowe B, Molyneaux CS, Obiero JO et al. (1997) Relation between severe malaria morbidity in children and level of *Plasmodium falciparum* transmission in Africa. Lancet 349: 1650-1654.

Soper FL, Wilson DB (1943) *Anopheles gambiae* in Brazil: 1930 to 1940. New York: The Rockefeller Foundation. 262 p.

Sousa CA, Pinto J, Almeida APG, Ferreira C, Do Rosario VE et al. (2001) Dogs as a favoured host choice of *Anopheles gambiae* sensu stricto (Diptera: Culicidae) of Sao Tome, West Africa. J Med Entomol 38(1): 122-125.

Staedke SG, Nottingham EW, Cox J, Kamya MR, Rosenthal PJ et al. (2003) Short report: proximity to mosquito breeding sites as a risk factor for clinical malaria episodes in an urban cohort of Ugandan children. Am J Trop Med Hyg 69(3): 244-246.

Stephens C, Masamu ET, Kiama MG, Keto AJ, Kinenekejo M et al. (1995) Knowledge of mosquitos in relation to public and domestic control activities in the cities of Dar es Salaam and Tanga. Bull World Health Organ 73(1): 97-104.

Surtees G (1970) Large-scale irrigation and Arbovirus epidemiology, Kano Plain, Kenya. I. Description of the area and preliminary studies on the mosquitoes. J Med Entomol 7(5): 509-517.

Sutherland DD, McNelly JJ, Hansen JA (1989) Evaluation of granular *Bacillus sphaericus* to control *Culex* in sewage treatments ponds in Cape May. New Jersey Mosqu Contr Assoc 76: 84-90.

Takken W (2002) Do insecticide-treated bednets have an effect on malaria vectors? Trop Med Int Health 7(12): 1022-1030.

Takken W, Charlwood JD, Billingsley PF, Gort G (1998) Dispersal and survival of *Anopheles funestus* and *A.gambiae* s.l. (Diptera: Culicidae) during the rainy season in southeast Tanzania. Bull Entomol Res 88: 561-566.

Thomas CJ, Lindsay SW (2000) Local-scale variation in malaria infection amongst rural Gambian children estimated by satellite remote sensing. Trans R Soc Trop Med Hyg 94: 159-163.

Thompson R, Begtrup K, Cuamba N, Dgedge M, Mendis C et al. (1997) The Matola malaria project: A temporal and spatial study of malaria transmission and disease in a suburban area of Maputo, Mozambique. Am J Trop Med Hyg 57(5): 550-559.

Trape JF (1987) Malaria and urbanization in Central Africa: the example of Brazzaville. Part IV: Parasitological and serological surveys in urban and surrounding areas. Trans R Soc Trop Med Hyg 81 (supplement 2): 26-33.

Trape JF, Zoulani A (1987) Malaria and urbanization in Central Africa: the example of Brazzaville. Part II: Results of entomological surveys and epidemiological analysis. Trans R Soc Trop Med Hyg 81 (Supplement 2): 10-18.

Trape JF, Zoulani A (1987) Malaria and urbanization in Central Africa: the example of Brazzaville. Part III: Relationship between urbanization and the intensity of malaria transmission. Trans R Soc Trop Med Hyg 81 (Supplement 2): 19-25.

Trape JF, Pison G, Spiegel A, Enel C, Rogier C (2002) Combating malaria in Africa. Trends Parasitol 18(5): 224-230.

Trape JF, Lefebvre-Zante E, Legros F, G. N, Bouganali H et al. (1992) Vector density gradients and the epidemiology of urban malaria in Dakar, Senegal. Am J Trop Med Hyg 47(2): 181-189.

UN (2004) World urbanization prospects: the 2003 revision. Data, tables and highlights (online). <http://www.un.org/esa/population/publications/wup2003/2003WUPHighlightspdf>.

Utzinger J, Tozan Y, Singer BH (2001) Efficacy and cost effectiveness of environmental management for malaria control. Trop Med Int Health 6(9): 677-687.

Utzinger J, Tozan Y, Doumani F, Singer BH (2002) The economic payoffs of integrated malaria control in the Zambian copperbelt between 1930 and 1950. Trop Med Int Health 7(8): 657-677.

Utzinger J, Tanner M, Kammen DM, Killeen GF, Singer BH (2002) Integrated programme is key to malaria control. Nature 419: 431.

van der Kolk M, Tebo AE, Nimpaye H, Ndombol DN, Sauerwein RW et al. (2003) Transmission of *Plasmodium falciparum* in urban Yaounde, Cameroon, is seasonal and age-dependent. Trans R Soc Trop Med Hyg 97(4): 375-379.

Vanek MJ, Shoo B, Mtasiwa D, Kiama M, Lindsay SW et al. (2006) Community-based surveillance of malaria vector larval habitats: a baseline study in urban Dar es Salaam, Tanzania. BMC Public Health 6: 154.

Walker K (2002) A review of control methods for African malaria vectors. Washington, DC: US Agency for International Development. 108 108. 54 p.

Wang SJ, Lengeler C, Smith TA, Vounatsou P, Cisse G et al. (2006) Rapid urban malaria appraisal (RUMA) III:Epidemiology of urban malaria in the municipality of Yopougon (Abidjan). Malar J 5(1): 28.

Wang SJ, Lengeler C, Smith TA, Vounatsou P, Akogbeto M et al. (2006) Rapid Urban Malaria Appraisal (RUMA) IV: epidemiology of urban malaria in Cotonou (Benin). Malar J 5: 45.

Wang SJ, Lengeler C, Mtasiwa D, Mshana T, Manane L et al. (2006) Rapid urban malaria appraisal (RUMA) II: Epidemiology of urban malaria in Dar es Salaam (Tanzania). Malar J 5(1): 29.

Wang SJ, Lengeler C, Smith TA, Vounatsou P, Diadie DA et al. (2005) Rapid urban malaria appraisal (RUMA) I: epidemiology of urban malaria in Ouagadougou. Malar J 4: 43.

Wang SJ, Lengeler C, Smith TA, Vounatsou P, Cisse G et al. (2005) Rapid urban malaria appraisal (RUMA) in sub-Saharan Africa. Malar J 4: 40.

Wanji S, Tanke T, Atanga SN, Ajonina C, Nicholas T et al. (2003) Anopheles species of the mount Cameroon region: biting habits, feeding behaviour and entomological inoculation rates. Trop Med Int Health 8(7): 643-649.

Watson M (1953) African highway: The battle for health in central Africa. London: John Murray. 294 p.

White GB (1974) *Anopheles gambiae* complex and disease transmission in Africa. Trans R Soc Trop Med Hyg 68(4): 279-301.

White NJ (2003) Malaria. In Manson's Tropical Diseases(21st edition. ed (GC Cook, & AI Zumla) Saunders): 1205-1295.

WHO (2004) Global Strategic Framework for Integrated Vector Management. Geneva: World Health Organization. 15 p.

WHO (2005) World Malaria Report. Available at http://rbmwhoint/wmr2005.

WHO RBM (2003) The Abuja declaration and the plan of action.

WHO/UNICEF (2003) The African Malaria Report 2003. Geneva: WHO/UNICEF. 120 p.

Yamagata Y (1996) Review of Tanzania-Japan Urban Malaria Control Project (UMCP) in Dar es Salaam and Tanga (1988-1996). Dar es Salaam, Tanzania: Japan International Cooperation Agency. 46 p.

Yapabandara AMGM, Curtis CF (2002) Laboratory and field comparisons of pyriproxifen, polystyrene beads and other larvicidal methods against malaria vectors in Sri Lanka. Acta Trop 81: 211-223.

Yapabandara AMGM, Curtis CF, Wickramasinghe MB, Fernando WP (2001) Control of malaria vectors with the insect growth regulator pyrproxyfen in a gem-mining area in Sri Lanka. Acta Trop 80: 265-276.

Ye-Ebiyo Y, Pollack RJ, Spielman A (2000) Enhanced development in nature of larval *Anopheles arabiensis* mosquitoes feeding on maize pollen. Am J Trop Med Hyg 63(1-2): 90-93.

Ye-Ebiyo Y, Pollack RJ, Kiszewski A, Spielman A (2003) Enhancement of development of larval *Anopheles arabiensis* by proximity to flowering maize (Zea mays) in turbid water and when crowded. Am J Trop Med Hyg 68(6): 748-752.

Yohannes M, Petros B (1996) Urban malaria in Nazareth, Ethiopia: parasitological studies. Ethiop Med J 34(2): 83-91.

Yohannes M, Haile M, Ghebreyesus TA, Witten KH, Getachew A et al. (2005) Can source reduction of mosquito larval habitat reduce malaria transmission in Tigray, Ethiopia? Trop Med Int Health 10(12): 1274-1285.

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.

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

This article has been published in:

Malaria Journal (2007), **6**(1): 126

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 km^2 [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 km^2 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].

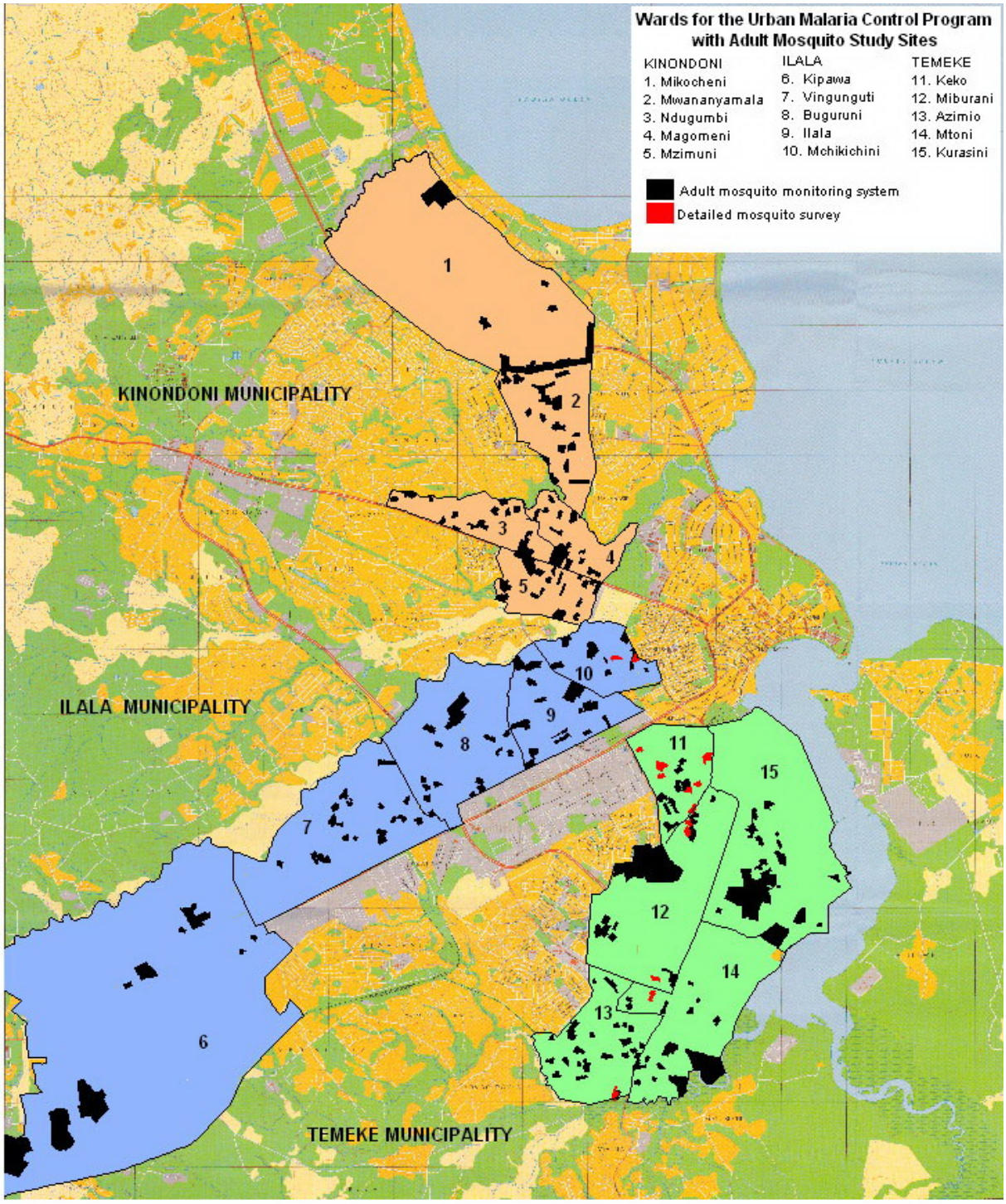


Figure 1. Wards included in the study area of the Urban Malaria Control Program in Dar es Salaam, showing the ten cell units (TCU) of the adult mosquito monitoring system as well as of the detailed survey.

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:

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

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:

$$B_u = \sum_{t=1}^{24} B_{u,t} \qquad \mathbf{2}$$

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:

$$B_p = \sum_{t=1}^{24} B_{p,t} = \sum_{t=1}^{24} [B_{o,t} (1-I_t) + B_{i,t} (I_t - S_t) + B_{i,t} S_t (1-P)] \quad \textbf{3}$$

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 (λ_p) as previously defined (See equations 8 and 14 in reference [95]), could be estimated. λ_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:

$$\lambda_p = B_p / B_u \quad \textbf{4}$$

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

$$P^* = 1 - \lambda_p \quad \textbf{5}$$

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 (π_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:

$$\pi_s = \sum_{t=10pm}^{6am} B_{u,t} / \sum_{t=1}^{24} B_{u,t} \quad \textbf{6}$$

Note that π_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, π_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 (π_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:

$$\pi_a = \sum_{t=1}^{24} [B_{i,t} (I_t - S_t)] \; / \sum_{t=1}^{24} [B_{o,t} (1-I_t) + B_{i,t} I_t] \qquad \mathbf{7}$$

The proportion of bites occurring indoors (π_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 π_a and π_s:

$$\pi_i = \pi_a + \pi_s = \sum_{t=1}^{24} [B_{i,t} I_t] \; / \sum_{t=1}^{24} [B_{o,t} (1-I_t) + B_{i,t} I_t] \qquad \mathbf{8}$$

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.

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

Table 2 Protection measures against mosquitoes in urban Dar es Salaam in the past and present, in rural Tanzania and in two Kenyan cities.

Location	Net usage		Net treatment status			Window screening					Other protection measures			
	%	N	In the last 6 months	More than 6 months	never	intact	Small holes	Big holes	Glass windows	none	Coil	repellent	spray	none
Urban Kenya and Tanzania														
Dar es Salaam; 2006[a]	78.8	1696	35.9	0.6	63.5	17.3	39.6	14.7	0.4	28	9.2	7.3	15.9	67.6
Dar es Salaam; 1994[34]	62										52	-	30	18
Kisumu, Kenya; 2001[99]	56	287					5			95	40	0.008	13	47
Malindi, Kenya; 2001 [99]	69	332					32			68	54	27	5	14
Rural Reference Site														
Kilombero, Valley Tanzania;2003 (Killeen et al. unpublished)	74.5	650	4.7	6.6	88.7								1.4[b]	98.6

[a] Data derived from the study presented here.
[b] 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 (π_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).

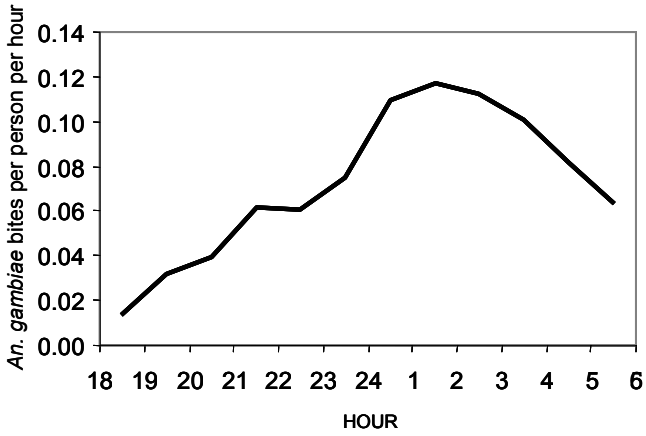


Figure 2. Hourly biting profile of *An. gambiae s.l.* based on averaged results of routine outdoor human landing catches from across the entire study area covered by the Urban Malaria Control Programme.

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.

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

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 *Mansonia*. 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 *Mansonia* 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.

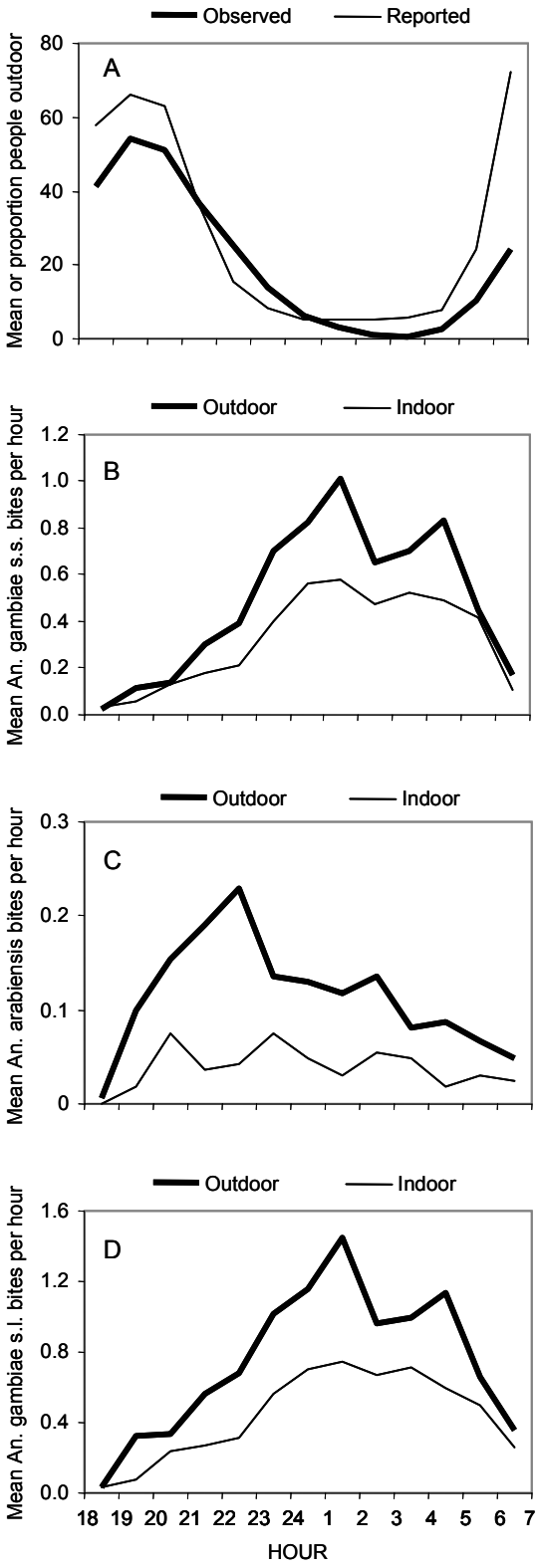


Figure 3. Human and mosquito behavioural patterns in Dar es Salaam, Tanzania. **A**. Number or proportion of time residents spend outdoors, comparing what they reported themselves with direct observations in the field. **B**. Mean numbers of *An. gambiae s.s* caught indoors and outdoors. **C**. Mean 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.

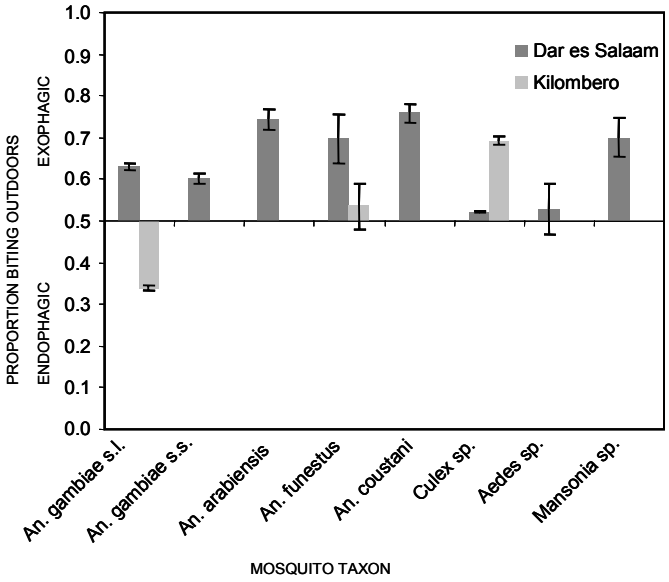


Figure 4. Comparison of exophagic and endophagic behaviour of different mosquito species in urban and rural Tanzania. Degree of exophagy or endophagy is presented as the proportion of mosquitoes caught outdoors so that all mosquitoes with a proportion of outdoor biting significantly greater than 0.5 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 (π_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 (π_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 (π_s; equation **6**).

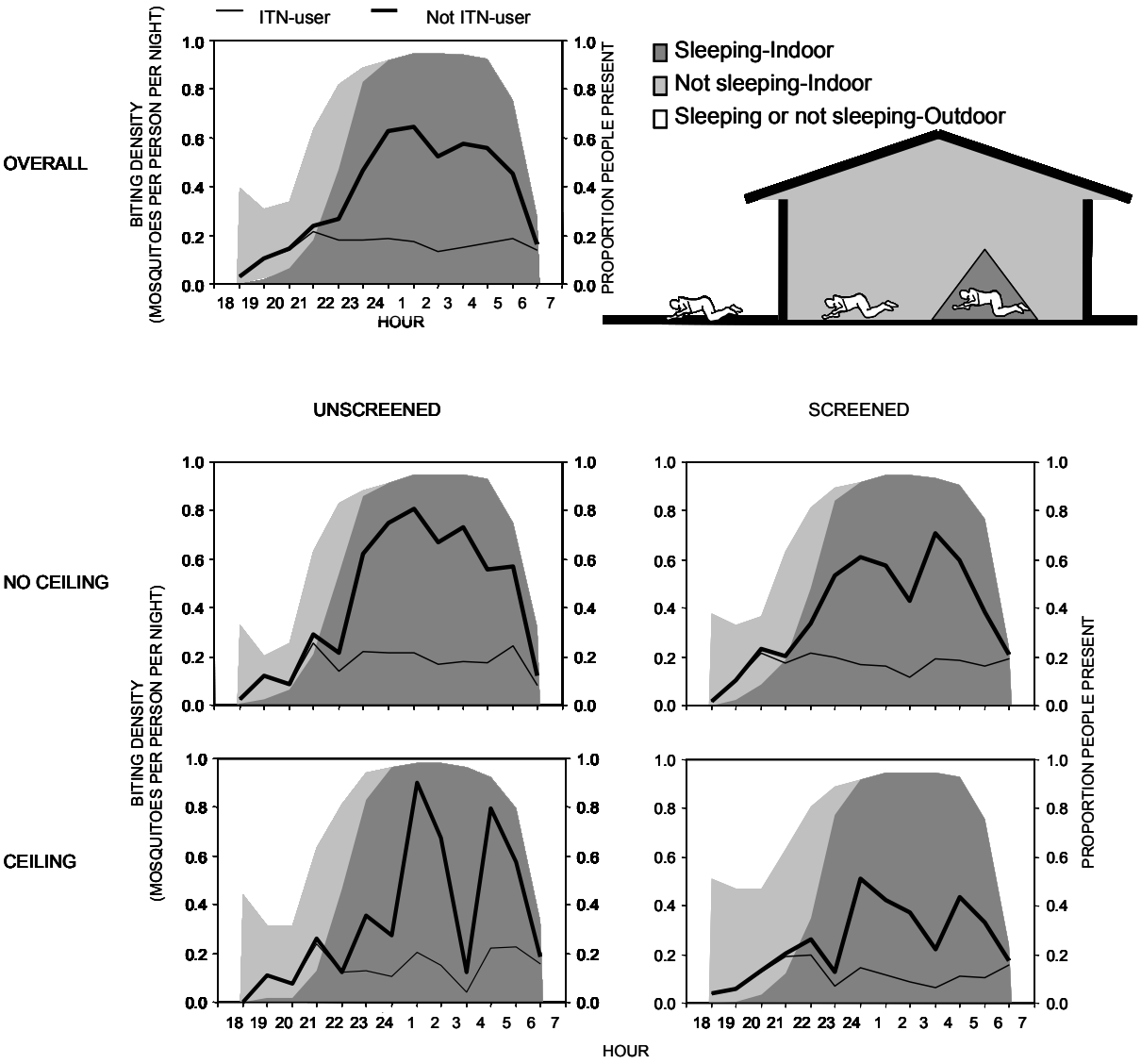


Figure 5. Exposure to biting of *An. gambiae s.s.* for ITN users and non-users. The shadings represent the proportion of time spend in each compartment (outdoor; $1-\pi_i$; equation **8**, indoor awake; π_a ; equation **7**, indoor asleep; π_s ; equation **6**). Exposure to biting is shown overall as 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).

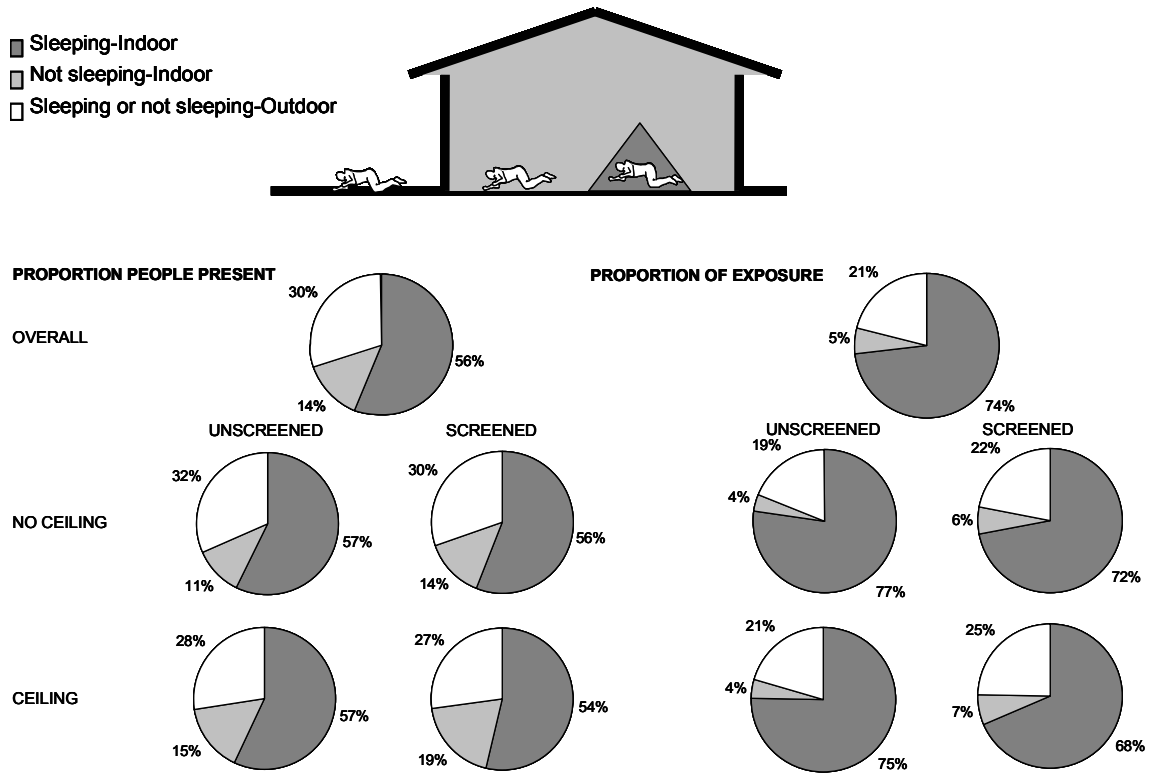


Figure 6. Proportion of people present in each compartment and their estimated exposure if not using a bednet (outdoor; $1-\pi_i$; equation **8**, indoor awake; π_a ; equation **7**, indoor asleep; π_s ; equation **6**), presented as an overall mean and for categories of different house qualities: Screened (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.

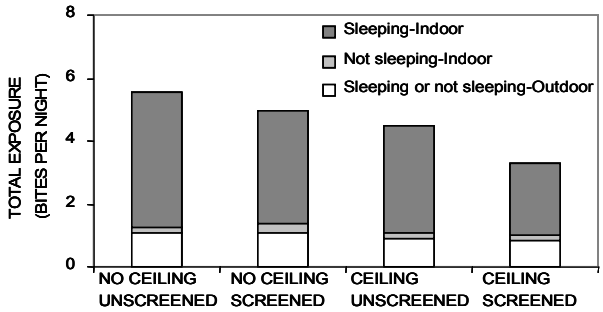


Figure 7. Mean number of bites received by a person in each of the three domestic and peri-domestic compartments (outdoor; $1-\pi_i$; equation **8**, indoor awake; π_a ; equation **7**, indoor asleep; π_s ; equation **6**).

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.

		Factor 1	Factor 2	Factor 3
	% of Variance	24.55	21.41	15.67
Scores	Complete ceiling[a]	- 0.477	0.646	0.039
	Good screening[b]	- 0.155	0.629	- 0.362
	Other personal protection[c]	- 0.528	0.392	0.374
	Bednet use	0.773	0.236	- 0.223
	ITN use	0.628	0.508	- 0.076
	Mean log (*An. gambiae s.l.*)	0.334	- 0.068	0.764
	Mean log Culex	0.289	0.463	0.430

[a] Complete and partly complete ceiling board
[b] Screening without holes, with small holes or glass windows
[c] Coils, spray and / or repellent

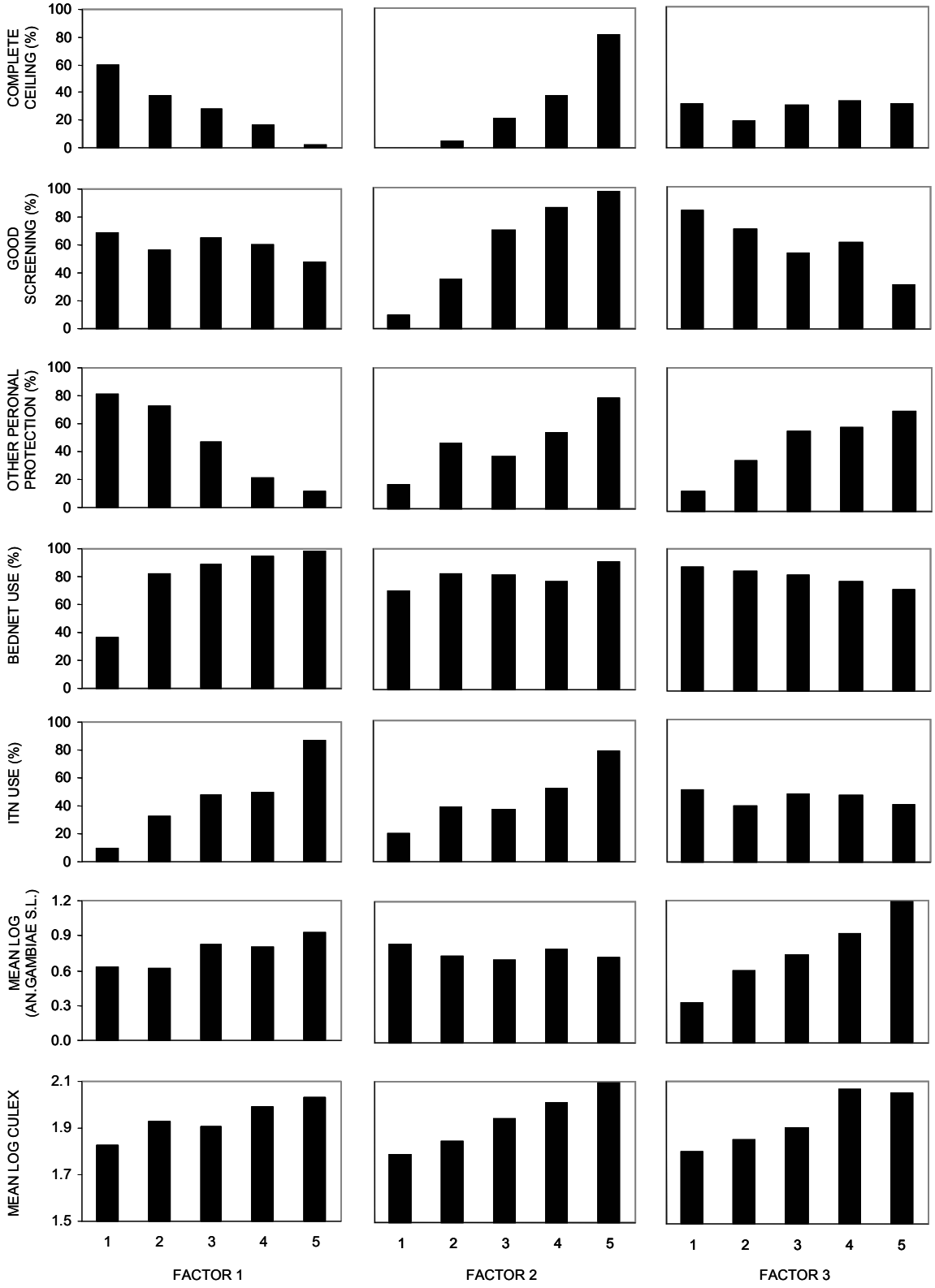


Figure 8. Three factors derived through principal component analysis and their association with different protective measures as well as mosquito densities.

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.

3.6 Acknowledgment

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 for their cooperation and facilitation during our regular visits. We thank A. Mtandanguo, A.

Mariwa, A. Hemedi and J.E. Msami in helping with the mosquito identification. We would also like to thank D.R. Nyika and C.B. Buberwa for drafting the map of the study area. This paper is published with kind permission of Dr. Andrew Kitua, Director of the National Institute for Medical Research, United Republic of Tanzania.

Research and ethical clearance were obtained from the Medical Research Coordination Committee of the National Institute for Medical Research through the Tanzanian commission for Science & Technology. This study was supported financially by the Swiss Tropical Institute, the Bill and Melinda Gates Foundation, Valent Biosciences and USAID through its Environmental Health Programme, the Tanzanian Mission at Dar es Salaam and the Presidents Malaria Initiative. GFK is supported by the Wellcome Trust through Research Career Development Fellowship number 076806.

3.7 References

1. WHO: **Global Strategic Framework for Integrated Vector Management**. Geneva: World Health Organization; 2004.
2. Snow RW, Guerra CA, Noor AM, Myint HY, Hay SI: **The global distribution of clinical episodes of *Plasmodium falciparum* malaria**. *Nature* 2005, **434**:214-217.
3. Hay SI, Guerra CA, Tatem TA, Noor AM, Snow RW: **The global distribution and population at risk of malaria: past, present and future**. *Lancet Infect Dis* 2004, **4**:327-336.
4. Roll Back Malaria Partnership: **World Malaria Report**. In. Geneva: World Health Organization; 2005.
5. Kiszewski A, Mellinger A, Spielman A, Malaney P, Sachs SE, Sachs J: **A global index representing the stability of malaria transmission**. *Am J Trop Med Hyg* 2004, **70**:486-498.
6. Robert V, MacIntyre K, Keating J, Trape JF, Duchemin JB, Warren M, Beier JC: **Malaria transmission in urban sub-Saharan Africa**. *Am J Trop Med Hyg* 2003, **68**:169-176.
7. Keiser J, Utzinger J, Castro MC, Smith TA, Tanner M, Singer BH: **Urbanization in sub-Saharan Africa and implication for malaria control**. *Am J Trop Med Hyg* 2004, **71**(2 Suppl):118-127.
8. Donnelly MJ, McCall PJ, Lengeler C, Bates I, D'Alessandro U, Barnish G, Konradsen F, Klinkenberg E, Townson H, Trape JF, Hastings IM, Mutero C: **Malaria and urbanization in sub-Saharan Africa**. *Malar J* 2005, **4**:12.
9. Wang SJ, Lengeler C, Smith TA, Vounatsou P, Cisse G, Diallo DA, Akogbeto M, Mtasiwa D, Teklehaimanot A, Tanner M: **Rapid urban malaria appraisal (RUMA) in sub-Saharan Africa**. *Malar J* 2005, **4**:40.

10. Hay SI, Guerra CA, Tatem AJ, Atkinson PM, Snow RW: **Urbanization, malaria transmission and disease burden in Africa.** *Nat Rev Microbiol* 2005, **3**:81-90.

11. Lines J, Harpham T, Leake C, Schofield C: **Trends, priorities and policy directions in the control of vector-borne diseases in urban environments**. *Health Policy Plann* 1994, **9**:113-129.

12. UN: **World urbanization prospects: the 2003 revision. Data, tables and highlights (online)**. *<http://www.un.org/esa/population/publications/wup2003/2003WUPHighlightspdf>* 2004.

13. Smith T, Maire N, Dietz K, Killeen GF, Vounatsou P, Molineaux L, Tanner M: **Relationship between the entomologic inoculation rate and the force of infection for *Plasmodium falciparum* malaria**. *Am J Trop Med Hyg* 2006, **75**(2 Suppl):11-18.

14. Ross A, Maire N, Molineaux L, Smith T: **An epidemiologic model of severe morbidity and mortality caused by *Plasmodium falciparum***. *Am J Trop Med Hyg* 2006, **75**(2 Suppl):63-73.

15. Ross A, Killeen G, Smith T: **Relationships between host infectivity to mosquitoes and asexual parasite density in *Plasmodium falciparum***. *Am J Trop Med Hyg* 2006, **75**(2 Suppl):32-37.

16. Trape JF, Pison G, Spiegel A, Enel C, Rogier C: **Combating malaria in Africa**. *Trends Parasitol* 2002, **18**:224-230.

17. Trape JF, Zoulani A: **Malaria and urbanization in Central Africa: the example of Brazzaville. Part III: Relationship between urbanization and the intensity of malaria transmission.** *Trans R Soc Trop Med Hyg* 1987, **81 (Supplement 2)**:19-25.

18. Trape JF, Zoulani A: **Malaria and urbanization in Central Africa: the example of Brazzaville. Part II: Results of entomological surveys and epidemiological analysis**. *Trans R Soc Trop Med Hyg* 1987, **81 (Supplement 2)**:10-18.

19. Trape JF, Lefebvre-Zante E, Legros F, G. N, Bouganali H, Druilhe P, Salem G: **Vector density gradients and the epidemiology of urban malaria in Dakar, Senegal**. *Am J Trop Med Hyg* 1992, **47**:181-189.

20. Eisele TP, Keating J, Swalm C, Mbogo CM, Githeko AK, Regens JL, Githure JI, Andrews L, Beier JC: **Linking field-based ecological data with remotely sensed data using a geographic information system in two malaria endemic urban areas of Kenya**. *Malar J* 2003, **2**:44.

21. Castro MC, Yamagata Y, Mtasiwa D, Tanner M, Utzinger J, Keiser J, Singer BH: **Integrated urban malaria control: a case study in Dar es Salaam, Tanzania**. *Am J Trop Med Hyg* 2004, **71 (Supplement 2)**:103-117.

22. Thompson R, Begtrup K, Cuamba N, Dgedge M, Mendis C, Gamage-Mendis A, Enosse SM, Barreto J, Sinden RE, Hogh B: **The Matola malaria project: A temporal and spatial study of malaria transmission and disease in a suburban area of Maputo, Mozambique**. *Am J Trop Med Hyg* 1997, **57**:550-559.

23. Staedke SG, Nottingham EW, Cox J, Kamya MR, Rosenthal PJ, Dorsey G: **Short report: proximity to mosquito breeding sites as a risk factor for clinical malaria episodes in an urban cohort of Ugandan children**. *Am J Trop Med Hyg* 2003, **69**:244-246.

24. Trape JF: **Malaria and urbanization in Central Africa: the example of Brazzaville. Part IV: Parasitological and serological surveys in urban and surrounding areas**. *Trans R Soc Trop Med Hyg* 1987, **81 (supplement 2)**:26-33.

25. Minakawa N, Seda P, Yan G: **Influence of host and larval habitat distribution on the abundance of African malaria vectors in Western Kenya**. *Am J Trop Med Hyg* 2002, **67**:32-38.

26. Service MW: **Mosquito (Diptera: Culicidae) dispersal--the long and short of it**. *J Med Entomol* 1997, **34**:579-588.

27. Killeen GF, Knols BG, Gu W: **Taking malaria transmission out of the bottle: implications of mosquito dispersal for vector-control interventions**. *Lancet Infect Dis* 2003, **3**:297-303.

28. Le Menach A, McKenzie FE, Flahault A, Smith DL: **The unexpected importance of mosquito oviposition behaviour for malaria: non-productive larval habitats can be sources for malaria transmission**. *Malar J* 2005, **4**:23.

29. Smith DL, Dushoff J, McKenzie FE: **The risk of a mosquito-borne infection in a heterogeneous environment**. *PLoS Biol* 2004, **2**:e368.

30. Stephens C, Masamu ET, Kiama MG, Keto AJ, Kinenekejo M, Ichimori K, Lines J: **Knowledge of mosquitos in relation to public and domestic control activities in the cities of Dar es Salaam and Tanga**. *Bull World Health Organ* 1995, **73**:97-104.

31. Curtis C, Maxwell C, Lemnge M, Kilama WL, Steketee RW, Hawley WA, Bergevin Y, Campbell CC, Sachs J, Teklehaimanot A, Ochola S, Guyatt H, Snow RW: **Scaling-up coverage with insecticide-treated nets against malaria in Africa: who should pay?** *Lancet Infect Dis* 2003, **3**:304-307.

32. Lines J, Lengeler C, Cham K, de Savigny D, Chimumbwa J, Langi P, Carroll D, Mills A, Hanson K, Webster J, Lynch M, Addington W, Hill J, Rowland M, Worrall E, MacDonald M, Kilian A: **Scaling-up and sustaining insecticide-treated net coverage**. *Lancet Infect Dis* 2003, **3**:465-468.

33. Wang SJ, Lengeler C, Mtasiwa D, Mshana T, Manane L, Maro G, Tanner M: **Rapid urban malaria appraisal (RUMA) II: Epidemiology of urban malaria in Dar es Salaam (Tanzania)**. *Malar J* 2006, **5**:29.

34. Evans PJ: **Community knowledge, attitudes and practices-urban mosquitoes and sustainable mosquito control**. *Degree of Doctor of Philosophy in Geography.* University of Exeter; 1994.

35. Wang SJ, Lengeler C, Smith TA, Vounatsou P, Akogbeto M, Tanner M: **Rapid Urban Malaria Appraisal (RUMA) IV: epidemiology of urban malaria in Cotonou (Benin)**. *Malar J* 2006, **5**:45.

36. Pates H, Curtis C: **Mosquito behavior and vector control**. *Annu Rev Entomol* 2005, **50**:53-70.

37. Carnevale P, Robert V, Boudin C, Halna JM, Pazart L, Gazin P, Richard A, Mouchet J: **La lutte contre le paludisme par des moustiquaires imprégnées de pyréthroides au Burkina Faso**. *Bull Soc Pathol Exot Filiales* 1988, **81**:832-846.

38. Curtis CF, Maxwell CA, Finch RJ, Njunwa KJ: **A comparison of use of a pyrethroid either for house spraying or for bednet treatment against malaria vectors**. *Trop Med Int Health* 1998, **3**:619-631.

39. Karch S, Garin B, Asidi N, Manzambi Z, Salaun JJ, Mouchet J: **Mosquito nets impregnated against malaria in Zaire**. *Ann Soc Belg Med Trop* 1993, **73**:37-53.

40. Jaenson TG, Gomes MJ, Barreto dos Santos RC, Petrarca V, Fortini D, Evora J, Crato J: **Control of endophagic Anopheles mosquitoes and human malaria in Guinea Bissau, West Africa by permethrin-treated bed nets**. *Trans R Soc Trop Med Hyg* 1994, **88**:620-624.

41. Knols BGJ, Takken W: **The widescale use of impregnated bednets for malaria control in Africa: impact on mosquitoes.** *Proc Exp Appl Entomol* 1998, **8**:15-20.

