EFFECTS OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* AND *BACILLUS SPHAERICUS* LARVICIDES, ON MOSQUITO ABUNDANCE, DIVERSITY AND DISTRIBUTION IN SELECTED AREAS OF LUSAKA URBAN DISTRICT, ZAMBIA

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A dissertation submitted to the University of Zambia in partial fulfillment of the requirements for the degree of Master of Science in Entomology of the University of Zambia.

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DECLARATION

I, **Alister Kandyata**, hereby declare that this dissertation represents my own work and that it has not been previously submitted for a degree at this or any other University.

SIGNATURE……

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APPROVAL

This dissertation of **Alister Kandyata** is approved as fulfilling partial requirements for the award of the degree of Master of Science in Entomology by the University of Zambia.

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DEDICATION

I dedicate this dissertation to my family and friends.

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ABBREVIATIONS AND ACRONYMS

ACTs	Artemisinin-based Combination Therapies
ANOVA:	Analysis of Variance
asl:	above sea level
Bs:	*Bacillus sphaericus*
Bti:	*Bacillus thuringiensis* var. *israelensis*
Cry:	Crystal Proteins
Cyt:	Cytoplasmic proteins
DDT:	Dichlorodiphenyltrichloroethane
DHO:	District Health Office
DNA	Deoxyribonucleic acid
EIR:	Entomological Inoculation Rates
EM:	Environmental Management
EST:	Expected Species Total
GPS:	Global Positioning System
ID:	Index of Distribution
IPCS	International Programme on Chemical Safety
IRHS	Indoor Residual House Spraying
ITN	Insecticide Treated Bed Net
IVM	Integrated Vector Management
KCM:	Konkola Copper Mines
LLIN	Long Lasting Insecticide Treated Bed Net
LSM:	Larval Source Management
M&E	Monitoring and Evaluation
MCM:	Mopani Copper Mines
MoH:	Ministry of Health
NLP:	National Larviciding Programme
NMCC:	National Malaria Control Centre
OST:	Observed Species Total
PC-ORD:	Personal Computer Ordination

PCR:	Polymerase Chain Reaction
Plc:	Private Limited Company/ Public Limited Company
PSC:	Pyrethrum Spray Sheet Collection Method.
RBM	Roll Back Malaria
RDT:	Rapid Diagnostic Test
SMT	Sterile Male Technique
SR	Source Reduction
UNEP:	United Nations Environmental Programme
UNZA:	University of Zambia
UV	Ultra Violet
var.	Variety
WHO:	World Health Organisation

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ABSTRACT

Effects of *Bacillus thuringiensis* var. *israelensis* (Bti) and *Bacillus sphaericus* (Bs) biolarvicides, on mosquito abundance, diversity and distribution in selected areas of Lusaka urban district, Zambia, were investigated. Four study areas including parts of the dam/stream systems of the Kalikiliki/Ibex hills area; Venta/Manzi valley area; Chelstone-Zambia airways marshy ponds area, and the Chamba valley brick factory area were selected, based on their being parts of areas where the Larval Source Management (LSM) programme using Bti and Bs was being implemented by the Ministry of Health in the district.

The study hypothesised that the anopheline mosquito species, *Anopheles gambiae* sensu stricto, was the dominant vector of malaria in Lusaka urban district; LSM using Bti and Bs reduced the abundance and diversity of anopheline mosquito larvae in their breeding sites; and that the use of Bti and Bs affected anopheline mosquito species distribution. Study Specific objectives were to: assess the ratio of mosquito colonised habitats to potentially available habitats in the study areas before and after larviciding; identify species of mosquito larvae and adults present in the study areas before and after larviciding; assess mosquito abundance, diversity, distribution patterns and species dominance in the study areas before and after larviciding; and to compare incidences of malaria prior to and after the larviciding programme in the study areas.

Biolarvicides (Bti and Bs) were sprayed on freshwater bodies in the study areas in June and July, 2011 using Hudson X-pert pressure pumps and a fixed-wing, single-engine aircraft. Incidences of malaria prior to and after larviciding were determined by reviewing and analysing health facility records of positive malaria cases attended to by health facilities in the study areas.

None of the major malaria vectors reported for Zambia in the literature from the *Anopheles gambiae* sensu lato (s.l) or the *An. funestus* s.l were identified from the study areas. Instead three anopheline mosquito species; *Anopheles coustani* Laveran, *An. squamosus* Theobald and *An. rufipes* Gough, were found in the study areas. *Anopheles coustani* (13.5%) and *An. squamosus* (9.5%) were collected from all four study areas, while *An. rufipes* (1.1%) was only found in one study area. Prior to larviciding, culicine mosquito larvae were the most abundant (75.9%) in the study areas. No Culicidae larvae of any species were found in the freshwater bodies after larviciding. Possible reasons for the absence of known major malaria vectors from the study areas are suggested. The potential of biolarviciding as malaria vector control intervention for integration with Indoor Residual Spraying (IRS) and Insecticide Treated bed Nets (ITNs) are discussed. Incidences of malaria were higher prior to larviciding (2-17%). Positive malaria cases dropped drastically, after larviciding (2-4%), in all four study areas. However, though numerically very small, percent-wise, rises in malaria positive rates, were observed in Chainda area by second month post-larviciding but continued declining in the other areas. Possible reasons for the slight rise in the incidences of malaria were due to case importation. This study recommended the integration of Bti and Bs LSM into the Malaria Control Programme in Zambia.

CHAPTER 1: INTRODUCTION

1.1 Background

Presently, malaria is one of the most important diseases globally, accounting for over two million fatalities annually, mostly in tropical countries in Africa and Asia (Resh and Carde, 2003). In Zambia, it accounts for 32% and 32-65% of hospital and health centre admissions yearly, respectively, and about 40% of all outpatient attendances (MoH-NMCC, 2007; Chanda et al., 2007c). The disease causes an estimated number of 4 million clinical cases and 8,000 deaths annually in the country (Kachimba, 2007; Hamainza *et al*., 2007). Globally, the WHO (2011) estimates 216 Million cases of malaria and 655 000 deaths with 81% and 91% occurring in Africa, respectively, in 2010.

Malaria in man is caused by four species of the protozoan, genus *Plasmodium,* namely: *Plasmodium falciparum, P. malariae, P. ovale* and *P. vivax.* It is vectored by species of *Anopheles* mosquitoes. Of the four parasites, P. *falciparum* is the greatest killer, the most prevalent (MoH-NMCC, 2007) and yet ironically, it causes the most easily cured form of malaria in affected areas of the world (Kettle, 1984). The documented mosquito vectors of Malaria in Zambia include *Anopheles gambiae* complex members (i.e. *An. gambiae* sensu stricto (s.s) *and An. arabiensis*) and *An. funestus* (Gillies and De Meillon, 1968; NMCC, 2007) of *Anopheles funestus* group. Among the prevalent mosquito species is *Anopheles gambiae s.s* which is an extremely efficient vector of malaria (MoH-NMCC, 2007; Basseri, 2010).

Mosquitoes belong to the family Culicidae in the insect order of true-flies or two-winged flies called Diptera. The family is large, occurring throughout the temperate and tropical regions of the world, and well beyond the Arctic Circle, and from lowlands to the peaks of high mountains (Harbach, 2008).

The mouthparts of female mosquitoes are long and adapted for piercing and sucking blood from vertebrate hosts (Schowalter, 2000; Verma and Jordan, 2003). The blood is required by female mosquitoes to supply essential proteins for egg development prior to the initial and for subsequent ovipositions (Chapman, 1982). During these blood meals, a female mosquito transmits agents of disease to man. These include, Arboviruses (disease agents for yellow fever, encephalitis, chikungunya, rift valley fevers) by female culicine mosquitoes; Protozoa (etiological agents for malaria) by female anopheline mosquitoes; and Nematodes (causal agents for filariasis) by females of both culicine and anopheline groups (Metcalf and Luckmann, 1994; CDC, 2007).

Mosquito control by man may be undertaken either to avert the transmission of mosquito-borne diseases or to protect humans and their livestock from the vicious bites or attacks by the insects. This entails both destroying mosquito breeding places, to reduce the number of available oviposition sites, terminate the larval and pupal stages of development as well as, using other intervention methods such as spraying of chemicals inside human habitations to kill endophilic mosquitoes (Goma, 1966).

Currently in Zambia, malaria vector control focuses on decreasing the number of infective bites from the malaria vector by deploying insecticide treated bed nets (ITNs) and Long Lasting Insecticide Treated nets(LLIN) since 2007 (Chanda et al., 2011) and/or through Indoor Residual House Spraying (IRS) of insecticides, coupled with prompt treatment of malaria cases by effective anti-malarial drugs in the context of Integrated Vector Management (IVM) (NMCC, 2009; WHO, 2009; Chanda et al., 2011).

Under terms of the Stockholm Convention on Persistent Organic Pollutants (WHO/UNEP, 2001; WHO, 2004), countries still using *Dichlorodiphenyltrichloroethane* (DDT) for malaria control including Zambia, are supported and encouraged to strengthen their vector control programmes through various other means such as:

(1) Implementation of alternative products, methods and strategies, including vector resistance management strategies to ensure that the DDT alternatives remain effective;

(2) Development of safe alternative chemical and non-chemical products , methods and strategies that are relevant to the conditions in those countries using DDT with the goal of reducing human and economic burden of disease; and

(3) Developing such DDT alternatives, where adequate consideration will be given to ensuring that viable alternatives present less risk to human health and the

environment and also that the alternatives are suitable for disease control within the particular context of each country.

It is in this vein that the malaria vector control programme in Zambia is implementing, on a small scale, a Larval Source Management (LSM) programme, which emphasises environment management (EM), and larviciding in some areas of some major towns of Zambia, namely; Livingstone, Lusaka, Kabwe, Ndola, Luanshya, Mufulira, Kitwe, Chingola and Chililabombwe. Recently, microbial mosquito larvicides have been used to control malaria vectors in Lusaka, Eastern, Northern, Muchinga and Luapula Provinces of the country.

The mosquito vector control efforts are complemented by private company efforts, such as in Mazabuka town, by Zambia Sugar Plc, in parts of the Copperbelt Province (i.e. Chingola and Chililabombwe towns) by Konkola Copper Mines Plc (KCM) and in Mufulira and Kitwe again on the Copperbelt, by Mopani Copper Mines plc (MCM), who conduct LSM activities in their industrial process water bodies. Currently, the country's Ministry of Health (MoH) is implementing a nation-wide community-based larviciding programme using the biolarvicides *Bacillus thuringiensis* var. *israelensis* (Bti) and *B. sphaericus* (Bs).