42. Magesa SM, Wilkes TJ, Mnzava AEP, Njunwa KJ, Myamba J, Kivuyo MDP, Hill N, Lines JD, Curtis CF: **Trial of pyrethroid impregnated bednets in an area of Tanzania holoendemic for malaria. Part 2 Effects on the malaria vector population**. *Acta Trop* 1991, **49**:97-108.

43. Maxwell CA, Chambo W, Mwaimu M, Magogo F, Carneiro IA, Curtis CF: **Variation of malaria transmission and morbidity with altitude in Tanzania and with introduction of alphacypermethrin treated nets**. *Malar J* 2003, **2**:28.

44. Maxwell CA, Msuya E, Sudi M, Njunwa KJ, Carneiro IA, Curtis CF: **Effect of community-wide use of insecticide-treated nets for 3-4 years on malarial morbidity in Tanzania**. *Trop Med Int Health* 2002, **7**:1003-1008.

45. Maxwell CA, Myamba J, Njunwa KJ, Greenwood BM, Curtis CF: **Comparison of bednets impregnated with different pyrethroids for their impact on mosquitoes and on re-infection with malaria after clearance of pre-existing infections with chlorproguanil-dapsone**. *Trans R Soc Trop Med Hyg* 1999, **93**:4-11.

46. Mbogo CNM, Baya NM, Ofulla AVO, Githure JI, Snow RW: **The impact of permethrin-impregnated bednets on malaria vectors of the Kenyan coast**. *Med Vet Entomol* 1996, **10**:251-259.

47. Njau RJA, Mosha FW, Nguma JFM: **Field trials of pyrethroid impregnated bednets in northern Tanzania. 1.Effects in malaria transmission**. *Insect Sci Appl* 1993, **5**:575-584.

48. Bogh C, Pedersen EM, Mukoko DA, Ouma JH: **Permethrin-impregnated bed net effects on resting and feeding behaviour of lymphatic filariasis vector mosquitoes in Kenya**. *Med Vet Entomol* 1998, **12**:52-59.

49. Lines JD, Myamba J, Curtis CF: **Experimental hut trials of permethrin-impregnated mosquito nets and eave curtains against malaria vectors in Tanzania**. *Med Vet Entomol* 1987, **1**:37-51.

50. Chinery WA: **Effects of ecological changes on the malaria vectors *Anopheles funestus* and *Anopheles gambiae* complex mosquitoes in Accra, Ghana**. *J Trop Meg Hyg* 1984, **87**:75-81.

51. Sattler MA, Mtasiwa D, Kiama M, Premji Z, Tanner M, Killeen GF, Lengeler C: **Habitat characterization and spatial distribution of Anopheles sp. mosquito larvae in Dar es Salaam (Tanzania) during an extended dry period**. *Malar J* 2005, **4**:4.

52. Vanek MJ, Shoo B, Mtasiwa D, Kiama M, Lindsay SW, Fillinger U, Kannady K, Tanner M, Killeen GF: **Community-based surveillance of malaria vector larval habitats: a baseline study in urban Dar es Salaam, Tanzania**. *BMC Public Health* 2006, **6**:154.

53. Keating J, MacIntyre K, Mbogo C, Githeko A, Regens JL, Swalm C, Ndenga B, Steinberg LJ, Kibe L, Githure JI, Beier JC: **A geographic sampling strategy for studying relationships between human activity and malaria vectors in urban Africa**. *Am J Trop Med Hyg* 2003, **68**:357-365.

54. Jacob B, Regens JL, Mbogo CM, Githeko AK, Keating J, Swalm CM, Gunter JT, Githure JI, Beier JC: **Occurrence and distribution of Anopheles (Diptera: Culicidae) larval habitats on land cover change sites in urban Kisumu and urban Malindi, Kenya**. *J Med Entomol* 2003, **40**:777-784.

55. Keating J, Macintyre K, Mbogo CM, Githure JI, Beier JC: **Characterization of potential larval habitats for Anopheles mosquitoes in relation to urban land-use in Malindi, Kenya**. *Int J Health Geogr* 2004, **3**:9.

56. Trape JF: **Malaria and urbanization in Central Africa: the example of Brazzaville. Part I: Description of the town and previous surveys**. *Trans R Soc Trop Med Hyg* 1987, **81 (Supplement 2)**:1-9.

57. Minakawa N, Mutero CM, Githure JI, Beier JC, Yan G: **Spatial distribution and habitat characterization of Anopheline mosquito larvae in Western Kenya**. *Am J Trop Med Hyg* 1999, **61**:1010-1016.

58. Roll Back Malaria Partnership: **Scaling up insecticide treated netting programmes in Africa: a strategic framework for coordinated national action.** In: *World Health Organization, Geneva.* Geneva: World Health Organization; 2005.

59. Lengeler C: **Insecticide-treated bed nets and curtains for preventing malaria**. *Cochrane Database Syst Rev* 2004:CD000363.

60. Lindsay SW, Jawara M, Paine K, Pinder M, Walraven GE, Emerson PM: **Changes in house design reduce exposure to malaria mosquitoes**. *Trop Med Int Health* 2003, **8**:512-517.

61. Lindsay SW, Emerson PM, Charlwood JD: **Reducing malaria transmission by mosquito-proofing homes**. *Trends Parasitol* 2002, **18**:510-514.

62. Rozendaal JA: **Vector Control. Methods for use by individuals and communities**. Geneva: WHO; 1997.

63. Rowland M, Freeman T, Downey G, Hadi A, Saeed M: **DEET mosquito repellent sold through social marketing provides personal protection against malaria in an area of all-night mosquito biting and partial coverage of insecticide-treated nets: a case-control study of effectiveness**. *Trop Med Int Health* 2004, **9**:343-350.

64. Snow RW, Peshu N, Forster D, Bomu G, Mitsanze E, Ngumbao E, Chisengwa R, Armstrong Schellenberg JRM, Hayes RJ, Newbold CI, Marsh K: **Environmental and entomological risk factors for the development of clinical malaria among children on the Kenyan coast**. *Trans R Soc Trop Med Hyg* 1998, **92**:381-385.

65. Koram KA, Bennett S, Adiamah JH, Greenwood BM: **Socio-economic risk factors for malaria in a peri-urban area of The Gambia**. *Trans R Soc Trop Med Hyg* 1995, **89**:146-150.

66. Muirhead-Thomson RC: **The significance of irritability, behaviouristic avoidance and allied phenomena in malaria eradication**. *Bull World Health Organ* 1960, **22**:721-734.

67. Muirhead-Thomson RC: **The winter activities of *An. gambiae* at high altitudes in Southern Rhodesia.** *unpublished WHO working document* 1960.

68. Faye O, Konate L, Gaye O, Fontenille D, Sy N, Diop A, Diagne M, Molez JF: **Impact of the use of permethrin pre-impregnated mosquito nets on malaria transmission in a hyperendemic village of Senegal**. *Med Trop (Mars)* 1998, **58**:355-360.

69. Cuzin-Ouattara N, Van den Broek AHA, Habluetzel A: **Wide-scale installation of insecticide-treated curtains confers high levels of protection against malaria transmission in a hyperendemic area of Burkina Faso.** *Trans R Soc Trop Med Hyg* 1999, **93**:473-479.

70. Ilboudo-Sanogo E, Cuzin-Ouattara N, Diallo DA, Cousens SN, Esposito F, Habluetzel A, Sanon S, Ouedraogo AP: **Insecticide-treated materials, mosquito adaptation and mass effect: entomological observations after five years of vector control in Burkina Faso**. *Trans R Soc Trop Med Hyg* 2001, **95**:353-360.

71. Takken W: **Do insecticide-treated bednets have an effect on malaria vectors?** *Trop Med Int Health* 2002, **7**:1022-1030.

72. Lindsay SW, Adiamah JH, Miller JE, Armstrong JRM: **Pyrethroid-treated bednet effects on mosquitoes of the *Anopheles gambiae* complex**. *Med Vet Entomol* 1991, **5**:477-483.

73. Miller JE, Lindsay SW, Armstrong JRM: **Experimental hut trials of bednet impregnated with synthetic pyrethroid and organophosphate insecticides for mosquito control in The Gambia**. *Med Vet Entomol* 1991, **5**:465-476.

74. Lindsay SW, Adiamah JH, Armstrong JRM: **The effect of permethrin-impregnated bed nets on house entry by mosquitoes in The Gambia**. *Bull Entomol Res* 1992, **82**:49-55.

75. Lindsay SW, Snow RW: **The trouble with eaves: house entry by vectors of malaria**. *Trans R Soc Trop Med Hyg* 1988, **82**:645-646

76. Adiamah JH, Koram KA, Thomson MC, Lindsay SW, Todd SJ, Greenwood BM: **Entomological risk factors for severe malaria in a peri-urban area of The Gambia**. *Ann Trop Med Parasitol* 1993, **87**:491-500.

77. Lindsay SW, Armstrong Schellenberg JRM, Zeiler HA, Daly RJ, Salum FM, Wilkins HA: **Exposure of Gambian children to *Anopheles gambiae* vectors in an irrigated rice production area**. *Med Vet Entomol* 1995, **9**:50-58.

78. Lindsay SW, Campbell H, Adiamah JH, Greenwood AM, Bangali JE, Greenwood BM: **Malaria in a periurban area of The Gambia**. *Ann Trop Med Parasitol* 1990, **84**:553-562.

79. Braimah N, Drakeley C, Kweka E, Mosha FW, Helinski M, Pates H, Maxwell CA, Massawe T, Kenward MG, Curtis C: **Tests of bednet traps (Mbita traps) for monitoring mosquito populations and time of biting in Tanzania and possible impact of prolonged ITN use.** *Int J Trop Insect Sci* 2005, **25**:208-213.

80. Oyewole IO, Awolola TS: **Impact of urbanization on bionomics and distribution of malaria vectors in Lagos, southwestern Nigeria**. *J Vector Borne Dis* 2006, **43**:173-178.

81. Mukabana WR, Kannady K, Kiama GM, Ijumba JN, Mathenge EM, Kiche I, Nkwengulila G, Mboera L, Mtasiwa D, Yamagata Y, van Schayk I, Knols BG, Lindsay SW, Caldas de Castro M, Mshinda H, Tanner M, Fillinger U, Killeen GF: **Ecologists can enable communities to implement malaria vector control in Africa**. *Malar J* 2006, **5**:9.

82. Castro MC, Singer B: **Migration, Urbanization and Malaria: A Comparative Analysis of Dar es Salaam, Tanzania and Machadinho, Rondônia, Brazil**. *Paper prepared for Conference on African Migration in Comparative Perspective Johnnesburg, South Africa* 2003.

83. Craig MH, Snow RW, le Sueur D: **A climate-based distribution model of malaria transmssion in sub-Saharan Africa**. *Parasitol Today* 1999, **15**:105-111.

84. Statistics NBo: **The 2002 population and housing census general report**. *http://www.tanzania.go.tz/census/dsm.htm* 2003.

85. Killeen GF, Kihonda J, Lyimo E, Oketch FR, Kotas ME, Mathenge E, Schellenberg JA, Lengeler C, Smith TA, Drakeley CJ: **Quantifying behavioural interactions between humans and mosquitoes: Evaluating the protective efficacy of insecticidal nets against malaria transmission in rural Tanzania**. *BMC Infect Dis* 2006, **6**:161.

86. Service MW: **A critical review of procedures for sampling populations of adult mosquitoes**. *Bull Entomol Res* 1977, **67**:343-382.

87. Gillies MT, DeMeillon B: **The Anophelinae of Africa South of the Sahara (Ethiopian zoogeographical region)**. Johannesburg: South African Institute for Medical Research; 1968.

88. Gillies MT, Coetzee M: **A supplement to the Anophelinae of Africa South of the Sahara (Afrotropical region)**. Johannesburg: South African Medical Research Institute; 1987.

89. Scott JA, Brogdon WG, Collins FH: **Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction**. *Am J Trop Med Hyg* 1993, **49**:520-529.

90. Burkot TR, Williams JL, Schneider I: **Identification of *Plasmodium falciparum*-infected mosquitoes by a double antibody enzyme-linked immunosorbent assay**. *Am J Trop Med Hyg* 1984, **33**:783-788.

91. Mukabana WR, Takken W, Knols BGJ: **Host-specific cues cause differential attractiveness of Kenyan men to the African malaria vector *Anopheles gambiae***. *Malar J* 2002, **1**:17.

92. Knols BG, de Jong R, Takken W: **Differential attractiveness of isolated humans to mosquitoes in Tanzania**. *Trans R Soc Trop Med Hyg* 1995, **89**:604-606.

93. Lindsay SW, Adiamah JH, Miller JE, Pleass RJ, Armstrong JRM: **Variation in the attractiveness of human subjects to malaria mosquitoes (Diptera: Culicidae) in The Gambia**. *J Med Entomol* 1993, **30**:368-373.

94. MacDonald G: **The epidemiology and control of malaria**. London: Oxford University Press; 1957.

95. Killeen GF, Smith TA: **Exploring the contributions of bednets, cattle, insecticides and excito-repellency to malaria control: A deterministic model of mosquito host-seeking behaviour and mortality.** *Trans R Soc Trop Med Hyg* 2007, **In press**.

96. Smith DL, McKenzie FE: **Statics and dynamics of malaria infection in Anopheles mosquitoes**. *Malar J* 2004, **3**:13.

97. Asidi AN, N'Guessan R, Koffi AA, Curtis CF, Hougard JM, Chandre F, Corbel V, Darriet F, Zaim M, Rowland MW: **Experimental hut evaluation of bednets treated with an organophosphate (chlorpyrifos-methyl) or a pyrethroid (lambdacyhalothrin) alone and in combination against insecticide-resistant *Anopheles gambiae* and *Culex quinquefasciatus* mosquitoes**. *Malar J* 2005, **4**:25.

98. Graham K, Kayedi MH, Maxwell C, Kaur H, Rehman H, Malima R, Curtis CF, Lines JD, Rowland MW: **Multicountry field trials comparing wash-resistance of PermaNet and conventional insecticide-treated nets against anopheline and culicine mosquitoes.** *Med Vet Entomol* 2005, **19**:72-83.

99. MacIntyre K, Keating J, Sosler S, Kibe L, Mbogo CM, Githeko A, Beier JC: **Examining the determinants of mosquito avoidance practcies in two Kenyan cities**. *Malaria J* 2002, **1**:14.

100. Roberts DR, Alecrim WD, Hshieh P, Grieco JP, Bangs M, Andre RG, Chareonviriphap T: **A probability model of vector behavior: effects of DDT repellency, irritancy, and toxicity in malaria control**. *J Vector Ecol* 2000, **25**:48-61.

101. Kulkarni MA, Kweka E, Nyale E, Lyatuu E, Mosha FW, Chandramohan D, Rau ME, Drakeley C: **Entomological evaluation of malaria vectors at different altitudes in Hai district, northeastern Tanzania**. *J Med Entomol* 2006, **43**:580-588.

102. Schellenberg JR, Abdulla S, Nathan R, Mukasa O, Marchant TJ, Kikumbih N, Mushi AK, Mponda H, Minja H, Mshinda H, Tanner M, Lengeler C: **Effect of large-scale social marketing of insecticide-treated nets on child survival in rural Tanzania**. *Lancet* 2001, **357**:1241-1247.

103. Abdulla S, Schellenberg JA, Nathan R, Mukasa O, Marchant T, Smith T, Tanner M, Lengeler C: **Impact on malaria morbidity of a programme supplying insecticide treated nets in children aged under 2 years in Tanzania: community cross sectional study**. *BMJ* 2001, **322**:270-273.

104. White GB: ***Anopheles gambiae*** **complex and disease transmission in Africa**. *Trans R Soc Trop Med Hyg* 1974, **68**:279-301.

105. Lindsay SW, Parson L, Thomas CJ: **Mapping the ranges and relative abundance of the two principle African malaria vectors, *Anopheles gambiae sensu stricto* and *An. arabiensis*, using climate data**. *Proc R Soc London Series B* 1998, **265**:847-854.

106. Gray EM, Bradley TJ: **Physiology of desiccation resistance in *Anopheles gambiae* and *Anopheles arabiensis***. *Am J Trop Med Hyg* 2005, **73**:553-559.

107. Charlwood JD, Pinto J, Ferrara PR, Sousa CA, Ferreira C, Gil V, Do Rosario VE: **Raised houses reduce mosquito bites**. *Malar J* 2003, **2**:45.

108. Laganier R, Randimby FM, Rajaonarivelo V, Robert V: **Is the Mbita trap a reliable tool for evaluating the density of anopheline vectors in the highlands of Madagascar?** *Malar J* 2003, **2**:42.

109. Wanji S, Tanke T, Atanga SN, Ajonina C, Nicholas T, Fontenille D: **Anopheles species of the mount Cameroon region: biting habits, feeding behaviour and entomological inoculation rates**. *Trop Med Int Health* 2003, **8**:643-649.

110. Shililu J, Ghebremeskel T, Seulu F, Mengistu S, Fekadu H, Zerom M, Asmelash GE, Sintasath D, Mbogo C, Githure J, Brantly E, Beier JC, Novak RJ: **Seasonal abundance, vector behavior, and malaria parasite transmission in Eritrea**. *J Am Mosq Control Assoc* 2004, **20**:155-164.

111. Afolabi BM, Amajoh CN, Adewole TA, Salako LA: **Seasonal and temporal variations in the population and biting habit of mosquitoes on the Atlantic coast of Lagos, Nigeria**. *Med Princ Pract* 2006, **15**:200-208.

112. Lengeler C: **Insecticide-treated nets for malaria control: real gains**. *Bull World Health Organ* 2004, **82**:84.

113. Hawley WA, Phillips-Howard PA, ter Kuile FO, Terlouw DJ, Vulule JM, Ombok M, Nahlen BL, Gimnig JE, Kariuki SK, Kolczak MS, Hightower AW : **Community-wide effects of permethrin-treated bednets on child mortality and malaria morbidity in western Kenya**. *Am J Trop Med Hyg* 2003, **68 (Supplement 4)**:121-127.

114. Le Menach A, Takala S, McKenzie FE, Perisse A, Harris A, Flahault A, Smith DL: **An elaborated feeding cycle model for reductions in vectorial capacity of night-biting mosquitoes by insecticide-treated nets**. *Malar J* 2007, **6**:10.

115. Fillinger U, Lindsay SW: **Suppression of exposure to malaria vectors by an order of magnitude using microbial larvicides in rural Kenya**. *Trop Med Int Health* 2006, **11**:1629-1642.

116. Killeen GF, Fillinger U, Kiche I, Gouagna LC, Knols BGJ: **Eradication of *Anopheles gambiae* from Brazil: lessons for malaria control in Africa?** *Lancet Infect Dis* 2002, **2**:618-627.

117. Keiser J, Singer BH, Utzinger J: **Reducing the burden of malaria in different eco-epidemiological settings with environmental management: a systematic review**. *Lancet Infect Dis* 2005, **5**:695-708.

118. Utzinger J, Tanner M, Kammen DM, Killeen GF, Singer BH: **Integrated programme is key to malaria control**. *Nature* 2002, **419**:431.

119. Utzinger J, Tozan Y, Singer BH: **Efficacy and cost effectiveness of environmental management for malaria control**. *Trop Med Int Health* 2001, **6**:677-687.

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 Fillinger[1*], Khadija Kannady[2], George William[2], Michael J. Vanek[3], Stefan Dongus[3,4], Dickson Nyika[5,6], Yvonne Geissbühler[2,3,5], Prosper P. Chaki[1,2,5], Nico J. Govella[1,2,5] Evan M. Mathenge[7], Burton H. Singer[8], Hassan Mshinda[5], Steven W. Lindsay[1], Marcel Tanner[3], Deo Mtasiwa[2], Marcia C. de Castro[9] and Gerry F. Killeen[1,3,5]

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

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

This article has been published in:

Malaria Journal (2008), **7**(1): 20

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 km^2 [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 m*itaa* (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 malariological situations as possible. In total an area of 55 km^2 is covered with wards ranging from 0.96 to 15 km^2 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.

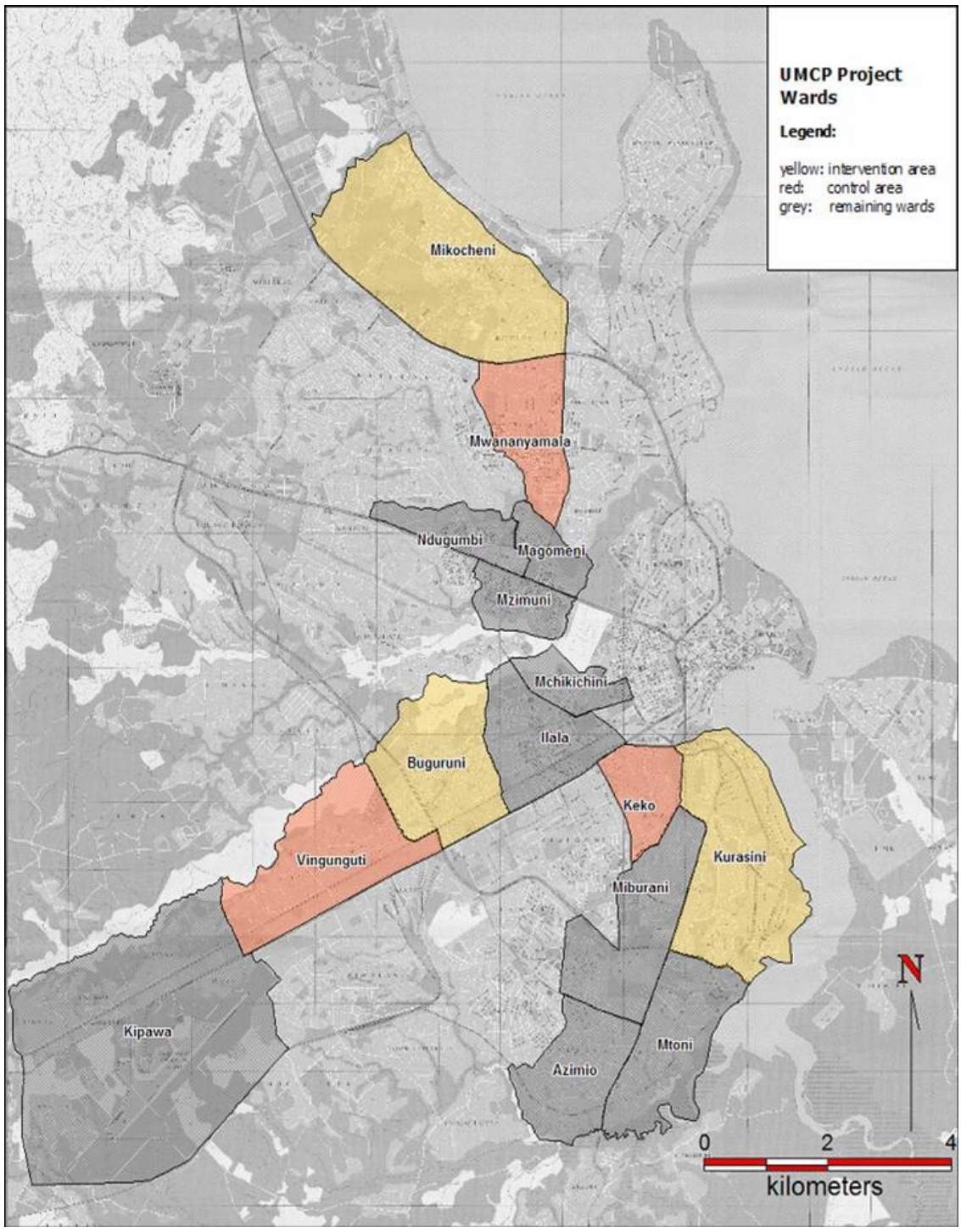


Figure 1. Wards included in the study area of the Dar es Salaam Urban Malaria Control Programme (UMCP), specifying those targeted for larviciding from March 2006 onwards (intervention), those considered to be the most comparable control (non-intervention wards) and those remaining.

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 allow 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 (mitaa) 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]

Rapid response	*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].
Community-based implementation	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].
Decentralization	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.
Comprehensive coverage	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].
Rigorous vertical management	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.
Adult mosquito densities as a priority performance indicator	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.
Separation of surveillance and treatment responsibilities	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]
Integration with existing infrastructure and governance mechanisms	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].
Full time staff	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.
Satisfactory evidence must precede scale up.	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.

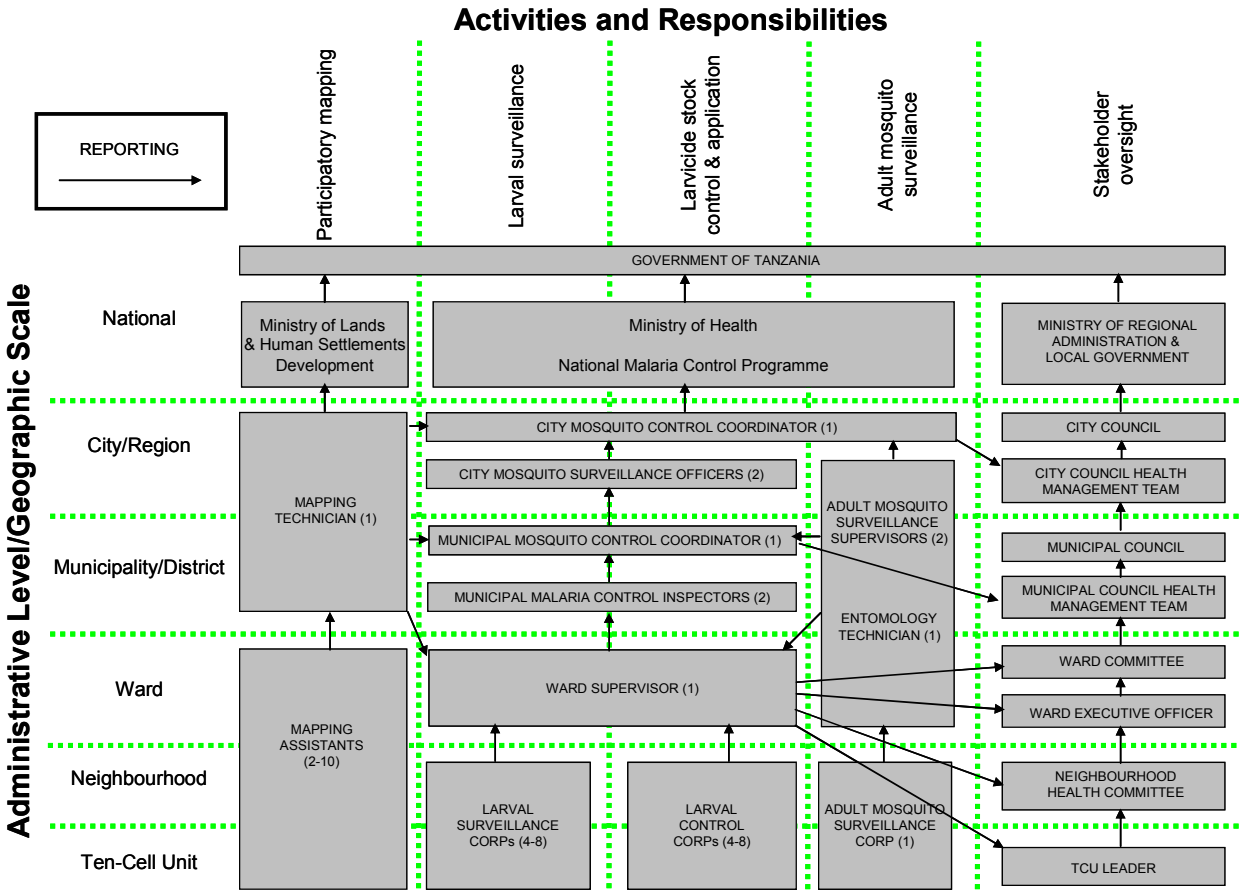


Figure 2. Reporting structure of the UMCP, presented as a matrix of activities which are hierarchically layered over a range of spatial and administrative scales. The numbers presented in brackets describe the number of personnel assigned to each post in each administrative subunit rather than level (e.g. 2 municipal inspectors at each of 3 municipalities means that a total of 6 should be working for the programme at any time).

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.

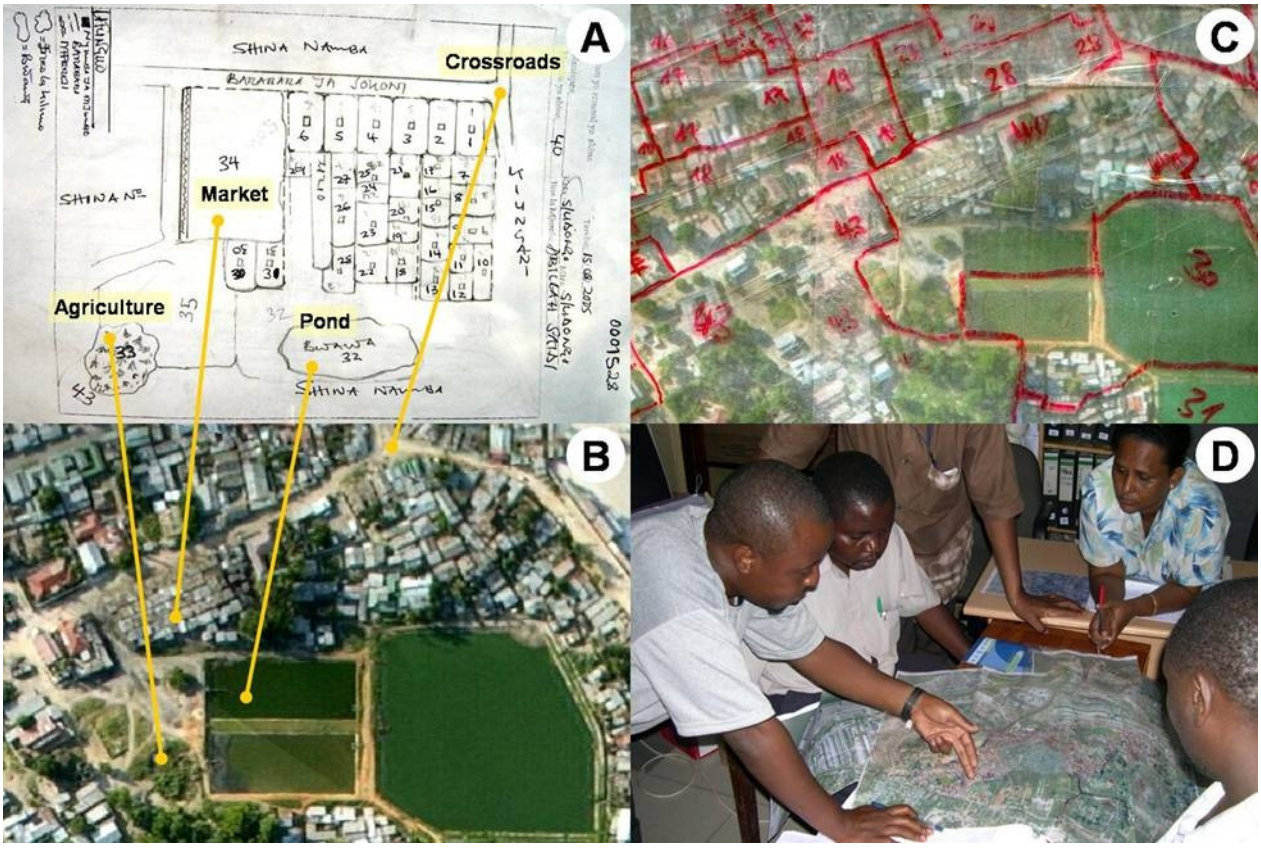


Figure 3. Example of a sketch map, aerial picture and field map.

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 (3^{rd} or 4^{th} 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 (1^{st} and 2^{nd} instars) or/and late (3^{rd} and 4^{th} 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.

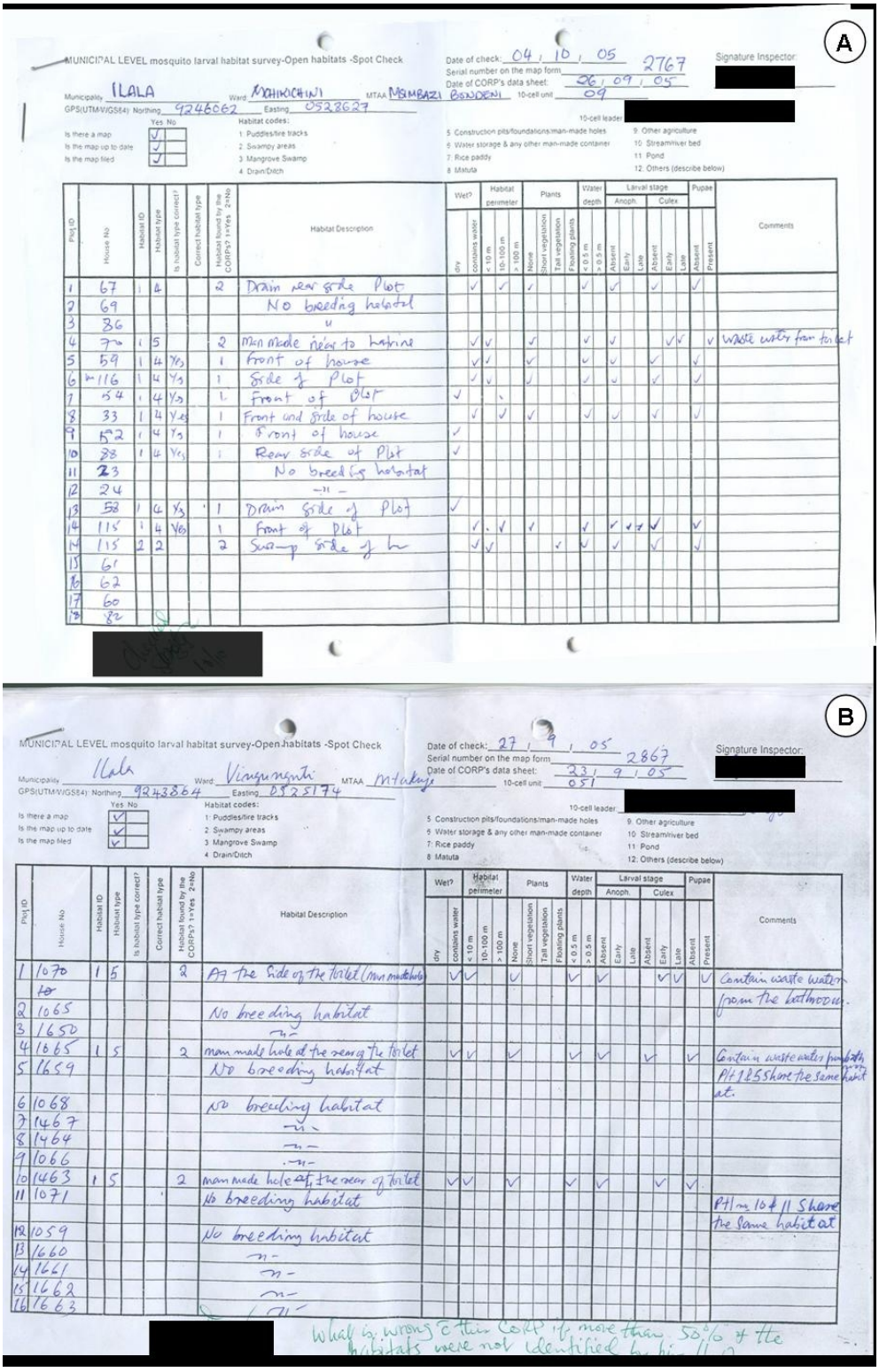


Figure 4. Examples of spot-checking forms [see Additional file 5] for Municipal Mosquito Control Inspectors. **A**. A typical example signed on the bottom left by a City Mosquito Surveillance Officer to show it has been checked for consistency and signs of problems requiring corrective action by management 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.

Product[a]	Active Ingredient[b]	Dosage		Application Cycle
		Kg/hectare	g/m^2	
Open habitats [c]				
VectoLex® WDG (650 ITU/mg)	Bs	2.0	0.20	1 week
VectoBac® WDG (3000 ITU/mg)	Bti	0.4	0.04	1 week
VectoLex® CG (50 ITU/mg)	Bs	30	3	1 week
VectoBac® CG (200 ITU/mg)	Bti	10	1	1 week
Closed habitats [c]				
VectoLex® CG (50 ITU/mg)	Bs	10	1	3 months

[a] 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
[b] *Bti* ; *Bacillus thuringiensis* var *israelensis*, *Bs* ; *Bacillus sphaericus*
[c] See 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.

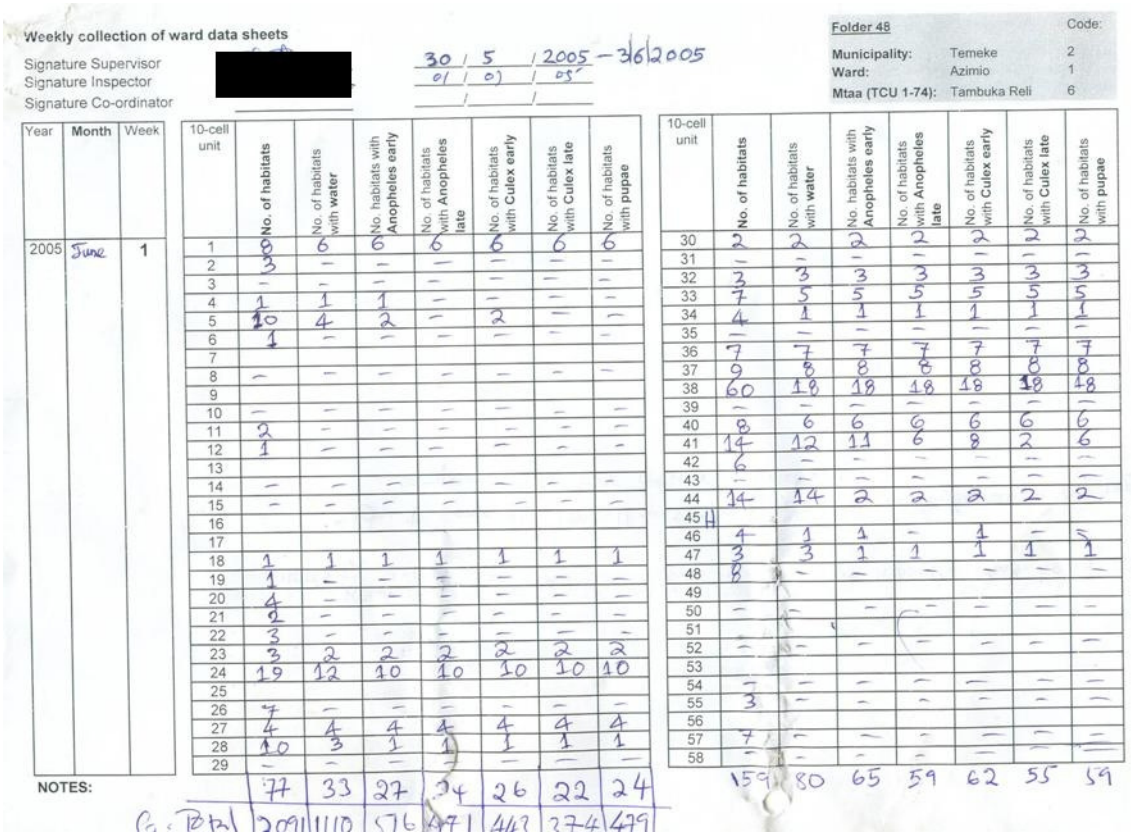


Figure 5. Example of a completed weekly ward summary form [see Additional file 9] filled out by the Ward Supervisor and totalled along the bottom with a pocket calculator to enable rapid entry into monthly report templates at the municipal level.

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.

A

Municipal larval survey- Monthly summary report — **Month:** 12 — **Year:** 2005 — **Ilala**

Mtaa/Tawi	Folder	10-cell units	Total habitats					Wet habitats					Habitats with early instar Anopheles					Habitats with late instar Anopheles					Habitats with early instar Culicines					Habitats with late instar Culex					Habitats with Pupae				
Week:			1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
BUGURUNI WARD																																				
Mnyamani	1	82	885	891	898	891	0	410	397	377	356	0	11	11	11	7	0	11	11	11	8	0	230	305	243	221	0	220	291	224	214	0	218	279	221	194	0
Malapa	2	66	183	194	194	194	0	73	81	117	81	0	24	25	26	35	0	25	27	26	36	0	47	49	53	57	0	47	49	53	57	0	46	48	54	56	0
Madenge	3	49	182	176	176	176	0	17	19	18	18	0	0	1	4	4	0	2	5	4	4	0	5	7	16	16	0	15	16	16	16	0	15	16	15	15	0
Kisiwani	4	91	220	150	200	334	0	230	147	182	302	0	133	5	7	17	0	130	5	7	13	0	156	59	71	144	0	145	51	87	150	0	103	28	38	98	0
TOTALS:		288	1470	1411	1468	1595	0	730	644	694	757	0	168	42	48	63	0	168	48	48	61	0	438	420	383	438	0	427	407	380	437	0	382	371	328	363	0
PROPORTION OF WET HABITATS OCCUPIED:													0.230	0.058	0.066	0.086	0.000	0.230	0.066	0.066	0.084	0.000	0.600	0.575	0.525	0.600	0.000	0.585	0.558	0.521	0.599	0.000	0.523	0.508	0.449	0.497	0.000
ILALA WARD																																					
Sharif Shamba	5	43	223	219	233	231	0	108	111	113	116	0	37	33	28	32	0	14	13	14	13	0	88	94	86	83	0	88	94	86	83	0	11	19	18	15	0
Mafuriko	6	35	204	230	230	481	0	85	99	109	229	0	6	4	1	93	0	6	4	1	1	0	60	74	71	9	0	76	74	71	68	0	74	69	69	65	0
Karume	7	33	57	58	67	78	0	8	9	14	26	0	2	5	3	4	0	2	5	3	4	0	7	7	3	9	0	7	7	3	9	0	6	7	3	9	0
Kasulu	8	39	126	129	131	131	0	47	43	52	50	0	0	0	0	0	0	0	0	0	0	0	29	18	27	27	0	29	18	27	27	0	29	18	26	23	0
TOTALS:		150	610	636	661	921	0	248	262	288	421	0	45	42	32	129	0	22	22	18	18	0	184	193	187	128	0	200	193	187	187	0	120	113	116	112	0
PROPORTION OF WET HABITATS OCCUPIED:													0.181	0.169	0.129	0.520	0.000	0.089	0.089	0.073	0.073	0.000	0.742	0.778	0.754	0.516	0.000	0.806	0.778	0.754	0.754	0.000	0.484	0.456	0.468	0.452	0.000
KIPAWA WARD:																																					
Kipawa	9	57	382	383	381	382	0	204	198	178	183	0	72	67	55	60	0	56	47	41	45	0	88	94	78	84	0	78	74	65	65	0	60	44	47	47	0
Karakata	10	55	1147	1215	776	1019	0	586	615	339	585	0	104	125	63	94	0	83	101	44	28	0	188	211	73	174	0	103	128	67	37	0	78	82	49	41	0
Mogo	11	63	806	893	949	928	0	338	337	333	335	0	162	173	192	172	0	93	108	110	97	0	159	173	186	169	0	143	137	138	115	0	95	75	93	83	0
Kipunguni	12	47	568	590	590	570	0	416	414	391	387	0	226	178	192	207	0	218	160	182	202	0	227	229	212	241	0	223	209	194	216	0	209	153	170	190	0
TOTALS:		222	2903	3081	2696	2899	0	1544	1564	1241	1490	0	564	543	502	533	0	450	416	377	372	0	662	707	549	668	0	547	548	464	433	0	442	354	359	361	0
PROPORTION OF WET HABITATS OCCUPIED:													0.365	0.352	0.325	0.345	0.000	0.291	0.269	0.244	0.241	0.000	0.429	0.458	0.356	0.433	0.000	0.354	0.355	0.301	0.280	0.000	0.286	0.229	0.233	0.234	0.000
MCHIKICHINI WARD:																																					
Ilala Kota	13	68	434	428	447	443	0	383	371	377	378	0	55	50	49	46	0	56	50	49	40	0	158	148	132	126	0	157	147	132	126	0	95	69	61	32	0
Mission Kota	14	29	117	118	117	117	0	96	94	92	86	0	6	3	2	1	0	6	3	2	1	0	69	62	59	49	0	69	62	59	49	0	69	62	59	45	0
Msimbazi Bondeni	15	68	796	809	810	820	0	538	544	541	529	0	88	69	46	40	0	88	69	47	40	0	435	406	362	358	0	434	406	362	358	0	97	24	7	4	0
TOTALS:		165	1347	1355	1374	1380	0	1017	1009	1010	993	0	149	122	97	87	0	150	122	98	81	0	662	616	553	533	0	660	615	553	533	0	261	155	127	81	0
PROPORTION OF WET HABITATS OCCUPIED:													0.147	0.120	0.095	0.086	0.000	0.147	0.120	0.096	0.080	0.000	0.651	0.606	0.544	0.524	0.000	0.649	0.605	0.544	0.524	0.000	0.257	0.152	0.125	0.080	0.000
VINGUNGUTI WARD:																																					
Mtakuja	16	75	324	326	329	352	0	96	85	74	103	0	6	5	5	9	0	4	5	5	6	0	21	26	32	46	0	20	24	30	32	0	19	24	30	32	0
Miembeni	17	56	257	267	271	277	0	109	110	102	88	0	9	10	10	8	0	17	15	8	7	0	17	24	24	22	0	35	29	23	22	0	36	29	23	23	0
Kombo	18	97	430	442	443	462	0	161	151	124	140	0	60	41	27	35	0	48	28	9	12	0	68	52	35	34	0	63	45	29	22	0	60	35	20	20	0
Mtambani	19 & 20	103	453	469	476	492	0	231	222	224	223	0	13	17	7	9	0	13	18	7	9	0	31	29	29	28	0	31	29	27	28	0	33	35	36	32	0
TOTALS:		331	1464	1504	1519	1583	0	597	568	524	554	0	88	73	49	61	0	82	66	29	34	0	137	131	120	130	0	149	127	109	104	0	148	123	109	107	0
PROPORTION OF WET HABITATS OCCUPIED:													0.147	0.122	0.082	0.102	0.000	0.137	0.111	0.049	0.057	0.000	0.229	0.219	0.201	0.218	0.000	0.250	0.213	0.183	0.174	0.000	0.248	0.206	0.183	0.179	0.000
OVERALL TOTALS		1156	7794	7987	7718	8378	0	4136	4047	3757	4215	0	1014	822	728	873	0	872	674	570	566	0	2083	2067	1792	1897	0	1983	1890	1693	1694	0	1353	1116	1039	1024	0
OVERALL PROPORTIONS OF WET HABITATS OCCUPIED													0.245	0.203	0.194	0.207	#####	0.211	0.167	0.152	0.134	#####	0.504	0.511	0.477	0.450	#####	0.479	0.467	0.451	0.402	#####	0.327	0.278	0.273	0.243	#####

BUGURUNI WARD

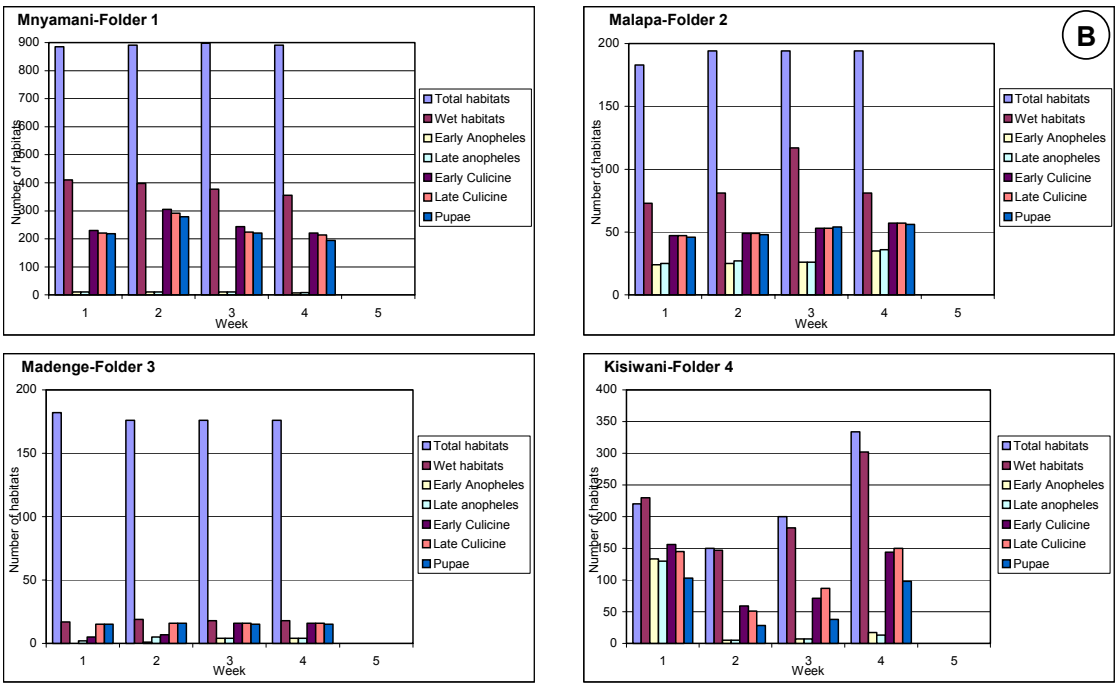


Figure 6. Example of a mosquito larval surveillance component in a municipal monthly report template [see Additional files 10 and 11]. **A**. The overall data entry table in which each row corresponds to one, or occasionally two (see bottom row for example of a very large neighbourhood) folders, each 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.

A

Adult mosquito survey summary																																
						Month:					April				Year:		2006				Ilala											
Mtaa/Tawi	*An gambiae*					*An funestus*					Other Anopheles					Culex					Aedes					Mansonia					Others				
Week (Site)	1	2	3	4	Avg	1	2	3	4	Avg	1	2	3	4	Avg	1	2	3	4	Avg	1	2	3	4	Avg	1	2	3	4	Avg	1	2	3	4	Avg
BUGURUNI WARD																																			
Mnyamani	0	0	0	0	0.0	0	0	0	1	0.3	0	0	0	0	0.0	56	0	68	42	41.5	0	0	0	0	0.0	0	0	0	0	0.0	0	0	0	0	0.0
Malapa	0	0	0	0	0.0	0	0	0	0	0.0	0	0	0	0	0.0	95	67	77	0	59.8	0	0	0	0	0.0	0	0	0	0	0.0	0	0	0	0	0.0
Madenge	0	0	1	0	0.3	0	0	0	0	0.0	0	0	0	0	0.0	0	81	38	29	37.0	0	0	0	0	0.0	0	0	0	0	0.0	0	0	0	0	0.0
Kisiwani	0	0	1	1	0.5	0	0	0	0	0.0	0	0	0	0	0.0	0	0	50	61	27.8	0	0	0	0	0.0	0	0	0	0	0.0	0	0	0	0	0.0
AVERAGES:	0.0	0.0	0.5	0.3	0.2	0.0	0.0	0.0	0.3	0.1	0.0	0.0	0.0	0.0	0.0	37.8	37.0	58.3	33.0	41.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TOTALS:	0	0	2	1	3	0	0	0	1	1	0	0	0	0	0	151	148	233	132	664	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ILALA WARD																																			
Sharif Shamba	0		5	0	1.7	0		0	0	0.0	0		0	0	0.0	0		344	387	###	0		0	0	0.0	0		0	0	0.0	0		0	0	0.0
Mafuriko	0		2	1	1.0	0		0	0	0.0	0		0	0	0.0	0		32	28	20.0	0		0	0	0.0	0		0	0	0.0	0		0	0	0.0
Karume	1		0	0	0.3	0		0	0	0.0	0		0	0	0.0	26		40	33	33.0	0		0	0	0.0	0		0	0	0.0	0		0	0	0.0
Kasulu	0		2	0	0.7	0		0	0	0.0	0		0	0	0.0	0		48	114	54.0	0		0	0	0.0	0		0	0	0.0	0			0	0.0
AVERAGES:	0.3		2.3	0.3	0.9	0.0		0.0	0.0	0.0	0.0		0.0	0.0	0.0	6.5		116.0	140.5	87.7	0.0		0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0		0.0	0.0	0.0
TOTALS:	1		9	1	11	0		0	0	0	0		0	0	0	26		464	562	1052	0		0	0	0	0		0	0	0	0		0	0	0
KIPAWA WARD:																																			
Kipawa	0	0	3		1.0	0	0	0		0.0	0	0	0		0.0	0	0	133		44.3	0	0	0		0.0	0	0	0		0.0	0	0	0		0.0
Karakata	6	0	1		2.3	0	0	0		0.0	0	0	0		0.0	55	68	61		61.3	0	0	0		0.0	0	0	0		0.0	0	0	0		0.0
Mogo	1	1	0		0.7	0	0	0		0.0	0	0	0		0.0	0	57	67		41.3	0	1	0		0.3	104	0	0		34.7	0	0	0		0.0
Kipunguni	3	0	10		4.3	0	0	0		0.0	0	0	0		0.0	0	61	102		54.3	40	0	4		14.7	0	0	0		0.0	0	0	0		0.0
AVERAGES:	2.5	0.3	3.5		2.1	0.0	0.0	0.0		0.0	0.0	0.0	0.0		0.0	13.8	46.5	90.8		50.3	10.0	0.3	1.0		3.8	26.0	0.0	0.0		8.7	0.0	0.0	0.0		0.0
TOTALS:	10	1	14		25	0	0	0		0	0	0	0		0	55	186	363		604	40	1	4		45	104	0	0		104	0	0	0		0
MCHIKICHINI WARD:																																			
Ilala Kota	6	16	4		8.7	0	0	0		0.0	0	10	7		5.7	64	432	183		###	0	0	0		0.0	2	0	0		0.7	3	0	0		1.0
Mission Kota	7	3	3		4.3	0	0	0		0.0	0	0	0		0.0	89	140	75		101.3	1	0	0		0.3	0	0	0		0.0	0	0	0		0.0
Msimbazi Bondeni	21	0	12		11.0	0	0	0		0.0	0	0	2		0.7	26	0	39		21.7	0	0	0		0.0	0	0	0		0.0	0	0	0		0.0
AVERAGES:	11.3	6.3	6.3		8.0	0.0	0.0	0.0		0.0	0.0	3.3	3.0		2.1	59.7	190.7	99.0		116.4	0.3	0.0	0.0		0.1	0.7	0.0	0.0		0.2	1.0	0.0	0.0		0.3
TOTALS:	34	19	19		72	0	0	0		0	0	10	9		19	179	572	297		1048	1	0	0		1	2	0	0		2	3	0	0		3
VINGUNGUTI WARD:																																			
Mtakuja	0		6		3.0	0		0		0.0	0		0		0.0	138		152		145.0	0		0		0.0	0		0		0.0	0		0		0.0
Miembeni	0		2		1.0	0		0		0.0	0		0		0.0	220		127		173.5	0		0		0.0	0		0		0.0	0		0		0.0
Kombo	6		0		3.0	0		0		0.0	0		0		0.0	34		63		48.5	0		0		0.0	0		0		0.0	0		0		0.0
Mtambani	0		13		6.5	0		0		0.0	0		0		0.0	0		279		139.5	0		0		0.0	0		0		0.0	0		0		0.0
AVERAGES:	1.5		5.3		3.4	0.0		0.0		0.0	0.0		0.0		0.0	98.0		155.3		126.6	0.0		0.0		0.0	0.0		0.0		0.0	0.0		0.0		0.0
TOTALS:	6	0	21		27	0		0		0	0		0		0	392		621		1013	0		0		0	0		0		0	0		0		0
OVERALL AVERAGES:	3.1	1.3	3.6	0.1	2.9	0.0	0.0	0.0	0.1	0.0	0.0	0.7	0.6	0.0	0.4	43.1	54.8	103.9	34.7	84.5	2.1	0.1	0.2	0.0	0.8	5.3	0.0	0.0	0.0	1.8	0.2	0.0	0.0	0.0	0.1
OVERALL TOTALS:	51	20	65	2	138	0	0	0	1	1	0	10	9	0	19	803	906	1978	694	4381	41	1	4	0	46	106	0	0	0	106	3	0	0	0	3

B

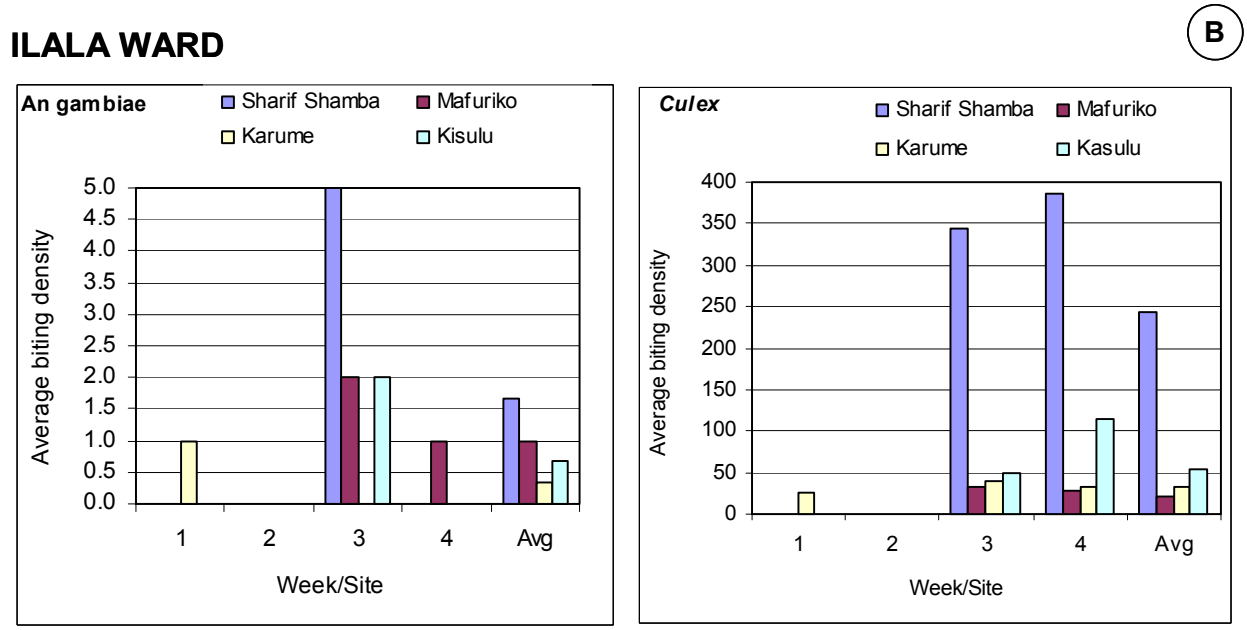


Figure 7. Example of a mosquito adult surveillance component in a municipal monthly report template. **A**. The overall data entry table (empty fields indicate missing data) **B**. A typical automatically generated chart summarizing the observed distribution of adult mosquitoes.

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).

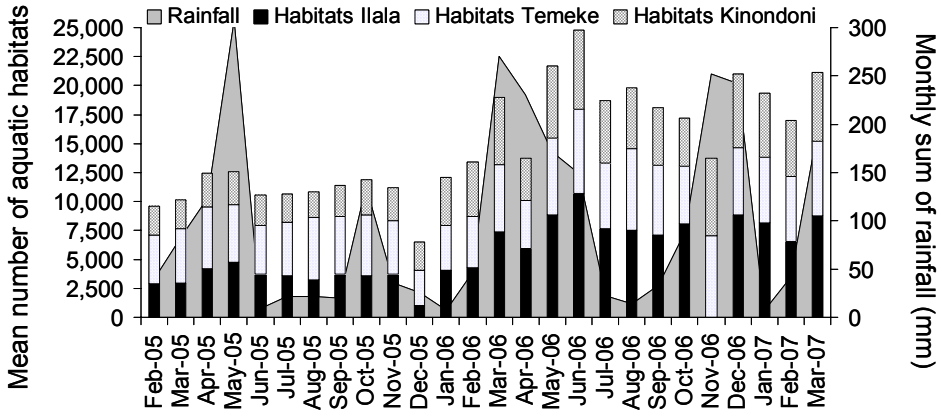


Figure 8. Monthly average of aquatic habitats surveyed in the three municipalities Kinondoni, Ilala and Temeke from February 2005 to March 2007 in relation to rainfall.

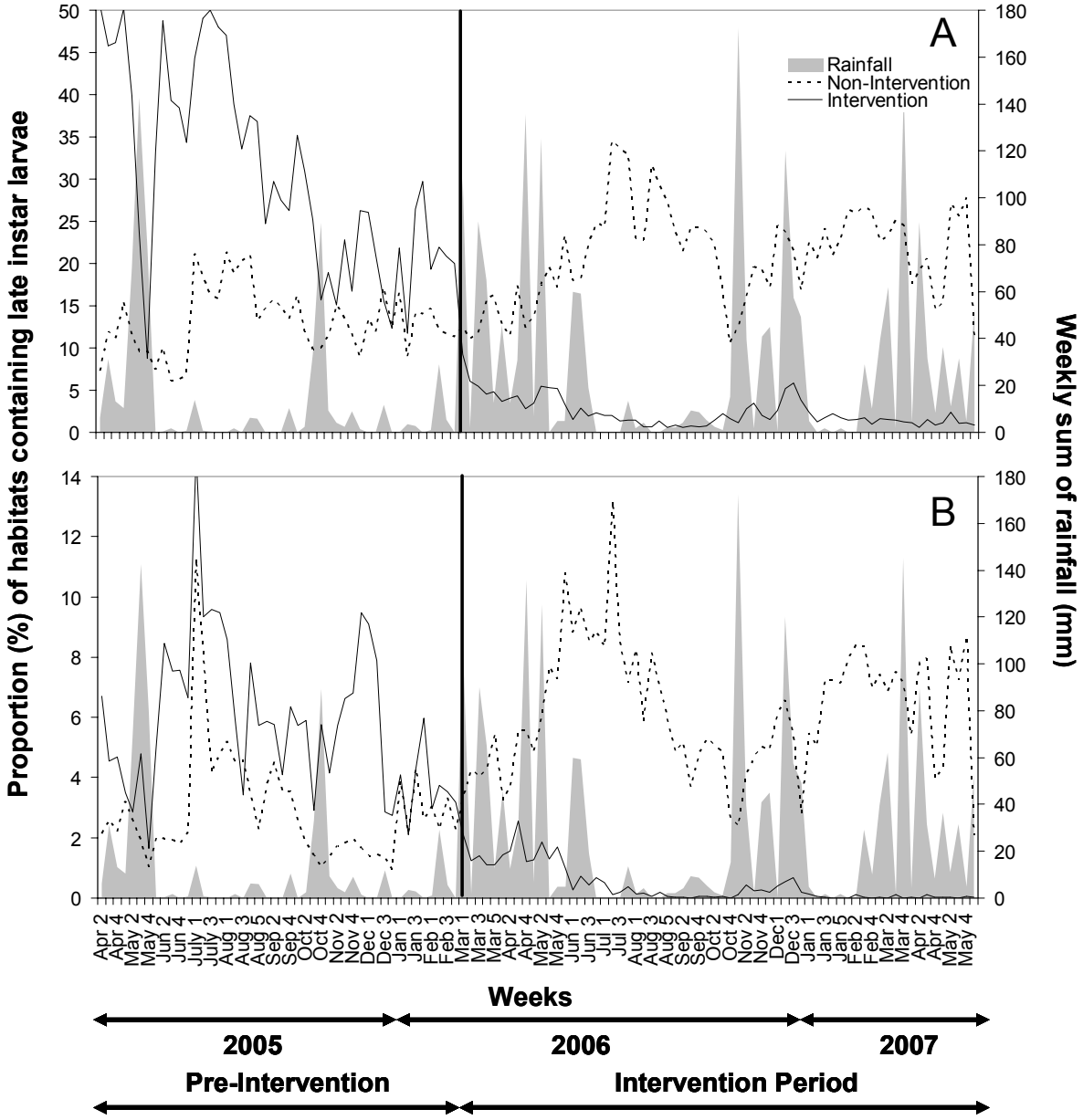


Figure 9. Impact of seasonal rainfall variation and larvicide application on aquatic-stage mosquito populations between April 2005 and June 2007. Larvicide application started in the intervention sites in March 2006 week number 1. **A**: Proportion of aquatic habitats containing late instar culicine larvae at weekly surveys. **B:** Proportion of aquatic habitats containing late instar anopheline larvae at weekly surveys.

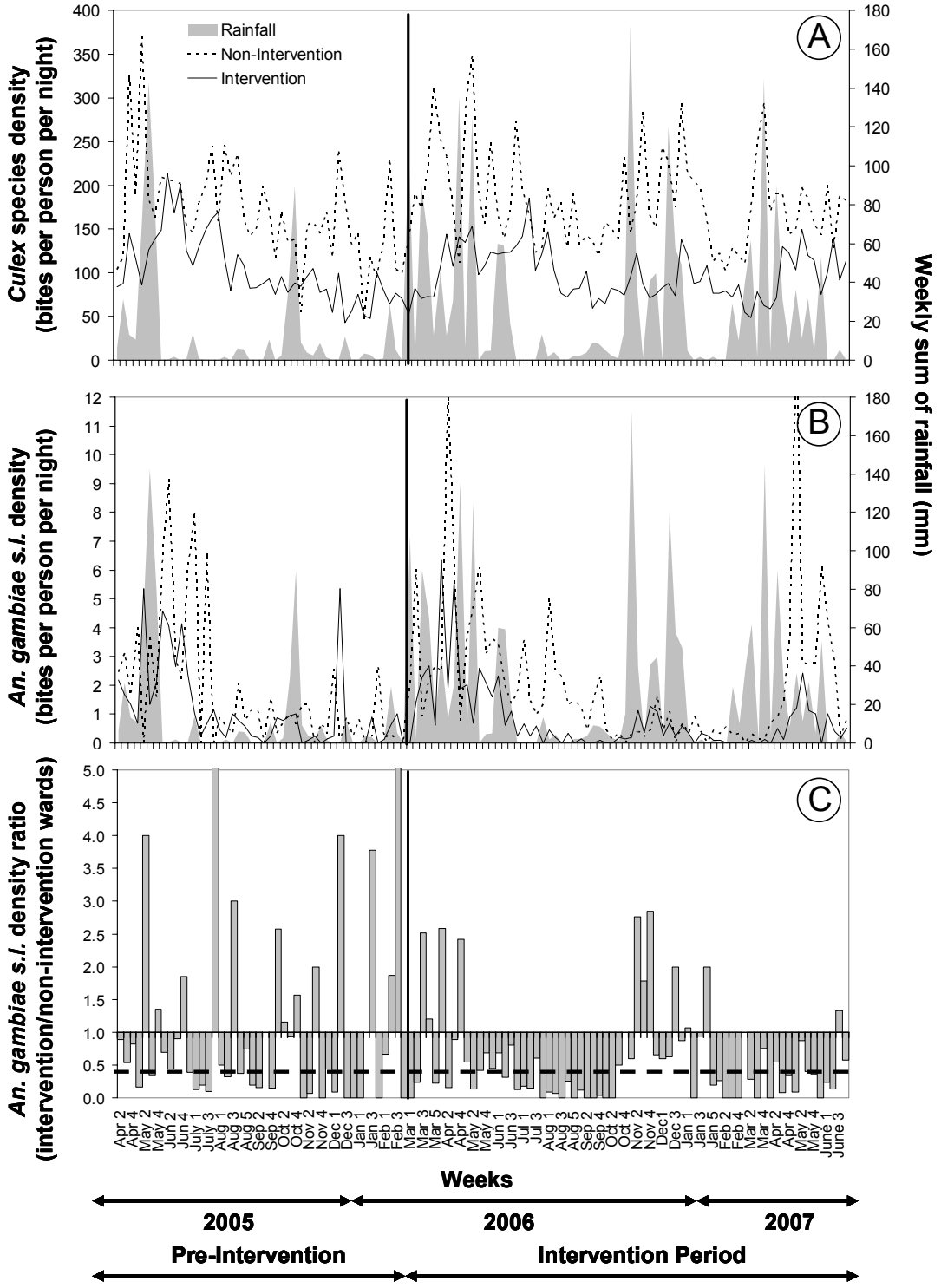


Figure 10. Impact of seasonal rainfall variation and larvicide application on weekly adult mosquito densities between April 2005 and June 2007. **A**. Rainfall and densities of adult *Culex* species, **B**. Rainfall and densities of adult *Anopheles gambiae s.l.*, **C**. The ratio of densities of *An. 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:

$$\% \text{ reduction} = 100 - (C_1/T_1 \times T_2/C_2) \times 100$$

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 (*An. gambiae*: 0.67, *An. funestus*: 0.725, *Anopheles coustani*: 0.448 and *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 artemisinin-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.

	Pre-Intervention [a]			First intervention year [b]			Percentage Reduction
	Non-Intervention Wards	Intervention Wards	*p* [c]	Non-Intervention Wards	Intervention Wards	*p*	
Annual mean							
Daily HBR *An. gambiae*	0.93 (0.60-1.46)	0.72 (0.51-1.02)	0.367	0.94 (0.57-1.56)	0.50 (0.38-0.68)	0.040	31.3%
Annual EIR *An. gambiae*	1.05 (0.68-1.65)	0.81 (0.58-1.15)		1.06 (0.64-1.77)	0.56 (0.43-0.77)		
Daily HBR *Culex sp.*	173.9 (140.7-214.9)	86.8 (72.7-103.7)	<0.001	171.5 (137.2-214.3)	86.1 (70.9-104.4)	<0.001	0%
Dry season mean (July-August-September)							
Daily HBR *An. gambiae*	0.59 (0.32-1.11)	0.46 (0.29-0.72)	0.505	1.17 (0.56-2.47)	0.12 (0.08-0.20)	<0.001	86.8%
EIR *An. gambiae*	0.67 (0.36-1.26)	0.52 (0.33-0.81)		1.32 (0.63-2.79)	0.14 (0.09-0.22)		
Daily HBR *Culex sp.*	196.3 (157.9-244.0)	98.4 (82.2-117.9)	<0.001	151.1 (125.3-192.0)	86.1 (67.1-110.6)	<0.001	0%

[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].
[b] April 2006 – March 2007.
[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 km^2 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).

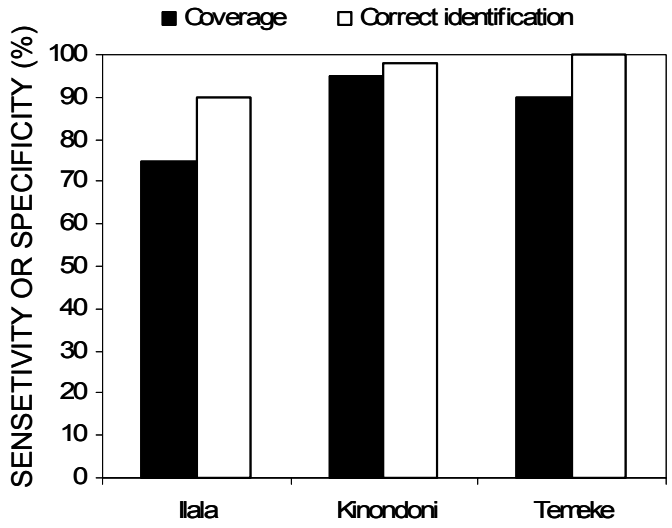


Figure 11. Proportion of habitats successfully detected (sensitivity) and correctly identified (specificity) by larval surveillance CORPs in November 2005, as determined from the random on-site spot checks of the Municipal Mosquito Control Inspectors using methodology essentially identical to earlier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

1. Hay SI, Guerra CA, Tatem AJ, Atkinson PM, Snow RW: **Urbanization, malaria transmission and disease burden in Africa.** *Nat Rev Microbiol* 2005, **3:**81-90.
2. Keiser J, Utzinger J, Caldas de Castro M, Smith TA, Tanner M, Singer BH: **Urbanization in sub-saharan Africa and implication for malaria control.** *Am J Trop Med Hyg* 2004, **71:**118-127.
3. Robert V, Macintyre K, Keating J, Trape JF, Duchemin JB, Warren M, Beier JC: **Malaria transmission in urban sub-Saharan Africa.** *Am J Trop Med Hyg* 2003, **68:**169-176.
4. Killeen GF: **Following in Soper's footsteps: northeast Brazil 63 years after eradication of *Anopheles gambiae*.** *Lancet Infect Dis* 2003, **3:**663-666.
5. Killeen GF, Fillinger U, Kiche I, Gouagna LC, Knols BG: **Eradication of *Anopheles gambiae* from Brazil: lessons for malaria control in Africa?** *Lancet Infect Dis* 2002, **2:**618-627.
6. Killeen GF, Seyoum A, Knols BG: **Rationalizing historical successes of malaria control in Africa in terms of mosquito resource availability management.** *Am J Trop Med Hyg* 2004, **71 (Suppl 2):**87-93.
7. Smith DL, McKenzie FE, Snow RW, Hay SI: **Revisiting the basic reproductive number for malaria and its implications for malaria control.** *PLoS Biol* 2007, **5:**e42.
8. Smith T, Maire N, Dietz K, Killeen GF, Vounatsou P, Molineaux L, Tanner M: **Relationship between the entomologic inoculation rate and the force of infection for *Plasmodium falciparum* malaria.** *Am J Trop Med Hyg* 2006, **75 (Suppl 2):**11-18.

9. Woolhouse ME, Dye C, Etard JF, Smith T, Charlwood JD, Garnett GP, Hagan P, Hii JL, Ndhlovu PD, Quinnell RJ, Watts CH, Chandiwana SK, Anderson RM: **Heterogeneities in the transmission of infectious agents: implications for the design of control programs.** *Proc Natl Acad Sci U S A* 1997, **94:**338-342.

10. Gu W, Novak RJ: **Habitat-based modeling of impacts of mosquito larval interventions on entomological inoculation rates, incidence, and prevalence of malaria.** *Am J Trop Med Hyg* 2005, **73:**546-552.

11. Gu W, Novak RJ: **In reply: habitat targeting for controlling aquatic stages of malaria vectors in Africa.** *Am J Trop Med Hyg* 2006, **74:**519-520.

12. Killeen GF, Tanner M, Mukabana WR, Kalongolela MS, Kannady K, Lindsay SW, Fillinger U, de Castro MC: **Habitat targeting for controlling aquatic stages of malaria vectors in Africa.** *Am J Trop Med Hyg* 2006, **74:**517-518; author reply 519-520.

13. Matthys B, Vounatsou P, Raso G, Tschannen AB, Becket EG, Gosoniu L, Cisse E, Tanner M, Utzinger J: **Urban farming and malaria risk factors in a medium-sized town in Cote D'Ivoire** *Am J Trop Med Hyg* 2006, **75:**1223-1231.

14. Watson M: *African Highway.* London: John Murray; 1953.

15. Utzinger J, Tozan Y, Singer BH: **Efficacy and cost-effectiveness of environmental management for malaria control.** *Trop Med Int Health* 2001, **6:**677-687.

16. Shousha AT, Pasha MD: **The eradication of *Anopheles gambiae* from Upper Egypt.** *Bull World Health Organ* 1948, **1:**309-333.

17. Soper FL, Wilson DB: *Anopheles gambiae in Brazil.* New York: The Rockefeller Foundation; 1943.

18. Barat LM: **Four malaria success stories: how malaria burden was successfully reduced in Brazil, Eritrea, India, and Vietnam.** *Am J Trop Med Hyg* 2006, **74:**12-16.

19. Impoinvil DE, Ahmad S, Troyo A, Keating J, Githeko AK, Mbogo CM, Kibe L, Githure JI, Gad AM, Hassan AN, Orshan L, Warburg A, Calderon-Arguedas O, Sanchez-Loria VM, Velit-Suarez R, Chadee DD, Novak RJ, Beier JC: **Comparison of mosquito control programs in seven urban sites in Africa, the Middle East, and the Americas.** *Health Policy* 2007, **83:**196-212.

20. Walker K, Lynch M: **Contributions of *Anopheles* larval control to malaria suppression in tropical Africa: review of achievements and potential.** *Med Vet Entomol* 2007, **21:**2-21.

21. Wang SJ, Lengeler C, Smith TA, Vounatsou P, Cisse G, Diallo DA, Akogbeto M, Mtasiwa D, Teklehaimanot A, Tanner M: **Rapid urban malaria appraisal (RUMA) in sub-Saharan Africa.** *Malar J* 2005, **4:**40.

22. de Castro C, Yamagata Y, Mtasiwa D, Tanner M, Utzinger J, Keiser J, Singer BH: **Integrated urban malaria control: a case study in Dar es Salaam, Tanzania.** *Am J Trop Med Hyg* 2004, **71 (Suppl 2):**103-117.

23. Sattler MA, Mtasiwa D, Kiama M, Premji Z, Tanner M, Killeen GF, Lengeler C: **Habitat characterization and spatial distribution of *Anopheles sp*. mosquito larvae in Dar es Salaam (Tanzania) during an extended dry period.** *Malar J* 2005, **4:**4.

24. Vanek MJ, Shoo B, Mtasiwa D, Kiama M, Lindsay SW, Fillinger U, Kannady K, Tanner M, Killeen GF: **Community-based surveillance of malaria vector larval habitats: a baseline study in urban Dar es Salaam, Tanzania.** *BMC Public Health* 2006, **6:**154.

25. Wang SJ, Lengeler C, Mtasiwa D, Mshana T, Manane L, Maro G, Tanner M: **Rapid urban malaria appraisal (RUMA) II: Epidemiology of urban malaria in Dar es Salaam (Tanzania).** *Malar J* 2006, **5:**29.

26. Geissbühler Y, Chaki P, Emidi B, Govella NJ, Shirima R, Mayagaya V, Mtasiwa D, Mshinda H, Fillinger U, Lindsay SW, Kannady K, De Castro MC, Tanner M, Killeen GF: **Interdependence of domestic malaria prevention measures and mosquito-human interactions in urban Dar es Salaam, Tanzania.** *Malar J* 2007, **6:**126.

27. NBS: *The 2002 population and housing census general report.* Dar es Salaam: National Bureau of Statistics, Government of Tanzania; 2003.

28. Dongus S, Nyika D, Kannady K, Mtasiwa D, Mshinda H, Fillinger U, Drescher A, Tanner M, Caldas De Castro M, Killeen GF: **Participatory mapping of target areas to enable routine comprehensive larviciding of malaria vector mosquitoes in Dar es Salaam, Tanzania.** *Int J Health Geogr* 2007, **6:**37.

29. Mukabana WR, Kannady K, Ijumba J, Mathenge E, Kiche I, Nkwengulila G, Mboera L, Mtasiwa D, Yamagata Y, van Schayk I, Knols BG, Lindsay SW, Caldas de Castro M, Mshinda H, Tanner M, Fillinger U, Killeen GF: **Ecologists can enable communities to implement malaria vector control in Africa.** *Malar J* 2006, **5:**9.

30. Bang YH, Mrope FM, Sabuni IB: **Changes in mosquito populations associated with urbanization in Tanzania.** *East Afr Med J* 1977, **54:**403-410.

31. Chavasse DC, Lines JD, Ichimori K: **The relationship between mosquito density and mosquito coil sales in Dar es Salaam.** *Trans R Soc Trop Med Hyg* 1996, **90:**493.

32. Stephens C, Masamu ET, Kiama MG, Keto AJ, Kinenekejo M, Ichimori K, Lines J: **Knowledge of mosquitos in relation to public and domestic control activities in the cities of Dar es Salaam and Tanga.** *Bull World Health Organ* 1995, **73:**97-104.

33. Chavasse DC, Lines JD, Ichimori K, Majala AR, Minjas JN, Marijani J: **Mosquito control in Dar es Salaam. II. Impact of expanded polystyrene beads and pyriproxyfen treatment of breeding sites on *Culex quinquefasciatus* densities.** *Med Vet Entomol* 1995, **9:**147-154.

34. Chavasse DC, Lines JD, Ichimori K, Marijani J: **Mosquito control in Dar es Salaam. I. Assessment of *Culex quinquefasciatus* breeding sites prior to intervention.** *Med Vet Entomol* 1995, **9:**141-146.

35. Fillinger U, Knols BG, Becker N: **Efficacy and efficiency of new *Bacillus thuringiensis var israelensis* and *Bacillus sphaericus* formulations against Afrotropical anophelines in Western Kenya.** *Trop Med Int Health* 2003, **8:**37-47.

36. Fillinger U, Lindsay SW: **Suppression of exposure to malaria vectors by an order of magnitude using microbial larvicides in rural Kenya.** *Trop Med Int Health* 2006, **11:**1629-1642.

37. Fillinger U, Sonye G, Killeen GF, Knols BG, Becker N: **The practical importance of permanent and semipermanent habitats for controlling aquatic stages of *Anopheles gambiae* sensu lato mosquitoes: operational observations from a rural town in western Kenya.** *Trop Med Int Health* 2004, **9:**1274-1289.

38. Opiyo P, Mukabana WR, Kiche I, Mathenge E, Killeen GF, Fillinger U: **An exploratory study of community factors relevant for participatory malaria control on Rusinga Island, western Kenya.** *Malar J* 2007, **6:**48.

39. Mushinzimana E, Munga S, Minakawa N, Li L, Feng CC, Bian L, Kitron U, Schmidt C, Beck L, Zhou G, Githeko AK, Yan G: **Landscape determinants and remote sensing of anopheline mosquito larval habitats in the western Kenya highlands.** *Malar J* 2006, **5:**13.

40. Service MW: *Mosquito Ecology.* Second Edition edn. London: Chapman & Hall; 1993.

41. Rozendaal JA: *Vector Control - Methods for use by individuals and communities.* Geneva: World Health Organization; 1997.

42. Majambere S, Lindsay SW, Green C, Kandeh B, Fillinger U: **Microbial larvicides for malaria control in The Gambia.** *Malar J* 2007, **6:**76.

43. Shililu JI, Tewolde GM, Brantly E, Githure JI, Mbogo CM, Beier JC, Fusco R, Novak RJ: **Efficacy of *Bacillus thuringiensis israelensis, Bacillus sphaericus* and temephos for managing *Anopheles* larvae in Eritrea.** *J Am Mosq Control Assoc* 2003, **19:**251-258.

44. WHO: **International programme on chemical safety (IPCS): Microbial pest control agent *Bacillus thuringiensis*.** *Environ Health Criteria* 1999, **217:**1-105.

45. Charles JF, Nicolas L: **Recycling of *Bacillus sphaericus* 2362 in mosquito larvae: a laboratory study.** *Ann Inst Pasteur Microbiol* 1986, **137:**101-111.

46. Hougard JM: **Formulation and persistence of *Bacillus sphaericus* in *Culex quinquefasciatus* larval sites in tropical Africa.** In *Bacteriological Control of Mosquitoes and Blackflies.* Edited by de Barjac H, Gutherland DJ. New Brunswick: Rutgers University Press; 1990: 295-306

47. Karch S, Monteny N, Jullien JL, Sinegre G, Coz J: **Control of *Culex pipiens* by *Bacillus sphaericus* and role of nontarget arthropods in its recycling.** *J Am Mosq Control Assoc* 1990, **6:**47-54.

48. Matanmi BA, Federici BA, Mulla MS: **Fate and persistence of *Bacillus sphaericus* used as a mosquito larvicide in dairy wastewater lagoons.** *J Am Mosq Control Assoc* 1990, **6:**384-389.

49. Pantuwatana S, Maneeroj R, Upatham ES: **Long residual activity of *Bacillus sphaericus* 1593 against *Culex quinquefasciatus* larvae in artificial pools.** *Southeast Asian J Trop Med Public Health* 1989, **20:**421-427.

50. Skovmand O, Bauduin S: **Efficacy of a granular formulation of *Bacillus sphaericus* against *Culex quinquefasciatus* and *Anopheles gambiae* in West African countries.** *J Vector Ecol* 1997, **22:**43-51.

51. Sutherland DD, McNelly JJ, Hansen JA: **Evaluation of granular *Bacillus sphaericus* to control *Culex* in sewage treatments ponds in Cape May.** *New Jersey Mosqu Contr Assoc* 1989, **76:**84-90.

52. Worral E: *Cost analyses for large scale use of larval source management in malaria control.* Washington DC: Prepared for RTI International by Liverpool Associates in Tropical Health under contract to the Bureau for Global Health, U.S. Agency for International Development (USAID). Contract no. GHS-I-01-03-00028-000-1, Integrated Vector Management Programs for Malaria Control; 2007.

53. Gunasekaran K, Shriram AN, Elangovan A, Narayanan RJ, Balaraman K: **Efficacy of *Bacillus sphaericus* in different breeding habitats of *Culex quinquefasciatus*.** *Southeast Asian J Trop Med Public Health* 1996, **27:**622-627.

54. Hougard JM, Mbentengam R, Lochouarn L, Escaffre H, Darriet F, Barbazan P, Quillevere D: **Campaign against *Culex quinquefasciatus* using *Bacillus sphaericus*: results of a pilot project in a large urban area of equatorial Africa.** *Bull World Health Organ* 1993, **71:**367-375.

55. Lago GM, Perez MC, Figueroa AM, Sonzalez FAC: **Results of a pilot application of the biolarvicide *Bacillus sphaericus* to larval habitats of mosquitoes in Santa Cruz del Norte municipality (La Habana Province).** *Rev Cubana Med Trop* 1991, **43:**39-44.

56. Booman M, Sharp BL, Martin CL, Manjate B, La Grange JJ, Durrheim DN: **Enhancing malaria control using a computerised management system in southern Africa.** *Malar J* 2003, **2:**13.

57. Mathenge EM, Killeen GF, Oulo DO, Irungu LW, Ndegwa PN, Knols BG: **Development of an exposure-free bednet trap for sampling Afrotropical malaria vectors.** *Med Vet Entomol* 2002, **16:**67-74.

58. Mathenge EM, Misiani GO, Oulo DO, Irungu LW, Ndegwa PN, Smith TA, Killeen GF, Knols BG: **Comparative performance of the Mbita trap, CDC light trap and the human landing catch in the sampling of Anopheles arabiensis, An. funestus and culicine species in a rice irrigation in western Kenya.** *Malar J* 2005, **4:**7.

59. Mathenge EM, Omweri GO, Irungu LW, Ndegwa PN, Walczak E, Smith TA, Killeen GF, Knols BG: **Comparative field evaluation of the Mbita trap, the Centers for Disease Control light trap, and the human landing catch for sampling of malaria vectors in western Kenya.** *Am J Trop Med Hyg* 2004, **70:**33-37.

60. Laganier R, Randimby FM, Rajaonarivelo V, Robert V: **Is the Mbita trap a reliable tool for evaluating the density of anopheline vectors in the highlands of Madagascar?** *Malar J* 2003, **2:**42.

61. Gillies MT, DeMeillon B: *The Anophelinae of Africa south of the Sahara (Ethiopian zoogeographical region).* 2nd edn. Johannesburg: South African Institute of Medical Research; 1968.

62. Scott JA, Brogdon WG, Collins FH: **Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction.** *Am J Trop Med Hyg* 1993, **49:**520-529.

63. Burkot TR, Williams JL, Schneider I: **Identification of Plasmodium falciparum-infected mosquitoes by a double antibody enzyme-linked immunosorbent assay.** *Am J Trop Med Hyg* 1984, **33:**783-788.

64. Mulla MS, Norland RL, Fanara DM, Darwazeh A, McKean D: **Control of chironomid midges in recreational lakes.** *J Econ Entomol* 1971, **64:**300-307.

65. Geissbühler Y, Kannady K, Chaki P, Emidi B, Govella NJ, Mayagaya V, Mtasiwa D, Mshinda H, Lindsay SW, Fillinger U, Tanner M, Castro MC, Killeen GF: **Integrated malaria control incorporating microbial larvicides in Dar es Salaam, United Republic of Tanzania.** *BMJ* 2007 submitted.

66. Lindsay SW, Egwang TG, Kabuye F, Mutambo T, Matwale GK: *Community-based environmental management program for malaria control in Kampala and Jinja, Uganda (EHP Activity Report 140).* Washington: Environmental Health Project; 2004.

67. Kouznetsov RL: **Malaria control by application of indoor spraying of residual insecticides in tropical Africa and its impact on community health.** *Trop Doct* 1977, **7:**81-91.