The use of microbial mosquito larvicides in LSM offers an alternative with very low risk to human health and the environment (WHO, 1982; Glare and O'Callaghan, 1998; WHO/IPCS, 1999) and has resulted into the eradication of mosquito species, malaria and other mosquito borne diseases in some countries of the world (Soper, 1943; Shousha, 1948;

Killeen, 2002). Specific biotoxin-producing strains of Bti and Bs have been used throughout the world to suppress or eliminate the larval stages of mosquitoes, particularly where malaria, filariasis or certain arboviruses are present (Fillinger et al., 2003; Fillinger and Lindsay, 2006; Maozami, 2007).

The Zambia Larviciding Programme is using the entomopathogenic bacteria Bs (2362-GRISELESF®; LABIOFAM-CUBA*)* and Bti (H14-BACTIVEC®; LABIOFAM-CUBA*)* to control mosquito larvae in aquatic habitats. The use of bacterial larvicides for pest and vector insect control has continuously increased in different regions of the world (Boisvert, 2005; Maozami, 2007). The success and continued use of these biological control agents in mosquito control programmmes hinges on their high efficacies, specificity, their feasibility to be mass produced at an industrial scale, their long shelf-life, transportability, and probably most importantly, due to the lack of known or documented field resistance by mosquito larval (Maozami, 2007).

An impact assessment of the Larviciding programme on malaria vectors where it is being implemented in Lusaka District in Zambia is thus an essential requirement in order to measure the success of this new intervention on malaria vector control in the country. This study evaluated the effects of the larviciding programme using Bs and Bti on the abundance, diversity and distribution of anopheline mosquito larvae in selected areas of Lusaka urban district. Further, the study considered the feasibility of large scale integration of this LSM method of malaria vector control with the existing IRS and ITNs malaria vector intervention strategies in Zambia and made appropriate recommendations.

1.2 Statement of the Problem

Malaria is a very serious health problem in tropical countries of the world, especially those in Africa including Zambia. A lot of efforts in the form of use of interventions like; IRS, ITNs and use of LLINs have been made by Government of the Republic of Zambia and its cooperating partners to control the disease through vector management. However, successful control of malaria requires the integration of all the available interventions in order to sustain the achieved gains in vector management. The Ministry of Health in its 2009 Action Plan prescribed Larviciding and EM as supplementary interventions for malaria vector control in Zambia (MoH-NMCC, 2009). In 2010, the Government of Zambia through its Ministry of Health implemented a community driven larviciding programme utilizing entomopathogenic bacteria Bti and Bs in four provinces namely; Eastern, Lusaka, Northern, Muchinga and Luapula Provinces. The question, however, was; could community-driven Larval Source Management (LSM) through larviciding contribute to the decline in Malaria vectors and the inherent Malaria incidence in the country?

A review of the literature showed that similar approaches in urban Dar-es-Salaam, Tanzania, recently and in Brazil in the 1930's yielded positive results (Soper et al., 1943; Killeen et al., 2002). However, whether this community-based vector control strategy could work in Zambia or not was not clear. This was the subject that this study aimed at elucidating. Further, the geographical distribution of disease vectors of malaria in Zambia is not yet well known. At the time of the study, it was assumed that the northern and wetter parts of the country (i.e. Copperbelt, Northern, Luapula, Muchinga, North-western and Central Provinces) were the domains of *Anopheles gambiae* sensu lato (s.l) and that the remaining

southern and drier parts of the country (i.e Western and Southern Provinces) were zones where *An. arabiensis* and *An. gambiae* sensu stricto (s.s) dominated in varying ratios depending on the time of the year, rainfall and temperature variations (Shretta, 2000; MoH-NMCC, 2007; Mwangangia et al., 2009).

This study also sought to ascertain the dominant malaria mosquito vector(s) of Lusaka Urban District and their spatial distribution.

1.3 Significance of the Study

The importance of this study was that it:

a) Provided the much needed information on which anopheline mosquito species were dominant vectors of human diseases in Lusaka urban district, and on their distribution patterns.

b) Served as feedback to the MoH and other stakeholders, in the country and abroad through its findings on the impact of the on-going larviciding programme on mosquito larvae abundance, diversity and distribution in relation to malaria disease incidences in the selected study areas.

c) Provided data needed by policy makers to make evidence-based decisions on the planned eventual integration of community-based mosquito LSM interventions, into the current strategies based on IRS and LLINs in the context of an Integrated Vector Control (IVC) programme in Zambia.

d) Recommended how to improve and scale-up the community-based LSM programme in the Lusaka urban district.

1.4 Study Objectives:

1.4.1 General Objective of the Study

The general objective of this study was to assess the effects of the Larviciding programme using biolarvicides *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus* recently introduced in Lusaka urban district on mosquito larvae abundance, diversity and distribution and on the incidences of malaria in the district.

1.4.2 Specific Objectives of the Study:

The specific objectives of this study were to:

a) Assess the ratio of mosquito colonized habitats to potentially available mosquito habitats in selected study areas before and after biolarvicide (Bti. and Bs.) application.

b) Identify species of mosquito larvae and adults present in the study area before and after biolarvicide applications.

c) Assess mosquito abundance, diversity, distribution patterns, and species

dominance in the study area before and after biolarvicide application.

d) Compare the incidences of malaria prior to and after the implementation of the malaria mosquito vector control Larviciding programme in the study area.

1.5 Study Hypotheses

This study tested the hypotheses that:

a) The anopheline mosquito species, *Anopheles gambiae* sensu stricto is the dominant vector of malaria in Lusaka Urban District.

b) Mosquito Larval Source Management (LSM) with biolarvicides *Bacillus thuringiensis* var. *israelensis* (Bti) and *Bacillus sphaericus* (Bs) reduces the abundance and diversity of mosquito larvae, in their breeding sites.

c) The use of the biolarvicides Bti and Bs affected the distribution of mosquitoes.

CHAPTER 2: LITERATURE REVIEW

Malaria is an infectious disease of humans and other animals caused by parasitic protozoa. Malaria in man is caused by four species of the protozoan genus *Plasmodium*, namely: *Plasmodium falciparum, P. malariae, P. ovale* and *P. vivax.* It is vectored by species of *Anopheles* mosquitoes. Of the four parasites, P. *falciparum* is the greatest killer, the most prevalent (MoH-NMCC, 2007) and yet ironically, it causes the most easily cured form of malaria in affected areas of the world (Kettle, 1984).

The mainstay malaria control interventions include; prompt and effective treatment using artemisinin-based combination therapies (ACTs), use of insecticides in indoor residual house spraying and on bed nets, and larval source management methods to control the vectors (mosquitoes).

2.1 Taxomomic Classification of Mosquitoes

Mosquitoes belong to the Kingdom; Animalia, Phylum; Arthropoda and family Culicidae, in the insect order of true-flies or two-winged flies called Diptera (Subfamily; Nematocera) (Harbach, 2008). The family is large, occurring throughout the temperate and tropical regions of the world, and well beyond the Arctic Circle, and from lowlands to the peaks of high mountains (Harbach, 2008). The family includes 3,524 species divided into three subfamilies and 113 genera. The mosquito subfamily Anophelinae has three genera, while the subfamily Culicinae has 110 genera, spread among 11 tribes. The third subfamily is Toxorhynchitinae (Harbach, 2008).

2.2 The Biology of the Mosquito

The mosquito genus *Anopheles*, has over 400 species that are widely distributed in the world. Like other mosquitoes, the head of an adult *Anopheles* mosquito is specialized for acquiring sensory information from the environment and for feeding. The head bears eyes and a pair of long, many-segmented antennae (Kettle, 1984). The latter are important for detecting host odours, as well as odours of breeding sites where females lay eggs. The head also has an elongate, forward-projecting proboscis used for feeding, and has two sensory palps.

The mosquito thorax is specialized for locomotion. Three pairs of legs and a pair of wings are attached to the thorax. The abdomen is specialized for food digestion and egg development. This segmented body region expands considerably when a female takes a blood meal. The blood is digested over time, serving as a source of protein for the production of eggs, which gradually fill the abdomen (Clements, 2000).

Anopheles mosquitoes can be distinguished from other mosquitoes by the size and ornamentation of their palps, which are as long as the proboscis, and by the presence of discrete blocks of black and white scales on the wings (Gillies and De Meillon, 1968). Adult *Anopheles* can also be identified by their typical resting position: males and females rest with their abdomens sticking up in the air rather than parallel to the surface on which they are resting (Gillies and De Meillon, 1968). Larval anopheline mosquitoes, on the other hand, rest horizontally from the surface of the water while those of culicines hang at an angle from the surface of the water.

2.2.1 Life Cycle

Mosquitoes undergo complete metamorphosis i.e. development is through four stages namely; the egg, four larval instars, and the pupa from which emerges the adult which deposits eggs thus completing the cycle (Fig. 2.1). Of the four life stages, only the host seeking adult is terrestrial but it frequents water bodies where it lays eggs from which hatch the larva (Goma, 1966; Clements, 2000).

The three stages egg; larva and pupa are all aquatic, however, eggs in some mosquito species may survive long periods out of water under humid conditions (James and Harwood, 1969). Eggs are deposited singly in *Anopheles*, or in rafts in *Culex*, on the surface of stagnant or slow flowing water, margins of flood water, in tree holes and in some the sea margins in some salt water species. The eggs hatch into larvae, which develops through four instars before pupating (Goma, 1966, James and Harwood, 1969). The pupa is a non-feeding but active stage from which the adult mosquito emerges. Figure 2.1 shows the life cycle of a *Culex* species mosquito as a general representation of the mosquito life cycle.