68. de Castro MC, Kannady K, Singer BH, Mtasiwa D, Mshinda H, Tanner M, Geissbühler Y, Lindsay SW, Fillinger U, Killeen GF: **Reduction in malaria prevalence in Dar es Salaam, Tanzania following vector control with microbial larvicides.** *PLoS Med* 2007 submitted.

69. de Savigny D, Binka F: **Monitoring future impact on malaria burden in sub-Saharan Africa.** *Am J Trop Med Hyg* 2004, **71 (Suppl 2):**224-231.

70. Holstein MH: *Biology of Anopheles gambiae.* Geneva: World Health Organization; 1954.

71. Mutuku FM, Alaii JA, Bayoh MN, Gimnig JE, Vulule JM, Walker ED, Kabiru E, Hawley WA: **Distribution, description, and local knowledge of larval habitats of *Anopheles gambiae* s.l. in a village in western Kenya.** *Am J Trop Med Hyg* 2006, **74:**44-53.

72. Townson H, Nathan MB, Zaim M, Guillet P, Manga L, Bos R, Kindhauser M: **Exploiting the potential of vector control for disease prevention.** *Bull World Health Organ* 2005, **83:**942-947.

73. van den Berg H, Knols BG: **The Farmer Field School: a method for enhancing the role of rural communities in malaria control?** *Malar J* 2006, **5:**3.

74. Utzinger J, Tozan Y, Doumani F, Singer BH: **The economic payoffs of integrated malaria control in the Zambian copperbelt between 1930 and 1950.** *Trop Med Int Health* 2002, **7:**657-677.

75. Killeen GF, Kihonda J, Lyimo E, Oketch FR, Kotas ME, Mathenge E, Schellenberg JA, Lengeler C, Smith TA, Drakeley CJ: **Quantifying behavioural interactions between humans and mosquitoes: evaluating the protective efficacy of insecticidal nets against malaria transmission in rural Tanzania.** *BMC Infect Dis* 2006, **6:**161.

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

Marcia C. de Castro[1], Khadija Kannady[2], Burton H. Singer[3], Deo Mtasiwa[2], Hassan Mshinda[4], Marcel Tanner[5], Yvonne Geissbühler[2,4,5], Steve W. Lindsay[6], Ulrike Fillinger[6], Gerry F. Killeen[4,5,6]

[1]Harvard School of Public Health, Department of Population and International Health, 665 Huntington Avenue, Bldg. I, Room 1113, Boston, MA 02115, USA

[2]City Medical Office of Health, Dar es Salaam City Council, P.O. Box 63320, Dar es Salaam, United Republic of Tanzania

[3]Princeton University, Office of Population Research, Wallace Hall, Princeton, NJ 08544, USA

[4]Ifakara Health Research and Development Centre, Coordination Office, P.O. Box 78373, Kiko Avenue, Mikocheni B, Dar es Salaam, United Republic of Tanzania

[5]Swiss Tropical Institute, Department of Public Health and Epidemiology, Socinstrasse 57, 4002 Basel, Switzerland

[6]Durham 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/km^2). 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 km^2 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].

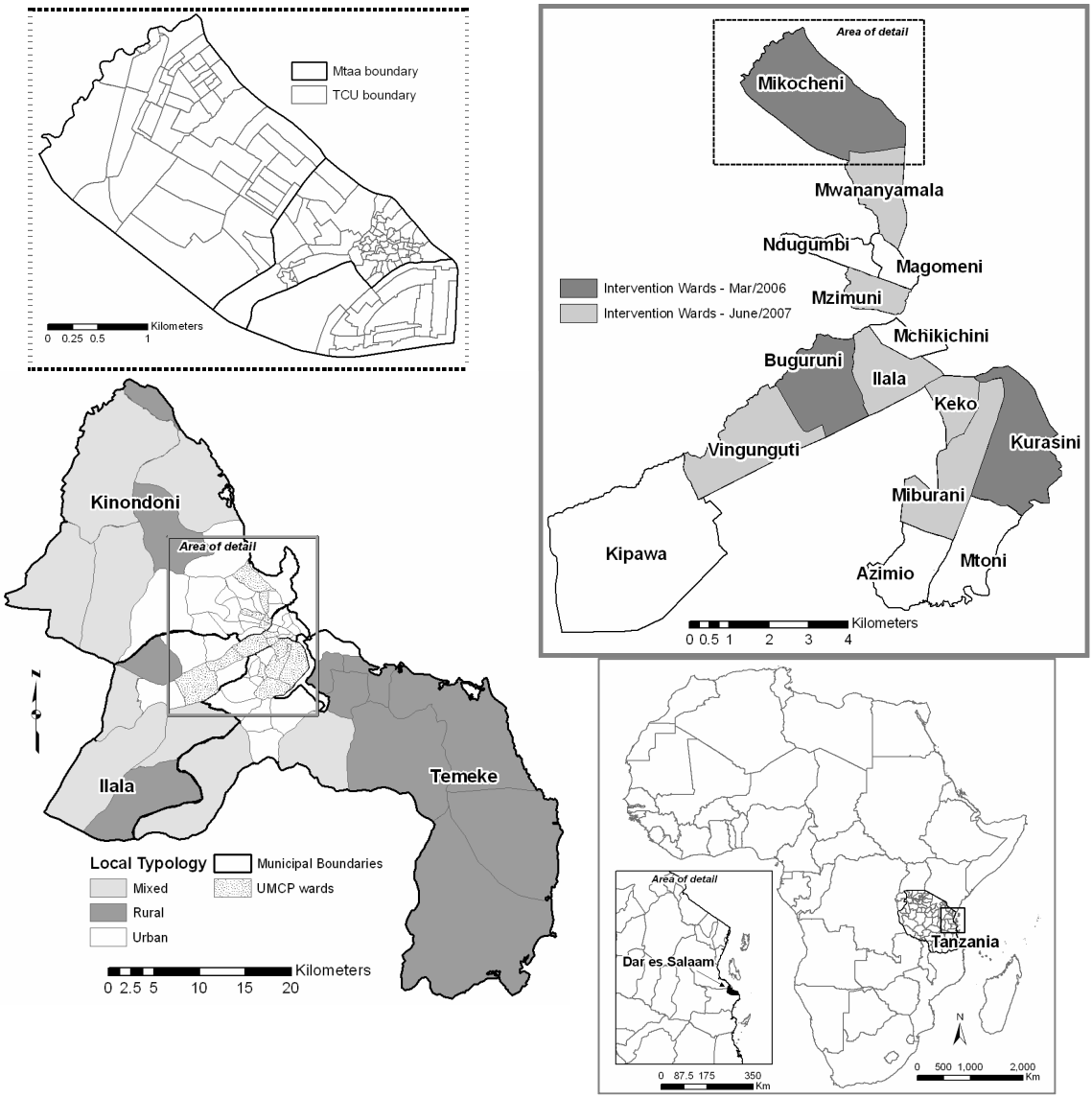


Figure 1. Administrative units of Dar es Salaam, UMCP targeted area, and intervention wards. Administratively, Dar es Salaam comprises three municipalities – Ilala, Kinondoni and Temeke – and is divided into 73 wards (22 in Ilala, 27 in Kinondoni, and 24 in Temeke), classified by the Tanzania National 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 –*Bacillus thuringiensis* var. *israelensis* (VectoBac®) or *B. sphaericus* (VectoLex®) – were applied weekly to all potential mosquito breeding sites as a control strategy in 3 wards, one in each municipality (Figure 1) [18]. Details of the methodology are presented elsewhere [18,19]. Antilarval mosquito control measures, although successfully implemented in the past [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 [29-33]. Indeed, data from Kenya indicate that areas treated with larvicides experienced a reduction of 95% in *Anopheles* larval density and a 92% decline in human exposure to mosquito bites [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 *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 *mitaa* (a Kiswahili word for street, written in the singular form as *mtaa*) [19]. Each *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 [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

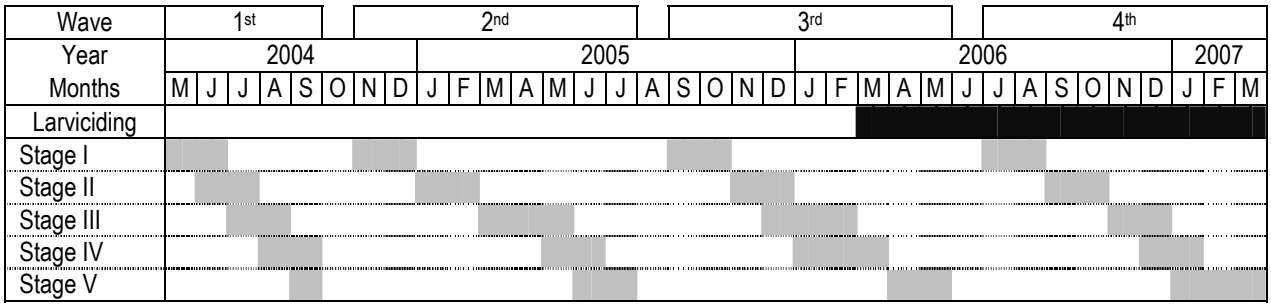
one occurring in November and December. The area is endemic for malaria and transmission is perennial [34].

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), Ndugumbi (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).



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.

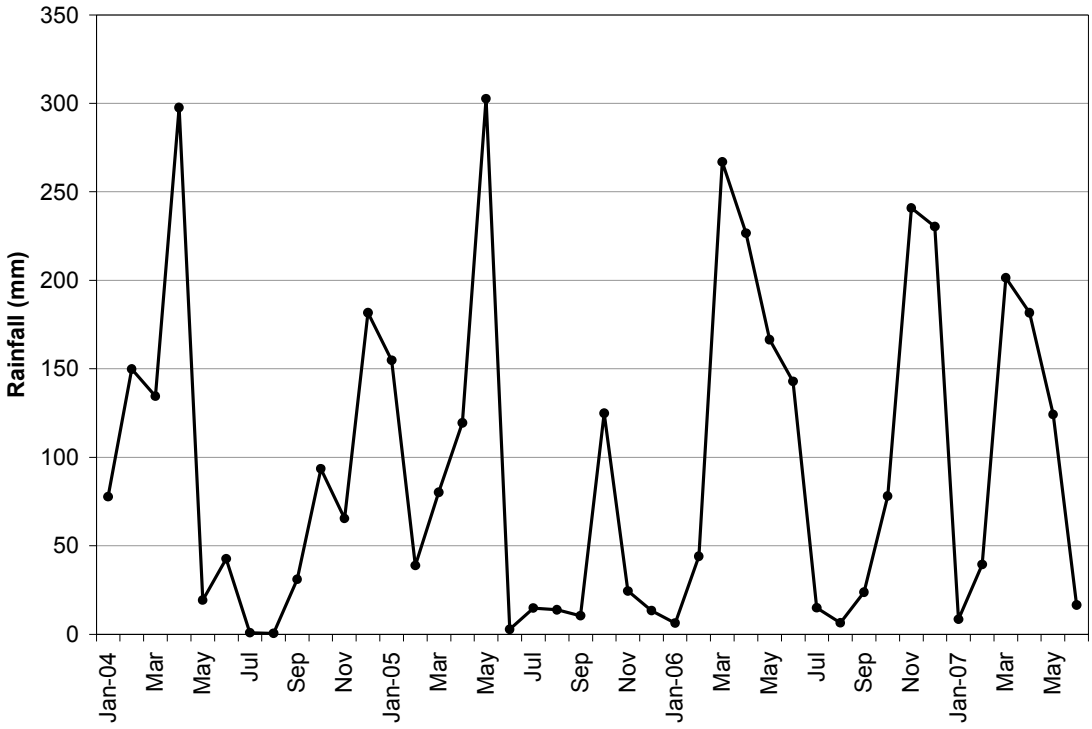


Figure 2. Monthly rainfall in Dar es Salaam, 2004-7. Rainfall measurements observed at the Dar es Salaam JK Nyerere airport station, and provided by the Tanzania Meteorological Agency, Ministry of Infrastructure and Development - http://www.meteo.go.tz. Dar es Salaam is characterized by two rainy seasons: 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)[1].

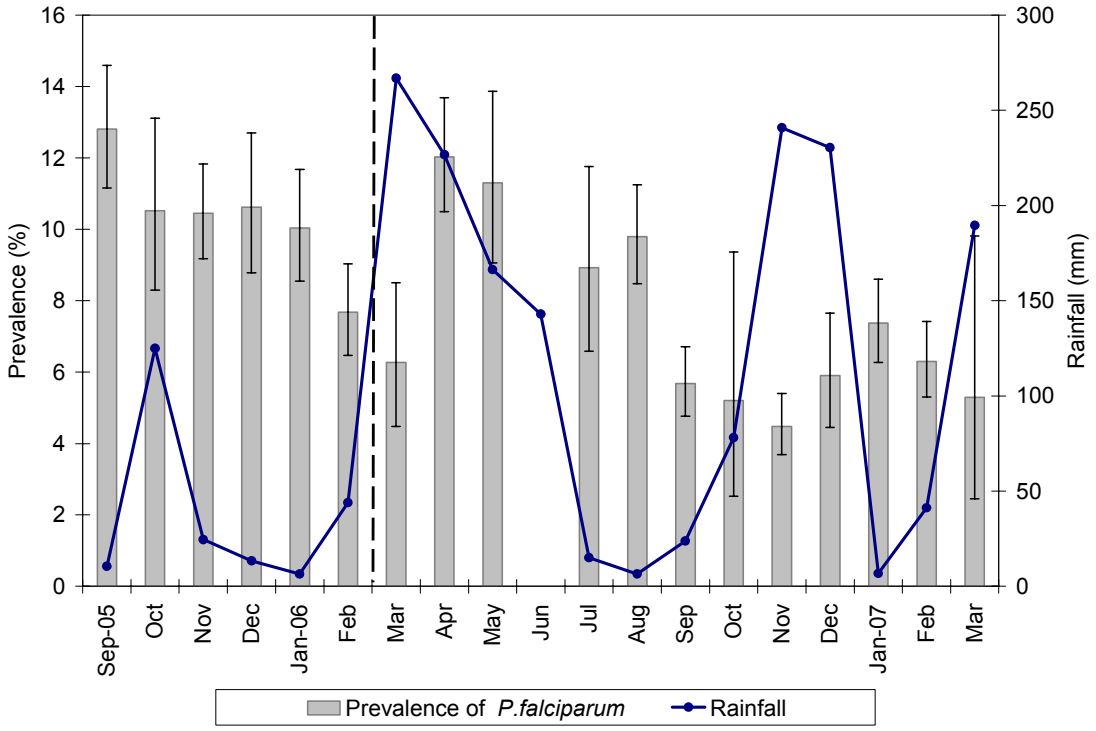


Figure 3. Prevalence of infection and rainfall by month. Error bars represent 95% confidence intervals for the prevalence of *P. falciparum* infection observed in all 15 UMCP wards. No data was collected in June 2006. Periods of heavy rains are usually associated to low prevalence of infection, while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

[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.

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

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.

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.

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

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.

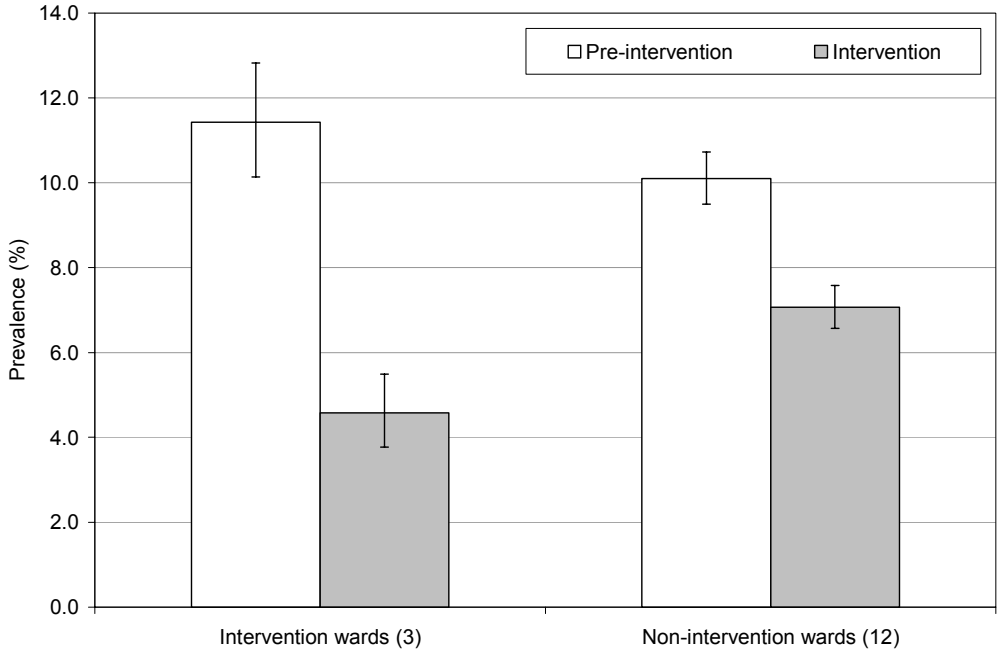


Figure 4. Prevalence of infection during pre-intervention and intervention periods. Error bars represent 95% confidence intervals for the prevalence of *P. falciparum* infection. Three wards were treated with microbial larvicides: Buguruni (I), Mikocheni (K), and Kurasini (T). Pre-intervention period 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,

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 *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). Wet season describe a period of heavy or moderate rainfall, while a dry season refer to absence of light rainfall. Average prevalence *P.falciparum* infection presented with the binomial confidence interval. 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.

Period and area	Prevalence of *P.falciparum* infection by precipitation seasonal pattern					
	Dry	95% CI		Wet	95% CI	
Pre-intervention						
Non-intervention wards	10.1	9.4	10.9	10.0	8.9	11.2
Intervention wards	10.7	9.1	12.4	12.7	10.5	15.1
Intervention						
Non-intervention wards	8.0	7.3	8.7	5.8	5.2	6.6
Intervention wards	5.1	3.8	6.8	4.2	3.3	5.4

5.5 Discussion

Recent studies in Africa have shown that the use of microbial larvicides reduced the *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 *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%

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

We are deeply grateful to the late Michael Kiama for his insights, contributions, and tireless dedication to the UMCP. We are also thankful to the people of Dar es Salaam, and to staff members at the council, municipal, and ward levels for their cooperation. MCC thanks the Department of Population and International Health, Harvard School of Public Health, and the Office of Population Research, Princeton University for financial support. This manuscript was published with the kind permission of Dr. Andrew Kitua, Director of the National Institute of Medical Research of the United Republic of Tanzania.

Funding

This research was funded by the Bill and Melinda Gates Foundation; the Swiss Tropical Institute; the United States Agency for International Development – USAID (Environmental Health Project, Dar es Salaam Mission and U.S. President's Malaria Initiative); the Wellcome Trust (through a Research Career Development Fellowship number 076806 awarded to GFK); Valent Biosciences Corporation; and the Japan International Cooperation Agency (JICA).

5.7 References

1. Ross R (1910) The prevention of malaria. London: J. Murray. 669 p.

2. Hay SI, Guerra CA, Tatem AJ, Atkinson PM, Snow RW (2005) Urbanization, malaria transmission and disease burden in Africa. Nature Reviews Microbiology 3: 81-90.

3. Keiser J, Utzinger J, Castro MC, A. ST, Tanner M, et al. (2004) Urbanization in sub-Saharan Africa and implications for malaria control. American Journal of Tropical Medicine and Hygiene 71(Suppl 2): 118-127.

4. Robert V, Macintyre K, Keating J, Trape J-F, Duchemin J-B, et al. (2004) Malaria transmission in urban sub-Saharan Africa. American Journal of Tropical Medicine and Hygiene 68: 169-176.

5. UNFPA (2007) State of World Population 2007: Unleashing the Potential of Urban Growth. New York, NY: United Nations Population Fund. 99 p.

6. United Nations (2006) World Urbanization Prospects: the 2005 Revision. New York: United Nations, Department of Economic and Social Affairs, Population Division.

7. Keating J, Macintyre K, Mbogo C, Githeko A, Regens JL, et al. (2003) A geographic sampling strategy for studying relationships between human activity and malaria vectors in urban Africa. American Journal of Tropical Medicine and Hygiene 68: 357-365.

8. Castro MC, Singer BH (2006) Migration, urbanization and malaria: a comparative analysis of Dar es Salaam, Tanzania and Machadinho, Rondônia, Brazil. In: Tienda M, Findley S, Tollman S, Preston-White E, editors. Africa on the move: African migration and urbanisation in comparative perspective. Johannesburg: Wits University Press. pp. 280-307.

9. Killeen GF, Mckenzie FE, Foy BD, Schieffelin C, Billingsley PF, et al. (2000) A simplified model for predicting malaria entomologic inoculation rates based on entomologic and parasitologic parameters relevant to control. American Journal of Tropical Medicine and Hygiene 62: 535-544.
10. Tanzania (2002) 2002 Population and Housing Census. (http://www.tanzania.go.tz/census/).
11. Kyessi AG (2002) Community Participation in Urban Infrastructure Provision. Servicing Informal Settlements in Dar es Salaam. Dortmund: University of Dortmund. 405 p.
12. Castro MC, Yamagata Y, Mtasiwa D, Tanner M, Utzinger J, et al. (2004) Integrated Urban Malaria Control: a Case Study In Dar es Salaam, Tanzania. American Journal of Tropical Medicine and Hygiene 71(Suppl 2): 103-117.
13. Sattler MA, Mtasiwa D, Kiama GM, Premji Z, Tanner M, et al. (2005) Habitat characterization and spatial distribution of Anopheles sp. mosquito larvae in Dar es Salaam (Tanzania) during an extended dry period. Malaria Journal 4(1): 4.
14. Vanek MJ, Shoo B, Mtasiwa D, Kiama GM, Lindsay SW, et al. (2006) Community-based surveillance of malaria vector larval habitats: a baseline study in urban Dar es Salaam, Tanzania. BMC Public Health 6:154.
15. Beck A (1977) Medicine and society in Tanganyika, 1890-1930: a historical inquiry. Philadelphia: American Philosophical Society. 59 p.
16. Clyde DF (1967) Malaria in Tanzania. London: Oxford University Press. 167 p.
17. Pomeroy AWJ (1920) The prophylaxis of malaria in Dar es Salaam, East Africa. Journal of the Royal Atrmy Medical Corps 35: 44-63.
18. Fillinger U, Kannady K, Kiama GM, William G, Ndaro J, et al. (2007) A practical management system for routine larviciding in African cities with preliminary results from Dar es Salaam, Tanzania.

19. Dongus S, Nyika D, Kannady K, Mtasiwa D, Mshinda H, et al. (2007) Participatory mapping of target areas to enable operational larval source management to suppress malaria vector mosquitoes in Dar es Salaam, Tanzania. International Journal of Health Geographics. In Press.
20. Mukabana WR, Kannady K, Kiama GM, Ijumba JN, Mathenge EM, et al. (2006) Ecologists can enable communities to implement malaria vector control in Africa. Malaria Journal 5: doi:10.1186/1475-2875-1185-1189.
21. NMCP, JICA (2004) Integrated Malaria Control Project in Tanzania. Project Document. Dar es Salaam: Tanzania National Malaria Control Programme; Japan International Cooperation Agency. 39 p.
22. Killeen GF, Fillinger U, Kiche I, Gouagna LC, Knols BG (2002) Eradication of Anopheles gambiae from Brazil: lessons for malaria control in Africa? Lancet Infectious Diseases 2: 618-627.
23. Kitron U, Spielman A (1989) Suppression of transmission of malaria through source reduction: anti-anopheline measures applied in Israel, the United States, and Italy. Reviews of Infectious Diseases 11: 391-406.
24. Russell PF (1955) Man's mastery of malaria. London, New York: Oxford University Press. 308 p.
25. Soper FL, Wilson DB (1943) Anopheles gambiae in Brazil, 1930 to 1940. New York City: The Rockefeller Foundation. 262 p.
26. Utzinger J, Tozan Y, Singer BH (2001) Efficacy and cost-effectiveness of environmental management for malaria control. Tropical Medicine and International Health 6: 677-687.
27. Watson M (1953) African highway: the battle for health in Central Africa. London: J. Murray. 294 p.

28. Watson M (1921) The prevention of malaria in the Federated Malay States: a record of twenty years' progress. London: J. Murray. 381 p.
29. Killeen GF, Fillinger U, Knols BG (2002) Advantages of larval control for African malaria vectors: low mobility and behavioural responsiveness of immature mosquito stages allow high effective coverage. Malaria Journal 1: 8.
30. Fillinger U, Sonye G, Killeen GF, Knols BG, Becker N (2004) The practical importance of permanent and semipermanent habitats for controlling aquatic stages of Anopheles gambiae sensu lato mosquitoes: operational observations from a rural town in western Kenya. Tropical Medicine & International Health 9: 1274–1289.
31. Fillinger U, Lindsay SW (2006) Suppression of exposure to malaria vectors by an order of magnitude using microbial larvicides in rural Kenya. Tropical Medicine & International Health 11: 1629–1642.
32. Shililu J, Mbogo C, Ghebremeskel T, Githure J, Novak R (2007) Mosquito larval habitats in a semiarid ecosystem in Eritrea: Impact of larval habitat management on *Anopheles Arabiensis* population. American Journal of Tropical Medicine and Hygiene 76: 103–110.
33. Sogoba N, Doumbia S, Vounatsou P, Baber I, Keita M, et al. (2007) Monitoring of larval habitats and mosquito densities in the Sudan savanna of Mali: Implications for malaria vector control. American Journal of Tropical Medicine and Hygiene 77: 82-88.
34. MARA/ARMA (2002) MARA LITe for Africa - Malaria. CD. Version 3.0.
35. World Health Organization. (1991) Basic malaria microscopy. Geneva: World Health Organization.
36. StataCorp (2005) Stata Statistical Software: Release 9.0. College Station, TX: StataCorp.
37. Champion AG, Hugo G (2004) New forms of urbanization: beyond the urban-rural dichotomy. Aldershot, Hants, England: Ashgate. 420 p.

38. Hugo G, Champion A, Lattes A (2003) Toward a New Conceptualization of Settlements for Demography. Population and Development Review 29: 277-297.

39. Phillips DR (1993) Urbanization and human health. Parasitology 106 (Suppl): S93-107.

40. Utzinger J, Tanner M, Kammen DM, Killeen GF, Singer BH (2002) Integrated programme is key to malaria control. Nature 419: 431.

41. Levin SA (1992) The problem of pattern and scale in ecology. Ecology 73: 1943-1967.

42. Lindsay SW, Birley M (2004) Rural Development and Malaria Control in Sub-Saharan Africa. EcoHealth 1: 129-137.

43. Mabaso MLH, Sharp B, Lengeler C (2004) Historical review of malarial control in southern African with emphasis on the use of indoor residual house-spraying. Tropical Medicine & International Health 9: 846-856.

44. Sharp B, van Wyk P, Sikasote JB, Banda P, Kleinschmidt I (2002) Malaria control by residual insecticide spraying in Chingola and Chililabombwe, Copperbelt Province, Zambia. Tropical Medicine & International Health 7: 732-736.

45. Utzinger J, Tozan Y, Doumani F, Singer BH (2002) The economic payoffs of integrated malaria control in the Zambian copperbelt between 1930 and 1950. Tropical Medicine & International Health 7: 657-677.

46. Kleinschmidt I, Sharp B, Benavente LE, Schwabe C, Torrez M, et al. (2006) Reduction in infection with Plasmodium Falciparum one year after the introduction of malaria control interventions on Bioko Island, Equatorial Guinea. American Journal of Tropical Medicine and Hygiene 74: 972-978.

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

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

Paper submitted to

British Medical Journal

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 [5-10]. Even though malaria transmission is generally lower in urban areas [5 6 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 [32]. Tanzania has emphasized widespread use of ITNs as a priority malaria vector control strategy [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 [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 [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 (*Bacillus thuringiensis* var. *israelensis (Bti)* and *B. sphaericus (Bs)*) through community-based but vertically managed delivery systems [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 [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.

6.3 Methods

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 [12]. It has around 2.5 million inhabitants and covers a total area of 1400 km^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 *mitaa* (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 [40] (Figure 1).

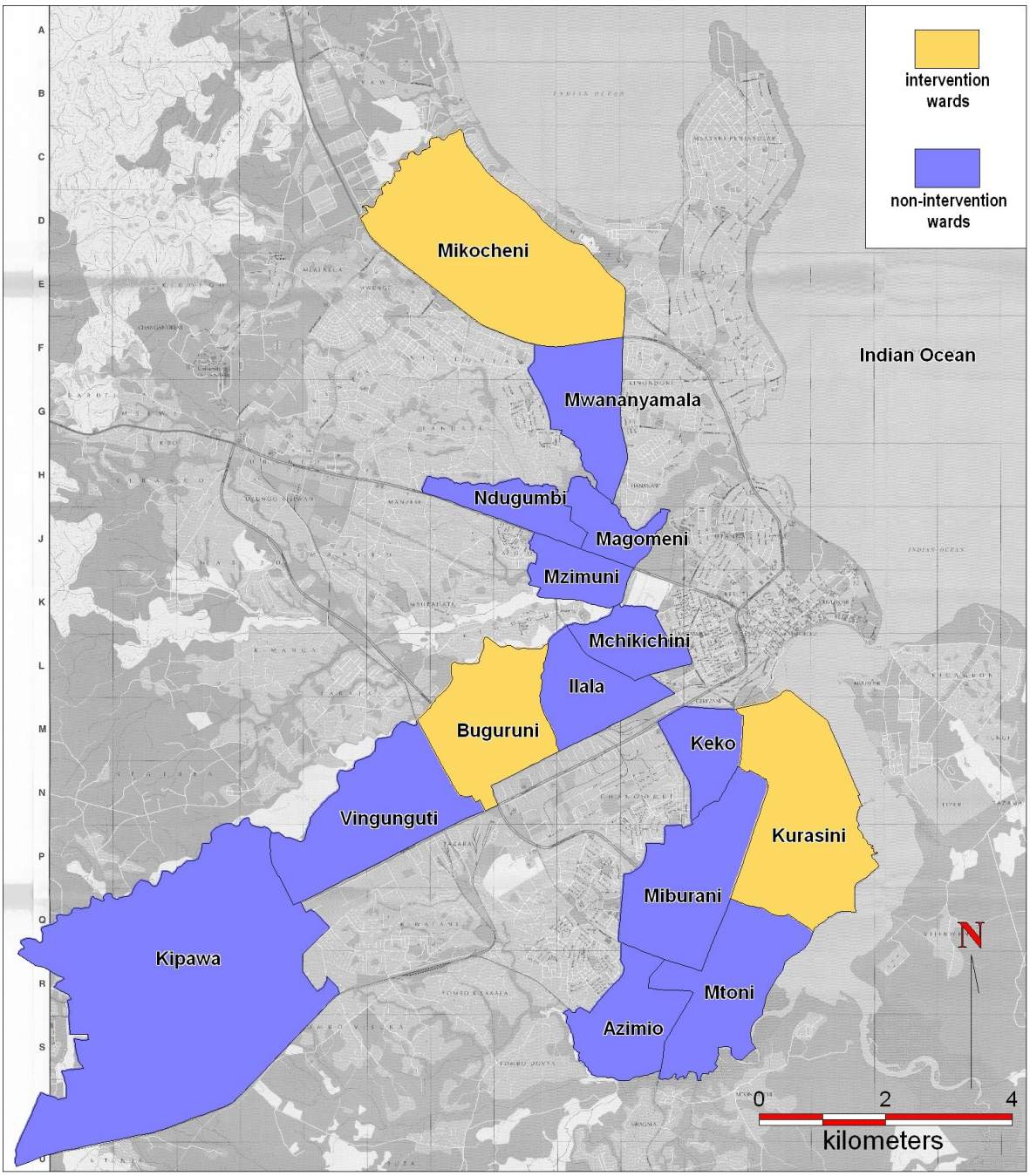


Figure 1. Wards included in the study area of the Dar es Salaam Urban Malaria Control Program (UMCP), specifying those targeted for larviciding from March 2006 onwards (intervention) and those which did receive any larviciding over the course of the study (non-intervention wards).

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 km^2 in which 611,871 people resided in 2002 [39]. The new management and delivery systems developed which underpin this program are described in detail elsewhere [38]. 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 [38 40], 2) Monitoring of adult mosquito densities [32 38], 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 [38], adding to existing interventions such as bednets, house screening, ceiling boards, repellents, coils and spray. Buguruni, Mikocheni and Kurasini wards in Ilala, Kindondoni and Temeke 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 [38 40]. 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 [38 40]. 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 [32]. 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 [32]. All mosquitoes were identified morphologically to genus and, in the case of *Anopheles*, to species complex level [42 43]. Members of the *An. gambiae* complex were further identified to sibling species level by polymerase chain reaction (PCR) [44]. The sporozoite infection status of each mosquito was determined by enzyme-linked immunoabsorbent assay as previously described [45].

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 [46]. 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 [38]. 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 [38].

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), χ^2=2.42, P<0.5) or *An. coustani* (0.53 % (5/936), χ^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).

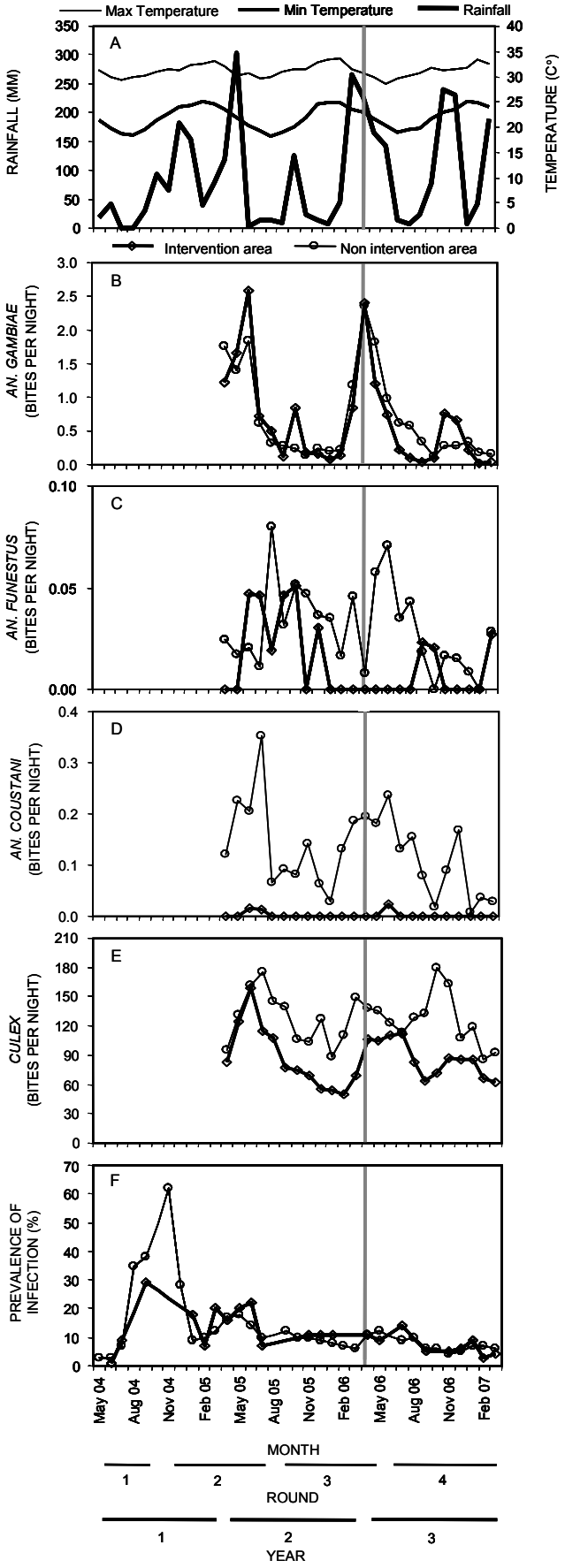


Figure 2. Monthly variations in rainfall, temperature (A), mosquito biting densities (B – E) and malaria prevalence (F) in the intervention and non-intervention areas over the first three years of the urban malaria control program (UMCP). Climatic and prevalence data was available from May 2004 till 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).

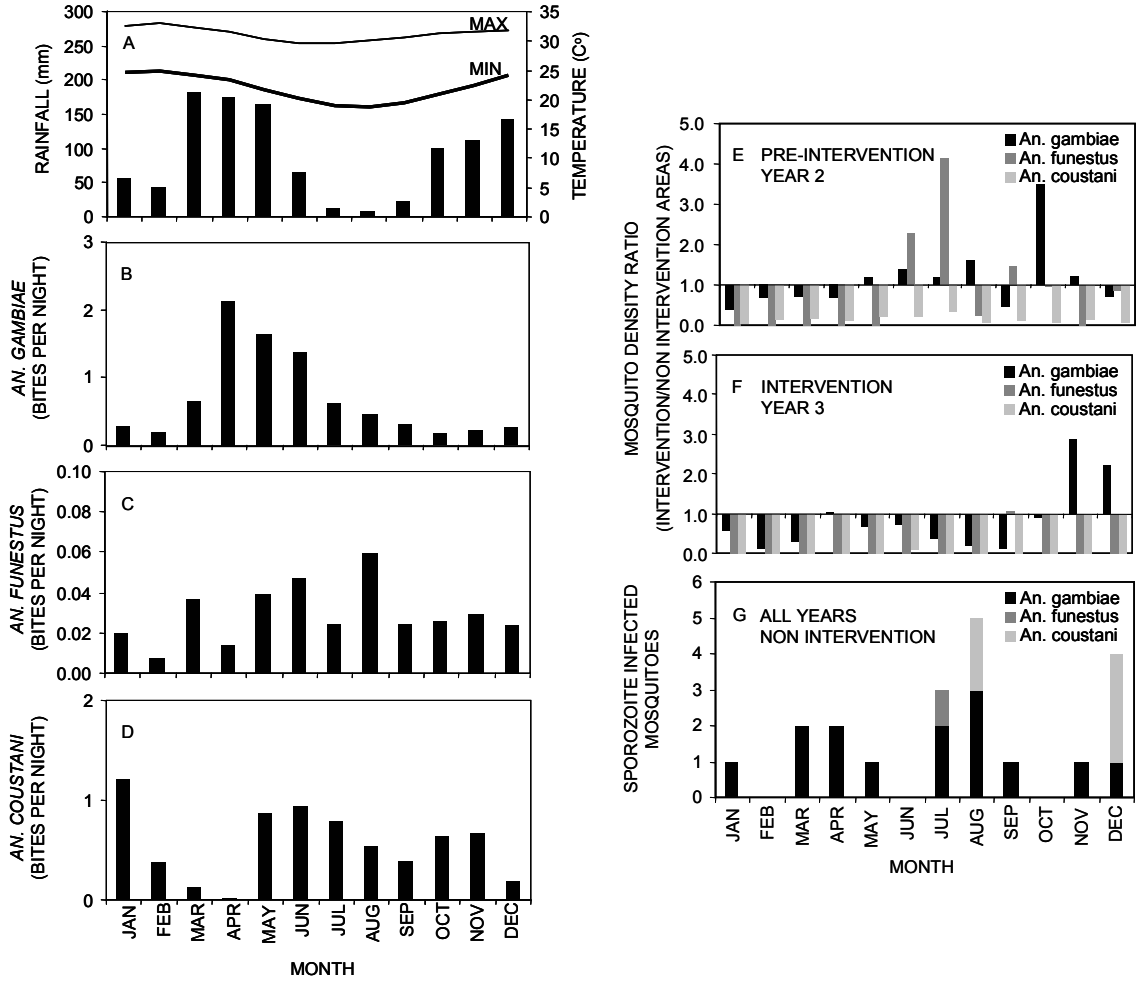


Figure 3. Seasonal patterns of rainfall and temperature (A), seasonal distribution of mosquito biting densities (B – D) and sporozoite-infected mosquitoes in the non-intervention areas (G), as well as relative biting rates in the pre-intervention and the intervention year (E, F). Relative biting densities 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 [23]. 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 [47-51]. 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).

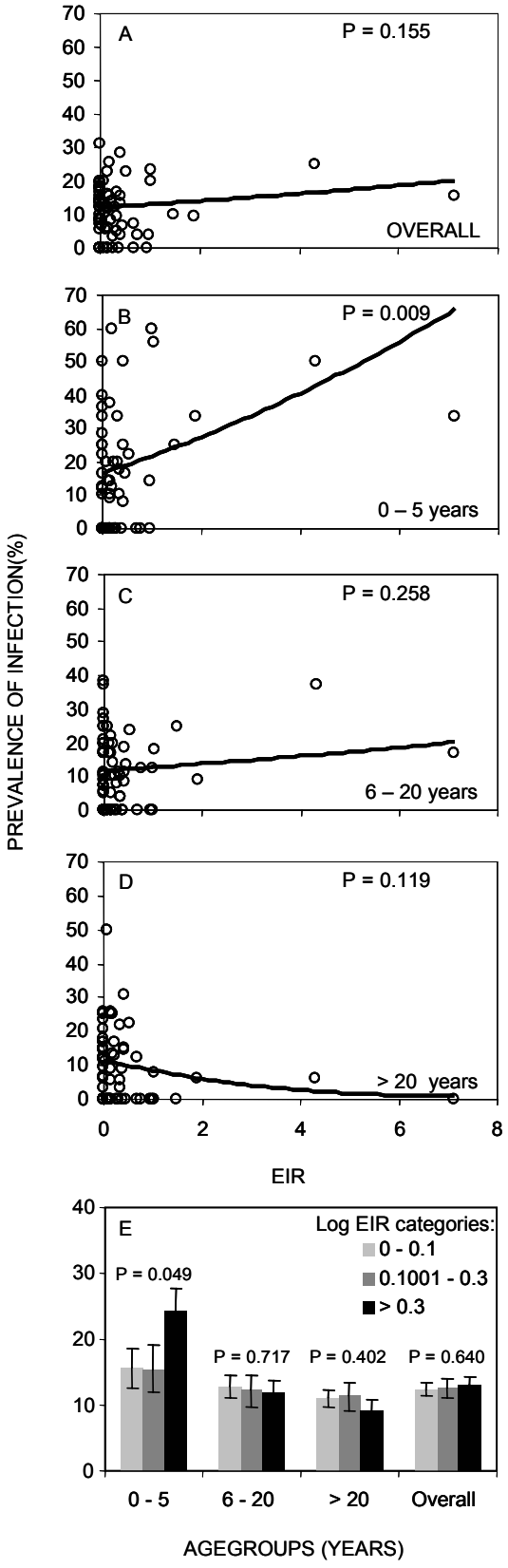


Figure 4. Association between malaria prevalence and entomological inoculation rate (EIR) as a function of age. The proportion of residents patently infected in each Ten Cell Unit (TCU) where EIR was also determined is presented as open circles in panels A-D for all ages (A), young children (B), older 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 [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 [56-58] in this urban setting with relatively well developed health services [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 [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 ($P \leq 0.001$). 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 [23]. 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.

Model details	Malaria Prevalence		Annual mean mosquito densities (bites per night) and malaria transmission intensity (infectious bites per person per year)									
Sample size	7173 human subjects over years 1 to 3		5228 catcher nights over years 2 and 3									
Geographical unit	Mtaa		TCU									
Response distribution	Binomial		Normal									
Response link function	Logit		Log									
			An. gambiae s.l.		*An. funestus*		*An. coustani*		Total EIR		*Culex*	
Parameters excluded		P-value	Parameter	P-value	Parameter	P-value	Parameter	P-value	Parameter	P-value	Parameter	P-value
Ceiling board		0.966	Year	0.635	Year	0.344	Year	0.236	Year	0.306	Year	0.835
ITN		0.902	Larviciding	0.356								
Repellent		0.676										
Sleep elsewhere		0.357										
Untreated bednets		0.344										
Window screening		0.301										
Coil		0.172										
Spray		0.107										
Parameters included	Estimate [95% CI]	P-value			Estimate [95% CI]	P-value	Estimate [95% CI]	P-value	Estimate [95% CI]	P-value	Estimate [95% CI]	P-value
Constant	0.388 [0.213, 0.706]	0.002			0.028 [0.016, 0.049]	<0.001	0.099 [0.055, 0.177]	<0.001	1.292 [1.058, 1.579]	0.012	122 [110, 136]	<0.001
	OR [95% CI]				OR [95% CI]		OR [95% CI]		OR [95% CI]		OR [95% CI]	
Fresh recruit	0.854 [0.712, 1.025]	0.090										
Location	NP	0.001										
Survey Round	NP	<0.001										
Larviciding	0.434 [0.263, 0.714]	0.001			0.320 [0.103, 0.992]	0.048	0.685 [0.645, 0.728]	<0.001	0.708 [0.505, 0.991]	0.044	0.834 [0.727, 0.956]	0.009

NP not presented

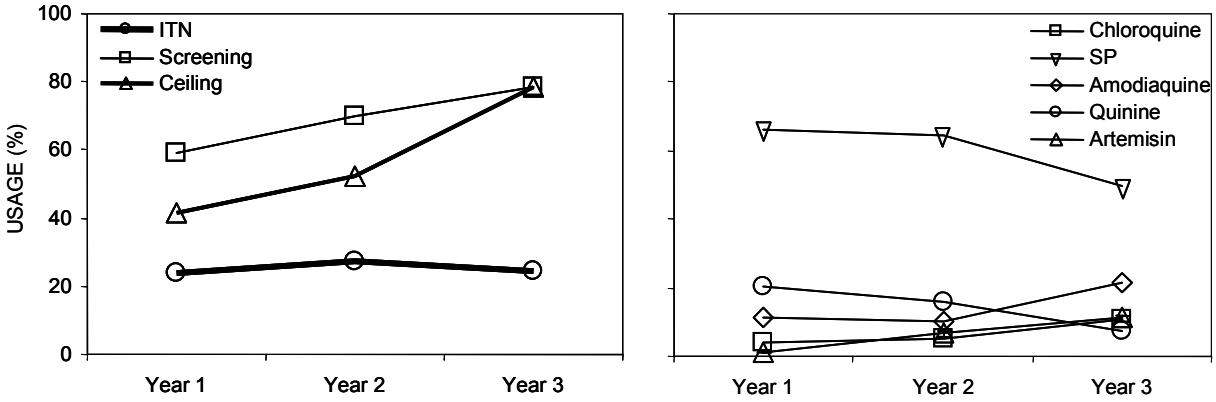


Figure 5. Time trends of protective measures and drug use in the survey areas of the Urban Malaria Control Program. The overall trends over time were calculated using a logistic regression model with the protection measures and drugs as an outcome. Except for ITN usage ($P = 0.507$), usage of other protective 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

(Figure 3G). 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 [62 63] and rural Tanzania [64 65]. Substantial delays have been observed between rainfall and peak malaria prevalence [66] 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 [67]

[68]. 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 [69-71].

Malaria prevalence is heavily influenced by EIR [18 72] but also by a number of interrelated non-entomological factors [19-21 28 73] including urbanization [20 28] that are difficult to dissect analytically [30]. 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 [74 75]. There was some evidence from year-by-year analyses (Appendix Table A3) that better established protective measures like ITNs [76 77] and well-protected houses [78 79], which not only have an individual but also community effects [32 35 76 77 79-86], modestly reduced malaria prevalence. Although window screening is also known to offer individual protection [32 35 79] 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 [32]. 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 [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 *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 [34-36 91 110-113], perhaps it is time to re-examine the theoretical considerations [109 114] which led to half a century of exclusive emphasis upon adult mosquito control for malaria prevention in Africa and beyond [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.

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.

Bacillus thuringiensis var. *israelensis* (*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'Sulivan 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.

Funding: This study was supported financially by the Swiss Tropical Institute, the Bill & Melinda Gates Foundation (Award number 23750), Valent Biosciences Corporation, USAID (Environmental Health Program, Dar es Salaam Mission and the President's Malaria Initiative, all administered through Research Triangle International) and a Wellcome Trust Research Career Development Fellowship (number 076806) to GFK.

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.

6.8 References

1. Kiszewski A, Johns B, Schapira A, Delacollette C, Crowell V, Tan-Torres T, et al. Estimated global resources needed to attain international malaria control goals. *Bull World Health Organ* 2007;85(8):623-630.

2. World Health Organization. Global Strategic Framework for Integrated Vector Management. Geneva: World Health Organization, 2004.

3. Roll Back Malaria Partnership. Roll Back Malaria Global strategic plan 2005 - 2015. Geneva: World Health Organization, 2005.

4. Roll Back Malaria Partnership. The Abuja declaration and the plan of action. Geneva: World Health Organization, 2003.

5. Robert V, MacIntyre K, Keating J, Trape JF, Duchemin JB, Warren M, et al. Malaria transmission in urban sub-Saharan Africa. *Am J Trop Med Hyg* 2003;68(2):169-176.

6. Keiser J, Utzinger J, Castro MC, Smith TA, Tanner M, Singer BH. Urbanization in sub-Saharan Africa and implication for malaria control. *Am J Trop Med Hyg* 2004;71(2 Suppl):118-27.

7. Donnelly MJ, McCall PJ, Lengeler C, Bates I, D'Alessandro U, Barnish G, et al. Malaria and urbanization in sub-Saharan Africa. *Malar J* 2005;4(1):12.

8. Wang SJ, Lengeler C, Smith TA, Vounatsou P, Cisse G, Diallo DA, et al. Rapid urban malaria appraisal (RUMA) in sub-Saharan Africa. *Malar J* 2005;4:40.

9. Hay SI, Guerra CA, Tatem AJ, Atkinson PM, Snow RW. Urbanization, malaria transmission and disease burden in Africa. *Nat Rev Microbiol.* 2005;3:81-90.

10. Lines J, Harpham T, Leake C, Schofield C. Trends, priorities and policy directions in the control of vector-borne diseases in urban environments. *Health Policy Plan* 1994;9(2):113-129.

11. Omumbo JA, Guerra CA, Hay SI, Snow RW. The influence of urbanisation on measures of *Plasmodium falciparum* infection prevalence in East Africa. *Acta Trop* 2005;93(1):11-21.

12. Castro MC, Yamagata Y, Mtasiwa D, Tanner M, Utzinger J, Keiser J, et al. Integrated urban malaria control: a case study in Dar es Salaam, Tanzania. *Am J Trop Med Hyg* 2004;71 (Supplement 2):103-117.

13. United Nations. World urbanization prospects: the 2003 revision. Data, tables and highlights <http://www.un.org/esa/population/publications/wup2003/2003WUPHighlights.pdf> 2004.

14. Smith T, Maire N, Dietz K, Killeen GF, Vounatsou P, Molineaux L, et al. Relationship between the entomologic inoculation rate and the force of infection for *Plasmodium falciparum* malaria. *Am J Trop Med Hyg* 2006;75(2 Suppl):11-8.

15. Ross A, Maire N, Molineaux L, Smith T. An epidemiologic model of severe morbidity and mortality caused by *Plasmodium falciparum*. *Am J Trop Med Hyg* 2006;75(2 Suppl):63-73.

16. Ross A, Killeen G, Smith T. Relationships between host infectivity to mosquitoes and asexual parasite density in *Plasmodium falciparum*. *Am J Trop Med Hyg* 2006;75(2 Suppl):32-7.

17. Trape JF, Pison G, Spiegel A, Enel C, Rogier C. Combating malaria in Africa. *Trends Parasitol* 2002;18(5):224-230.

18. Beier JC, Killeen GF, Githure J. Short report: Entomologic inoculation rates and *Plasmodium falciparum* malaria prevalence in Africa. *Am J Trop Med Hyg* 1999;61(1):109-113.

19. Koram KA, Bennett S, Adiamah JH, Greenwood BM. Socio-economic risk factors for malaria in a peri-urban area of The Gambia. *Trans R Soc Trop Med Hyg* 1995;89(2):146-50.

20. Mensah OA, Kumaranayake L. Malaria incidence in rural Benin: does economics matter in endemic area? *Health Policy* 2004;68(1):93-102.

21. Klinkenberg E, McCall PJ, Wilson MD, Akoto AO, Amerasinghe FP, Bates I, et al. Urban malaria and anaemia in children: a cross-sectional survey in two cities of Ghana. *Trop Med Int Health* 2006;11(5):578-88.

22. Ronald LA, Kenny SL, Klinkenberg E, Akoto AO, Boakye I, Barnish G, et al. Malaria and anaemia among children in two communities of Kumasi, Ghana: a cross-sectional survey. *Malar J* 2006;5:105.

23. Wang SJ, Lengeler C, Mtasiwa D, Mshana T, Manane L, Maro G, et al. Rapid urban malaria appraisal (RUMA) II: Epidemiology of urban malaria in Dar es Salaam (Tanzania). *Malar J* 2006;5(1):29.

24. Wang SJ, Lengeler C, Smith TA, Vounatsou P, Cisse G, Tanner M. Rapid urban malaria appraisal (RUMA) III:Epidemiology of urban malaria in the municipality of Yopougon (Abidjan). *Malar J* 2006;5(1):28.

25. Ng'andu N, Watts TE, Wray JR, Chela C, Zulu B. Some risk factors for transmission of malaria in a population where control measures were applied in Zambia. *East Afr Med J* 1989;66(11):728-37.

26. Govere J, Durrheim D, la Grange K, Mabuza A, Booman M. Community knowledge and perceptions about malaria and practices influencing malaria control in Mpumalanga Province, South Africa. *S Afr Med J* 2000;90(6):611-6.

27. Doannio JM, Konan YL, Amalaman K, Attiah J. [Knowledge, attitudes and practices of populations towards mosquitoes in urban and rural area (Cote d'Ivoire--West Africa)]. *Bull Soc Pathol Exot* 2004;97(4):295-301.

28. MacIntyre K, Keating J, Sosler S, Kibe L, Mbogo CM, Githeko A, et al. Examining the determinants of mosquito avoidance practices in two Kenyan cities. *Malaria J* 2002;1:14.

29. Stephens C, Masamu ET, Kiama MG, Keto AJ, Kinenekejo M, Ichimori K, et al. Knowledge of mosquitoes in relation to public and domestic control activities in the cities of Dar es Salaam and Tanga. *Bull World Health Organ* 1995;73(1):97-104.

30. Bates I, Fenton C, Gruber J, Lalloo D, Medina Lara A, Squire SB, et al. Vulnerability to malaria, tuberculosis and HIV/AIDS infection and disease. Part 1:determinants operating at individual and household level. *Lancet Infect Dis* 2004;4:267-277.

31. Bates I, Fenton C, Gruber J, Lalloo D, Medina Lara A, Squire SB, et al. Vulnerability to malaria, tuberculosis and HIV/AIDS infection and disease. Part 2:determinants operating at environmental and institutional level. *Lancet Infect Dis* 2004;4:368-375.

32. Geissbühler Y, Chaki P, Emidi B, Govella NJ, Shirima R, Mayagaya V, et al. Interdependence of domestic malaria prevention measures and mosquito-human interactions in urban Dar es Salaam, Tanzania. *Malar J* 2007;6(1):126.

33. Ministry of Health. National malaria medium term strategic plan, 2002-2007. Dar es Salaam: Ministry of Health, United Republic of Tanzania & World Health Organization, 2002:55.

34. Utzinger J, Tozan Y, Singer BH. Efficacy and cost effectiveness of environmental management for malaria control. *Trop Med Int Health* 2001;6(9):677-687.

35. Keiser J, Singer BH, Utzinger J. Reducing the burden of malaria in different eco-epidemiological settings with environmental management: a systematic review. *Lancet Infect Dis* 2005;5(11):695-708.

36. Killeen GF, Fillinger U, Kiche I, Gouagna LC, Knols BGJ. Eradication of *Anopheles gambiae* from Brazil: lessons for malaria control in Africa? *Lancet Infect Dis* 2002;2:618-627.

37. Mukabana WR, Kannady K, Kiama GM, Ijumba JN, Mathenge EM, Kiche I, et al. Ecologists can enable communities to implement malaria vector control in Africa. *Malar J* 2006;5:9.

38. Fillinger U, Kannady K, William G, Vanek MJ, Dongus S, Nyika D, et al. A tool box for operational mosquito larval control: preliminary results and early lessons from the Urban Malaria Control Programme in Dar es Salaam, Tanzania. *Malar J* 2008;7(1):20.

39. National Bureau of Statistics. The 2002 population and housing census general report. *http://www.tanzania.go.tz/census/dsm.htm* 2003.

40. Dongus S, Nyika D, Kannady K, Mtasiwa D, Mshinda H, Fillinger U, et al. Participatory mapping of target areas to enable operational larval source management to suppress malaria vector mosquitoes in Dar es Salaam, Tanzania. *Int J Health Geogr* 2007;6(1):37.

41. Service MW. A critical review of procedures for sampling populations of adult mosquitoes. *Bull Entomol Res* 1977;67:343-382.

42. Gillies MT, DeMeillon B. The Anophelinae of Africa South of the Sahara (Ethiopian zoogeographical region). Johannesburg: South African Institute for Medical Research, 1968.

43. Gillies MT, Coetzee M. A supplement to the Anophelinae of Africa South of the Sahara (Afrotropical region). Johannesburg: South African Medical Research Institute, 1987.

44. Scott JA, Brogdon WG, Collins FH. Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. *Am J Trop Med Hyg* 1993;49:520-529.

45. Burkot TR, Williams JL, Schneider I. Identification of *Plasmodium falciparum*-infected mosquitoes by a double antibody enzyme-linked immunosorbent assay. *Am J Trop Med Hyg* 1984;33:783-788.

46. Filmer D, Pritchett LH. Estimating wealth effects without expenditure data--or tears: an application to educational enrolments in states of India. *Demography* 2001;38(1):115-32.

47. Snow RW, Omumbo JA, Lowe B, Molyneaux CS, Obiero JO, Palmer J, et al. Relation between severe malaria morbidity in children and level of *Plasmodium falciparum* transmission in Africa. *Lancet* 1997;349:1650-1654.

48. Marsh K. Malaria--a neglected disease? *Parasitology* 1992;104 Suppl:S53-69.

49. Smith T, Beck HP, Kitua A, Mwankusye S, Felger I, Fraser-Hurt N, et al. Age dependence of the multiplicity of *Plasmodium falciparum* infections and of other malariological indices in an area of high endemicity. *Trans R Soc Trop Med Hyg* 1999;93 Suppl 1:15-20.

50. Molineaux L, Gramiccia G. The Garki Project. Geneva: World Health Organisation, 1980.

51. Snow RW, Marsh K. The consequences of reducing transmission of *Plasmodium falciparum* in Africa. *Adv Parasitol* 2002;52:235-64.

52. Mbogo CN, Snow RW, Khamala CP, Kabiru EW, Ouma JH, Githure JI, et al. Relationships between *Plasmodium falciparum* transmission by vector populations and the incidence of severe disease at nine sites on the Kenyan coast. *Am J Trop Med Hyg* 1995;52(3):201-6.

53. Mbogo CN, Snow RW, Kabiru EW, Ouma JH, Githure JI, Marsh K, et al. Low-level *Plasmodium falciparum* transmission and the incidence of severe malaria infections on the Kenyan coast. *Am J Trop Med Hyg* 1993;49(2):245-53.

54. Marsh K, Otoo L, Hayes RJ, Carson DC, Greenwood BM. Antibodies to blood stage antigens of *Plasmodium falciparum* in rural Gambians and their relation to protection against infection. *Trans R Soc Trop Med Hyg* 1989;83(3):293-303.

55. Greenwood BM, Bradley AK, Greenwood AM, Byass P, Jammeh K, Marsh K, et al. Mortality and morbidity from malaria among children in a rural area of The Gambia, West Africa. *Trans R Soc Trop Med Hyg* 1987;81(3):478-86.

56. Gu W, Mbogo CM, Githure JI, Regens JL, Killeen GF, Swalm CM, et al. Low recovery rates stabilize malaria endemicity in areas of low transmission in coastal Kenya. *Acta Trop* 2003;86(1):71-81.

57. Vercruysse J, Jancloes M, Van de Velden L. Epidemiology of seasonal *falciparum* malaria in an urban area of Senegal. *Bull World Health Organ* 1983;61:821-831.

58. Gu W, Killeen GF, Mbogo CM, Regens JL, Githure JI, Beier JC. An individual-based model of *Plasmodium falciparum* malaria transmission on the coast of Kenya. *Trans R Soc Trop Med Hyg* 2003;97(1):43-50.

59. Lorenz N, Mtasiwa D. Health in the urban environment: experience from Dar es Salaam/Tanzania. *Ann N Y Acad Sci* 2004;1023:159-63.

60. Few R, Harpham T, Atkinson S. Urban primary health care in Africa: a comparative analysis of city-wide public sector projects in Lusaka and Dar es Salaam. *Health Place* 2003;9(1):45-53.

61. Harpham T, Few R. The Dar Es Salaam Urban Health Project, Tanzania: a multi-dimensional evaluation. *J Public Health Med* 2002;24(2):112-9.

62. Koenraadt CJ, Githeko AK, Takken W. The effects of rainfall and evapotranspiration on the temporal dynamics of *Anopheles gambiae s.s.* and *Anopheles arabiensis* in a Kenyan village. *Acta Trop* 2004;90(2):141-53.

63. Mbogo CM, Mwangangi JM, Nzovu J, Gu W, Yan G, Gunter JT, et al. Spatial and temporal heterogeneity of *Anopheles* mosquitoes and *Plasmodium falciparum* transmission along the Kenyan coast. *Am J Trop Med Hyg* 2003;68(6):734-42.

64. Kulkarni MA, Kweka E, Nyale E, Lyatuu E, Mosha FW, Chandramohan D, et al. Entomological evaluation of malaria vectors at different altitudes in Hai district, northeastern Tanzania. *J Med Entomol* 2006;43(3):580-8.

65. Oesterholt MJ, Bousema JT, Mwerinde OK, Harris C, Lushino P, Masokoto A, et al. Spatial and temporal variation in malaria transmission in a low endemicity area in northern Tanzania. *Malar J* 2006;5:98.

66. Drakeley C, Sutherland C, Bousema JT, Sauerwein RW, Targett GA. The epidemiology of *Plasmodium falciparum* gametocytes: weapons of mass dispersion. *Trends Parasitol* 2006;22(9):424-30.

67. Craig MH, Snow RW, le Sueur D. A climate-based distribution model of malaria transmssion in sub-Saharan Africa. *Parasitol Today* 1999;15(3):105-111.

68. Beier JC. Malaria parasite development in mosquitoes. *Annu Rev Entomol* 1998;43:519-43.

69. Charlwood JD, Kihonda J, Sama S, Billingsley PF, Hadji H, Verhave JP, et al. The rise and fall of *Anopheles arabiensis* (Diptera: Culicidae) in a Tanzanian village. *Bull. Entomol. Res.* 1995;85:37-44.

70. Shililu J, Ghebremeskel T, Seulu F, Mengistu S, Fekadu H, Zerom M, et al. Seasonal abundance, vector behavior, and malaria parasite transmission in Eritrea. *J Am Mosq Control Assoc* 2004;20(2):155-64.

71. Shiff CJ, Minjas JN, Hall T, Hunt RH, Lyimo S, Davis JR. Malaria infection potential of anopheline mosquitoes sampled by light trapping indoors in coastal Tanzanian villages. *Med Vet Entomol* 1995;9:256-262.

72. van der Kolk M, Tebo AE, Nimpaye H, Ndombol DN, Sauerwein RW, Eling WM. Transmission of *Plasmodium falciparum* in urban Yaounde, Cameroon, is seasonal and age-dependent. *Trans R Soc Trop Med Hyg* 2003;97(4):375-9.

73. Clarke SE, Bogh C, Brown RC, Pinder M, Walraven GEL, Lindsay SW. Untreated nets protect against malaria infection. *Trans R Soc Trop Med Hyg* 2001;95:457-462.

74. Le Goff G, Robert V, Carnevale P. Evaluation of a DEET-based repellent on 3 vectors of malaria in central Africa. *Santé* 1994;4(4):269-73.

75. Manga L, Robert V, Carnevale P. Effectiveness of coils and mats for protection against malaria vectors in Cameroon. *Santé* 1995;5(2):85-8.

76. Lengeler C. Insecticide-treated bed nets and curtains for preventing malaria. *Cochrane Database Syst Rev* 2004(2):CD000363.

77. Lengeler C. Insecticide-treated nets for malaria control: real gains. *Bull World Health Organ* 2004;82(2):84.

78. Lindsay SW, Jawara M, Paine K, Pinder M, Walraven GE, Emerson PM. Changes in house design reduce exposure to malaria mosquitoes. *Trop Med Int Health* 2003;8(6):512-7.

79. Lindsay SW, Emerson PM, Charlwood JD. Reducing malaria transmission by mosquito-proofing homes. *Trends Parasitol* 2002;18(11):510-514.

80. Schellenberg JR, Abdulla S, Nathan R, Mukasa O, Marchant TJ, Kikumbih N, et al. Effect of large-scale social marketing of insecticide-treated nets on child survival in rural Tanzania. *Lancet* 2001;357(9264):1241-7.

81. Abdulla S, Schellenberg JA, Nathan R, Mukasa O, Marchant T, Smith T, et al. Impact on malaria morbidity of a programme supplying insecticide treated nets in children aged under 2 years in Tanzania: community cross sectional study. *BMJ* 2001;322(7281):270-3.

82. Killeen GF, Kihonda J, Lyimo E, Oketch FR, Kotas ME, Mathenge E, et al. Quantifying behavioural interactions between humans and mosquitoes: Evaluating the protective efficacy of insecticidal nets against malaria transmission in rural Tanzania. *BMC Infect Dis* 2006;6(1):161.

83. Maxwell CA, Msuya E, Sudi M, Njunwa KJ, Carneiro IA, Curtis CF. Effect of community-wide use of insecticide-treated nets for 3-4 years on malarial morbidity in Tanzania. *Trop Med Int Health* 2002;7(12):1003-8.

84. Hawley WA, Phillips-Howard PA, ter Kuile FO, Terlouw DJ, Vulule JM, Ombok M, et al. Community-wide effects of permethrin-treated bednets on child mortality and malaria morbidity in western Kenya. *Am J Trop Med Hyg* 2003;68 (Supplement 4):121-127.

85. Killeen GF, Smith TA, Ferguson HM, Mshinda H, Abdulla S, Lengeler C, et al. Preventing childhood malaria in Africa by protecting adults from mosquitoes with insecticide-treated nets. *PLoS Med* 2007;4(7):e229.

86. Killeen GF, Smith TA. Exploring the contributions of bed nets, cattle, insecticides and excitorepellency to malaria control: a deterministic model of mosquito host-seeking behaviour and mortality. *Trans R Soc Trop Med Hyg* 2007;101(9):867-880.

87. Fillinger U, Lindsay SW. Suppression of exposure to malaria vectors by an order of magnitude using microbial larvicides in rural Kenya. *Trop Med Int Health* 2006;11(11):1629-42.

88. Fillinger U, Knols BG, Becker N. Efficacy and efficiency of new *Bacillus thuringiensis* var *israelensis* and *Bacillus sphaericus* formulations against Afrotropical anophelines in Western Kenya. *Trop Med Int Health* 2003;8(1):37-47.

89. Majambere S, Lindsay SW, Green C, Kandeh B, Fillinger U. Microbial larvicides for malaria control in The Gambia. *Malar J* 2007;6:76.

90. Shililu JI, Tewolde GM, Brantly E, Githure JI, Mbogo CM, Beier JC, et al. Efficacy of *Bacillus thuringiensis israelensis*, *Bacillus sphaericus* and temephos for managing *Anopheles* larvae in Eritrea. *J Am Mosq Control Assoc* 2003;19(3):251-8.

91. Soper FL, Wilson DB. *Anopheles gambiae* in Brazil: 1930 to 1940. New York: The Rockefeller Foundation, 1943.

92. Shousha AT. Species-eradication. the eradication of *Anopheles gambiae* from Upper Egypt, 1942-1945. *Bull World Health Organ* 1948;1:309-353.

93. Rozendaal JA. Vector Control. Methods for use by individuals and communities. Geneva: World Health Organization, 1997.

94. Gopaul R. Surveillance entemologique a Maurice. *Santé* 1995;5:401-405.

95. Fletcher M, Teklehaimanot A, Yemane G. Control of mosquito larvae in the port city of Assab by an indigenous larvivorous fish, *Aphanius dispar*. *Acta Tropica* 1992;52:155-166.

96. Sabatinelli G, Blanchy S, Majori G, Papakay M. Impact of the use of the larvivorous fish, *Poecilia reticulata* in the transmission of malaria in the Federal Islamic Republic of Comoros. *Ann Parasitol Hum Comp* 1991;66(2):84-88.

97. Louis JP, Albert JP. Malaria in the Republic of Djibouti. Strategy for control using a biological antilarval campaign: indigenous larvivorous fishes (*Aphanius dispar*) and bacterial toxins. *Med Trop* 1988;48(2):127-131.

98. Ragavoodoo C. Situation du paludimse a Maurice. *Santé* 1995;5:371-375.

99. Julvez J. Historique du paludime insulaire dans l'ocean Indien (sud-Ouest). Une approche eco-epidemiologique. *Santé* 1995;5:353-357.

100. Watson M. African highway: The battle for health in central Africa. London: John Murray, 1953.

101. Kitron U, Spielman A. Suppression of transmission of malaria through source reduction: antianopheline measures applied in Israel, the United States, and Italy. *Rev Infect Dis* 1989;11(3):391-406.

102. Barbazan P, Baldet T, Darriet F, Escaffre H, Djoda DH, Hougard JM. Impact of treatments with *Bacillus sphaericus* on *Anopheles* populations and the transmission of malaria in Maroua, a large city in a savannah region of Cameroon. *J Am Mosq Control Assoc* 1998;14(1):33-9.

103. Gu W, Novak RJ. Habitat-based modeling of impacts of mosquito larval interventions on entomological inoculation rates, incidence, and prevalence of malaria. *Am J Trop Med Hyg* 2005;73(3):546-52.

104. Killeen GF, Tanner M, Mukabana WR, Kalongolela MS, Kannady K, Lindsay SW, et al. Habitat targeting for controlling aquatic stages of malaria vectors in Africa. *Am J Trop Med Hyg* 2006;74(4):517-8; author reply 519-20.

105. Gu W, Utzinger J, Novak RJ. Habitat-based larval interventions: a new perspective for malaria control. *Am J Trop Med Hyg* 2008;78(1):2-6.

106. Worrall E. Cost analyses for large scale use of larval source management in malaria control. Washington DC: Prepared for RTI International by Liverpool Associates in Tropical Health under contract to the Bureau for Global Health, U.S. Agency for International Development (USAID). Contract no. GHS-I-01-03-00028-000-1, Integrated Vector Management Programs for Malaria Control; 2007.

107. Yukich J, Tedioso F, Lengeler C. Operations, costs and Cost-Effectiveness of Five Insecticide-Treated Net Programs (Eritrea, Malawi, Tanzania, Togo, Senegal) and Two Indoor Residual Spraying Programs (Kwa-Zulu-Natal, Mozambique). *USAID report* 2007.

108. Killeen GF, Fillinger U, Knols BGJ. Advantages of larval control for African malaria vectors: Low mobility and behavioural responsiveness of immature mosquito stages allow high effective coverage. *Malar J* 2002;1:8.

109. MacDonald G. The epidemiology and control of malaria. London: Oxford University Press, 1957.

110. Killeen GF. Following in Soper's footsteps: northeast Brazil 63 years after eradication of *Anopheles gambiae*. *Lancet Infect Dis* 2003;3:663-666.

111. Utzinger J, Tozan Y, Doumani F, Singer BH. The economic payoffs of integrated malaria control in the Zambian copperbelt between 1930 and 1950. *Trop Med Int Health* 2002;7(8):657-677.

112. Barat LM. Four malaria success stories: how malaria burden was successfully reduced in Brazil, Eritrea, India, and Vietnam. *Am J Trop Med Hyg* 2006;74(1):12-6.

113. Ross R. An address on the prevention of malaria in British possessions, Egypt, and parts of America. *Lancet* 1907;2:879-87.

114. Garrett-Jones C. Prognosis for interruption of malaria transmission through assessment of the mosquito's vectorial capacity. *Nature* 1964;204:1173-1175.

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

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.

2005). 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).

Surprislingly, *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

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

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.

7.7 References

Afrane YA, Klinkenberg E, Drechsel P, Owusu-Daaku K, Garms R et al. (2004) Does irrigated urban agriculture influence the transmission of malaria in the city of Kumasi, Ghana? Acta Trop 89(2): 125-134.

Antonio-Nkondjio C, Kerah CH, Simard F, Awono-Ambene P, Chouaibou M et al. (2006) Complexity of the malaria vectorial system in Cameroon: contribution of secondary vectors to malaria transmission. J Med Entomol 43(6): 1215-1221.

Barat LM (2006) Four malaria success stories: how malaria burden was successfully reduced in Brazil, Eritrea, India, and Vietnam. Am J Trop Med Hyg 74(1): 12-16.

Bates I, Fenton C, Gruber J, Lalloo D, Medina Lara A et al. (2004) Vulnerability to malaria, tuberculosis and HIV/AIDS infection and disease. Part 1:determinants operating at individual and household level. Lancet Infect Dis 4: 267-277.

Beier JC, Killeen GF, Githure J (1999) Short report: Entomologic inoculation rates and *Plasmodium falciparum* malaria prevalence in Africa. Am J Trop Med Hyg 61(1): 109-113.

Booman M, Sharp BL, Martin CL, Manjate B, La Grange JJ et al. (2003) Enhancing malaria control using a computerised management system in southern Africa. Malar J 2(1): 13.

Boyd MF (1926) The influence of obstacles unconsciously erected against anophelines (housing and screening) upon the incidence of malaria. Am J Trop Med Hyg 6: 157-160.

Byrd I (1914) Mosquitoes: role of certain species in prevalence of malaria. New Orleans Med Surg J 67: 1417.

Castro MC, Yamagata Y, Mtasiwa D, Tanner M, Utzinger J et al. (2004) Integrated urban malaria control: a case study in Dar es Salaam, Tanzania. Am J Trop Med Hyg 71 (Supplement 2): 103-117.

Celli A (1901) Sulla nuova profilassi della malaria. Società Editrice Dante Alighieri.

Celli A (1901) The new preventative treatment of malaria in Latium.: London School of Hygiene and Tropical Medicine. 1-12 p.

Chinery WA (1969) A survey of mosquito breeding in Accra, Ghana, during a two-year period of larval mosquito control. I. The mosquitoes collected and their breeding places. Ghana Medical Journal 8: 266-275.

Chinery WA (1984) Effects of ecological changes on the malaria vectors *Anopheles funestus* and *Anopheles gambiae* complex mosquitoes in Accra, Ghana. J Trop Meg Hyg 87: 75-81.

Doannio JM, Konan YL, Amalaman K, Attiah J (2004) [Knowledge, attitudes and practices of populations towards mosquitoes in urban and rural area (Cote d'Ivoire--West Africa)]. Bull Soc Pathol Exot 97(4): 295-301.

Dongus S, Nyika D, Kannady K, Mtasiwa D, Mshinda H et al. (2007) Participatory mapping of target areas to enable operational larval source management to suppress malaria vector mosquitoes in Dar es Salaam, Tanzania. Int J Health Geogr 6(1): 37.

Fillinger U, Lindsay SW (2006) Suppression of exposure to malaria vectors by an order of magnitude using microbial larvicides in rural Kenya. Trop Med Int Health 11(11): 1629-1642.

Fillinger U, Kannady K, William G, Vanek MJ, Dongus S et al. (2008) A tool box for operational mosquito larval control: preliminary results and early lessons from the Urban Malaria Control Programme in Dar es Salaam, Tanzania. Malar J 7(1): 20.

Geissbühler Y, Chaki P, Emidi B, Govella NJ, Shirima R et al. (2007) Interdependence of domestic malaria prevention measures and mosquito-human interactions in urban Dar es Salaam, Tanzania. Malar J 6(1): 126.

Geissbühler Y, Kannady K, Chaki P, Emidi B, Govella NJ et al. (2008) Urban malaria epidemiology and the impact of microbial larvicides upon infection prevalence in Dar es Salaam, United Republic of Tanzania. Submitted.

Gillies MT (1964) The Role Of Secondary Vectors Of Malaria In North-East Tanganyika. Trans R Soc Trop Med Hyg 58: 154-158.

Gillies MT, DeMeillon B (1968) The Anophelinae of Africa South of the Sahara (Ethiopian zoogeographical region). Johannesburg: South African Institute for Medical Research.

Gillies MT, Coetzee M (1987) A supplement to the Anophelinae of Africa South of the Sahara (Afrotropical region). Johannesburg: South African Medical Research Institute.

Gilroy AB, Bruce-Chwatt LJ (1945) Mosquito-control by swamp drainage in the coastal belt of Nigeria. Croydon: HR Grubb.

Grabowsky M, Nobiya T, Selanikio J (2007) Sustained high coverage of insecticide-treated bednets through combined Catch-up and Keep-up strategies. Trop Med Int Health 12(7): 815-822.

Graham K, Kayedi MH, Maxwell C, Kaur H, Rehman H et al. (2005) Multicountry field trials comparing wash-resistance of PermaNet and conventional insecticide-treated nets against anopheline and culicine mosquitoes. Med Vet Entomol 19: 72-83.

Gray EM, Bradley TJ (2005) Physiology of desiccation resistance in *Anopheles gambiae* and *Anopheles arabiensis*. Am J Trop Med Hyg 73(3): 553-559.

Gu W, Novak RJ (2005) Habitat-based modeling of impacts of mosquito larval interventions on entomological inoculation rates, incidence, and prevalence of malaria. Am J Trop Med Hyg 73(3): 546-552.

Haddow AJ (1943) Measurement of temoerature and light in artificial pools with reference to the larval habitat of *Anopheles (Myzomia) gambiae* Giles abd *A. (M.) funestus* Giles. Bull Entomol Res 34: 89.

Hawley WA, Phillips-Howard PA, ter Kuile FO, Terlouw DJ, Vulule JM et al. (2003) Community-wide effects of permethrin-treated bednets on child mortality and malaria morbidity in western Kenya. Am J Trop Med Hyg 68 (Supplement 4): 121-127.

Hemingway J, Field L, Vontas J (2002) An overview of insecticide resistance. Science 298(5591): 96-97.

Holstein MH (1954) Biology of *Anopheles gambiae*. Geneva: World Health Organization. 173 p.

Howard SC, Omumbo J, Nevill CG, Some ES, Donnelly CA et al. (2000) Evidence for a mass community effect of insecticide treated bednets on the incidence of malaria on the Kenyan coast. Trans R Soc Trop Med Hyg 94(4): 357-360.

Impoinvil DE, Ahmad S, Troyo A, Keating J, Githeko AK et al. (2007) Comparison of mosquito control programs in seven urban sites in Africa, the Middle East, and the Americas. Health Policy 83(2-3): 196-212.

Jacob B, Regens JL, Mbogo CM, Githeko AK, Keating J et al. (2003) Occurrence and distribution of Anopheles (Diptera: Culicidae) larval habitats on land cover change sites in urban Kisumu and urban Malindi, Kenya. J Med Entomol 40(6): 777-784.

Jacob BG, Arheart KL, Griffith DA, Mbogo CM, Githeko AK et al. (2005) Evaluation of environmental data for identification of Anopheles (Diptera: Culicidae) aquatic larval habitats in Kisumu and Malindi, Kenya. J Med Entomol 42(5): 751-755.

Keating J, Macintyre K, Mbogo CM, Githure JI, Beier JC (2004) Characterization of potential larval habitats for Anopheles mosquitoes in relation to urban land-use in Malindi, Kenya. Int J Health Geogr 3(1): 9.

Keating J, MacIntyre K, Mbogo C, Githeko A, Regens JL et al. (2003) A geographic sampling strategy for studying relationships between human activity and malaria vectors in urban Africa. Am J Trop Med Hyg 68(3): 357-365.

Keiser J, Singer BH, Utzinger J (2005) Reducing the burden of malaria in different eco-epidemiological settings with environmental management: a systematic review. Lancet Infect Dis 5(11): 695-708.

Keiser J, Utzinger J, Castro MC, Smith TA, Tanner M et al. (2004) Urbanization in sub-Saharan Africa and implication for malaria control. Am J Trop Med Hyg 71(2 Suppl): 118-127.

Kiker CC (1941) Housing with special reference to mosquito-proofing for malaria control. In *Symposium on Human Malaria*: 308-314, American Association for the Advancement of Science.

Killeen GF (2003) Following in Soper's footsteps: northeast Brazil 63 years after eradication of *Anopheles gambiae*. Lancet Infectious Diseases 3: 663-666.

Killeen GF, Smith TA (2007) Exploring the contributions of bed nets, cattle, insecticides and excitorepellency to malaria control: a deterministic model of mosquito host-seeking behaviour and mortality. Trans R Soc Trop Med Hyg 101(9): 867-880.

Killeen GF, Fillinger U, Knols BGJ (2002) Advantages of larval control for African malaria vectors: Low mobility and behavioural responsiveness of immature mosquito stages allow high effective coverage. Malar J 1: 8.

Killeen GF, Knols BG, Gu W (2003) Taking malaria transmission out of the bottle: implications of mosquito dispersal for vector-control interventions. Lancet Infect Dis 3(5): 297-303.

Killeen GF, Seyoum A, Knols BGJ (2004) Rationalizing historical successes of malaria control in Africa in terms of mosquito resource availability management. Am J Trop Med Hyg 71 (Supplement 2): 87-93.

Killeen GF, Fillinger U, Kiche I, Gouagna LC, Knols BGJ (2002) Eradication of *Anopheles gambiae* from Brazil: lessons for malaria control in Africa? Lancet Infect Dis 2: 618-627.

Killeen GF, Tanner M, Mukabana WR, Kalongolela MS, Kannady K et al. (2006) Habitat targeting for controlling aquatic stages of malaria vectors in Africa. Am J Trop Med Hyg 74(4): 517-518; author reply 519-520.

Killeen GF, Kihonda J, Lyimo E, Oketch FR, Kotas ME et al. (2006) Quantifying behavioural interactions between humans and mosquitoes: Evaluating the protective efficacy of insecticidal nets against malaria transmission in rural Tanzania. BMC Infect Dis 6(1): 161.

Kitron U (1987) Malaria, agriculture, and development: lessons from past camapigns. International Journal of Health Services 17(2): 295-326.

Klinkenberg E, McCall PJ, Hastings IM, Wilson MD, Amerasinghe FP et al. (2005) Malaria and irrigated crops, Accra, Ghana. Emerg Infect Dis 11(8): 1290-1293.

Klinkenberg E, McCall PJ, Wilson MD, Akoto AO, Amerasinghe FP et al. (2006) Urban malaria and anaemia in children: a cross-sectional survey in two cities of Ghana. Trop Med Int Health 11(5): 578-588.

Koram KA, Bennett S, Adiamah JH, Greenwood BM (1995) Socio-economic risk factors for malaria in a peri-urban area of The Gambia. Trans R Soc Trop Med Hyg 89(2): 146-150.

Kouznetsov RL (1977) Malaria control by application of indoor spraying of residual insecticides in tropical Africa and its impact on community health. Tropical Doctor 7: 81-93.

Le Menach A, Takala S, McKenzie FE, Perisse A, Harris A et al. (2007) An elaborated feeding cycle model for reductions in vectorial capacity of night-biting mosquitoes by insecticide-treated nets. Malar J 6: 10.

Lengeler C (2004) Insecticide-treated bed nets and curtains for preventing malaria. Cochrane Database Syst Rev(2): CD000363.

Lengeler C, Grabowsky M, McGuire D, DeSavigny D (2007) Quick Wins Versus Sustainability: Options for the Upscaling of Insecticide-Treated Nets. Am J Trop Med Hyg 77(6 Suppl): 222-6.

Lindsay SW, Parson L, Thomas CJ (1998) Mapping the ranges and relative abundance of the two principle African malaria vectors, *Anopheles gambiae sensu stricto* and *An. arabiensis*, using climate data. Proceedings of the Royal Society of London Series B 265(1399): 847-854.

Lindsay SW, Emerson PM, Charlwood JD (2002) Reducing malaria transmission by mosquito-proofing homes. Trends Parasitol 18(11): 510-514.

MacIntyre K, Keating J, Sosler S, Kibe L, Mbogo CM et al. (2002) Examining the determinants of mosquito avoidance practcies in two Kenyan cities. Malar J 1: 14.

Matthys B, N'Goran EK, Kone M, Koudou BG, Vounatsou P et al. (2006) Urban agricultural land use and characterization of mosquito larval habitats in a medium-sized town of Cote d'Ivoire. J Vector Ecol 31(2): 319-333.

Matthys B, Vounatsou P, Raso G, Tschannen AB, Becket EG et al. (2006) Urban farming and malaria risk factors in a medium-sized town in Cote d'Ivoire. Am J Trop Med Hyg 75(6): 1223-1231.

Maxwell CA, Msuya E, Sudi M, Njunwa KJ, Carneiro IA et al. (2002) Effect of community-wide use of insecticide-treated nets for 3-4 years on malarial morbidity in Tanzania. Trop Med Int Health 7(12): 1003-1008.

Maxwell CA, Myamba J, Magoma J, Rwegoshora RT, Magesa SM et al. (2006) Tests of Olyset nets by bioassay and in experimental huts. J Vector Borne Dis 43(1): 1-6.

Mensah OA, Kumaranayake L (2004) Malaria incidence in rural Benin: does economics matter in endemic area? Health Policy 68(1): 93-102.

Mukabana WR, Kannady K, Kiama GM, Ijumba JN, Mathenge EM et al. (2006) Ecologists can enable communities to implement malaria vector control in Africa. Malar J 5: 9.

N'Guessan R, Corbel V, Akogbeto M, Rowland M (2007) Reduced efficacy of insecticide-treated nets and indoor residual spraying for malaria control in pyrethroid resistance area, Benin. Emerg Infect Dis 13(2): 199-206.

Nafo Traore F (2005) Rolling back malaria: opportunities and challenges. Trans R Soc Trop Med Hyg 99(6): 403-406.

Ng'andu N, Watts TE, Wray JR, Chela C, Zulu B (1989) Some risk factors for transmission of malaria in a population where control measures were applied in Zambia. East Afr Med J 66(11): 728-737.

Oyewole IO, Awolola TS (2006) Impact of urbanization on bionomics and distribution of malaria vectors in Lagos, southwestern Nigeria. J Vector Borne Dis 43: 173-178.

Robert V, MacIntyre K, Keating J, Trape JF, Duchemin JB et al. (2003) Malaria transmission in urban sub-Saharan Africa. Am J Trop Med Hyg 68(2): 169-176.

Roll Back Malaria Partnership (2005) Global strategic plan 2005 - 2015.

Roll Back Malaria Partnership b (2005) Scaling up insecticide treated netting programmes in Africa: a strategic framework for coordinated national action. Geneva: World Health Organization.

Ronald LA, Kenny SL, Klinkenberg E, Akoto AO, Boakye I et al. (2006) Malaria and anaemia among children in two communities of Kumasi, Ghana: a cross-sectional survey. Malar J 5: 105.

Ross R (1907) An address on the prevention of malaria in British possessions, Egypt, and parts of America. Lancet 2: 879-887.

Sattler MA, Mtasiwa D, Kiama M, Premji Z, Tanner M et al. (2005) Habitat characterization and spatial distribution of Anopheles sp. mosquito larvae in Dar es Salaam (Tanzania) during an extended dry period. Malar J 4(1): 4.

Service MW (1997) Mosquito (Diptera: Culicidae) dispersal--the long and short of it. J Med Entomol 34(6): 579-588.

Service MW (2000) Medical entomology for students. Liverpool: Cambridge University Press.

Sharp BL, Kleinschmidt I, Streat E, Maharaj R, Barnes KI et al. (2007) Seven years of regional malaria control collaboration--Mozambique, South Africa, and Swaziland. Am J Trop Med Hyg 76(1): 42-47.

Shililu J, Mbogo C, Ghebremeskel T, Githure J, Novak R (2007) Mosquito larval habitats in a semiarid ecosystem in Eritrea: impact of larval habitat management on *Anopheles arabiensis* population. Am J Trop Med Hyg 76(1): 103-110.