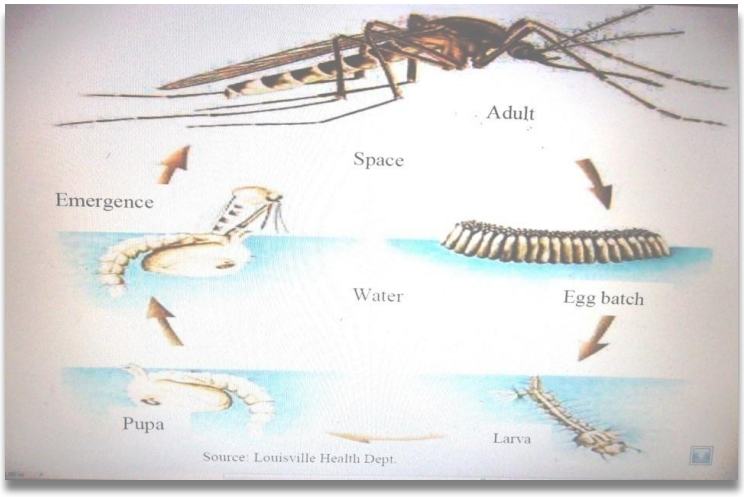

Figure 2.1 Mosquito life cycle (*Culex sp*.). (Source: Louisville Health Dept.)

2.1.2 Feeding Habits

As larvae, mosquitoes are generally filter feeders and their mouthparts are structurally adapted for this mode of feeding. They are able to feed at all levels of the water body i.e. at the water/air interface, in the water column and at the substratum. The principal feeding habit is particle capture, but can extend to predation on invertebrates of their own size (Clements, 2000).

As adults, the major sources of food for mosquitoes are plant sugars in the form of floral nectars, as well as extra floral nectarines such as; damaged fruit, damaged and intact vegetative tissues. In addition, adult mosquitoes feed on honeydew an elimination product collected from plant organs and produced by other insects mainly the Homoptera, such as aphids and scale insects. The plant juices and honey dew provide energy for both sexes of the adult mosquito (Hocking, 1953, 1968). Female mosquitoes however, additionally take blood meals from a range of vertebrate hosts including humans, during which event they transmit

disease pathogens to the hosts. The blood meal is principally to provide protein needs for egg production (Clements, 1955, 2000).

2.2.3 Mosquito Ecology

Female mosquitoes lay their eggs in water only; some species lay their eggs in running water, others in woodland pools, marshes, swamps, estuaries, or in containers such as rain barrels (Verma and Jordan, 2003). The majority of mosquito larvae live in standing fresh-water. Only a minority of species utilize brackish water or certain situations in flowing streams. Mosquito larvae require some protection from wind or waves at the water surface and so do not occur in such places as the open waters of lakes and rivers. The favoured habitats are themselves extremely varied, although most are characterized by small size and relative impermanence. According to Goma (1966), mosquito habitats may be classified into four major groups namely:

a) Permanent or semi permanent standing water.
b) Flowing water (Stream associations).
c) Transient ground pools.
d) Container habitats.

Different species of mosquito larvae inhabit different situations. Some species make use of a wide range of habitats, while others are very limited in their choice of habitats, preferring one type to all others. Thus, a mosquito like Anopheles gambiae s.s., the principal vector of malaria in tropical Africa, may be found breeding in temporary small sunlit pools, drains and

ditches, in running water backwaters of streams, ponds in grassy swamps, rice fields, and wells. On the other hand, mosquitoes such as *Malaya taeniorostris* and some *Eretmapodites* spp. appear to breed exclusively in leaf axils of plants (Goma, 1966; Verma and Jordan, 2003; Majambere et al., 2007).

A large number of mosquito eggs and larvae are consumed and hence destroyed by mosquito fish of the genera *Gambusia* and *Minnows* in tropical freshwater bodies where these fish are found. The fish species thus afford natural control of mosquitoes. Mosquitoes may also be controlled by spraying these breeding places with petroleum oil and with insecticides **(**Kettle, 1984; Verma and Jordan, 2003).

2.2.4 Mosquito distribution and feeding habits

Mosquitoes are distributed worldwide except in areas that are permanently frozen. They have a terrestrial adult stage but require a diversity of water bodies for the development of their immature stages i.e., the egg, larva and pupa (Goma, 1966; Clements, 2000). Mosquito population dynamics are dependent on a number of biotic factors such as predation by larvivorous fish, competition for resources with other mosquito species in the habitat, aquatic plant species/ hydrophytes, host choice and feeding preference, and abiotic factors including availability of suitable oviposition sites, physicochemical qualities of the breeding site water like; dissolved substances, pH, conductivity and water temperature (Service, 1989; Robert et al., 1998).

2.3 Mosquitoes and Disease Transmission in Man

Both male and female mosquitoes are primarily fluid feeders, naturally consuming plant nectars, other exudates and water (Chapman, 1982). In both anopheline and culicine mosquito families, males feed exclusively on plant juices and water, and hence do not vector agents of human diseases (Chapman, 1982; Clements, 2000). However, anautogenous females of both mosquito subfamilies in addition to their plant juice and water diet, require vertebrate blood meals which provide the necessary proteins for the process of öogenesis before the initial and for subsequent ovipositions (Chapman, 1982).

When in search of a blood meal, female mosquitoes bite their vertebrate hosts including man. They inject some of their saliva into the wounds they create, causing swelling and irritation. Further, they may inject infectious agents into the hosts including viruses and protozoa, and thus transmit such diseases as malaria (anopheline mosquitoes), yellow fever (culicine mosquitoes; *Aedes aegypti, Haemagogus* sp. and *Sabethes* sp.), dengue fever (*Aedes aegypti)*, Japanese encephalitis (*Culex tritaeniorhynchus*, *Culex pseudovishnui* or *Culex gelidus)* and filariasis (*Culex quinquefasciatus*- found primarily in urban and suburban areas) (Clements, 2000). *Anopheles, Mansonia* and *Aedes* species can also vector filariasis (WHO, 1988; Verma and Jordan, 2003; Harbach, 2007, 2008). In the tropical Africa, malaria is dorminantly vectored by members of two mosquito complexes, namely, *Anopheles gambiae* sensu lato (s.l) and the *Anopheles funestus* group. The *Anopheles gambiae* complex (i.e. sibling species) comprises the following: *Anopheles gambiae* sensu stricto (s.s.), *Anopheles arabiensis*, *Anopheles quadriannulatus*, *Anopheles bwambae*, *Anopheles merus*, and

Anopheles melas. The *Anopheles funestus* group comprises nine members: *Anopheles funestus, Anopheles vaneedeni, Anopheles leesoni, Anopheles rivulorum, Anopheles parensis, Anopheles fuscivenosus, Anopheles aruni, Anopheles brucei,* and *Anopheles confuses* (Gillies and De Meillon, 1968; Harbach, 2008).

Mosquitoes are the most serious indirect causes of morbidity and mortality among humans when compared to other groups of organisms (Verma and Jordan, 2003; Harbach, 2008). Malaria and other vector-borne diseases contribute substantially to the global burden of diseases and disproportionately affect poor and under-served populations living in tropical and sub-tropical regions of the world (Beier et al., 2008).

2.4 Mosquito Identification

Mosquito species identifications can be done at both larval and adult stages. These identifications are either morphological (Gillies and De Meillon, 1968) or use of molecular techniques such as Polymerase Chain Reaction (PCR). The morphological methods are employed as preliminary identifications into the broad sibling species complexes, while PCR is used to separate the specific individual members of the group. Well developed PCR methods are credited to the works of Scott et al., (1993) and Koekemoer and Coetzee (2002). These are procedures for the identification of the *An. gambiae* s.l and *An. funestus s.l* respectively. The morphological methods can be variously modified employing from simple hand lenses in field settings to dissection and compound light microscopes in the laboratory. The PCR based methods are however very expensive cost-wise and are

therefore mostly only used in research settings rather than in general programmatic mosquito /vector control laboratories.

The morphological species identification keys for adult mosquitoes use body features as;

(i) The Head and Head Appendages

In *Anopheles* species, the palps are usually as long as the proboscis and are each divided into five (5) segments. The papal segment joints are marked with rings of white scales. The proportion of the different segments may be of characteristic length in different species. The scales on the basal segment of palps are also important distinguishing features which are either upstanding (termed shaggy) or appressed (termed smooth) (Gillies and De Meillon, 1968).

(ii) The Thorax and Thoracic Appendages

The thoracic region exhibits various colouration in different mosquito species and the distribution of mesonotal scales is another important feature. The wings are another very important appendage used in morphological mosquito identifications. The scaling on the veins forms species characteristic colour markings on the wings. The vein scaling in males is less pronounced when compared to the female counterparts. The scales are either standing or decumbent, the decumbent scales being closely pared hence giving more definite markings. Other diagnostic colourations are found on the tarsi of the hind legs (Gillies and De Meillon, 1968).

(iii) The Abdomen and Abdominal Appendages

The abdomen consists of 8 visible segments and 2 highly modified retractile terminal segments. The abdominal tergites are usually clothed in hairs only, but in many species, the last two sternites are ornamented with a few scales. The abdomens of some species are densely covered with broad scales forming lateral projecting tufts (Gillies and De Meillon, 1968).

2.5 Mosquito Vector Control

Mosquito control maybe undertaken either to avert the transmission of mosquito-borne diseases or to protect man and his livestock from the vicious bites of the insects by minimizing the man-mosquito contact. Control is targeted at the two ecologically distinct life stages of the insect, namely; the immature (egg, larva and pupa) and the adult mosquito. At aquatic stages, the control of mosquitoes is broadly termed LSM.

The control of mosquitoes takes a variety of forms including; Physical, Chemical and Biological Methods.

2.5.1 Physical methods of mosquito control

Physical methods of mosquito control utilize mechanical barriers to prevent the entry of host seeking mosquitoes into the human dwellings or access to human host. These barriers include; window screens, sealing of heaves and openings in the roof, doorways, and

windows (WHO, 1988). These mosquito control methods target adult mosquito and will additionally work as deterrent entry measures for other arthropods and non-arthropod household pests e.g. rats and bats. These physical methods are also advantageous in that they do not use chemical insecticides, and are thus environmentally friendly. The barriers are however limited in the sense that they are only effective in the protection of man against endophilic/endophagic inside the dwellings and will not affect outdoor feeding vector species.

Another physical control measure against mosquito is source reduction which is a component of a broader category of larval source management.