Shililu J, Ghebremeskel T, Seulu F, Mengistu S, Fekadu H et al. (2004) Seasonal abundance, vector behavior, and malaria parasite transmission in Eritrea. J Am Mosq Control Assoc 20(2): 155-164.

Shousha AT (1948) Species-eradication. the eradication of *Anopheles gambiae* from Upper Egypt, 1942-1945. Bull World Health Organ 1: 309-353.

Sina BJ, Aultman K (2001) Resisting resistance. Trends Parasitol 17(7): 305-306.

Smith DL, Dushoff J, Snow RW, Hay SI (2005) The entomological inoculation rate and *Plasmodium falciparum* infection in African children. Nature 438(7067): 492-495.

Smith DL, McKenzie FE, Snow RW, Hay SI (2007) Revisiting the basic reproductive number for malaria and its implications for malaria control. PLoS Biol 5(3): e42.

Soper FL, Wilson DB (1943) *Anopheles gambiae* in Brazil: 1930 to 1940. New York: The Rockefeller Foundation. 262 p.

Staedke SG, Nottingham EW, Cox J, Kamya MR, Rosenthal PJ et al. (2003) Short report: proximity to mosquito breeding sites as a risk factor for clinical malaria episodes in an urban cohort of Ugandan children. Am J Trop Med Hyg 69(3): 244-246.

Stephens C, Masamu ET, Kiama MG, Keto AJ, Kinenekejo M et al. (1995) Knowledge of mosquitos in relation to public and domestic control activities in the cities of Dar es Salaam and Tanga. Bull World Health Organ 73(1): 97-104.

Thompson R, Begtrup K, Cuamba N, Dgedge M, Mendis C et al. (1997) The Matola malaria project: A temporal and spatial study of malaria transmission and disease in a suburban area of Maputo, Mozambique. Am J Trop Med Hyg 57(5): 550-559.

Townson H, Nathan MB, Zaim M, Guillet P, Manga L et al. (2005) Exploiting the potential of vector control for disease prevention. Bull World Health Organ 83(12): 942-947.

Trape JF, Lefebvre-Zante E, Legros F, G. N, Bouganali H et al. (1992) Vector density gradients and the epidemiology of urban malaria in Dakar, Senegal. Am J Trop Med Hyg 47(2): 181-189.

Utzinger J, Tozan Y, Singer BH (2001) Efficacy and cost effectiveness of environmental management for malaria control. Trop Med Int Health 6(9): 677-687.

Utzinger J, Tozan Y, Doumani F, Singer BH (2002) The economic payoffs of integrated malaria control in the Zambian copperbelt between 1930 and 1950. Trop Med Int Health 7(8): 657-677.

Utzinger J, Tanner M, Kammen DM, Killeen GF, Singer BH (2002) Integrated programme is key to malaria control. Nature 419: 431.

van der Kolk M, Tebo AE, Nimpaye H, Ndombol DN, Sauerwein RW et al. (2003) Transmission of *Plasmodium falciparum* in urban Yaounde, Cameroon, is seasonal and age-dependent. Trans R Soc Trop Med Hyg 97(4): 375-379.

Vanek MJ, Shoo B, Mtasiwa D, Kiama M, Lindsay SW et al. (2006) Community-based surveillance of malaria vector larval habitats: a baseline study in urban Dar es Salaam, Tanzania. BMC Public Health 6: 154.

Walker K, Lynch M (2007) Contributions of Anopheles larval control to malaria suppression in tropical Africa: review of achievements and potential. Med Vet Entomol 21(1): 2-21.

Wang SJ, Lengeler C, Smith TA, Vounatsou P, Akogbeto M et al. (2006) Rapid Urban Malaria Appraisal (RUMA) IV: epidemiology of urban malaria in Cotonou (Benin). Malar J 5: 45.

Wang SJ, Lengeler C, Smith TA, Vounatsou P, Cisse G et al. (2006) Rapid urban malaria appraisal (RUMA) III:Epidemiology of urban malaria in the municipality of Yopougon (Abidjan). Malar J 5(1): 28.

Wang SJ, Lengeler C, Mtasiwa D, Mshana T, Manane L et al. (2006) Rapid urban malaria appraisal (RUMA) II: Epidemiology of urban malaria in Dar es Salaam (Tanzania). Malar J 5(1): 29.

Watson M (1953) African highway: The battle for health in central Africa. London: John Murray. 294 p.

Worrall E (2007) Integrated vector management programs for malaria vector control. Cost analysis for large-scale use of larval source management in malaria control. USAID report.

Yates A, N'Guessan R, Kaur H, Akogbeto M, Rowland M (2005) Evaluation of KO-Tab 1-2-3: a wash-resistant 'dip-it-yourself' insecticide formulation for long-lasting treatment of mosquito nets. Malar J 4: 52.

Yukich J, Tedioso F, Lengeler C (2007) Operations, costs and Cost-Effectiveness of Five Insecticide-Treated Net Programs (Eritrea, Malawi, Tanzania, Togo, Senegal) and Two Indoor Residual Spraying Programs (Kwa-Zulu-Natal, Mozambique). USAID report.

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:

This document has been produced and made available by the Dar es Salaam Urban Malaria Control Programme. Contact: Urban Malaria Control Programme, City Medical Office of Health, City Council, P.O. Box 63320, Dar es Salaam, Tanzania, Phone: +255 22 212 1649

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 intent 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

10-cell unit plots map form

Municipality:_____ Ward:_____ MTAA:_____ 10-cell unit:_____

Date_____

Plot ID	House Number	Owner's name	Plot location decription (where is it in the 10-cell unit) and its basic characteristics	GPS (UTM/WGS84)	
				Northing	Easting

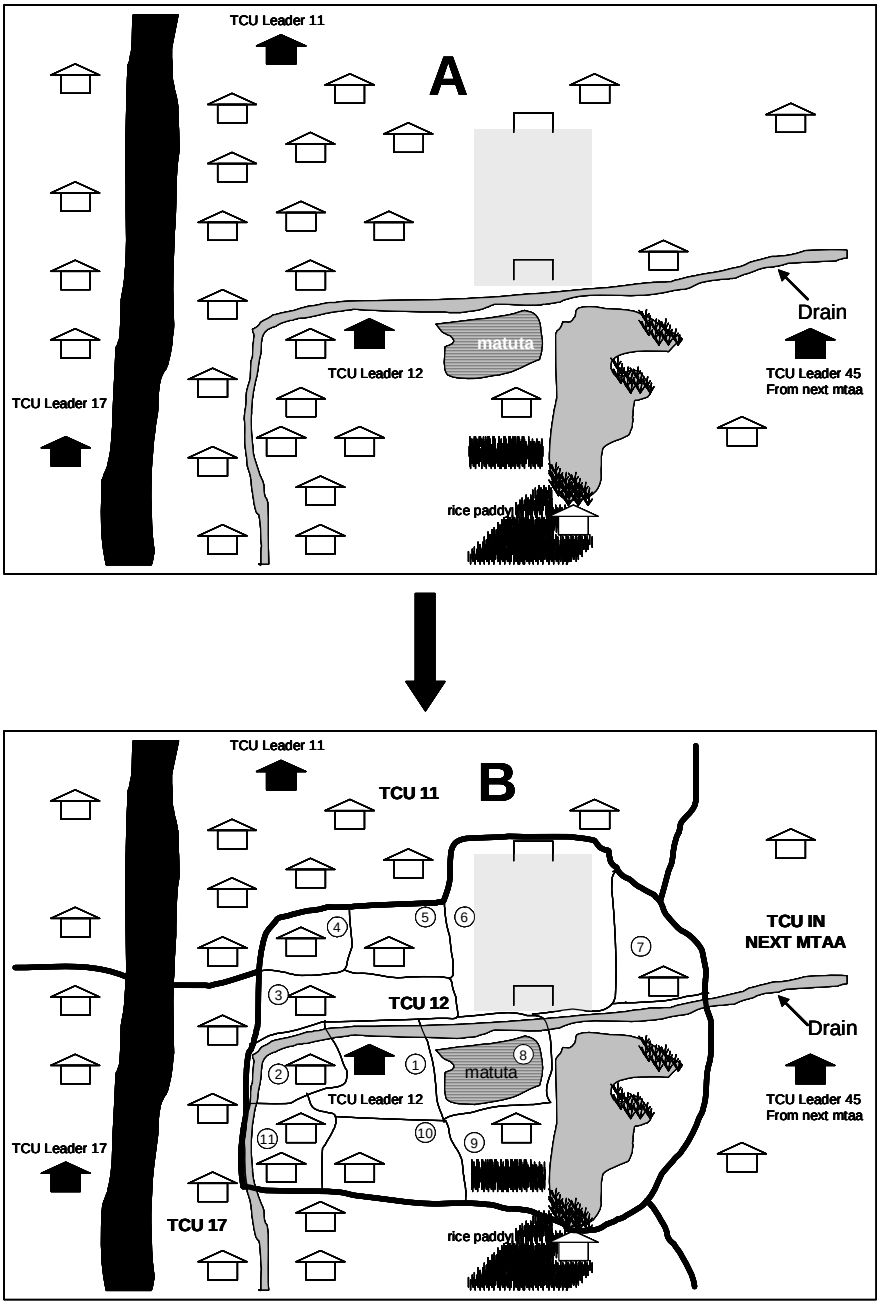
Signatures: Spervisor_____

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**).
NB: See how the drain has been associated with specific plots.
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

Plot Description Form

Municipality: Ilala Ward: Vingunguti MTAA: Mtambani 10-cell unit: 12

Date:24/06/04 10-cell unit leader:Abdalla Mwasiba

Plot ID	House Number	GPS	Owner's name	Short name for the plot	Plot location decription (where is it in the 10-cell unit	Basic Characteristics
1	Vngt/Mtb/13		Abdalla Mwasimba	Kwa Mjumbe	Next to the drain and large matuta farm	This is gerally a dry plot but there is a permanent drain running near it
2	Vngt/Mtb/10		Jonh Kahama	Corner	At the corner of the drain and next to Mjumbe's hous	A small plot and much is covered by the open drain often with water water
3	Vngt/Mt/75		Joseph Kibwana	Plot ndefu	A long plot from TCU 17 boundary to footbal pitch	Dry but near a permanently wet drain
4	Vngt/Mtb/22		Mwanahawa Ali	Dukani	At the corner bordering TCU 11 & TCU 17	Has a burst pipe most of the time with water filling most of the small drains
5	Vngt/Mtb/13		Juma Kajembe	Mwembeni	Bordering the football pitch and TCU11	Prone to flooding during the wet season. Even in the dry season parts of it are wet
6	No house		Vijana wa Vingunguti FC	Uwanjani	Football pitch bordering TCU 11 and the drain	Flooded during wet season. Has several house-construction foundations the fringes
7	Vngt/Mbt/103		Bakari Jaku	Pembeni	Bordered by the drain, the footbal pitch and TCU11	Flooded most of the times with a big part covered by leeds and tall grass
8	No house		Mukurima Mashuhuri	Shama safi	Bordered by Mjumbes plot, pond, and drain	Usually cultivated, has several irrigation channells & man-made holes & matuta
9	Vngt/Mtb/44		Edith Ikupa	Kwenye bwawa	Bordered by drain, footbal pitch, TCU17 & TCU45	Wet throughout the year with regular rice cultivation, has a big pond.
10	Vngt/Mtb/65		Cyrus Makumi	Mgema	South of Mjumbe's plot & bordering TCU 17	Half of it normally wet and the other half normally dry
11	Vngt/Mtb/07		Mwanahamisi Juma	Nyuma Mbili	Bordering drain on the West & TCU17 on the South	Gerally a dry plot but there is a permanent drain running near it

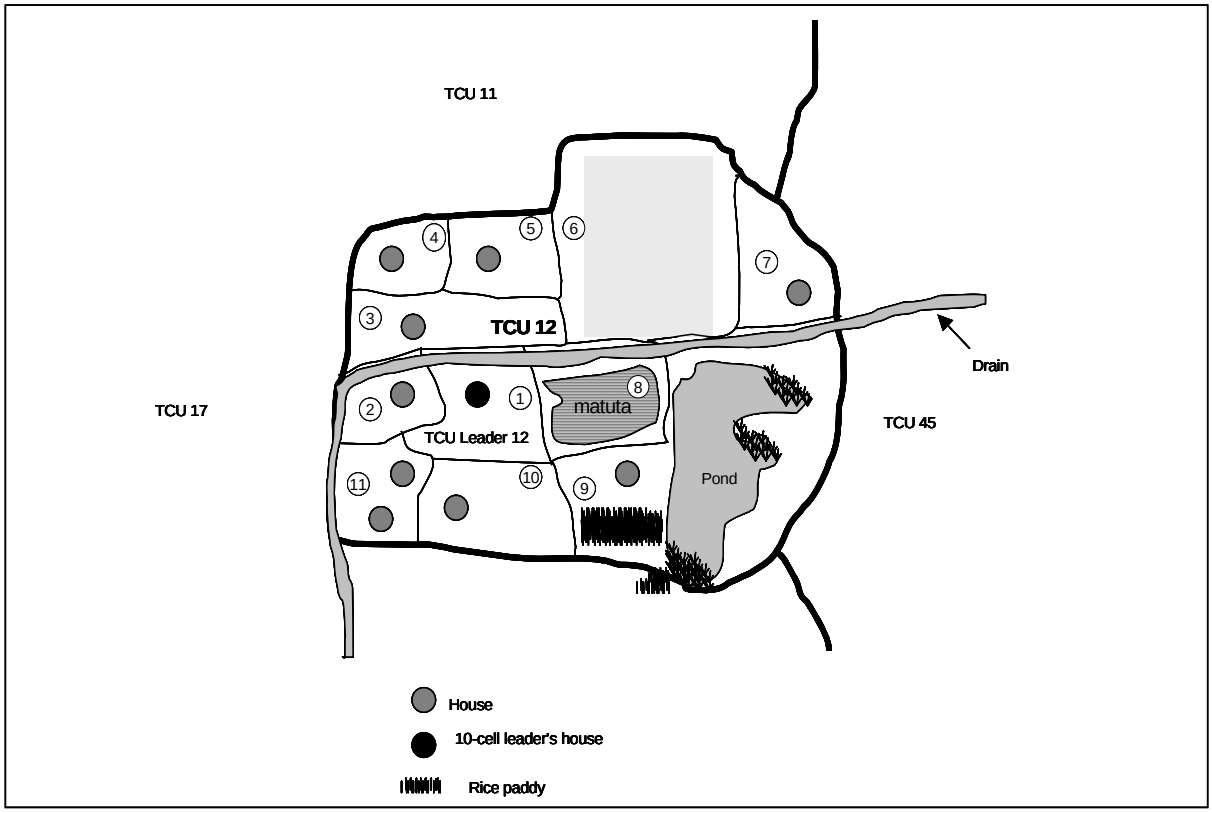
Signatures: Spervisor_____
CORP_____
10-cell leader_____

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



Additional file 2:

This document has been produced and made available by the Dar es Salaam Urban Malaria Control Programme. Contact: Urban Malaria Control Programme, City Medical Office of Health, City Council, P.O. Box 63320, Dar es Salaam, Tanzania, Phone: +255 22 212 1649

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)

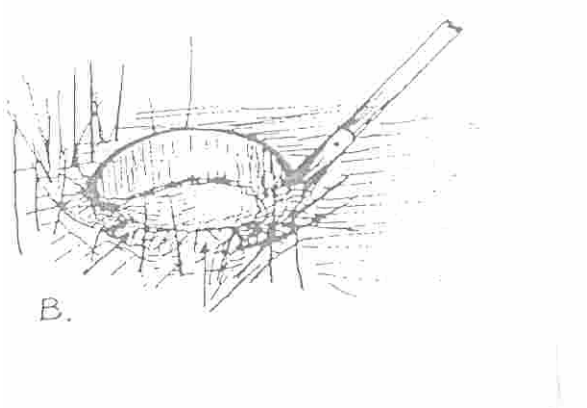
Where to do a mosquito larval search in the habitat (stagnant water body)

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 tuffs, 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:

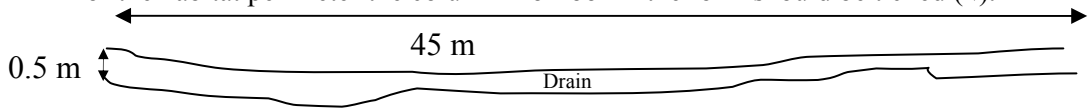
Open Habitats (these are visited once every week)

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 (√)**.**



   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**, b**ad** 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)

1: Puddles and Tyre Tracks

Puddles
- small to medium sized
- stagnant water
- water source = rain water and water run off
- shallow water = less than 0.5 m deep (<0.5 m)

Tyre tracks
- made by wheels of vehicles
- these tracks/depressions hold water longer than the surrounding areas, these are potential mosquito breeding grounds


2: Swampy Areas

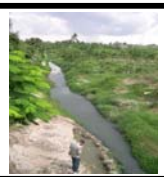
- very high ground water table, but can be fed by rainwater
- can border a large water body like a river or creek
- tall reeds and/or floating plants (left photo)
- short grassy vegetation with water seepage (right photo)

3: Mangrove swamp

- near the sea only
- salty water
- water is tidal
- small pools
- mangrove trees growing with water underneath

4: Drains and Ditches

- man-made for getting rid of water or to irrigate an area
- the water should flow, but if blocked with litter, the drains and ditches can become stagnant water bodies
- cement lined or just dug in the ground




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

- small to medium sized
- man-made habitats to collect water
- open stagnant water
- in the ground and not moveable
- 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


6: Water storage or other Man-made containers:

- any container that holds water for more than one week
- examples: open water storage tanks, barrels, tyres, buckets, clay pots, livestock feeding trays
- can be moved from one place to another (except big open cemented water tanks etc.)


7: Rice paddy (Rice field)

- plots where rice grows
- when fields are drying up the mosquito larvae can then be concentrated in very small water pools

8: Matuta

- raised ridges on agricultural plots
- furrows created to hold water for longer duration

9: Other Agriculture

- other agricultural area that might provide stagnant water bodies for mosquito larvae
- water source = irrigation or high water table or rainfall


10: Streams and River beds

- flowing water bodies
- the edge of the stream or river, where the water is slow moving or stagnant and when streams and rivers are drying up leave stagnant pooling water that can serve as larval habitats

11: Ponds

- medium to large in size
- open water
- permanent water or present for several months in the year
- tall vegetation and floating plants

12: Others (please describe them)

- any other stagnant water bodies that could be mosquito larval habitats and do not fit under any of the habitat categories 1 to 11
- In the comment section of the data sheet, the description should be as detailed as possible

This document has been produced and made available by the Dar es Salaam Urban Malaria Control Programme. Contact: Urban Malaria Control Programme, City Medical Office of Health, City Council, P.O. Box 63320, Dar es Salaam, Tanzania, Phone: +255 22 212 1649

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

Ward level mosquito larval habitat survey - 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:_____

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)

Plot ID	Habitat ID	Habitat type	Same habitat type from last visit? 1=Yes 2=No 3=First visit	Previous habitat type	Habitat description	House number	Wet?		Habitat perimeter			Plants				Water depth		Larval stage						Pupae		Comments
																		Anoph.			Culex					
							dry	Contains water	< 10 m	10-100 m	> 100 m	None	Short vegetation	Tall vegetation	Floating plants	< 0.5 m	> 0.5 m	Absent	Early	Late	Absent	Early	Late	Absent	Present	

CORPS Signature:_____ _____ Date:_____/_____/_____ Supervisors Signature:_____ Date of check :_____/_____/_____

Ward level mosquito larval habitat survey - 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:_____

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

Plot ID	House number	Habitat ID	Habitat type	Habitat description	Wet?		Condition of the latrine			Habitat perimeter			Water depth		Larval stage			Pupae		Comments
					dry	contains water	Good	Bad	Full	< 10 m	10-100 m	> 100 m	< 0.5 m	> 0.5 m	None	Early	Late	Present	Absent	

CORPS signature:_____ _____ Date:_____/_____/_____ Supervisors signature:_____ Date of check :_____/_____/_____

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

Serial namba ya fomu hii ____________________

Serial namba ilioko kwenye fomu ya ramani ya shina hili ____________________

Namba ya shina ya pekee ____________________

Manisipaa:____________________ Kata:____________________ Mtaa:________________________ Namba ya shina:________________ Jina la Mjumbe:____

Aina ya mazalio:
1: Dimbwi na kashata za matairi ya magari
2: Mbojimboji/ziwa la matope
3: Bwawa maji chumvi/Mikoko
4: Mfereji/Kijito
5: Shimo/Ujenzi/Mchanga
6: Chombo cha kuhifathia maji
7: Mpunga
8: Matuta
9: Aina nyingine ya kilimo
10: Mto
11: Bwawa maji baridi
12: Aina Nyingine (Elezea)

Namba ya utambulisho wa ploti	Namba ya zalio	Aina ya zalio	Aina ya zalio inafanana na ulipo zuru awali 1=Ndio 2=Hapana 3=jipya	Aina ya zalio la awali	Maelezo kuhusu zalio	Namba ya Nyumba.	Maji?		Ukumbwa wa zalio			Mimea				Kiina cha maji		Hatua za viluwiluwi						Mabuu		Maoni
																		Anoph.			Culex					
							Hakuna	Yapo	< 10 m	10-100 m	> 100 m	Hakuna	Majani mafupi	Majani marefu	Majani elea	< 0.5 m	> 0.5 m	Hakuna	Wodogo	Wakubwa	Hakuna	Wadogo	Wakubwa	Hakuna	Wapo	

Jina La CORP: __________________________ ________________ Tarehe:______/______/______ Jina La Supervisor: __________________________________ Tarehe:_______/_______/_________

Fomu ya mazalio ya mbu kwenye vyoo na makaro Ngazi ya kata

Serial namba ya fomu hii _____

Serial namba ilioko kwenye fomu ya ramani ya shina hili _____

Namba ya shina ya pekee _____

Manisipaa:_____ Kata:_____ Mtaa:_____ Namba ya shina:_____ Jina la Mjumbe:_____

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

Namba ya Utambulisho wa ploti	Namba ya nyumba.	Namba ya zalio	Aina ya zalio	Maelezo kuhusu zalio	Maji yapo?		Hali ya choo			Ukubwa wa zalio			Kina cha maji		Hatua za viluwiluwi			Mabuu		Maoni
					kavu	kina maji	Nzuri	Mbaya	Kimejaa	< 10 m	10-100 m	> 100 m	< 0.5 m	> 0.5 m	Hakuna	Wadogo	Wakubwa	Yapo	Hakuna	

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?		
Is the map accurate?		
Does map match city copy?		

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 & any other man-made container
7: Rice paddy
8: Matuta
9: Other agriculture
10: Stream/river bed
11: Pond
12: Others (describe below)

Plot ID	House No.	Habitat ID	Habitat type	Is habitat type correct?	Correct habitat type	Habitat found by the CORPs? 1=Yes 2=No	Habitat Description	Wet?		Habitat perimeter			Plants				Water depth		Larval stage						Pupae		Comments
																			Anoph.			Culex					
								dry	contains water	< 10 m	10-100 m	> 100 m	None	Short vegetation	Tall vegetation	Floating plants	< 0.5 m	> 0.5 m	Absent	Early	Late	Absent	Early	Late	Absent	Present	

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_____

	Yes	No
Is there a map?		
Is the map accurate?		
Does map match city copy?		

Serial number on the map form_____

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

Plot ID	House No.	Habitat ID	Is habitat type correct?	Correct habitat type	Habitat found by the CORPs? 1=Yes 2=No	Habitat Description	Wet?		Condition of the latrine			Habitat perimeter			Water depth		Culicine stage			Pupae		Comments
							dry	contains water	Good	Bad	Full	< 10 m	10-100 m	> 10 m	< 0.5 m	> 0.5 m	None	Early	Late	Absent	Present	

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:_____
GPS(UTM/WGS84): Northing_____ Easting_____ 10-cell leader:_____

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: Matuta
9: Other agriculture
10: Stream/river bed
11: Pond
12: Other (describe below)

New

Plot ID	Habitat ID	Habitat type	Same habitat type from last visit? 1=Yes 2=No 3=First visit	Previous habitat type	Habitat description	House number	Wet?		Habitat perimeter			Plants				Water depth		Larval stage						Pupae		Comments
																		Anoph.			Culex					
							dry	Contains water	< 10 m	10-100 m	> 100 m	None	Short vegetation	Tall vegetation	Floating plants	< 0.5 m	> 0.5 m	Absent	Early	Late	Absent	Early	Late	Absent	Present	

How to fill in the data sheets

- Plot No.
- House No.
- Habitat ID.

All **unique** and **continuous**

- example

2 habitat types in same plot

Ward level - data sheet

WARD LEVEL mosquito larval habitat survey - Open habitats

Serial number of this form_____0793_____
Serial number on the map form__0891_____
Date:___**7**____/__**4**_____/___**05**_____

Municipality:___Kinondoni_____ Ward:____Ndgumbi_____ MTAA:___Vigaeni_____ 10-cell unit:_____38_____
GPS(UTM/WGS84): Northing_____ Easting_____ 10-cell leader:____Omary Bauari_____

	Yes	No
Is there a map	X	
Is the map up to d	X	
Is the map filed	X	

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: Matuta
9: Other agriculture
10: Stream/river bed
11: Pond
12: Other (describe below)

Plot ID	Habitat ID	Habitat type	Same habitat type from last visit? 1=Yes 2=No 3=First visit	Previous habitat type	Habitat description	House number	Wet?		Habitat perimeter			Plants				Water depth		Larval stage						Pupae		Comments
																		Anoph.			Culex					
							dry	Contains water	< 10 m	10-100 m	> 100 m	None	Short vegetation	Tall vegetation	Floating plants	< 0.5 m	> 0.5 m	Absent	Early	Late	Absent	Early	Late	Absent	Present	
7	1	4	1	4	large drain with flowing water			X			X		X	X			X	X			X			X			Irrigating local agriculture
7	2	1	1	1	small open shallow puddle			X	X			X				X			X		X			X			beside the drain

Habitat type = 1 to 12 codes

Habitat ID = how many different habitats in one plot

Dry or No habitat ???

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

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

Serial number of this form_____0723_____
Serial number on the map form__0333_____
Date:___1____/__4_____/___05_____

Municipality:___Kinondoni_____ Ward:____Ndgumbi_____ MTAA:___Vigaeni_____ 10-cell unit:_____38_____
GPS(UTM/WGS84): Northing_____ Easting_____ 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: Matuta
9: Other agriculture
10: Stream/river bed
11: Pond
12: Other (describe below)

Plot ID	Habitat ID	Habitat type	Same habitat type fr last visit? 1=Yes 2: 3=First visit	Previous habitat ty	Habitat description	House number	Wet?		Habitat perimeter			Plants				Water depth		Larval stage						Pupae		Comments
							dry	Contains water	< 10 m	10-100 m	> 100 m	None	Short vegetation	Tall vegetation	Floating plants	< 0.5 m	> 0.5 m	Anoph.			Culex			Pupae		
																		Absent	Early	Late	Absent	Early	Late	Absent	Present	
3	1	4	1	4	water flowing = water tap on	22		X		X			X			X		X				X		X		inbetween the houses

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

WARD LEVEL mosquito larval habitat survey - Open habitats

Serial number of this form_____0456_____
Serial number on the map form__0333_____
Date:___7____/__4_____/___05_____

Municipality:___Kinondoni_____ Ward:____Ndgumbi_____ MTAA:___Vigaeni_____ 10-cell unit:_____38_____
GPS(UTM/WGS84): Northing_____ Easting_____ 10-cell leader:____Omary Bauari_____

Habitat codes:

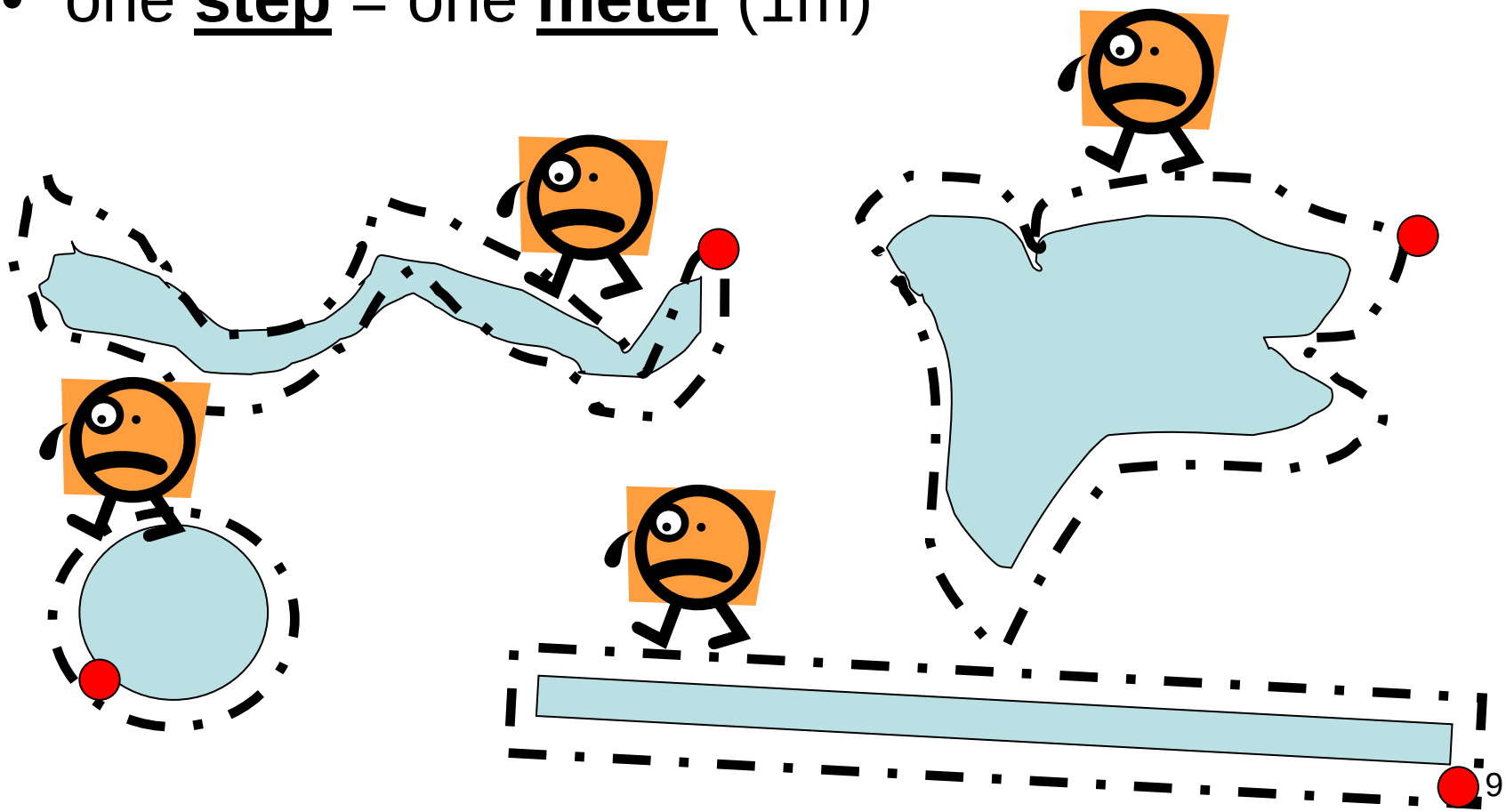
1: Puddles&tire tracks
2: Swampy areas
3: Mangrove Swamp
4: Drain/Ditch
5: Construction pits/foundations/man-made holes
6: Water storage container
7: Rice paddy
8: Matuta
9: Other agriculture
10: Stream/river bed
11: Pond
12: Other (describe below)

Full habitat description even if dry

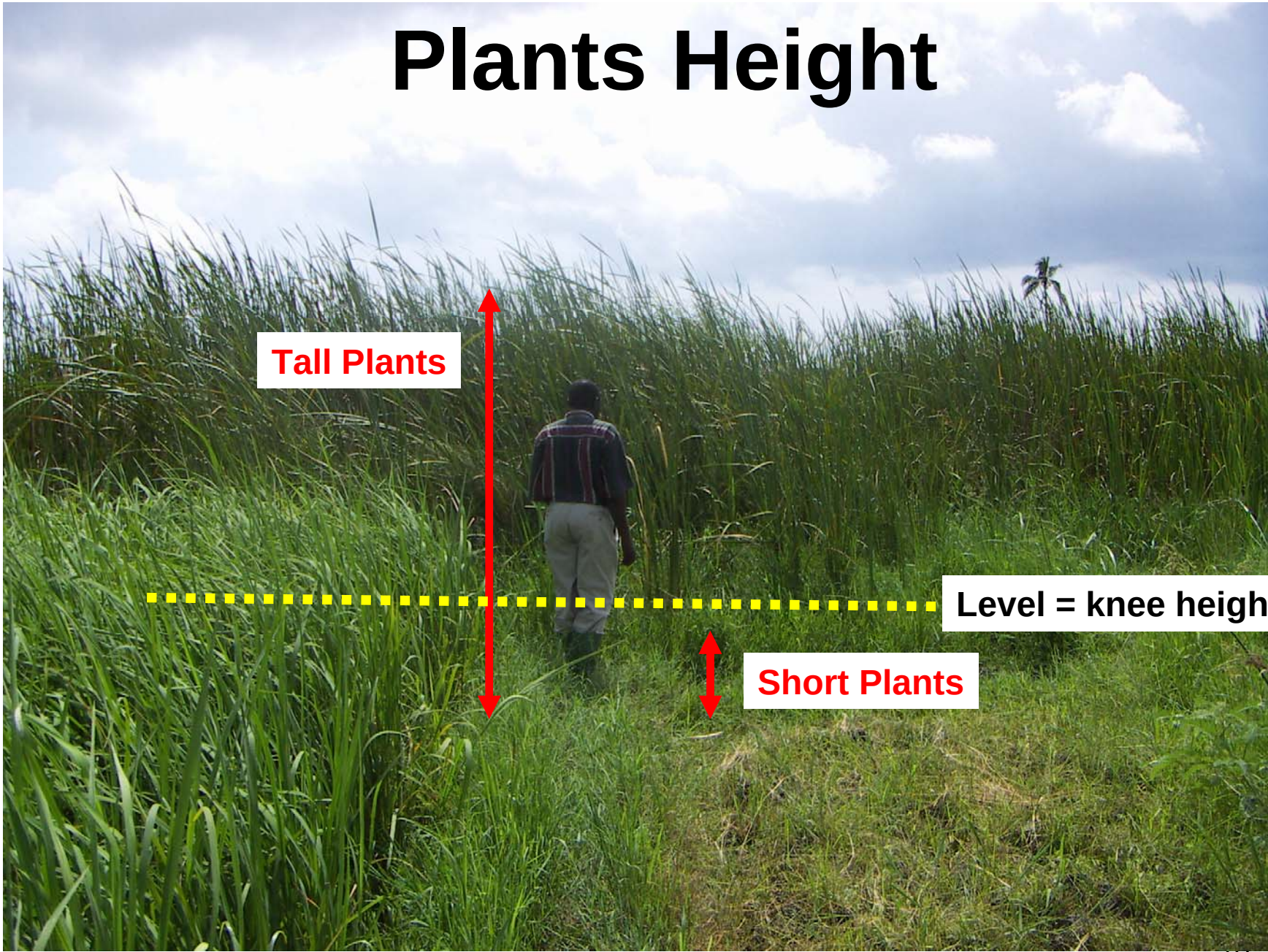
Plot ID	Habitat ID	Habitat type	Same habitat type last visit? 1=Yes 3=First visit	Previous habitat	Habitat description	House number	Wet?		Habitat perimeter			Plants				Water depth		Larval stage						Pupae		Comments
							dry	Contains water	< 10 m	10-100 m	> 100 m	None	Short vegetation	Tall vegetation	Floating plants	< 0.5 m	> 0.5 m	Anoph.			Culex			Pupae		
																		Absent	Early	Late	Absent	Early	Late	Absent	Present	
3	1	4	2	4	water tap turned off this week	22	X																			inbetween the houses

Habitat perimeter (m)

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



Plants Height

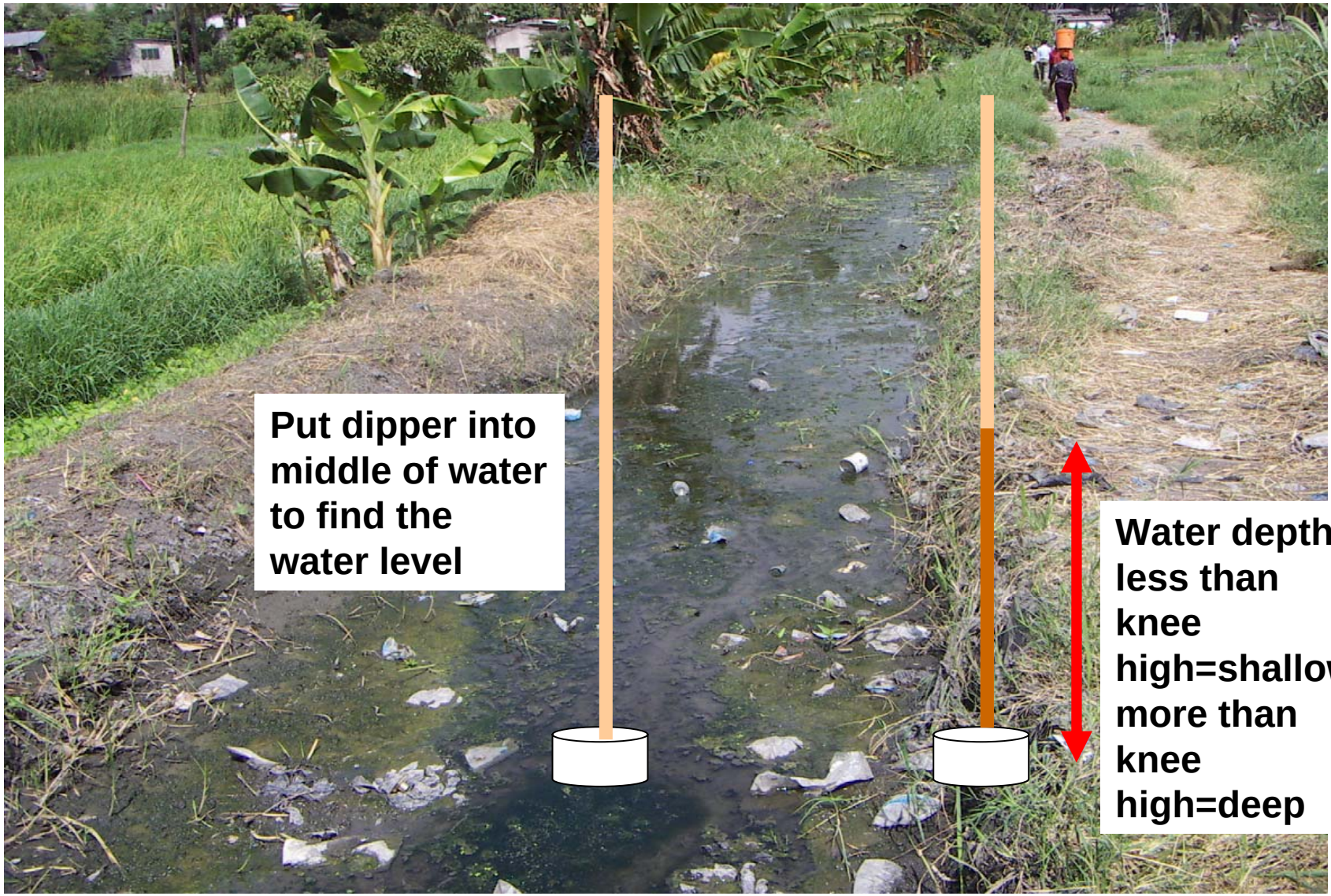


Floating Plants

Floating Plants

Floating Plants

Water depth

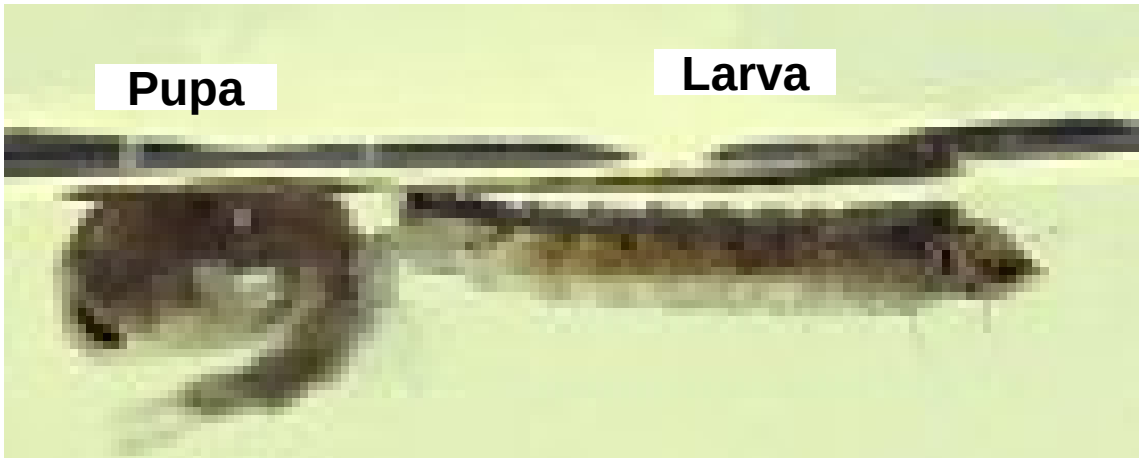


Mosquito types

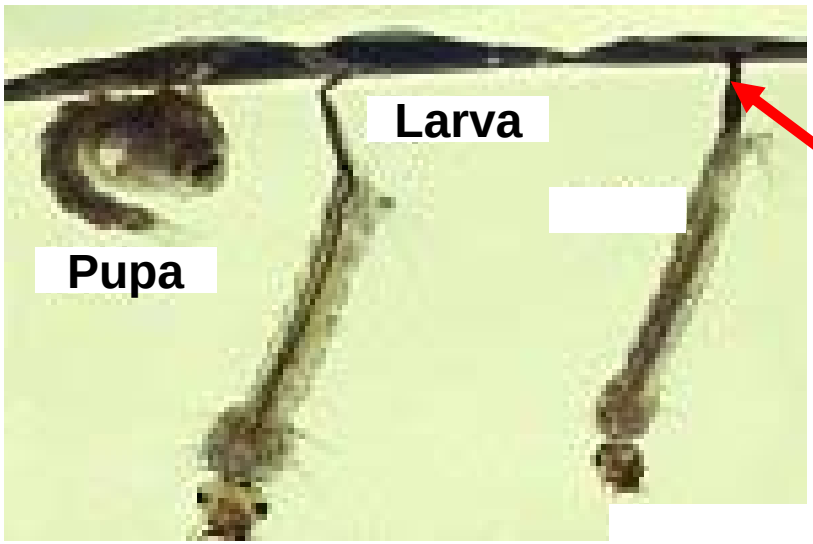
- Mosquitoes breed in **all types of water**, it is important to **check all** water bodies during a larval survey.