Larval Source Management, a strategy that utilizes methods such as larviciding (i.e. the use of mosquito larvicides to prevent the emergence of adult mosquito vectors) and source reduction (i.e. environmental manipulation, modification and elimination of aquatic habitats for mosquito larval control) has historically been used as a measure for malaria control in many parts of the tropical world (Fillinger et al., 2004, 2009). The World Health Organization defines environmental management (EM) for vector control as "The planning, organizing, carrying out and monitoring of activities for the modification and/or manipulation of environmental factors or their interaction with man with a view to preventing or minimizing vector propagation and reducing man-vector-pathogen contact" (Castrol et al., 2009). The objective of EM for vector control is the reduction of the population density of target vector species below disease transmission threshold levels (WHO, 1988). Research into the biology of disease vectors has shown that each species has

a defined geographical distribution and the availability of optimal biotic and abiotic ecological conditions correlate with their high abundance occurrences. Environmental management methods demand for a thorough understanding of vector ecology and population dynamics, as well as an understanding of vector-borne disease epidemiology (WHO, 1988).

2.5.2 Chemical methods of mosquito control

Chemical insecticides are an important armory in the control of mosquitoes, which are employed in IRS, LLINs and Larviciding mosquito vector control strategies.

The main thrust interventions presently being applied in Zambia to control mosquito vectors include the IRS intervention with insecticides and the use of LLINs, in an integrated vector management fashion (WHO, 2006b; Chanda et al., 2007a/b; MoH-NMCC, 2009; WHO, 2009; Chanda et al., 2011). Throughout Africa, national malaria control programmes have recently embarked on emphasizing vector control as an essential component in the fight against malaria disease (Yakob and Yan, 2009). Most programmes are using ITNs and/or IRS incentives. When optimally employed, these vector control measures can reduce malaria parasite transmission by 90% (Utzinger et al., 2001; WHO, 2006a; Beier et al., 2008) or more and can correspondingly reduce malaria disease incidence, malaria disease prevalence, parasite density, and clinical malaria (WHO, 2006a; Chanda et al., 2008, 2011; Geissbühler et al., 2009).

Despite their great success in the fight against insect pests and insect vectors of disease, synthetic chemical insecticides have given birth to serious ecological problems, which have led to development of insecticide resistance in the target insect pests and vectors (Shiff, 2002; Maozami, 2007; Majambere et al.,, 2007; Chanda et al., 2011). Their use has also resulted in the killing of non-target beneficial insects/animals and insect pest/vector natural enemies. Consquently, environments have been polluted by the insecticides which have also accumulated in food chains (Maozami, 2007; Poopath et al., 2009).

However, the following questions remain unanswered regarding insecticide use and mosquito vector control: What should be done to avert these negative impacts of chemical insecticides on the environment? Are malaria vector control approaches involving ITNs and IRHS sufficient? Where and when are they effective? What else is needed to eliminate the remaining low-levels of malaria transmission? (Killeen et al., 2002; WHO, 2006a)

With the increasing development of insecticide resistance by the adult malaria mosquito vector (Biosvert, 2005; Chanda et al., 2011) and its avoidance behaviour to many interventions by its ability to readily detect and escape through its high mobility by flight, characters that are lacking in mosquito eggs, larvae and pupae, which remain confined within relatively small aquatic habitats, it is easier to target the latter mosquito developmental stages by control measures (Killeen et al., 2002; Fillinger and Lindsay, 2006). Thus the development and evaluation of complementary vector control strategies remains a priority.

2.5.3. Biological methods of mosquito control.

Biological control methods include but are not limited to the following;

a) Sterile Male Technique (SMT) - This involves the release of genetically modified males of a mosquito species into the environment to competitively mate with the wild female mosquitoes, and in the process, limiting the reproductivity of a vector population.
b) Release of other organisms that predate on mosquito larvae in the aquatic environment; e.g. larvivorous fish species of *Minnows* sp and *Gambusia affinis*.
c) Microbials and microbial products with larvicidal efficacy.

Larviciding may play an important supportive or even leading role in some special settings such as in arid environments where mosquito breeding sites, often a result of human activity, are few, and are well identified and easily accessible (Gu et al., 2006). Larviciding may also be used to reduce receptivity in recent foci (WHO, 2007, 2008). Larval control can only be applied as a single method of vector control if a high proportion of the breeding sites within the vector's flight range of the community to be protected can be located, accessed and managed. Larval control may also be undertaken to supplement or synergize the effects of other vector control interventions. Larval control affects only the vector density and requires high area coverage to be significantly effective (Castrol et al., 2009, Geissbühler et al, 2009). It is thus felt that it is imperative to introduce Larval Source Management as a primary alternative method and strategy to further reduce and maintain malaria transmissions at low levels in Lusaka Urban, Zambia (MoH-NMCC, 2006).

Reviews of the early 20th century programmes in Brazil, Zambia and Egypt have highlighted dramatic reductions of malaria burden achieved by integrated vector management generally and mosquito larval control specifically (Utzinger et al., 2002; Yakob and Yan, 2009; Fillinger et al., 2009). Application of microbial larvicides, such as Bti, to larval habitats offers a control option that is effective (Nicholas et al., 1987; Fillinger et al., 2003; Chanda et al., 2007b; Chaki et al., 2009) and is less likely to be avoided by adult mosquitoes. Further, the complex mode of action of the larvicide formulations present low probability of development of larvicide resistance (Chaki et al., 2009). The integration of LSM into malaria vector control programmes offers the potential to considerably augment the protection afforded by existing strategies (Killeen, 2002).

More recently larval control has been shown to be highly effective at reducing malaria transmission in Eritrea, Kenya and Tanzania (Fillinger et al, 2009; Chaki et al., 2009) and historically in other parts of the world (Soper, 1943; Shousha, 1948; Xu et al., 1992; Kumar et al., 1994; Barbazan, 1998; Yapabandara, 2001; De Castro et al., 2004; Gu et al., 2006). General vector population suppression afforded by LSM may also reduce selection pressure towards resistance to chemical insecticides in ITNs and IRS by reducing the rate of insect-chemical interactions and substantially reduce adult *Anopheles* mosquito densities in various settings (Kitron and Spielman, 1989; Yu and Shen, 1990; Kroeger et al., 1995; Fillinger and Lindsay, 2006) and enhance effects of IRS (Soekirno et al., 1983). Entomological Inoculation Rates (EIR) based studies analysing impacts of different vector control interventions, IRS, ITNs and Source Reduction (SR) have shown that no single intervention alone could eradicate malaria transmission (Shaukati et al., 2010). The EIR is

the number of infectious bites per person per unit time, frequently measured or expressed per year.

Currently, it is commonly felt that there is a strong case for implementing larval control to target immature stages of anopheline mosquitoes to supplement the adult mosquito control interventions and further reduce malaria transmission (Killeen et al., 2002; Gu et al., 2006; Beier et al., 2008). Further, WHO recommends that all countries aiming for elimination of malaria disease, eventually need to create or enhance legislation supporting the identification and notification of malaria cases and mosquito breeding sites, (WHO, 2007).

2.5.3.1 Bacterial-control Agents in Insect Control: Mosquito Vector Control

Insect pest control with biological agents date back to the 19th century and is credited to the works of Bassi and Pastuer (Maozami, 2007). Mechnikoff in 1879 and Krassilnikow in 1888 pioneered the microbial control of insects when they documented the mass production and field application of entomopathogenic fungi *Metarrhizium anisopliae* against sugar beet pests (Maozami, 2007).

Discovered in the 1970s, mosquitocidal bacteria *Bacillus thuringiensis* var. *israelensis* (Bti) and *Bacillus sphaericus* (Bs) have proven to be efficacious and as a preferred option against aquatic stages of insect vectors of malaria, dengue and filariasis diseases (Kalfon et al., 1983; Boisvert, 2005). The mosquitocidal and blackfly killing Bti was initially isolated

from a mosquito breeding site in Israel and the Bs strain 2362 was isolated from the blackfly *simulium damnosum* adult.

The insecticidal properties of the bacteria are primarily due to proteins produced during sporulation namely; Cyt1A, Cry11A, Cry4A and Cry4B in Bti and a single Binary toxin (Bin) in Bs (Federici et al., 2003). Other promising candidate bacteria with mosquitocidal proteins have been identified including; *Bacillus thuringiensis* var. *jegathesan* (in Malaysia), *Bacillus thuringiensis* var. *morrisoni* (in Phillipines) and other bacterial species like, *Clostridium bifermentans* (Federici et al., 2003; Maozami, 2007).

Larvicidal efficacy of the bacterial products depends on a number of factors such as type of insecticide used to control mosquito larvae in the area, larval age, larvicide formulation type, water quality, temperature, physical conditions and culture media used in the production of the bacteria in question. Other factors include; hampering and synergistic factors as, larval food intake, depth of habitat water, availability of food, and concentration and mode of action of larvicide (Maozami, 2007). The target spectrum of Bs is narrower than that of Bti, being restricted only to mosquitoes and with greatest activity in *Culex* and certain *Anopheles* species. However, Bs has demonstrated better initial and residual activity against mosquitoes in polluted waters (Federici et al., 2003).

Studies have demonstrated development of resistance and even cross resistance by larvae of *Culex quinquefasciatus* Say to *Bacillus sphaericus* strains used in mosquito control (Adak et al., 1995; Pei et al., 2002; Federici et al., 2003; Poopath et al., 2009) in many

countries including; Brazil, China, France and India (Federici et al, 2003). This resistance has developed due to selection pressure conferred by continued use of Bs products in breeding sites (Pei et al., 2002). However, interruption with Bti for a six (6) months period restored Bs suscpetibility in *Culex quinquefasciatus* Say (Pei at al., 2002). To date, no field resistance of mosquito larvae to Bti has been reported (Federici et al., 2003).

Toxicological and biochemical variation of the larvicidal activity of Bti and Bs provoked efforts in the last two to three decades to create recombinant bacteria with the potential of integrating the individual qualities of the two bacteria. However, these efforts have not yielded products exhibiting enhanced larvicidal efficacy when compared to the natural candidates. Recently, greater understanding of properties of cyt1A, knowledge of genetic elements for improving endotoxin synthesis and a greater number of mosquitocidal proteins give hope for commercial development of products with enhanced efficacy (Federici et al., 2003). The Cyt1A protein synergise and delay resistance to mosquitocidal Cry protein, hence can be employed to avert larval Bs resistance and extend its activity to *Aedes aegypti*. This makes these microbial products, Bti and Bs potential components in the rational management of insecticide resistance in malaria vector control programmes (Kitron and Spielman, 1989; Yu and Shen, 1990).

The malaria control programme in Zambia has introduced the Larval Source Management intervention for vector control and recently, larviciding with microbial products Bti and Bs as a primary alternative method in parts of the country without any control measures. This is a supporting intervention in IRS and ITN/LLIN protected areas, and is a strategy to further

reduce and maintain transmissions at low levels in low disease incidence areas e.g. Lusaka Urban, Zambia with malaria incidences of less than 2 % in the year 2010 (Fig. 2.2).

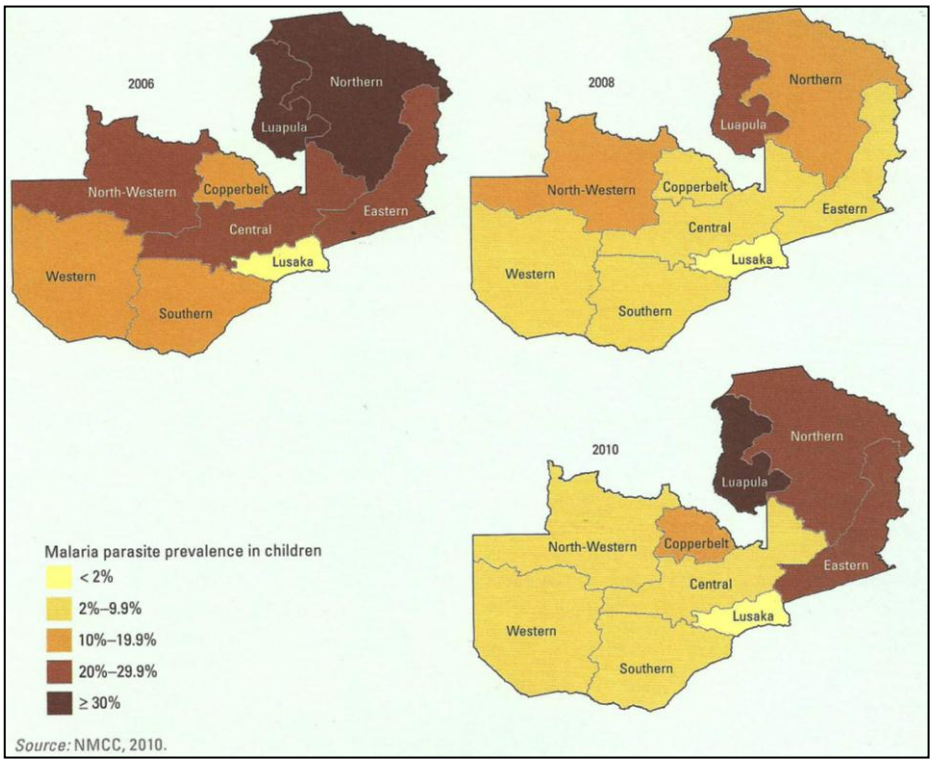

Figure 2.2 Malaria prevalence rates by Province among under five children, in Zambia (2006-2010) (Modified from WHO/RBM, 2011)

2.5.3.2 The Zambian Larviciding Programme.

The larviciding programme for malaria vector control in Zambia is as a result of an intergovernmental agreement between the Governments of Zambia and Cuba, signed in

Havana, Cuba on 15th October, 1985. It included the establishment of the intergovernmental commission for the economic, scientific and technical cooperation between the two countries. At the 11th session of the Commission held in Havana, Cuba on 28-30 September, 2009, the two governments agreed to pursue the programme for the period, 2009-2011 which included malaria control. The Cuban government suggested the use of the biological products Bactivec® and Griselesf® in the control of malaria in Zambia during this programme, and indicated that it would offer technical assistance for the implementation of the programme.

2.5.3.2.1 Implementation of the Zambian Larviciding Programme.

The implementation process of the larviciding for malaria (vector) control programme in Zambia is a community-driven intervention which requires participation and ownership by the local people, in the target areas. The National Larviciding Programme (NLP) team working with District Health Offices (DHO) conducts information exchange discussions with community members (leaders and general) members at Health Facility level, to introduce the Biolarvicides (Griselef® and Bactivec®) concept and the application methods. The community members are very instrumental in the identification of potential mosquito breeding sites around their residences in the programme and for subsequent "self-application" of the biolarvicides in these potential mosquito breeding sites in the environment. The appropriateness and feasibility of the community self application of biolarvicides is based on the environmental sociability, safety and non-technical requirements of the larvicides (Griselef® and Bactivec®) i.e. the biolarvicides' specific

activity against mosquito larvae, lack of special disposal requirements and direct application without complicated dilutions.

2.5.3.2.2 Monitoring and Evaluation (M&E) of the Zambian Larviciding Programme.

The Monitoring and Evaluation (M&E) Programme involves the assessment of various entomological parameters e.g. larval/adult mosquito densities and malaria incidences in target areas (MoH-Programme brochure, 2011c). Data are collected at scheduled time intervals, with the initial monitoring visit being made at least one week to a month following completion of the application of larvicides around the catchment area. The purpose of the M&E is to assess the effects of the applied control measure on mosquito vector parameters and disease trends. It is also used to identify breeding sites that continue producing mosquito larvae or new breeding sites that require retreatment and treatment respectively.

2.5.3.2.3 The Biolarvicides used in the Zambian Larviciding Programme

Among the most promising biological control agents of the mosquito that have been tested for the fight against mosquito-borne diseases are the sporogenous bacteria, *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus*. Dependent on the test results obtained and their safety to humans, the WHO recommends the use of both agents, even for the treatment of water supplies for human consumption (Kaflon et al., 1983; WHO/IPCS, 1999; Maozami, 2007; WHO, 2009). Their larvicidal action has been demonstrated in laboratory and in field trials for the control of mosquitoes and black flies (Chanda et al.,

2007b). They are target specific for mosquito larvae and are hence considered to be environmentally friendly (Gu et al., 2006).

The aqueous biolarvicides which are manufactured in Cuba by LABIOFAM$^{(T)}$, are Griselesf® which contains Bs and Bactivec® which contain Bti. They have been effective in the control of malaria, dengue, yellow fever and encephalitis vectors in different countries in Americas, Africa, Asia and Europe (MoH- Programme brochure, 2011c).

CHAPTER 3: MATERIALS AND METHODS

3.1 Study Areas

This study was conducted in Lusaka urban district, Zambia (Latitude, 15º - 16ºS; Longitude, 28º - 30ºE.) for a period of 1 year (August, 2010- August, 2011) covering pre- and post-larviciding periods. The selection criterion used for the study areas was based on which part of Lusaka urban district the LSM programme using Bti and Bs was being implemented. This included parts of the following water systems: the Dam/Stream systems of the Kalikiliki/Ibex Hills area; the Venta/Manzi Valley area, the Chelstone marshy ponds area; and the Chamba Valley Brick Factory site area (Fig. 3.1). The mosquito breeding sites in the selected study areas were predominantly man-made in nature with some varying degrees of natural streams and marshy areas.

Four separate study areas with clearly defined geographic, hydrologic, land use and demographic characteristics were chosen in the study areas and the mosquito larval habitats in their environments distinguished from each other in order to assess distinct mosquito populations in each of the study areas.

Data on incidences of malaria in the study areas were collected from health facilities nearest the chosen water systems for the study and it was assumed that human populations around the study areas attended those health facilities namely Chelstone, Chainda, Ng'ombe and Mtendere Health facilities.

3.1.1 The Dam/Stream Systems of the Ibex Hills/Kalikiliki Study Area

The Ibex Hills/Kalikiliki mosquito breeding habitat (Latitude, 15^{o} 24.707′ S and Longitude 28^{o} 22.296′ E; Elevation, 1266 m above sea level (asl)) has a man-made brick making quarry, approximately 100-150 m^2 in size, 5-20m deep, surrounded by thick grass vegetation (reeds), and drained by a natural stream (Appendix B; Figs.B1-B and C). During the rainy reasons when the dam part is flooded, water collects in the shade area and result into sunlit, shallow waters and the stream part is segmented into numerous micro and macro mosquito habitats, sunlit with submergent grass and suitable for anopheline mosquito breeding. A trench for a wall fence construction foundation (Appendix B; Figs.B1-A) creats a temporal reserviour of water and is good ground for anopheles mosquito breeding.

3.1.2 The Venta/Manzi Valley Study Area

The Venta Dam area (Latitude, 15^{o} 22.564′ S, Longitude, 28^{o} 24.148′ E, Elevation, 1214 m asl) is a man-made quarry with submergent vegetation. It is 500-1000 m^2 in size, surrounded by grassy vegetation (Appendix B; Figs.B2-A and B). The area has an independent smaller pond, sunlit with submergent grass and suitable for anopheline mosquito breeding. Anopheline mosquito larvae were present in some parts of the main dam as well.

3.1.3 The Chelstone-marsh Ponds Study Area

The Chelstone-Zambia airways marsh area (Latitude, 15^{o} 21.924′ S, Longitude, 28^{o} 23.836′ E; Elevation, 1212 m asl) is a natural marshland resulting from the emergence of an underground stream passing across the eastern part of Lusaka city. Man has however, modified the marshland by constructing a cement block water reserviour and digging of house construction furrows/ trenches (Appendix B; Figs.B3-A, B and C). The marshland is 100-200 m^2 in size, surrounded by grassy vegetation. Anthropogenic activity has fragmented the marshland into numerous micro and macro mosquito habits, sunlit with submergent grass and suitable for anopheline mosquito breeding. These activities have caused disappearance of some parts of the marshland reducing the overall size of mosquito breeding sites.

3.1.4 The Chamba Valley Brick Factory Site Study Area

Chamba Valley area (Latitude, 15^{o} 21.587′ S; Longitude, 28^{o} 20.01′ E; Elevation, 1213 m asl) has a man-made brick-making quarry, larger than 300x400 m^2 in size and 10-20m deep, surrounded by thick, grassy vegetation (reeds), drained by a natural stream (Appendix B; Figs.B3-A). In drier months (April to November), the stream part segmented into numerous micro and macro mosquito habits, sunlit with submergent grass and suitable for anopheline mosquito breeding (Appendix B; Figs.B4-B).

Figure 3.1. Study areas in Lusaka urban district.

Estimates of location of study areas; 1= Chamba valley study area and 2= Chelstone Zambia airways ponds study area, 3= Venta area and 4= Ibex hills/Kalikiliki areas (Source: Google Earth, 2012).