- **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

Signature and date from inspectors

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:_____

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: Matuta
9: Other agriculture
10: Stream/river bed
11: Pond
12: Other (describe below)

Plot ID	Habitat ID	Habitat type	Same habitat type from last visit? 1=Yes 2=No 3=First visit	Previous habitat type	Habitat description	House number	Wet?		Habitat perimeter			Plants			Water depth		Larval stage						Pupae		Comments
																	Anoph.			Culex					
							dry	Contains water	< 10 m	10-100 m	> 100 m	None	Tall vegetation	Floating plants	< 0.5 m	> 0.5 m	Absent	Early	Late	Absent	Early	Late	Absent	Present	

CORPS signature Date

Inspectors signature Date

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

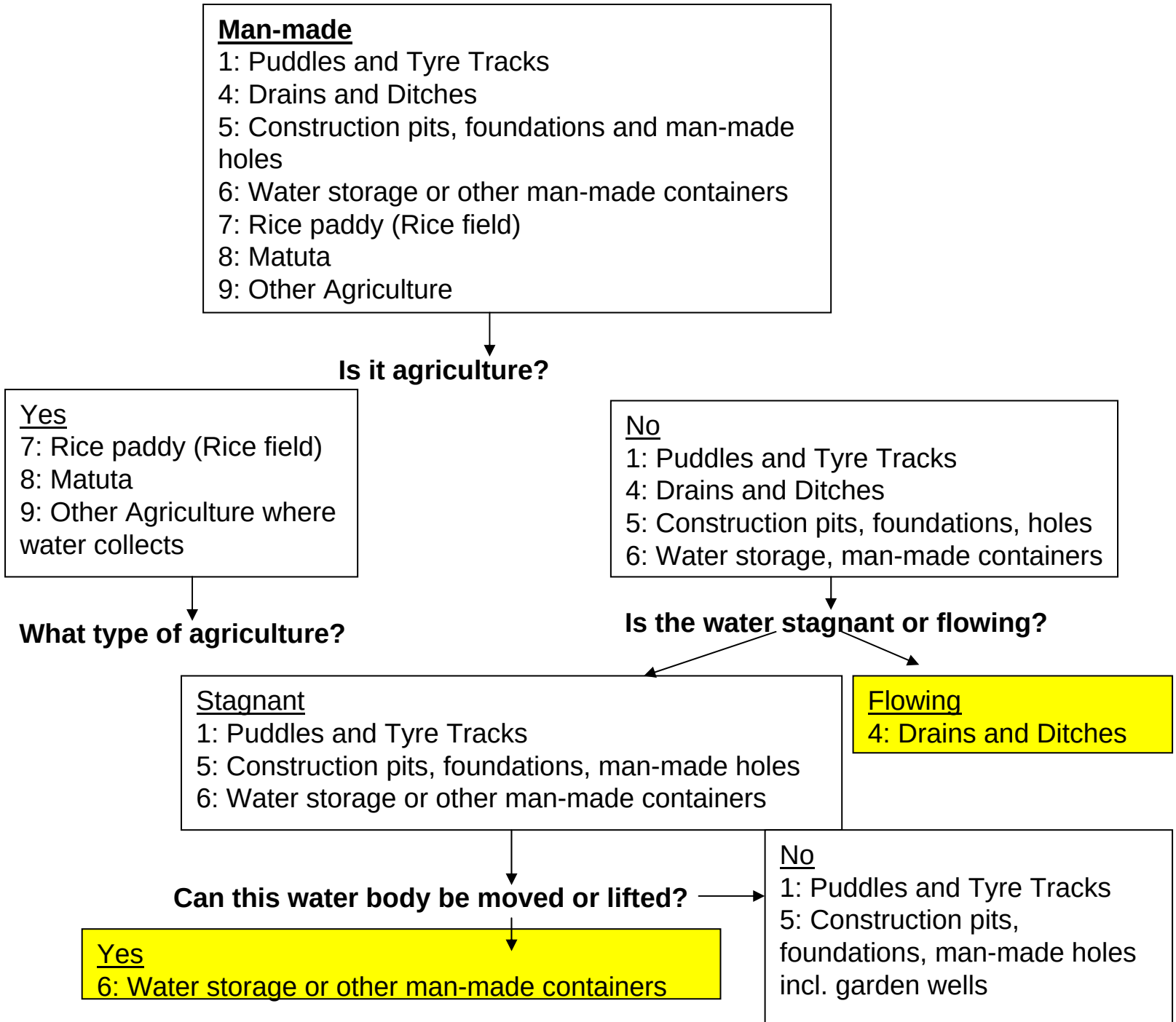
Is the water stagnant or flowing or should it be flowing?

Stagnant
2: Swampy Areas
11: Ponds

Flowing
10: Stream and River

Next slide

Drain is straight and man-made and river meanders and is natural


Man-made
1: Puddles and Tyre Tracks
4: Drains and Ditches
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
Is it agriculture?
Yes
7: Rice paddy (Rice field)
8: Matuta
9: Other Agriculture where water collects
What type of agriculture?
No
1: Puddles and Tyre Tracks
4: Drains and Ditches
5: Construction pits, foundations, holes
6: Water storage, man-made containers
Is the water stagnant or flowing?
Stagnant
1: Puddles and Tyre Tracks
5: Construction pits, foundations, man-made holes
6: Water storage or other man-made containers
Flowing
4: Drains and Ditches
Can this water body be moved or lifted?
Yes
6: Water storage or other man-made containers
No
1: Puddles and Tyre Tracks
5: Construction pits, foundations, man-made holes incl. garden wells

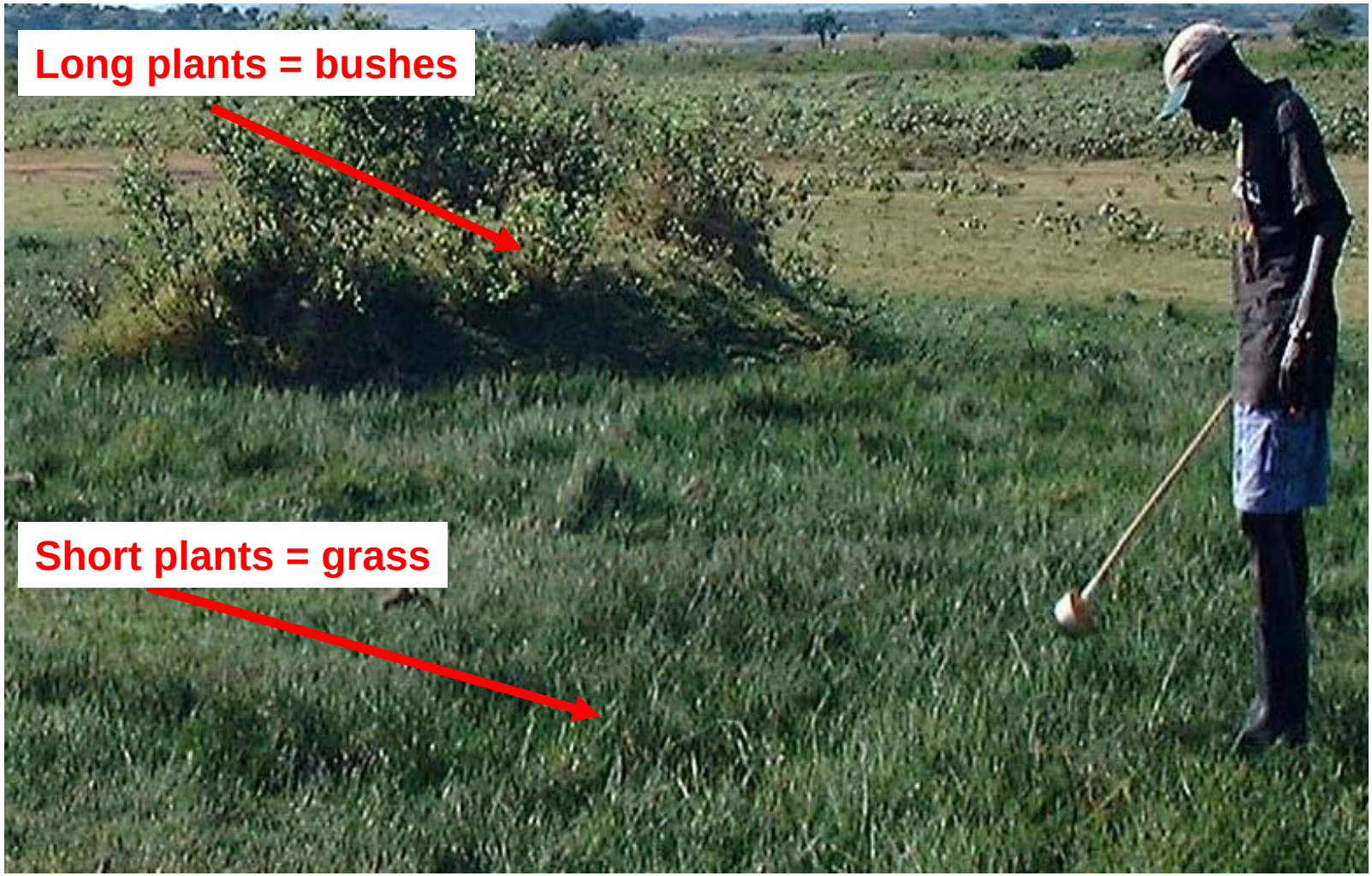
1: Puddles and Tyre Tracks

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



2: Swampy Areas

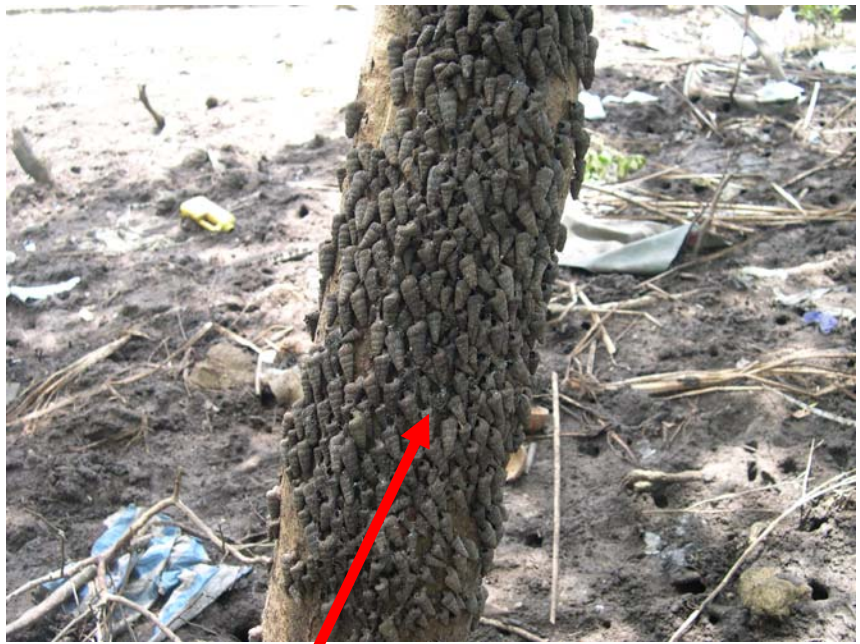
3: Mangrove swamp

- 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

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

4: Drains and Ditches

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

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:

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

10: Stream and River beds

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

Additional file 7:

This document has been produced and made available by the Dar es Salaam Urban Malaria Control Programme. Contact: Urban Malaria Control Programme, City Medical Office of Health, City Council, P.O. Box 63320, Dar es Salaam, Tanzania, Phone: +255 22 212 1649

Larval Control Interventions in Dar es Salaam

GUIDELINES

Background Information and Operational Procedures
March 2006

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

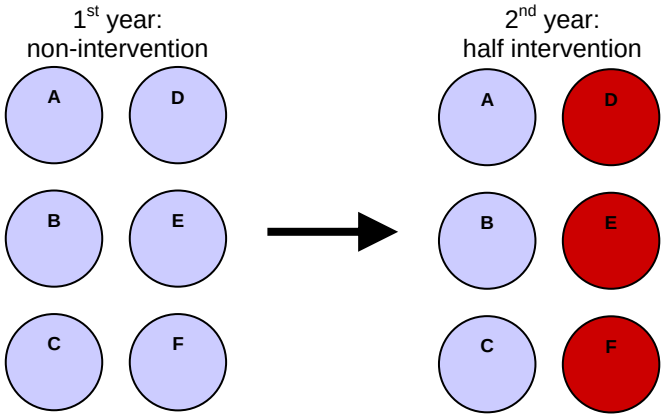
Study Hypothesis
Larval mosquito control in urban Dar es Salaam where malaria transmission is relatively low and focal will decrease densities of adult mosquitoes to such an extent that malaria transmission will also decline and reduce the level of malaria infection prevalence in local communities/wards 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 blacklfy 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:		
VectoLex WDG (650 ITU/mg)	2.0 kg/ha	0.20 g/m2
VectoBac WDG (3000 ITU/mg)	0.4 kg/ha	0.04 g/m2
VectoLex CG (50 ITU/mg)	30 kg/ha	3 g/m2
VectoBac CG (200 ITU/mg)	10 kg/ha	1 g/m2

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
VectiBac (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^{o} 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 (m^2), equivalent to 10 Kg per hectare. For medium to large areas (>9m^2 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 m^2 (3m ×4m), 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 cm^2 or 10 cm x 10 cm area.
- Application rate for small to medium habitats: 1 teaspoon full per 2 m^2
- Swath width for habitats > 9 m^2 in size: 3 meters
- Application rate for swaths across habitats > 9 m^2 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 1st 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 spayman 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):_____

Day	Date	TCU number	Wet habitats present?		Larvicide applied?		Comments
			YES	NO	YES	NO	
MON	01.01.06	001	x		x		
		002	x		x		
		005		x		x	
		007		x		x	
		008		x		x	
		009	x			x	no application because access was denied by residents

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 the 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**.

Larvicide Release Records

MUNICIPALITY: WARD: Mtaa: Sprayman's name:

Supervisor's name:

Week:	Day:	Date:	Amount of Granule received (in kg):	Signature Sprayman	Amount of granule returned (in kg)	Amount of granule used	Total number of TCUs treated (as per Mosquito Control CORP data sheet)	Signature Supervisor
1	Mon							
1	Tue							
1	Wed							
1	Thu							
1	Fri							
1	**Weekly Total:**							
2	Mon							
2	Tue							

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 chairmen 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

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?		
Is the map accurate?		
Does map match city copy?		

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 & any other man-made container
7: Rice paddy
8: Matuta
9: Other agriculture
10: Stream/river bed
11: Pond
12: Others (describe below)

Plot ID	House No.	Habitat ID	Habitat type	Is habitat type correct?	Correct habitat type	Habitat found by the CORPs? 1=Yes 2=No	Habitat Description	Wet?		Habitat perimeter			Plants				Water depth		Larval stage						Pupae		Last application of larvicide as per timetable?	Comments
								dry	contains water	< 10 m	10-100 m	> 100 m	None	Short vegetation	Tall vegetation	Floating plants	< 0.5 m	> 0.5 m	Anoph.			Culex			Absent	Present		
																			Absent	Early	Late	Absent	Early	Late				

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).

Intervention Spot Checks

MUNICIPALITY: _____ INTERVENTION WARD: _____

checked by (name and position): _____

Date	Mtaa	TCU number	Plot ID	Habitat ID	Date of larviciding	Larval stage						Pupae		Sprayman seen?		Signs of CG?		Comments
						Anoph.			Culex									
						Absent	Early	Late	Absent	Early	Late	Absent	Present	Yes	No	Yes	no	

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

Dar UMCP
Dar es Salaam
January 2006

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

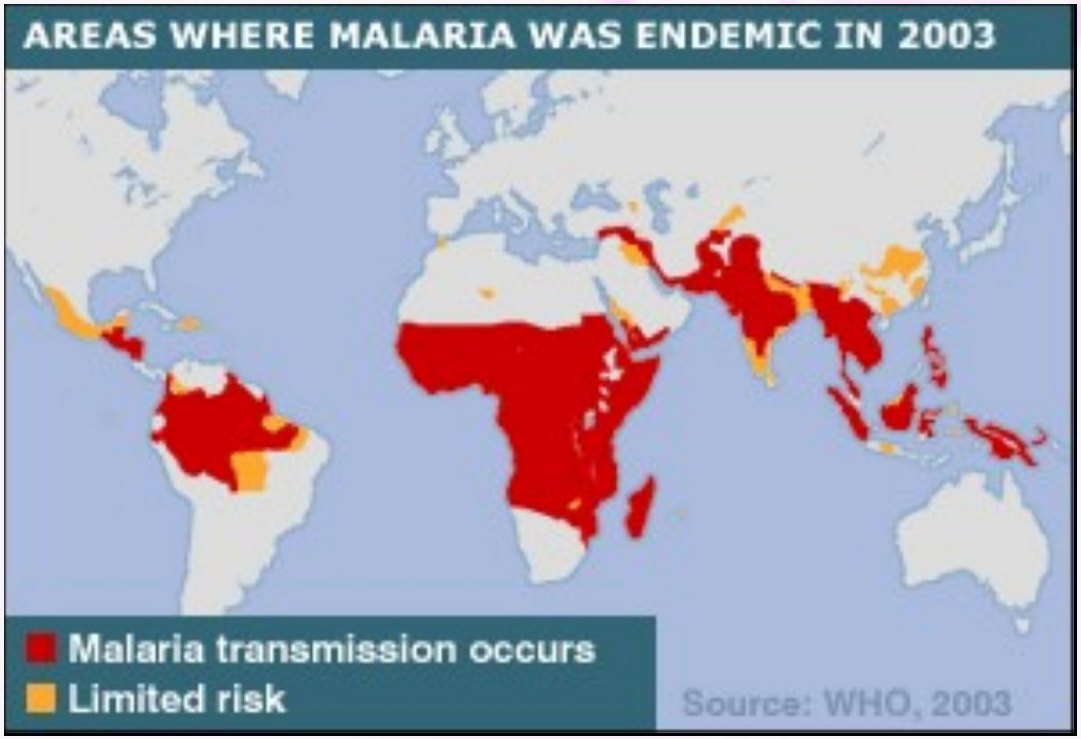


Image courtesy of The World Health Organization

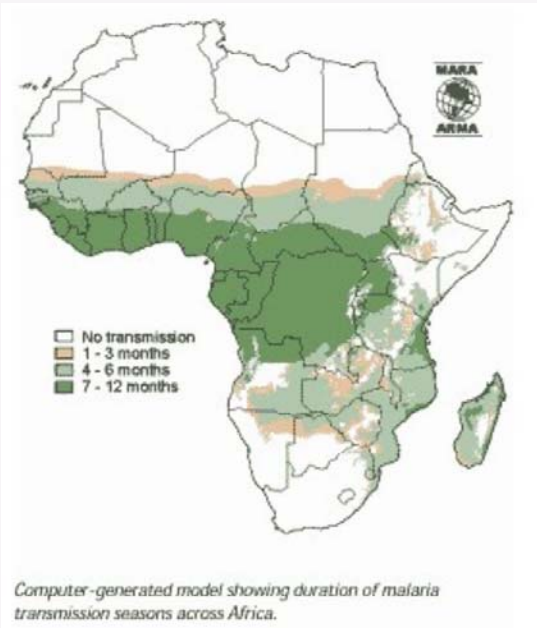


Image courtesy of Roll Back Malaria, World Health Organization

Dar UMCP
Dar es Salaam
January 2006

VALENT BIOSCIENCES CORPORATION

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)

Dar UMCP
Dar es Salaam
January 2006

VALENT BIOSCIENCES CORPORATION

Malaria Control Challenges

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

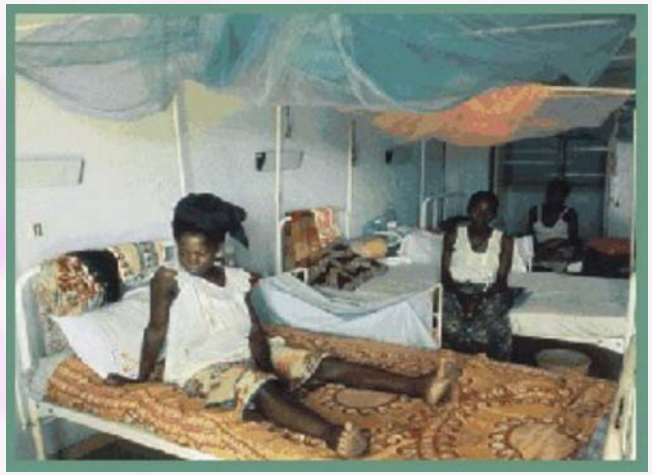
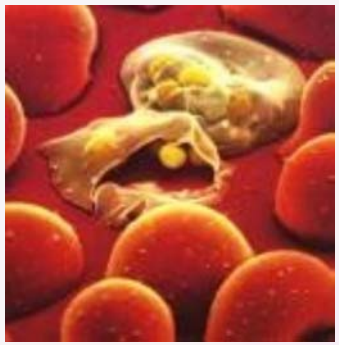
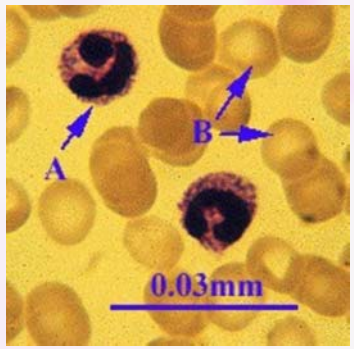


Image courtesy of The World Health Organization



Copyright (c) traveldoctor.co.uk



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

Image courtesy of The Ohio State University College of Biological Sciences

Dar UMCP
Dar es Salaam
January 2006

VALENT BIOSCIENCES CORPORATION

Agenda

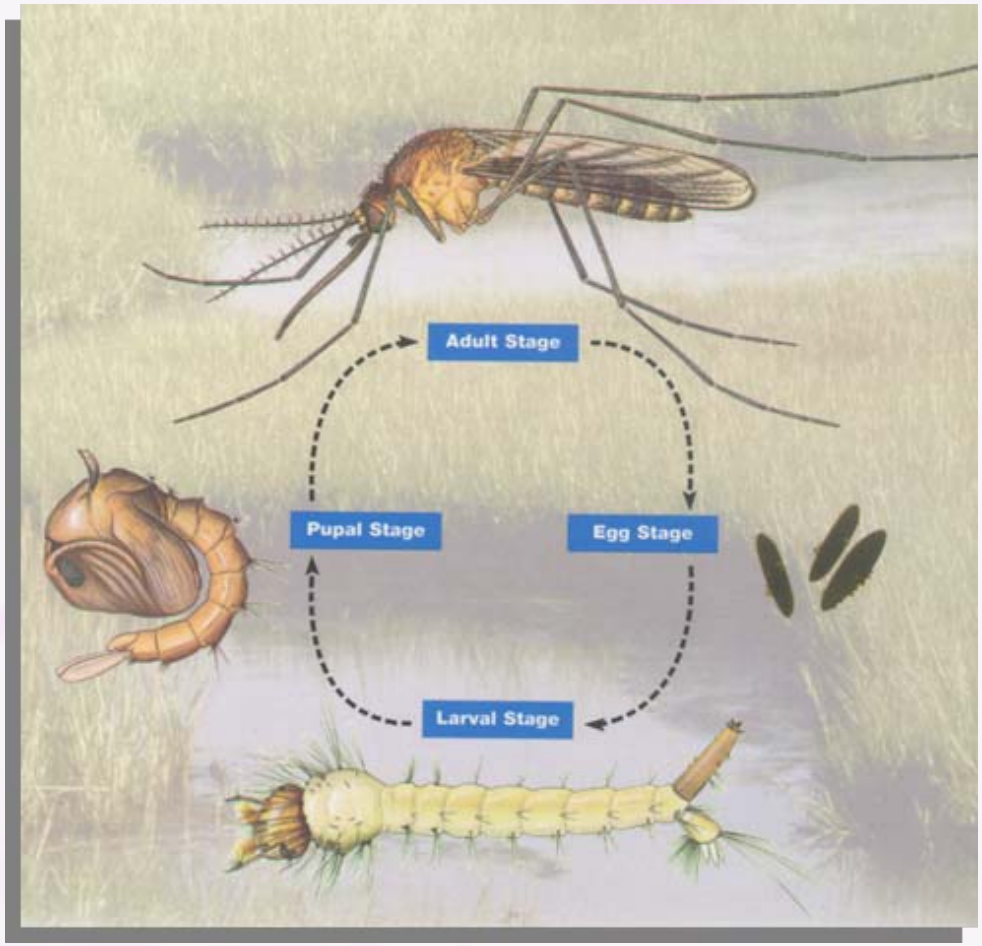
- ✓ The global malaria problem
- ❑ **Current strategies in malaria control**
- ❑ **Mosquito life cycle and control strategies**
- ❑ Microbial mosquito larvicides
- ❑ Microbial mosquito larvicide formulations
- ❑ Application Equipment
- ❑ Calibration methods

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



Methods of Mosquito Control

Source Reduction

Larviciding

Adulticiding

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

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

Dar UMCP
Dar es Salaam
January 2006

VALENT BIOSCIENCES CORPORATION

Source Reduction

(Environmental Management)

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

Larviciding

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

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

VALENT BIOSCIENCES CORPORATION

Adulticiding

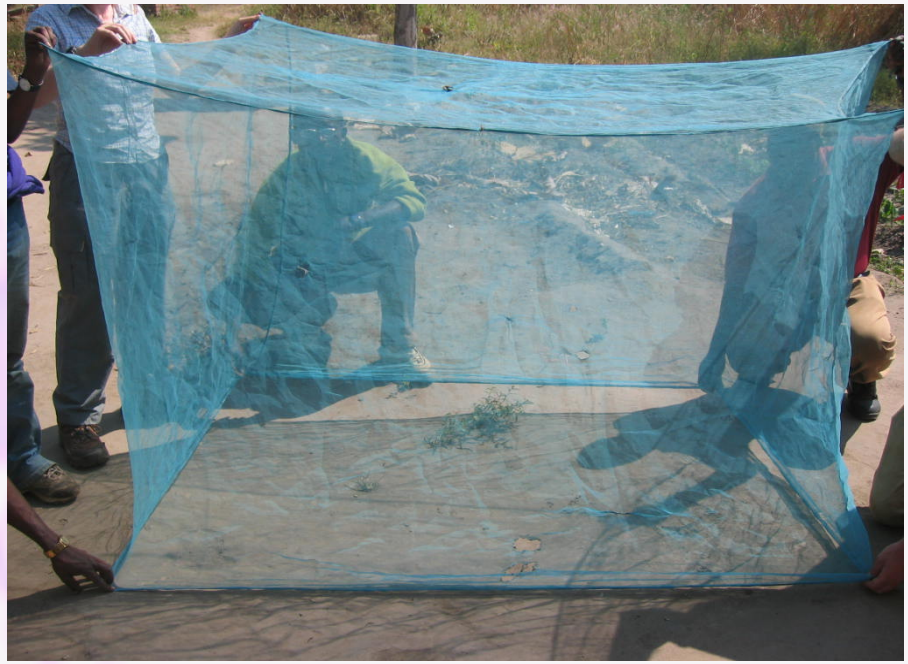
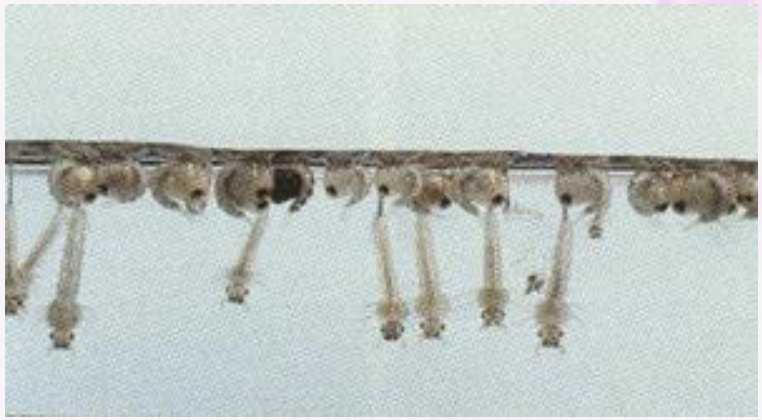


PHOTO COURTESY OF C.F. CURTIS - U of M Website

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

Larviciding Philosophy (CIA)

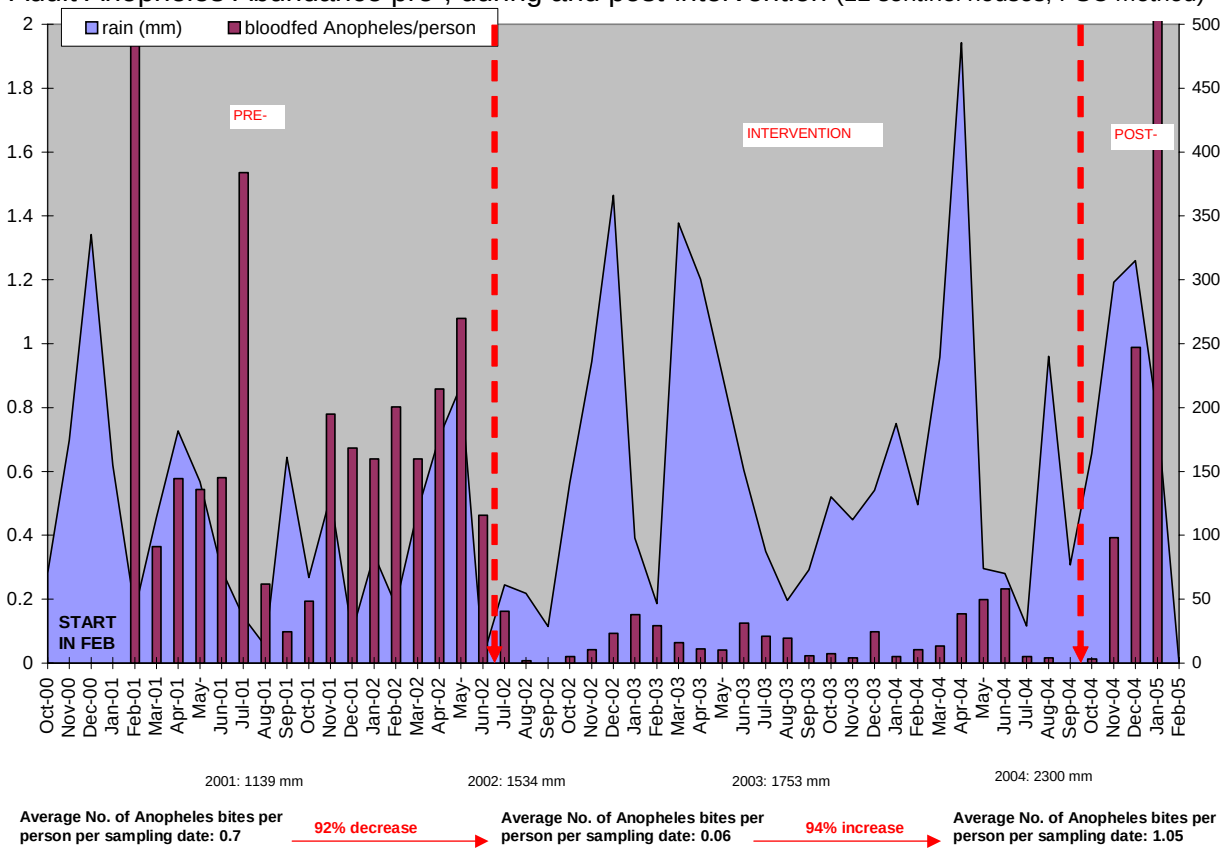
PHOTO COURTESY OF C.F. CURTIS - U of M Website



- Mosquito Larvae are generally:
  - **C**oncentrated
  - **I**mmobile
  - **A**ccessible
- Adult mosquitoes spread out over a much larger area.
- **CIA** = efficiency of larval control.

New Look at Environmental Management and Larval Control

Courtesy of U Fillinger



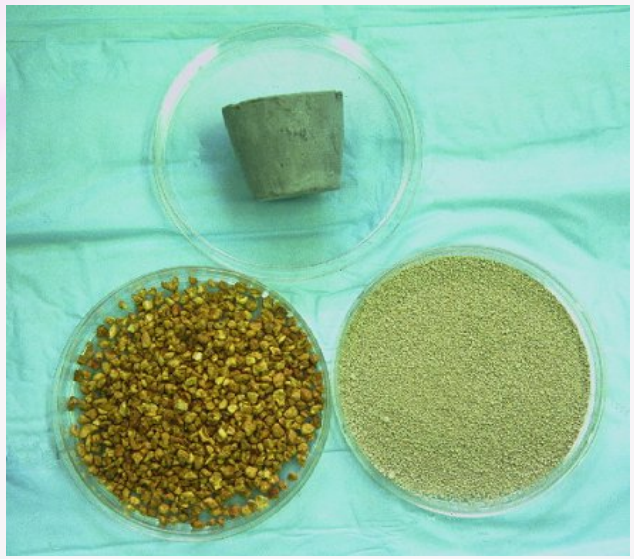
Dar UMCP
Dar es Salaam
January 2006

VALENT BIOSCIENCES CORPORATION

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

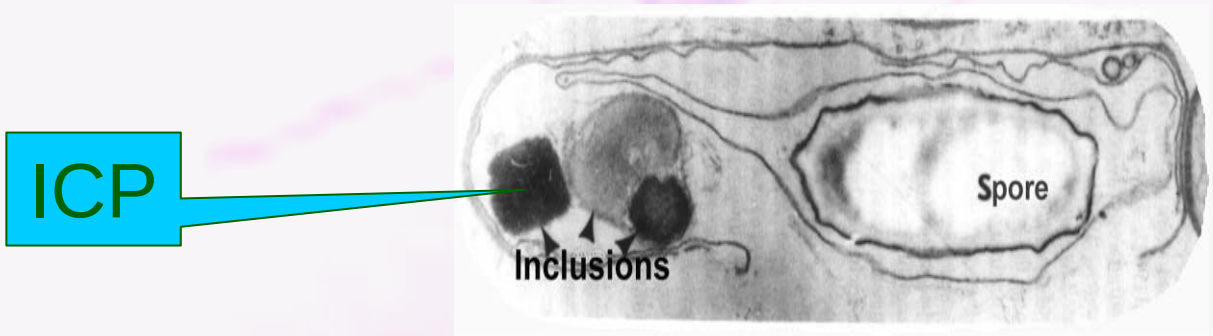


- Chemicals
  - OP's (temephos)
- Surface Agents
  - oils, monomolecular films
- **Microbials**
  - ***Bacillus thuringiensis israelensis (Bti)***
  - ***Bacillus sphaericus (Bs)***

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



Bti

VALENT BIOSCIENCES CORPORATION

Bacillus sphaericus (Bs)

VectoLex = *Bs*

Bacteria that produces 2 toxins (ICP)
ICP = Insecticidal Crystal Protein
Protein is not toxic until digested by larvae

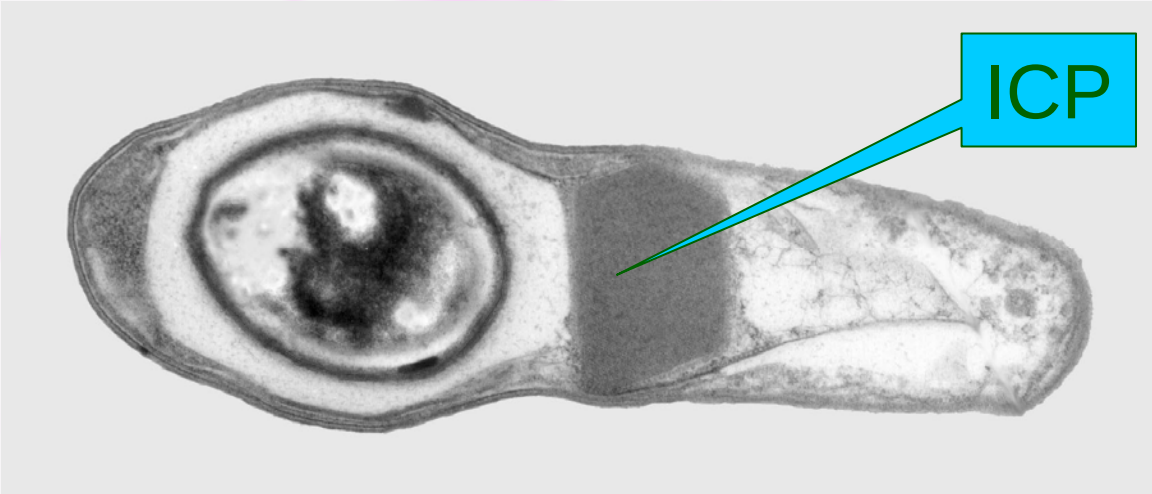
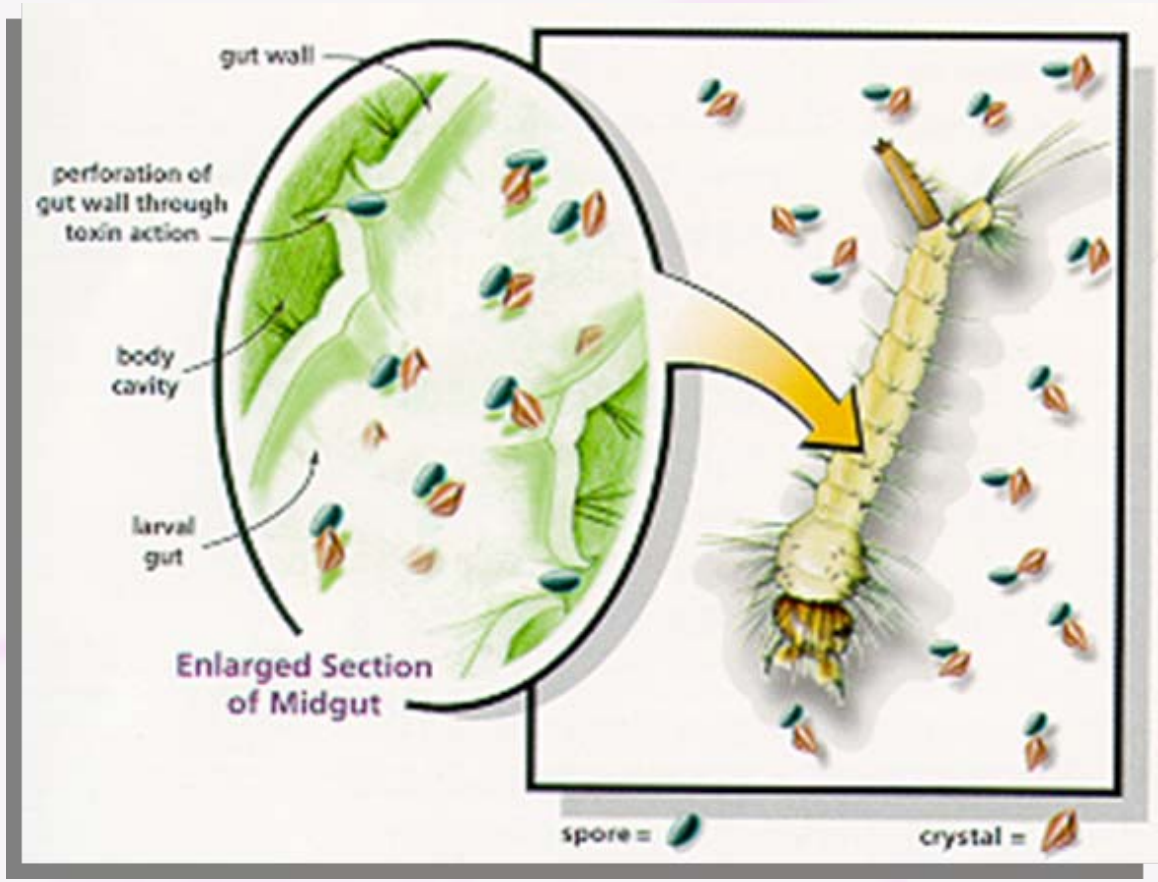


Figure courtesy of Jean-François Charles

VALENT BIOSCIENCES CORPORATION

Mode of Action



ICP

VALENT BIOSCIENCES CORPORATION

Insecticidal Crystal Protein

The larvae's Last Meal

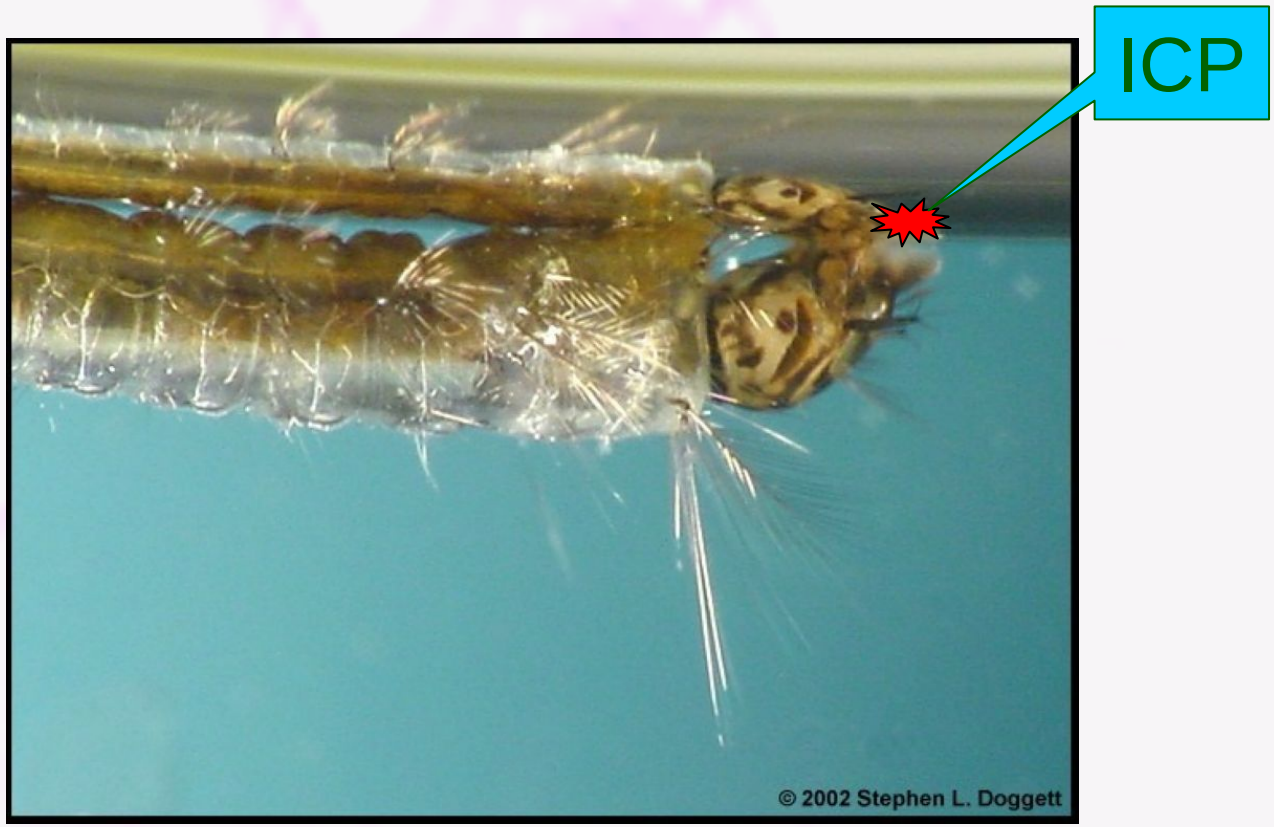


Figure courtesy of Stephen L. Doggett

Dar UMCP
Dar es Salaam
January 2006

VALENT BIOSCIENCES CORPORATION

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

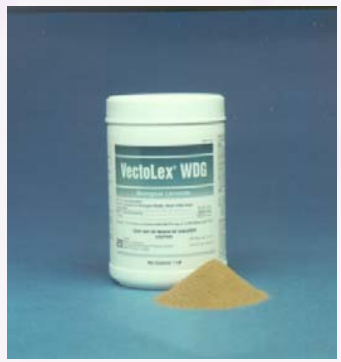
Agenda

- ✓ 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



Dar UMCP
Dar es Salaam
January 2006

VALENT BioSciences Corporation

VectoLex® and VectoBac® CG

Granular formulations for dry application

Dar UMCP
Dar es Salaam
January 2006

VALENT BIOSCIENCES CORPORATION

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

VALENT BIOSCIENCES CORPORATION

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.

VALENT BIOSCIENCES CORPORATION

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)

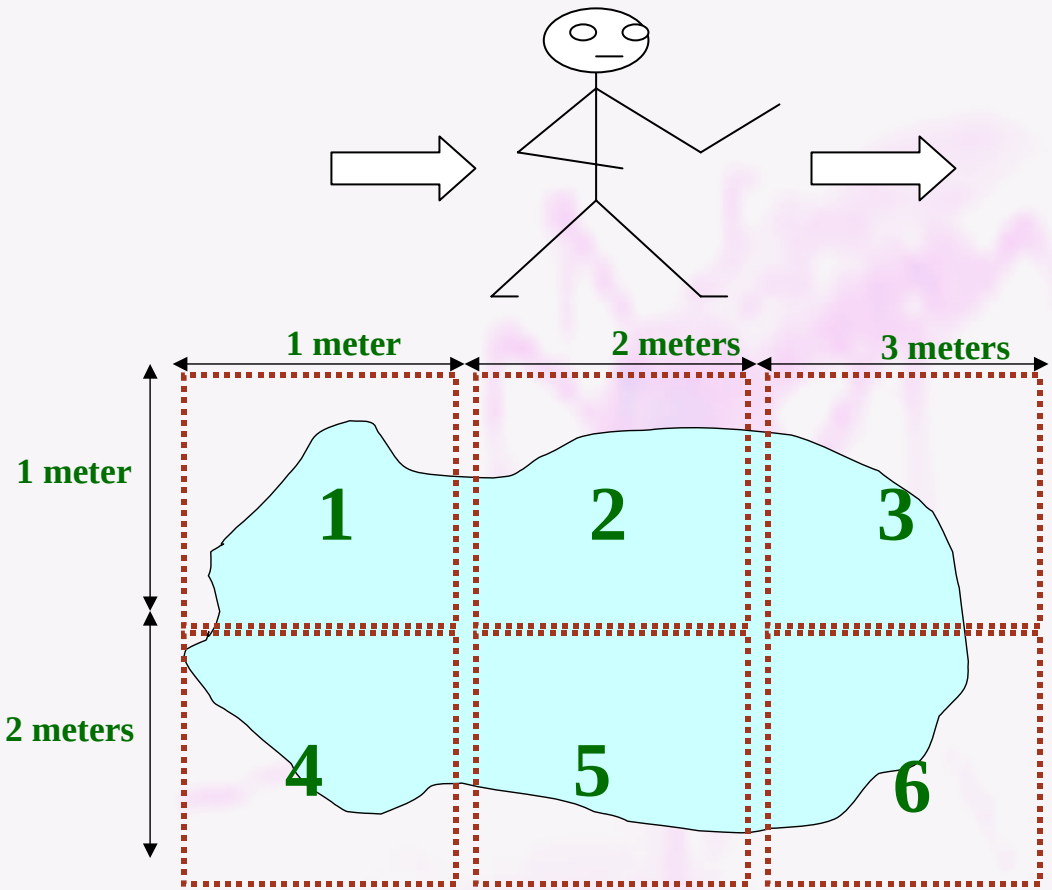
Dar UMCP
Dar es Salaam
January 2006

VALENT BIOSCIENCES CORPORATION

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 x 3 = 6 square meters

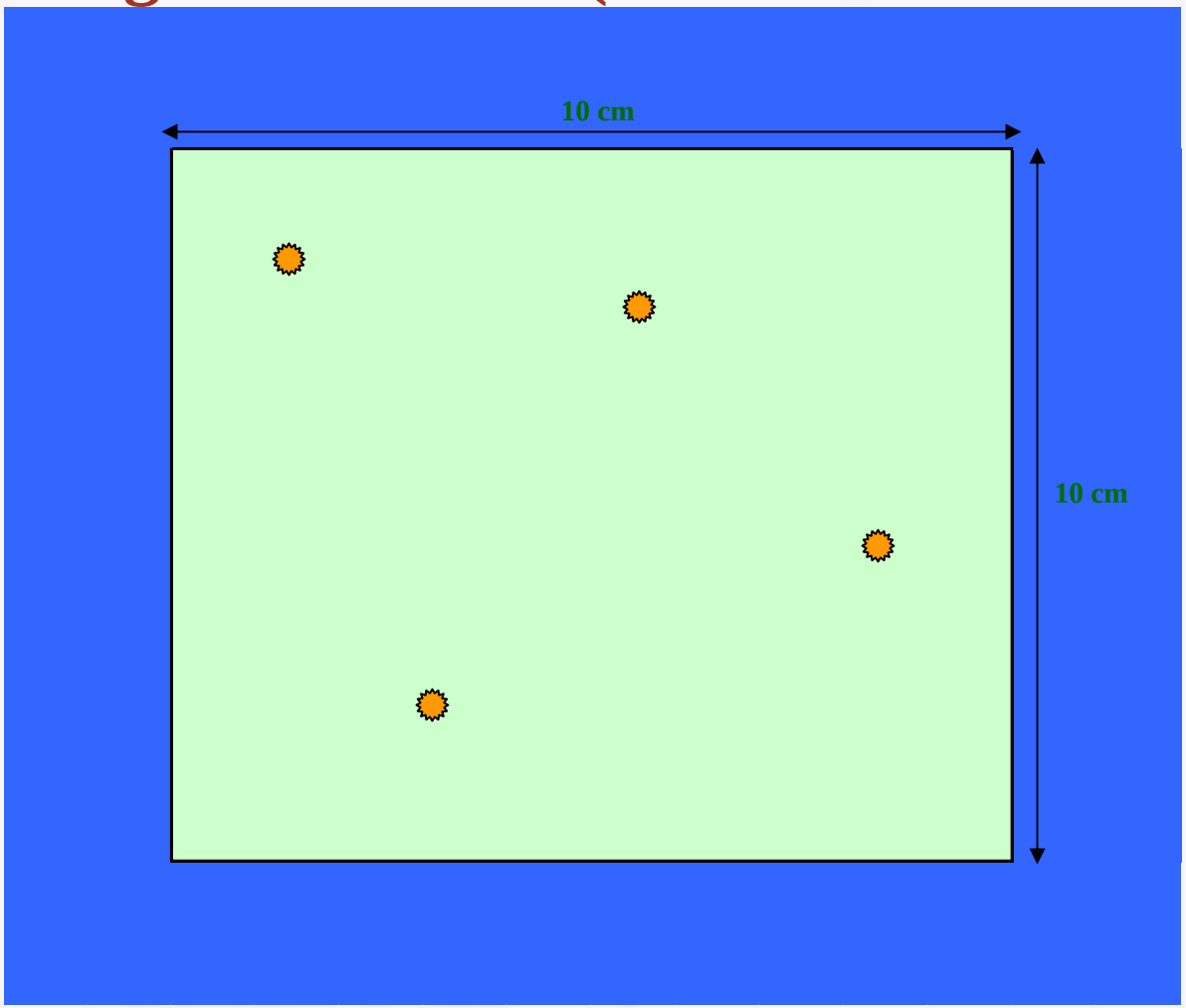
Needs 6 grams

Hand Application Rates

KG/HA	VectoBac CG 410 granules/gram	
	# PER M2	10 cm x 10 cm
5	205	2
10	**410**	**4**
15	615	6
20	820	8

Good Hand Application Rate

10 Kilogram/Hectare (AT LEAST FOUR)



VALENT BIOSCIENCES CORPORATION

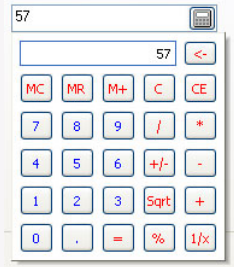
HAND APPLICATION RATES of CG

To Large Areas (> 3 meters x 3 meters)

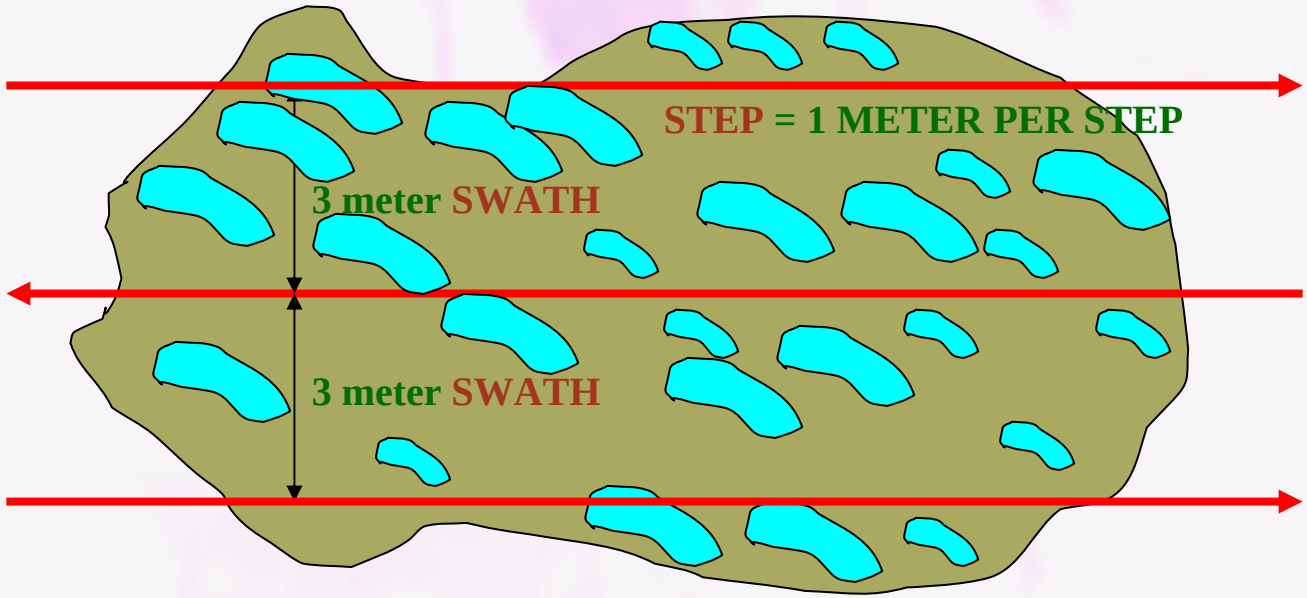
- Your Walking STEP (meters per step)
- SWATH (3 meters wide)
- Weight of your HANDFUL (of granules)

- 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



VALENT BIOSCIENCES CORPORATION

Hand Calibration for CG

RATE = (HANDFUL)/ (STEPS PER HADFUUL x SWATH x STEP)

STEPS PER HANDFUL = HANDFUL/(STEP x SWATH)

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

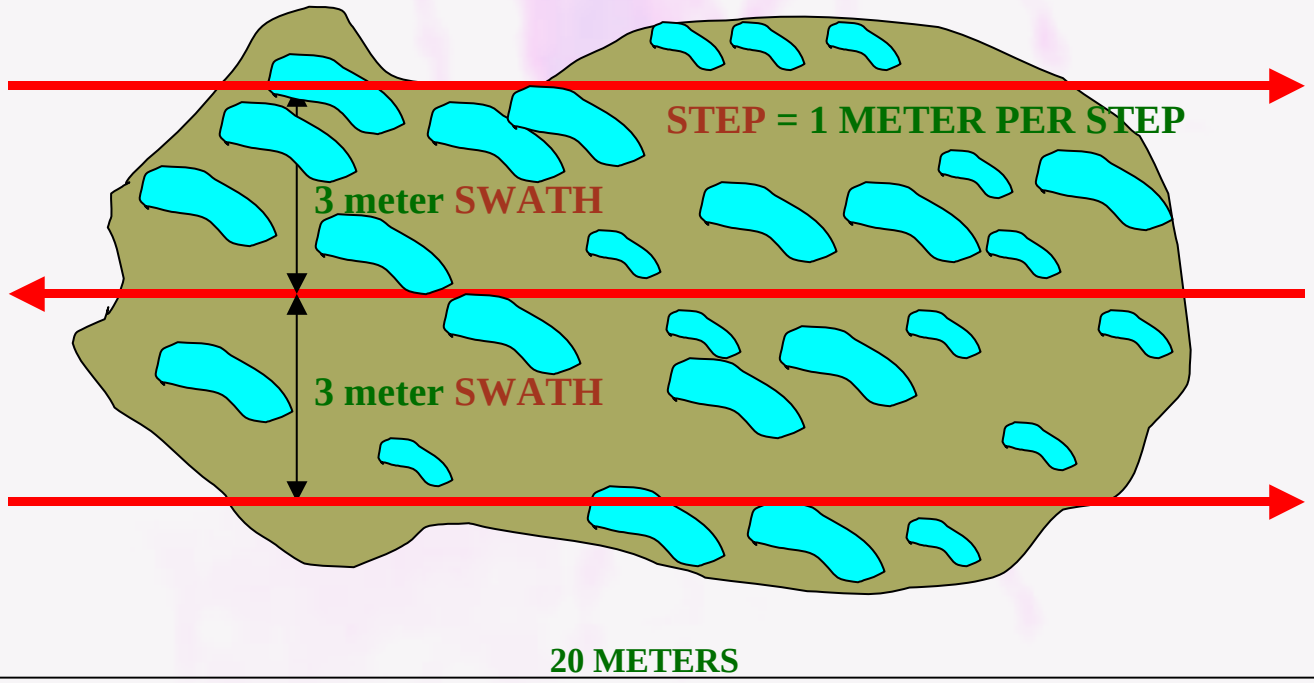
Dar UMCP
Dar es Salaam
January 2006

VALENT BIOSCIENCES CORPORATION

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



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.

Dar UMCP
Dar es Salaam
January 2006

VALENT BIOSCIENCES CORPORATION

Let’s all push!

Dar UMCP
Dar es Salaam
January 2006

VALENT BIOSCIENCES CORPORATION

Calibration For Application of VectoBac WDG

Peter DeChant
Valent BioSciences Corporation
Libertyville, IL

Dar UMCP
Dar es Salaam
January 2006

VALENT BIOSCIENCES CORPORATION

Objective

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

Agenda

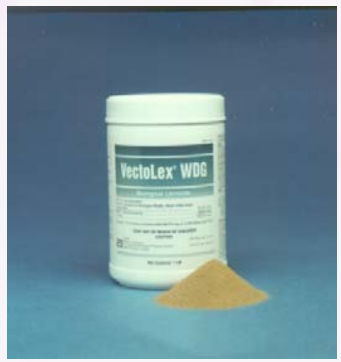
- **Microbial mosquito larvicide formulations**
- **VectoBac WDG**
- Application Equipment
- Calibration methods
- Verification

VALENT BIOSCIENCES CORPORATION

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



Dar UMCP
Dar es Salaam
January 2006

VALENT BioSciences Corporation

VectoBac® WDG

The stability of a granule with the application flexibility of a liquid.

Dar UMCP
Dar es Salaam
January 2006

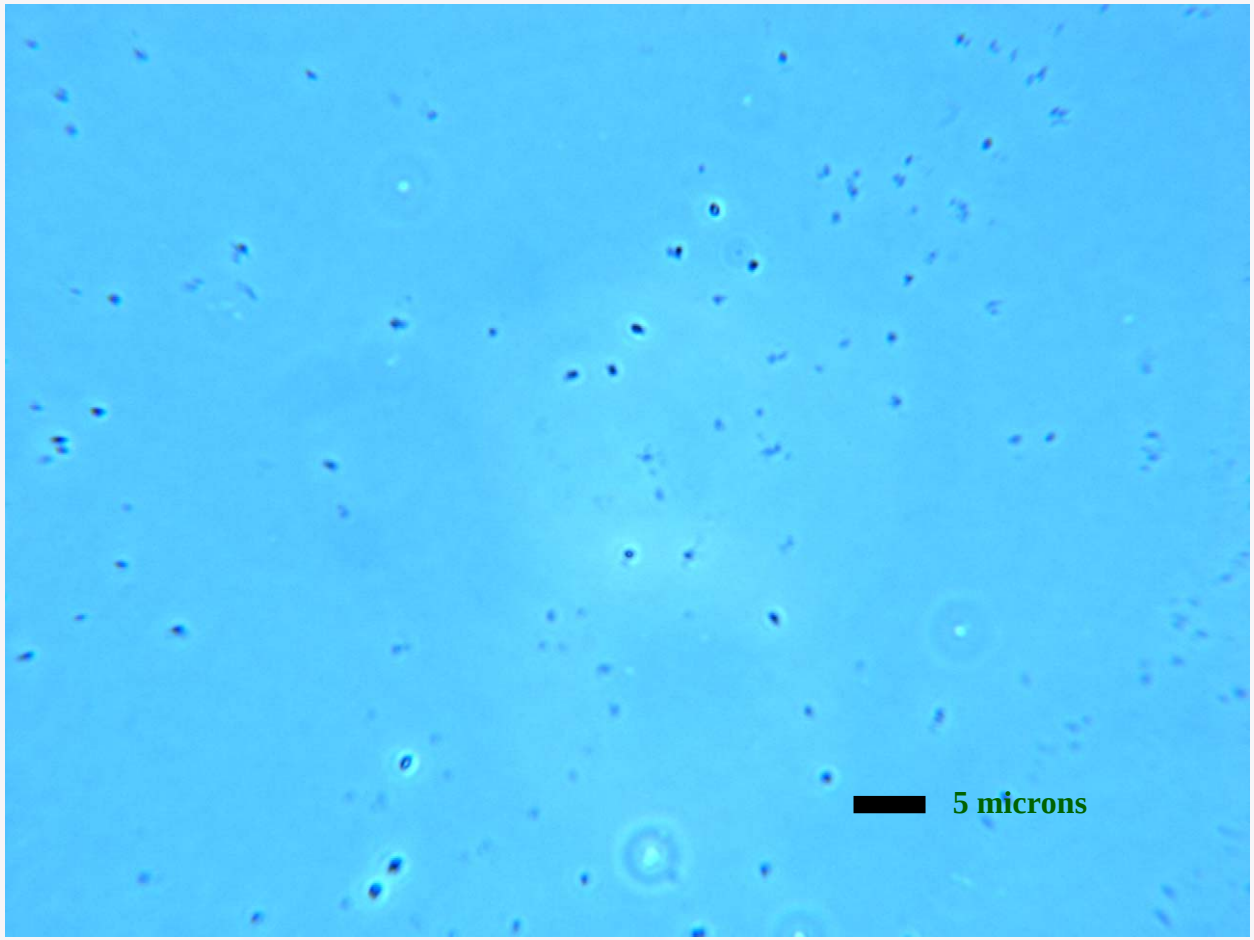
VALENT BIOSCIENCES CORPORATION

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



Dar UMCP
Dar es Salaam
January 2006

VALENT BIOSCIENCES CORPORATION

Insecticidal Crystal Protein - The larvae's Last Meal

Small particles suspend in feeding zone

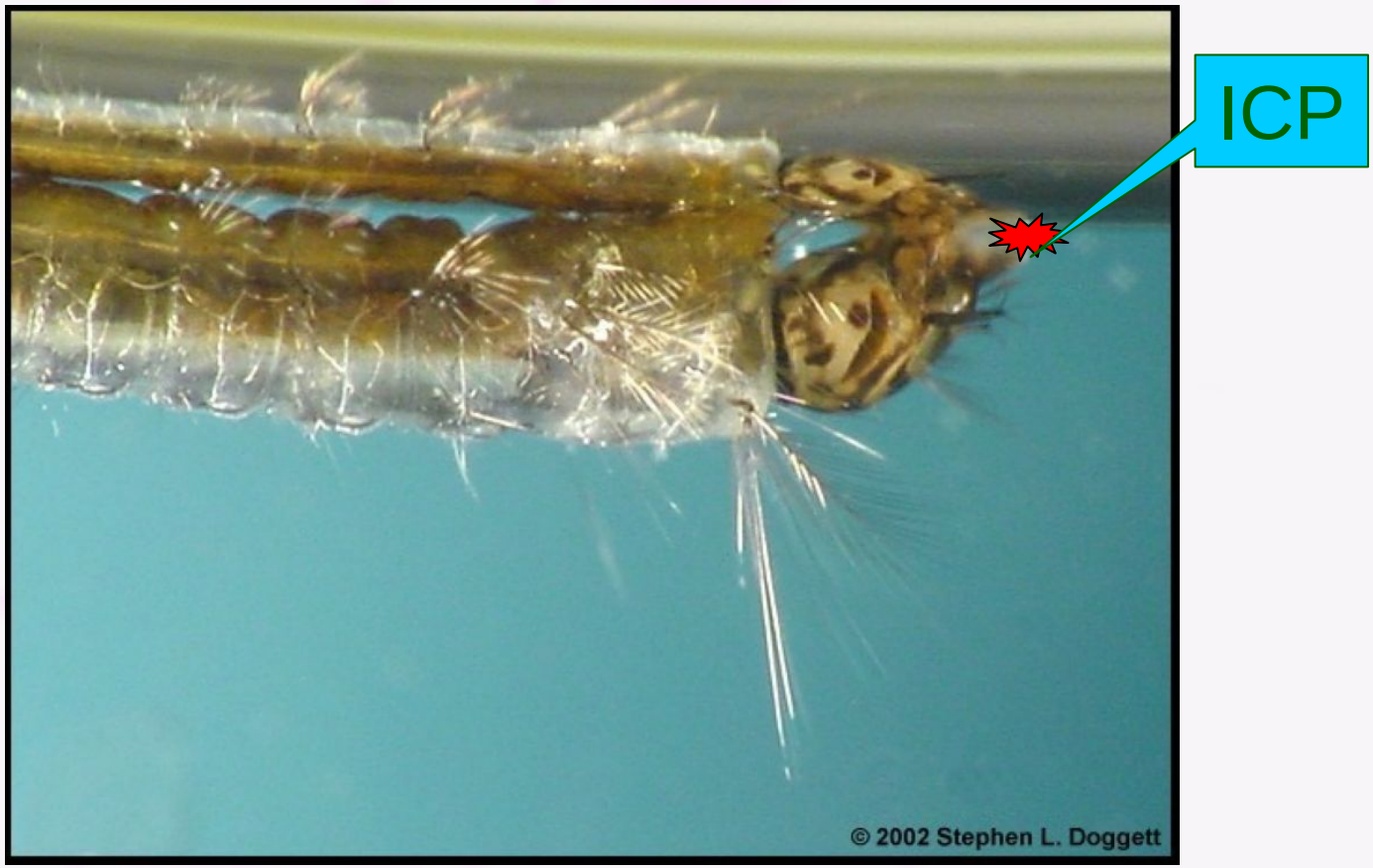


Figure courtesy of Stephen L. Doggett

VALENT BIOSCIENCES CORPORATION

Agenda

- ✓ Microbial mosquito larvicide formulations
- ✓ VectoBac WDG
- ❑ **Application Equipment**
- ❑ **Calibration methods**
- ❑ Verification

Examples of Spray Equipment for WDG Application

Dar UMCP
Dar es Salaam
January 2006

VALENT BIOSCIENCES CORPORATION

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

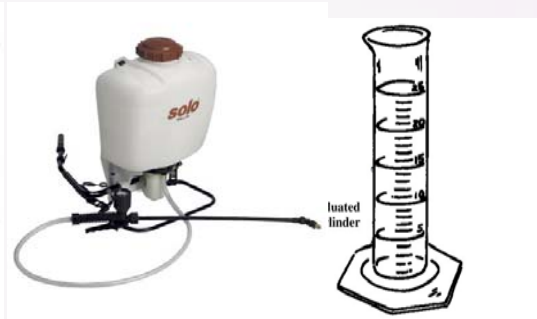
We aim to achieve this dose.

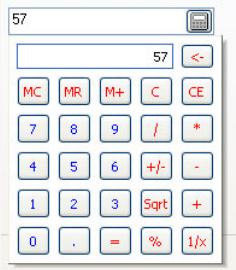
Factors That Determine Application Rate

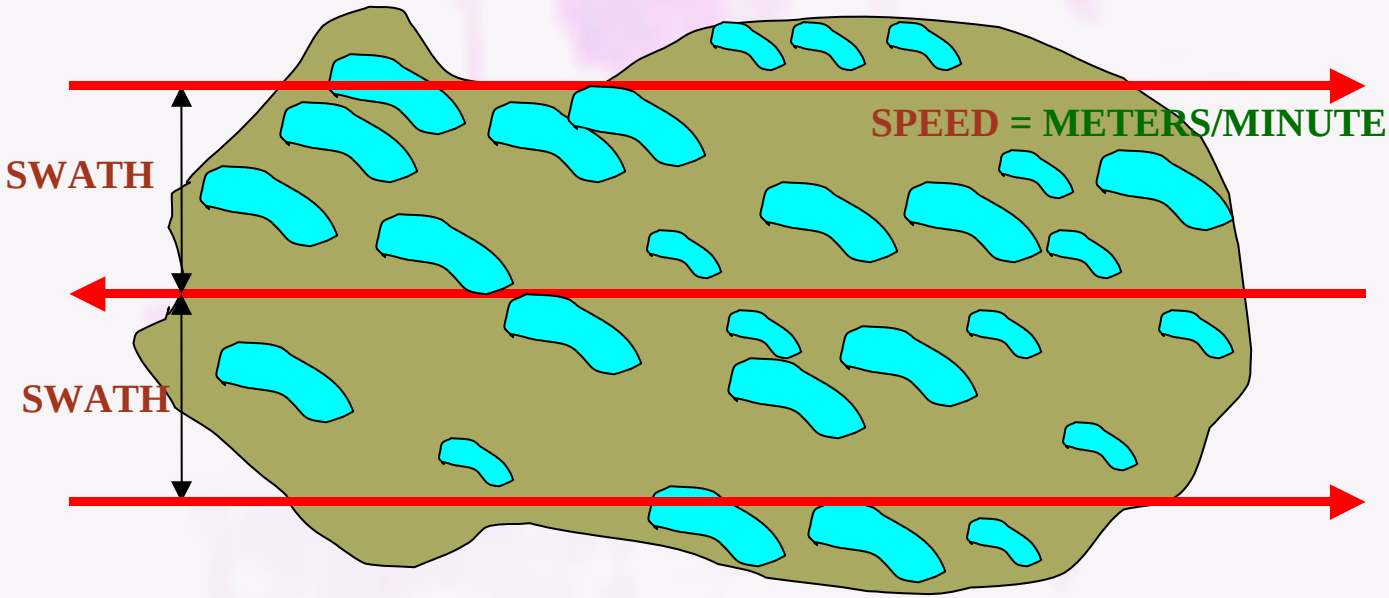
- SPEED of travel (meters per minute)
- width of SWATH (meters wide)
- FLOW rate of sprayer (liters per minute)
- DILUTION rate of product (grams per liter)

Calibration Steps for WDG

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







VALENT BIOSCIENCES CORPORATION

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

Dar UMCP
Dar es Salaam
January 2006

VALENT BIOSCIENCES CORPORATION

Measure the walking speed to determine the required dilution

Dar UMCP
Dar es Salaam
January 2006

VALENT BIOSCIENCES CORPORATION

Measure the flow rate

Dar UMCP
Dar es Salaam
January 2006

VALENT BIOSCIENCES CORPORATION

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)

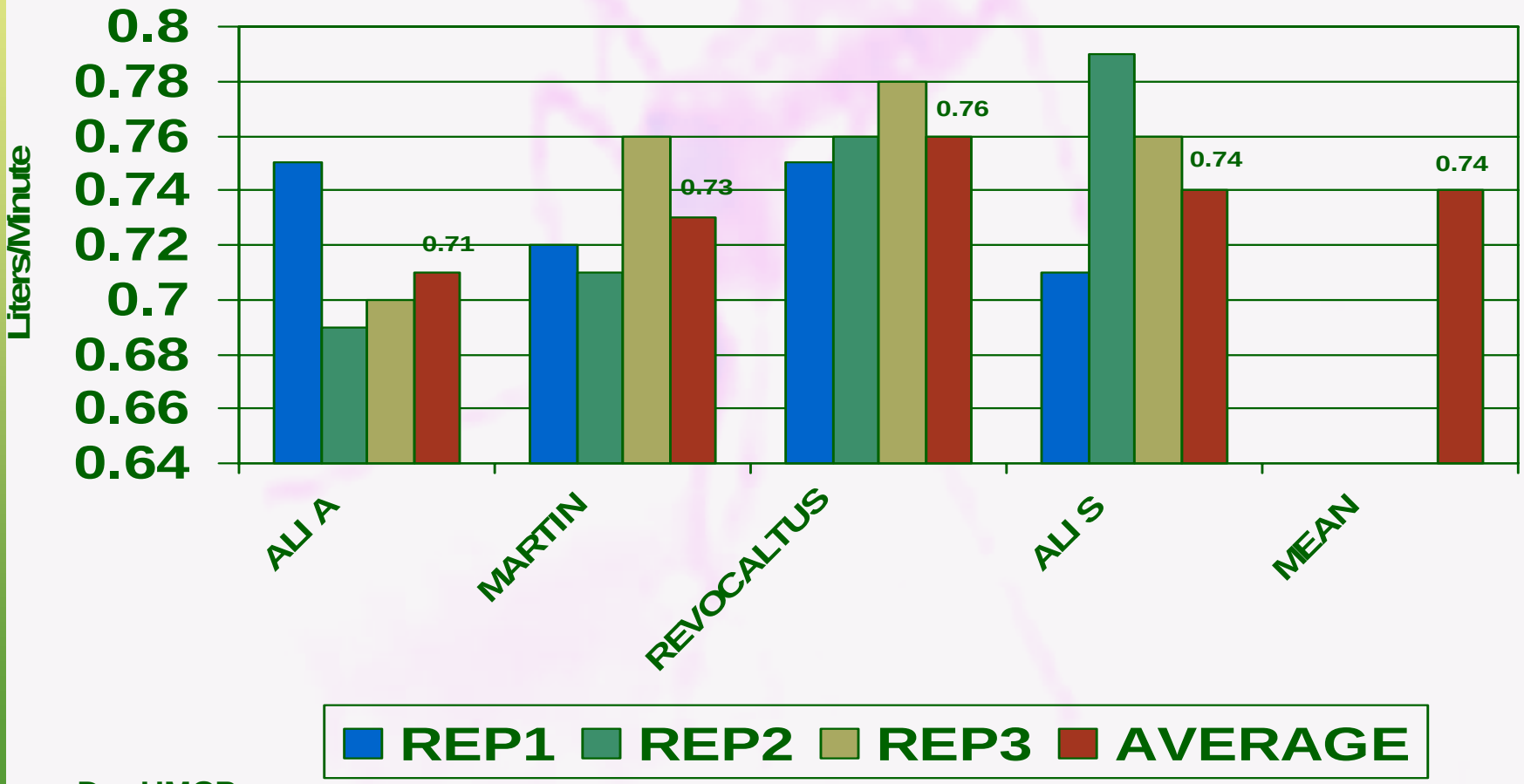
VALENT BIOSCIENCES CORPORATION

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



Dar UMCP
Dar es Salaam
January 2006

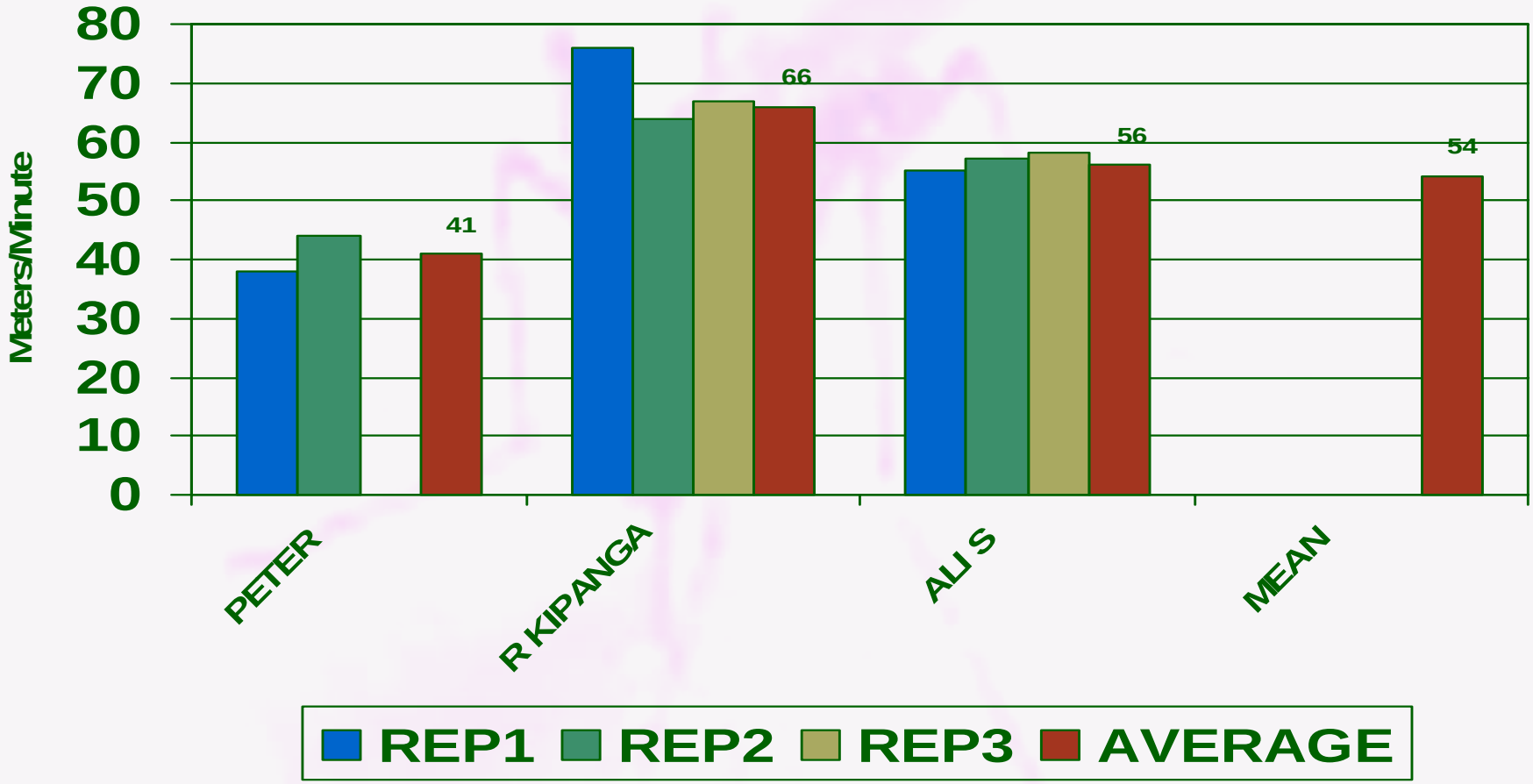
VALENT BIOSCIENCES CORPORATION

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 =



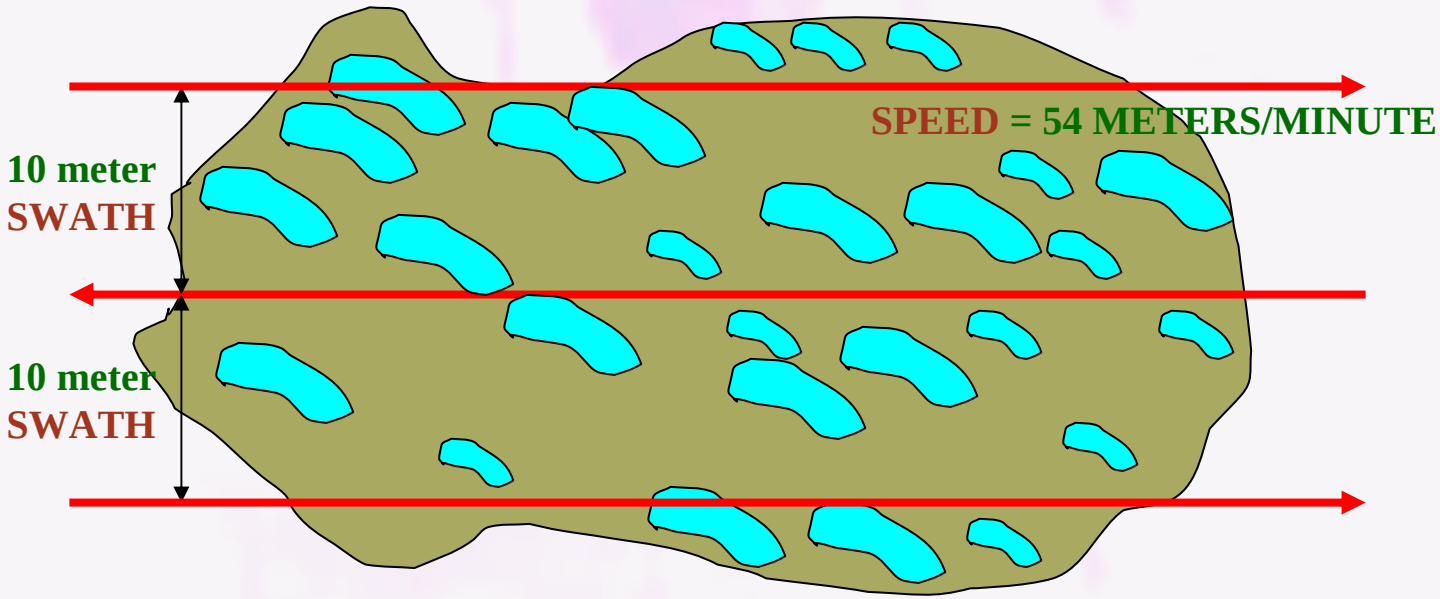
Dar UMCP
Dar es Salaam
January 2006

VALENT BIOSCIENCES CORPORATION

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 = (0.74 x 10000) / 54 x 10 = 14 LITERS PER HECTARE
DILUTION = 400 GRAMS / 14 LITERS or 200 GRAMS / 7 LITERS



One Tank (14 l) = One Hectare

VALENT BIOSCIENCES CORPORATION

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

Dar UMCP
Dar es Salaam
January 2006

VALENT BIOSCIENCES CORPORATION

Apply evenly and consistently

Dar UMCP
Dar es Salaam
January 2006

VALENT BIOSCIENCES CORPORATION

Treat the entire surface area with 10 meter-wide swaths

Dar UMCP
Dar es Salaam
January 2006

VALENT BIOSCIENCES CORPORATION

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

Folder number:		
		Code:
Municipality:		
Ward:		
Mtaa:		

Signature Supervisor ______________________ Date: _______/_________/________
Signature Inspector ______________________ Date: _______/_________/________
Signature Co-ordinator ______________________ Date: _______/_________/________

Year	**Month**	**Week**	10-cell unit	**No. of habitats**	No. of habitats with **water**	No. habitats with **Anopheles early**	No. of habitats with **Anopheles late**	No. of habitats with **Culex early**	No. of habitats with **Culex late**	No. of habitats with **pupae**	10-cell unit	**No. of habitats**	No. of habitats with **water**	No. habitats with **Anopheles early**	No. of habitats with **Anopheles late**	No. of habitats with **Culex early**	No. of habitats with **Culex late**	No. of habitats with **pupae**
2007																		

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

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

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 Comparsion 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).

	Year 2			Year 3		
Variables	Non-intervention area Mean [95% CI]	Intervention area Mean [95% CI]	P	Non-intervention area Mean [95% CI]	Intervention area Mean [95% CI]	P
An. gambiae (bites per night)	0.664 [0.538 – 0.819]	0.719 [0.507 – 1.019]	0.702	0.614 [0.482 – 0.783]	0.513 [0.383 – 0.686]	0.350
An. funestus (bites per night)	0.036 [0.021 – 0.061]	0.021 [0.007 – 0.065]	0.400	**0.026 [0.012 – 0.057]**	**0.005 [0.002 – 0.016]**	**0.021**
An. coustani (bites per night)	**0.143 [0.077 – 0.267]**	**0.003 [0.001 – 0.010]**	**< 0.001**	**0.103 [0.059 – 0.178]**	**0.002 [0.0003 – 0.015]**	**< 0.001**
Total EIR (infectious bites per year)	1.435 [1.137 – 1.813]	1.178 [0.804 – 1.725]	0.386	**1.236 [0.974 – 1.568]**	**0.796 [0.599 – 1.059]**	**0.020**
Culex (bites per night)	**130 [115 – 146]**	**87 [73 – 104]**	**< 0.001**	**126 [112 – 143]**	**86 [71 – 104]**	**0.001**

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).

	Year 1				**Year 2**				**Year 3**			
Overall prevalence	23.1 % (534/2310)				16.2 % (392/2418)				10.2 % (242/2371)			
Explanatory variables	n	%	OR [95% CI]	P-value	n	%	OR [95% CI]	P-value	n	%	OR [95% CI]	P-value
Constant			0.347 [0.296, 0.408]	< 0.001			0.202 [0.162, 0.253]	< 0.001			0.113 [0.083, 0.154]	< 0.001
Round												
Fresh[f]	1948	84.3	1.000		1492	61.7	1.000		1349	56.9	1.000	
Follow up	**362**	**15.7**	**1.356 [1.051, 1.749]**	**0.019**	926	38.3	0.957[0.764, 1.198]	0.701	1022	43.1	0.821[0.626, 1.076]	0.153
Net usage												
No net[a, f]	1704	73.8	1.000		1771	73.2	1.000		1713	72.2	1.000	
ITN	606	26.2	0.805 [0.642, 1.009]	0.060	647	26.8	0.814[0.629, 1.053]	0.117	658	27.8	1.206[0.903, 1.611]	0.205
Window screening												
No[c, f]	1108	48.0	1.000		942	39.0	1.000		816	34.4	1.000	
Complete[b]	1202	52.0	0.900 [0.727, 1.115]	0.335	1476	61.0	1.122[0.893, 1.409]	0.324	1555	65.6	1.290[0.969, 1.718]	0.081
Ceiling board												
No[e, f]	1518	65.7	1.000		1353	56.0	1.000		1148	48.4	1.000	
Complete[d]	792	34.3	0.879 [0.699, 1.104]	0.268	**1065**	**44.0**	**0.776 [0.620, 0.970]**	**0.026**	1223	51.6	0.926[0.707, 1.211]	0.573
Larviciding area												
No[f]	1896	82.1	1.000		1969	81.4	1.000		1907	80.4	1.000	
Yes	414	17.9	0.771 [0.590, 1.009]	0.058	**449**	**18.6**	**1.350 [1.036, 1.759]**	**0.026**	**464**	**19.6**	**0.540[0.365, 0.798]**	**0.002**

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.

[a] no or untreated net

[b] Complete screening, screening with small holes, glass windows

[c] No screening or badly damaged screening

[d] Complete and partly complete ceiling board

[e] No ceiling board

[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.

	Year 1						Year2						Year 3					
Variables	Non-intervention area		Intervention area				Non-intervention area		Intervention area				Non-intervention area		Intervention area			
	n/N	%	n/N	%	χ^2	P	n/N	%	n/N	%	χ^2	P	n/N	%	n/N	%	χ^2	P
Personal protection																		
ITN	2481/10507	23.6	479/2058	23.3	1.0	0.741	3026/10932	27.7	708/2695	26.3	0.9	0.142	**3237/13137**	**24.6**	**732/3264**	**22.4**	**0.9**	**0.008**
Window screening																		
Complete[a]	758/1252	60.5	163/270	60.4	1.0	0.946	**537/811**	**66.2**	**255/325**	**78.5**	**1.9**	**< 0.001**	**1394/1834**	**76.0**	**462/527**	**87.7**	**2.2**	**< 0.001**
Ceiling board																		
Complete[b]	526/1252	42.0	101/271	37.3	0.8	0.151	**406/811**	**50.1**	**186/326**	**57.1**	**1.3**	**0.033**	**1141/1834**	**62.2**	**289/527**	**54.8**	**0.7**	**0.002**
Drug use																		
Chloroquine	30/721	4.2	1/119	0.8	0.2	0.110	31/603	5.1	6/136	4.4	0.9	0.725	**179/1493**	**12.0**	**7/193**	**3.6**	**0.3**	**0.001**
SP	**461/721**	**63.9**	**93/119**	**78.2**	**2.0**	**0.003**	**373/603**	**61.9**	**104/136**	**76.5**	**2.0**	**0.001**	**698/1493**	**46.8**	**138/193**	**71.5**	**2.9**	**< 0.001**
Amodiaquine	86/721	11.9	8/119	6.7	0.5	0.100	69/603	11.4	8/136	5.9	0.5	0.060	**355/1493**	**23.8**	**11/193**	**5.7**	**0.2**	**< 0.001**
Quinine	151/721	20.9	21/119	17.6	0.8	0.410	101/603	16.7	14/136	10.3	0.6	0.063	116/1493	7.8	12/193	6.2	0.8	0.445
Artemisin	11/721	1.5	0/119	0.0	0.0	0.996	43/603	7.1	5/136	3.7	0.5	0.147	161/1493	10.8	26/193	13.5	1.3	0.264
Traditional	1/721	0.1	0/119	0.0	0.0	0.997	2/603	0.3	0/136	0	0.0	0.996	4/1493	0.3	0/193	0	0.0	0.996

[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