3.1.5 Hydrology and Land use in the Study Areas

The four selected study areas were diverse in hydrology and land use profiles. They ranged from irrigated agricultural cultivation to gardening of mostly food crops for both commercial and home consumption purposes in terms of land use (Appendix B: Fig.B5-A and B). The crops cultivated included maize in the rainy season and vegetable crops such as tomato, cabbage, and rape in the dry season months by irrigation, and sugarcane and bananas as perennial plants. These small scale farmers used agricultural chemicals to control insect pests and weeds in the gardens. These were characteristic of two of the four study sites namely; Chamba valley brick factory quarry dam/stream and the Ibex Hills dam/stream.

Fish breeding and farming in small soil lined ponds dug alongside the course of the stream was another type of land use (Appendix B: Fig.B6-A). Other domestic activities in the water systems included, water drawing washing of household kitchen utensils, laundry (Appendix B: Fig.B6-B) and making of cement dough for construction (Appendix B: Fig.B6-C).

3.1.6 House Structure in the Study Area

The study area was subdivided into two parts based on the structure of houses. One portion encompassed the Chelstone-Zambia Airways and Ng'ombe Compound sampling

units and had houses that were formal, Iron or asbestos roofed, plastered only or plastered with painted walls, and with very little to no openings in the heaves between the wall and the roof (Appendix B: Fig.B7-A and B). The second category which included the Venta Compound and Ibex Hills areas had similar structures of housing units, i.e. mostly informal with open heaves, unplastered or plustered but unpainted (Appendix B: Fig.B7-C,D,E and F).

The type of housing structure has implications on the entry and exit behaviour of endophilic mosquito species and on the efficiency of the application of IRS products and the resulting wall residual retention of these applied insecticides (NMCC, 2009). For example housing units with openings in the heaves and door/windows would allow free entry and exit of mosquitoes accessing blood meals from people sleeping in the house. Informal housing units without closable, transparent windows tend to be dark for a long period of the day, long after sunrise providing good conditions for endophilic blood fed mosquitoes. Unplastered walls pose a challenge to the IRS programme as such surfaces are unsuitable and rather absorb sprayed insecticides.

3.1.7 Application Methods for Biolarvicides in the Zambian Programme.

The spraying of target areas with the biolarvicides on the Zambia programme was done using Hudson X-pert pressure manual spray pumps (Appendix B: Fig.B8-A), motor knapsacks or by fixed-wing aerorplanes (Appendix B: Fig.B8-B), depending on physical conditions and geographic characteristics of the target mosquito breeding places of interest.

Biolarvicides are not compatible with the use of chemical insecticides on mosquito larvae and care is therefore taken since they cannot be applied where these chemicals had been applied recently (MoH- Programme brochure, 2011c). At least two months should pass following application of insecticdes (e.g Temephos) before biolarvicides can be applied in the same area (MoH- Programme brochure, 2011c). Only one of the two larvicides is applied at a time and at intervals of 6-9 months depending on the realised efficacy in field settings.

The large breeding sites with high submergent, emergent and surrounding vegetation are aerial sprayed (Appendix B: Fig.B8-B), while the smaller and more accessible water bodies are sprayed by hand using the Hudson X-pert pressure spray pump (Appendix B: Fig.B8-A). In manual applications using hand sprayers, an initial dose of 5 ml per square meter of surface of the active breeding site is applied (MoH- Programme brochure, 2011c). If mosquito larvae are still present during the M&E sampling, the spraying is repeated at a higher rate of 10 ml per square meter. For Aerial application a dose of 15 litres per hectare of Griselesf® or Bactivec® is used (MoH- Programme brochure, 2011c).

The spraying of the breeding sites in the study areas was done in June, 2011, with the Chamba valley and Venta dam sites receiving initial aerial application and hand-spray after M&E sampling in July, 2011. The Ibex and Chelstone marshy ponds were hand sprayed in June, 2011.

Following the application of the larvicides in breeding sites, weekly surveys for mosquito larvae are done to check on the success of the control effort of mosquito larvae in treated breeding sitres.

3.2 Mosquito Sampling

3.2.1 Mosquito Habitat Identification and Characterization, and Mosquito Larvae/ Pupae Sampling.

Mosquito larvae/ pupae sampling was conducted at monthly intervals from the selected aquatic habitats for a total period of 13 months. Some sampling was done before and a week or so after the biolarvicide applications. During each larvae/pupae sampling occasion, each selected mosquito habitat was visited and three sampling spots (each of the dimensions 2x4m) were selected at random and geo-referenced using a hand-held Geographical Positioning System (GPS) instrument. Ten scoops of water using a standard dipper (350 ml capacity) were made from each sampling spot to collect mosquito larvae and pupae (Appendix B: Fig.B9-A and B).

Some collected anopheline mosquito larvae and pupae (90 %) from each sampling spot were preserved in 70% ethanol in screw-cap vials following a little heating and were transported to the laboratory for enumeration. About 10% of collected larvae and pupae from the spots were taken to the laboratory live for rearing, for adult emergences used in mosquito identifications.

In the laboratory, the preserved larvae were enumerated and categorized according to their developmental stages. Their frequencies were calculated for the two periods i.e. before and after biolarvicides application.

3.2.2 Adult Mosquito Sampling

Adult mosquito sampling was also done at monthly intervals during the period of the study. A sampling was purposefully conducted a month before and after the biolarvicides application in the study areas. A total of 120 houses located within 500m of the identified mosquito habitats in the study areas, following the guidelines of WHO (1975) were selected randomly for adult mosquito sampling and their locations mapped using the GPS instrument (Appendix D).

Adult mosquito sampling was conducted in the morning between 04:30hours and 8:30 hours using the WHO (1975) protocol i.e. the Pyrethrum Spray Sheet Collection (PSC) method. A non-residual Pyrethroid aerosol insecticide (Target®) was used as the knock-down agent for adult mosquito sampling in selected houses (Appendix B: Fig.B10-A and B).

Knocked down adult mosquitoes (Appendix B: Fig.B10-C) were collected from white sheets of cloth spread on floors of each selected house at the beginning of sampling (Fig. 3.9 D), preserved in separate, labeled specimen vials containing silica gel (Appendix B: Fig.B10-E) and transported to the laboratory for enumeration and identification.

During sampling, the following data on occupants of each sampled house was also recorded: Number of occupants in the house, head of the household's, age, sex, occupation; ITN usage and IRS status using a questionnaire (see Appendix A).

3.3 Mosquito Identifications

3.3.1 Larval Mosquito Morphological Identifications

Collected mosquito larvae did not undergo species morphological identifications; instead, 10% of collected mosquito larvae were transported to the laboratory live, and reared to adult stages and used for species identifications. Only the resting position on the surface of the water was used to distinguish between anopheline (parallel) and culicine (hanging at an angle) mosquito larvae.

3.3.2 Adult Mosquito Morphological Identifications.

Live third and fourth instar anopheline mosquito larvae and pupae collected from the field and brought to the laboratory, were reared for adult emergencies later used in mosquito identifications. Emerged adults were identified morphologically using the computer software, "Anopheline mosquitoes of the Afro tropical region" by Hervey et al., (1998) and confirmed using the manual mosquito identification keys, "The Anopheline of Africa South of the Sahara" by Gillies and De Meillon, (1968), and "Mosquitoes of the Ethiopian Region-Adults and Pupae" by Edwards, (1941).

3.4 Mosquito Parameter Assessments

3.4.1 Mosquito Larval Habitat Colonisation Rates in the Study Areas.

Mosquito larval habitat colonisation rates (percentage) in the selected study areas prior to and after the application of biolarvicides were determined using the formula.

$$\% \text{ Colonisation} = \frac{\text{Number of mosquito colonised habitats}}{\text{Total number of potentially available habitats}} \text{X } 100.$$

3.4.2 Larval and Adult Mosquito Abundance, Diversity and Species Distribution and Dominance.

Larval and adult mosquito diversity was determined using the Alpha (α) Index of diversity to compare differences among communities in the study areas. This index was read off William's nomograph for determining α diversity indices using observed species totals (OSTs) and total numbers of individual mosquito species (N) in samples from the study area (Southwood, 1978; Southwood and Henderson, 2000).

The following expression was then used to calculate the Expected Species Totals in the study areas (Southwood, 1978; Southwood and Henderson, 2000; Mbata and Lumbwe, 2005).

$$\text{EST} = \frac{\alpha X}{(1 - X)}$$

Where, EST = Expected Species Total

α = Alpha index of biodiversity

X = Sampling factor

[$X = 1\text{-}e^{-N/\alpha}$ (and e = 2.7182818)]

The sampling factor indicates the magnitude of population fluctuation in the study area.

The beta (β) diversity index was used to give the inter-community population diversity differences (i.e. post-LSM period to Pre-Larviciding period). Mosquito Abundance was determined as frequencies of the different species (Southwood and Henderson, 2000).

Larval and adult mosquito densities were calculated as an indicator of larval survival and emergence. The species dorminance (H) was computed using the statistical software PC-ORD 5.0 (McCune and Mefford, 1995).

Southwood and Hendersen (2000) explains three types of species distribution, all based on the comparison of the sample variance (S^2) to its arithmetic mean ($\bar{x}$). This method was used to identify the distribution patterns of larvae and adult mosquito species in the study areas.

The sample variance was calculated as:

$$S^2 = \frac{\Sigma x^2 - [(\Sigma x)^2 / n]}{n-1}$$

Where; Σ denotes summation of all factors to the right.

x = value of number of mosquitoes per house/ larval habitat.

n = total number of samples (habitats for mosquito larvae or houses for adult mosquitoes).

S^2 = Sample variance.

The departure of the coefficient of distribution ($S^2/\bar{x}$) from Poisson randomness was tested by calculating the index ID given by the expression:

$$ID = \frac{S^2 (n-1)}{\bar{x}}$$

Where;

n = number of mosquito samples.

ID = index approximately distributed as Chi-square with n-1 degrees of freedom.

$\bar{x}$ = the arithmetic mean number of mosquitoes (Larvae or Adults).

S^2 = Sample variance.

Random distribution will have the ID falling within the limits 0.95 and 0.05 of the Chi-square for n-1 as given in statistics tables (Southwood and Henderson, 2000). The conclusion of the type of spatial distribution pattern exhibited by the organism depends on a comparison of the variance (S^2) to the mean ($\bar{x}$). Random (Poisson) distribution is described by a variance equal to the mean. When the variance is less than the mean, the population is said to have a regular (or even) distribution pattern, while a variance larger than the mean implies that the distribution is contagious (or aggregated or clumped) in pattern.

A two way analysis of variance was conducted to compare the mosquito species, abundance, diversity and distribution among the four study areas at the pre- and post-larviciding times respectively, and to assess the effect of biolarvicides on these mosquito parameters in the study areas. The calculated p-values are tabulated in the ANOVA tables and conclusions drawn at 0.05 confidence level.

3.4.3 Determination of Malaria Incidences in the Study Area.

The malaria incidences were determined by review of records of the Health Centers in the study areas for the periods prior to and after the implementation of the Larviciding programme. The review period was January to August, 2010 and January to August, 2011. These were the centrally located Health Facilities assumed to be attended by the people in the study areas. These were the; Chelstone, Mtendere, N'gombe and Chainda Health facilities. Only laboratory confirmed malaria cases with either Rapid Diagnostic Tests (RDTs) or Microscopy were considered for the study. The health facility record data included records of travel history of patients that tested positive for malaria. The malaria positive rates were calculated as a percentage of the positives cases to the total number of diagnostic tests done in the period (month).

CHAPTER 4: RESULTS

4.1 Mosquito Identification (Larval and Adult Mosquito Morphological Identifications).

No morphological species identifications were done for the collected mosquito larvae from the area. Instead, larvae were reared and emerged adults were used for species identifications. A total of 816 mosquitoes were collected from the four study areas and identified using morphological methods. Three species of *Anopheles* mosquitoes were identified while the rest of collected mosquitoes (75%) were culicines (Table 4.1) and since these were not the centre of focus of the study, they were not processed any further. *Anopheles coustani* s.l. Laveran (13.5%) and *Anopheles squamosus* Theobald (9.5%) were collected from all four study areas, while *Anopheles rufipes* Gough (1.1%) was only found in the Chamba valley study area. No members of the *Anopheles gambiae* complex or the *Anopheles funestus* group which are reported as principal vectors of malaria in Zambia (Chanda, 2007a; Chanda et al., 2011; Gillies and De Meillon, 1968) were identified among the mosquitoes in the collections made from the study areas.

No mosquito larvae were collected/ identified after biolarviciding in the study areas.

Table 4.1 Mosquito species identification from the four study areas.

	STUDY SITE			
TAXA	IBEX HILLS	VENTA	CHAMBA VALLEY	CHELSTONE
An. coustani s.l.	+	+	+	+
An. squamosus	+	+	+	+
An. rufipes	-	-	+	-
Culex spp.	+	+	+	+

Legend: + = species was present, - = species was absent in the study area and *An*= *Anopheles*.

4.2 Mosquito Parameter Assessments.

4.2.1 Mosquito Larval Habitat Colonisation Rates in the Study Areas.

The identification and characterisation of breeding sites in the study areas showed a variety in the types of water bodies ranging from: man-made to natural streams and dams; temporal status ranging from temporal, semi-permanent to permanent; sizes from a few centimeters to over a kilometer; water quality from clear to heavy organic scammed surface/algae. The hydrological and land use activities included; vegetable farming, construction, fish breeding and domestic water collection. The Chamba valley and Chelstone study areas had the highest number of identified potential mosquito breeding sites with nine water bodies each, while the Ibex hills and Venta areas had six and eight potential mosquito breeding sites, respectively (Table 4.2a). The larval habitat colonisation rates in the study areas ranged from 33% to 50%, with the Ibex hills stream/ dam and the Chelstone/ Zambia-Airways areas showing the highest and lowest rates respectively as presented in table 4.2a.

There was a reduction in the number of potentially available breeding sites post-larviciding compared to the initial pre-larviciding period in all four sites. The post-larviciding mosquito larval habitat colonisation rates were zero in all the four study areas, i.e. all mosquito larvae were cleared from the breeding sites by the applied biolarvicides (Table 4.2b).

Table 4.2a Mosquito larval habitat colonisation rates in the study areas (Pre-larviciding)

	Ibex hills stream/ dam	Venta quarry dam	Chamba valley quarry/stream	Chelstone Zambia Airways marsh ponds	Total (Avg)
Total breeding sites identified	6	8	9	9	32(8)
Total positive breeding sites	3	3	4	3	13(3)
% Colonisation	50 %	38 %	44 %	33 %	41 %

Table 4.2b Mosquito Larval Habitat Colonisation rates in the study areas (Post-larviciding)

	Ibex Hills Stream/ Dam	Venta Quarry Dam	Chamba Valley Quarry Stream	Chelstone Zambia Airways Marsh ponds	Totals
Total Breeding Sites Identified	3	5	6	6	20
Total Positive	0	0	0	0	0
% Colonisation	0%	0%	0%	0%	0

4.2.2 Expected Species Totals (ESTs).

The estimated expected species totals of mosquito larvae for the four study areas (Table 4.3) were very close to what was actually collected from the study areas. A species total of three was expected in the Ibex hills, Venta and Chelstone but the number of species found in each of these was two anophelines. The expected species number for Chamba valley was four but only three anopheline species were collected from the area.

In the indoor collections of adult mosquitoes, only adult culicine mosquitoes were found, therefore the adult mosquito ESTs could not be computed for the study areas.

After biolarviciding, no mosquito larvae were found in the breeding sites and only a few culicines were collected as adults from indoors.

Table 4.3 Mosquito larvae expected species totals.

	STUDY SITE			
Index	Ibex hills	Venta	Chamba valley	Chelstone
*EST	3	3	4	3

4.2.3 Larvae and Adult Mosquito Species Abundance.

The largest numbers of mosquito larvae in the study areas were those of *Culex* mosquito species. Among the three anopheline species collected, *Anopheles coustani*

larvae (111) were the most abundant in all four study areas (Table 4.4a). *Anopheles squamosus* (78), were second in abundance, and *Anopheles rufipes* (9) were the least abundant and were only collected from the Chamba valley study area breeding sites, while the other mosquitoes were found in all study sites.

No *Anopheles* or *Culex* species larvae were collected in the post-larviciding sampling efforts in the study areas as all mosquito larvae were completely cleared from the breeding sites.

Table 4.4a Mosquito Larvae Abundance.

	STUDY SITE				
Taxa	Ibex hills stream/ dam	Venta quarry Dam	Chamba Valley quarry/ stream	Chelstone Zambia Airways marshy ponds	Totals
An. coustani s.l.	56	10	25	20	111
An. squamosus	48	4	14	12	78
An. rufipes	0	0	9	0	9
Culex spp.	138	59	70	54	321
Total larval no.	242	73	118	86	(519)
Mean Larval density per scoop.	24	7	12	9	

There was no significant difference ($p \geq 0.05$) in the mosquito larval abundance among the different study areas at both the pre- and post-larviciding sampling times (Table 4.4b) i.e. the four study sites had similar species abundances of mosquito larvae when

compared to each other in each of the two periods. There was however, a significant difference ($p < 0.05$) in the mosquito larval abundance between the pre- and post-larviciding periods within each study area (Table 4.4b).

Table 4.4b Analysis of variance for mosquito larvae abundance.

Source	DF	SS	MS	F	P
Study area	3	8936.4	2978.8	1.00	0.500
Treatment	1	33670.1	33670.1	11.30	0.0437*
Error	3	8936.4	2978.8		
Total	7	51542.9			

* Significant at p = 0.05 significance level.

Before biolarviciding, no adult anopheline mosquitoes were collected indoors in all the selected 120 houses in the study area. Instead only *Culex* mosquitoes were found indoors in all four sampling areas. The Ibex Hills and Venta sampling areas showed higher abundances of adult mosquitoes than the N'gombe and Chelstone sites (Table 4.5a).

Again, no indoor resting adult *Anopheles* mosquito species were collected from the 120 selected houses during the post-larviciding sampling efforts in the study areas. Only *Culex* mosquitoes were collected from three of the four study areas except Chelstone area (Table 4.5b).

Table 4.5a Indoor adult mosquito abundance.

	STUDY AREA				
Taxa	Ibex hills	Venta	Chamba valley	Chelstone	Totals
Anopheles	0	0	0	0	0
Culex	106	104	22	28	260
Total	106	104	22	28	(260)

Table 4.5b Indoor adult mosquito abundance.

	STUDY AREA				
Taxa	Ibex hills	Venta	Chamba valley	Chelstone	Totals
Anopheles	0	0	0	0	0
Culex	32	2	3	0	37
Total	32	2	3	0	(37)

There was no significant difference ($p > 0.05$) in the indoor adult mosquito species abundances between the different study areas at both the pre- and post-larviciding sampling times (Table 4.5c). There was also no significant difference ($p > 0.05$) in the indoor adult mosquito species abundance between the pre- and post-larviciding periods in the study areas (Table 4.5c).

Table 4.5c Analysis of variance for indoor mosquito abundance.

Source	DF	SS	MS	F	P
Study area	3	4818.4	1606.12	2.10	0.2792
Treatment	1	6216.1	6216.12	8.12	0.0651
Error	3	2296.4	765.46		
Total	7	13330.9			

4.2.4 Mosquito Species Diversity.

4.2.4.1 Larval Mosquito Species Diversity.

The Chamba valley study area had the highest mosquito species diversity value of four mosquito species collected from the breeding sites, while the other three study areas i.e. Ibex hills, Venta and Chelstone had a mosquito species diversity of three species each as shown in Table 4.3. The calculated Beta diversity index was Zero for the three sites and one for Chamba valley with four mosquito species collected (Table 4.6a).

No *Anopheles* or *Culex* species larvae were collected in the breeding sites during the post-larviciding sampling efforts in the study areas as mosquito larvae were completely cleared from the breeding sites by the applied biolarvicides Bti and Bs.

Table 4.6a Mosquito larvae species diversity.

	STUDY SITE			
Index	Ibex Hills	Venta	Chamba Valley	Chelstone
β-diversity	-	-	1	-

There was no significant difference ($p \geq 0.05$) in the mosquito larval diversity between the different sites at both the pre- and post-larviciding sampling times (Table 4.6b). There was however a significant difference ($p < 0.05$) in the mosquito larval diversity between the pre- and post-larviciding periods within each of the study areas (Table 4.6b).

Table 4.6b Analysis of variance for mosquito larvae species diversity.

Source	DF	SS	MS	F	P
Study area	3	0.03089	0.01030	1.00	0.5000
Treatment	1	2.74131	2.74131	266.25	0.0005*
Error	3	0.03089	0.01030		
Total	7	2.80309			

*significant at p = 0.05 Significance level.

4.2.4.2 Indoor Adult Mosquito Species Diversity.

No adult anopheline mosquitoes were collected from indoors in the selected 120 houses in the study area in both the pre- and post-larviciding periods. Indoor collected adult mosquitoes were all identified as culicine species therefore, the calculated inter-site mosquito species diversity was zero. The calculated Beta diversity among the four sites was zero. Only a single species (*Culex* spp) was collected at both the pre- and post-larviciding sampling times. Therefore, analysis of variance for the indoor adult mosquito species diversity between the pre- and post-larviciding periods in the study areas could not be computed.

4.2.5 Mosquito Species Distribution in the Study Areas.

4.2.5.1 Mosquito Larvae Species Distribution Patterns.

In the pre-larviciding period, the calculated distribution index (ID) was larger than the mean number of mosquito larvae collected from the study areas (Table 4.7), implying that the mosquito larvae were contagiously distributed (over dispersion) i.e. the population was clumped or aggregated in the study areas. This implied that the larvae tended to aggregate in certain parts of the water bodies. An ID index less than one or unit is indicative of under dispersion while that equal to one means random distribution.

Table 4.7 Mosquito larvae species distribution.

Variance (S^2)	Mean	Number of sites (n)	Distribution Index (ID)
5957.6	129.75	4	137.75

No *Anopheles* or *Culex species* larvae were collected in the post-larviciding sampling efforts in the study areas as mosquito larvae were completely cleared from the breeding sites. The mosquito species distribution index (ID) could not be calculated since all mosquito larvae died after larviciding.

4.2.5.2 Indoor Adult Mosquito Species Distribution Patterns.

Prior to larviciding, the calculated distribution index (ID) was larger than the mean number of indoor collected culicine adult mosquitoes from the study areas (Table 4.8a), implying that the culicine mosquitoes were contagiously distributed in the study areas.

After larviciding, the calculated distribution index (ID) was larger than unit (Table 4.8b), implying that the culicine mosquitoes were contagiously distributed (over dispersion) i.e. the population was clumped or aggregated.

Compared to the pre-larviciding figures, the mean number of mosquitoes and calculated index of distribution reduced in value.

Table 4.8a Indoor adult mosquito species distribution.

Variance (S^2)	Mean $\bar{x}$	Number of Houses (n)	Distribution Index (ID)
52.10	2.50	120	2479.96

Table 4.8b Indoor adult mosquito species distribution patterns.

Variance (S^2)	Mean	Number of Houses (n)	Distribution Index (ID)
38.54	2.2	120	2134.46

4.2.6 Mosquito Species Dominance in the Study Areas.

4.2.6.1 Mosquito Larvae Species Dominance.

At larval stages, *Culex species* were the dominant ($d_{Culicine}$ = 0.62) to all mosquito species while *Anopheles coustani* larvae were the dominant *Anopheles* mosquitoes ($d_{Anopheline}$ = 0.56) in the study areas (Table 4.9).

Table 4.9 Mosquito Larval Species Dominance.

	Nmax	Ntotal	Dominance Index (d)
$d_{Culicine}$	321	519	0.62
$d_{An.\ coustani}$	111	198	0.56

Nmax = number of individuals of species, Ntotal = total number of mosquitoes.

No Anopheles or Culex species larvae were collected in the post-larviciding sampling efforts in the study areas as mosquito larvae were completely cleared from the breeding sites by the treatment with biolarvicides. The mosquito species dominance index (d) could not be calculated since mosquito larvae were cleared from the breeding sites after larviciding.

4.2.6.2 Indoor Adult Mosquito Species Dominance.

Only culicine mosquito species were collected from indoors in the 120 sampled houses of the study areas in both the pre- and post larviciding periods hence the calculation of the dominant mosquito species could not be undertaken.

4.3 Incidences of Malaria in the Study Areas.

The malaria disease rates in the Chainda compound from a review of the health facility records for the period January to August, 2010 before the implementation of the larviciding programme in the study areas showed the highest peak in April-May (Fig 4.1a). The facility however lacked microscopy services and hence, only Rapid Diagnostic Test (RDT) data were used where available. The Zeros in the graphs show times of RDT stock-out or lack of microscopy services in the month at the health facility.

Biolarviciding in the study area was conducted in June, 2011. As shown in figure 4.1b, malaria incidences peaked in February and May of 2011. Following biolarviciding, the malaria incidences were reduced to below 4%.

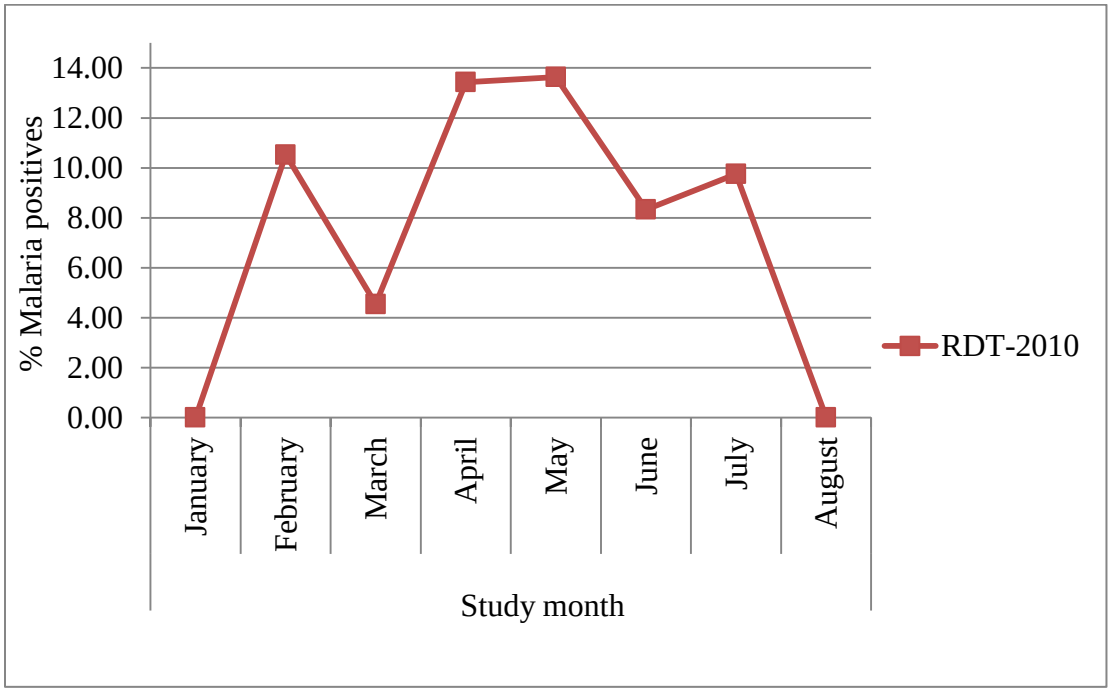

Figure 4.1a Incidences of malaria in Chainda, January-August 2010.

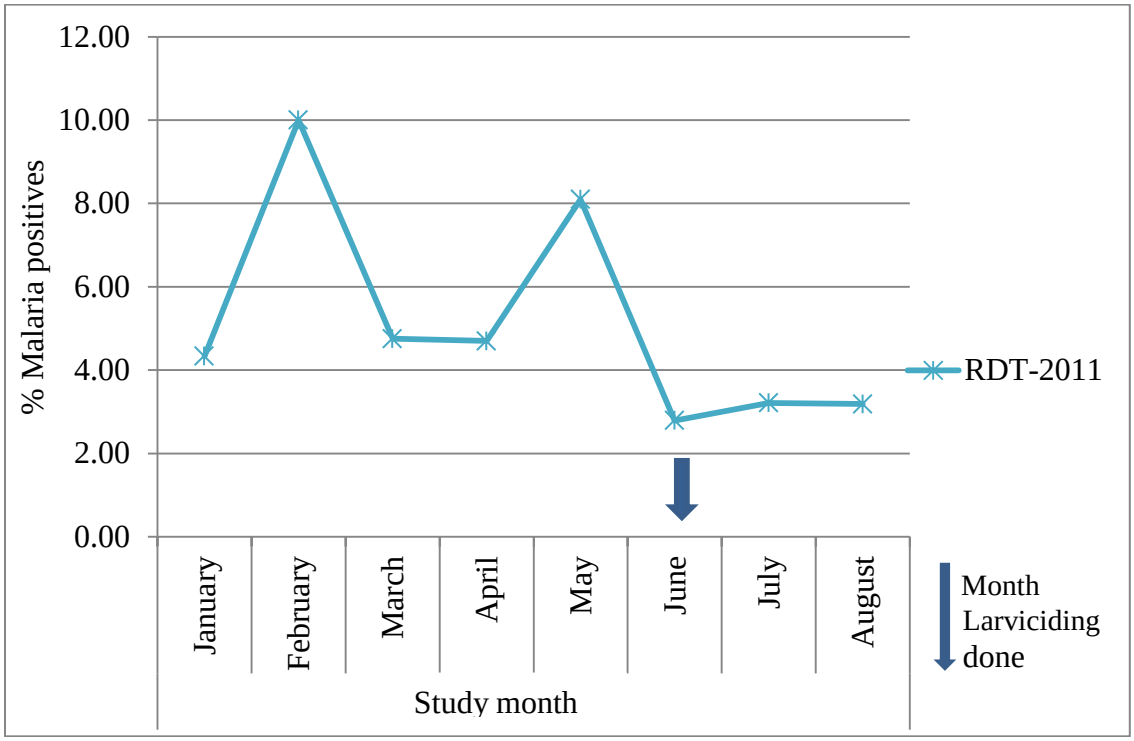

Figure 4.1b Malaria rates in Chainda compound, January- August, 2011

The incidence of malaria disease in Chelstone area for the same period, January to August, 2010 i.e. before the implementation of the larviciding programme in the study areas showed the highest peak in May, 2010 (Fig 4.2a). Again, the Zeros in the graphs show periods of RDT stock-out or lack of microscopy services at the health facility.

The malaria disease rates in the Chelstone compound for the period January to August, 2011 after the implementation of the larviciding programme in the study areas in June, 2011, showed a highest peak in January, March and June (Fig 4.2b). The microscopy determined malaria rates show a downward trend in June to August in both the pre (2010) and post (2011) larviciding periods. The rates reduced to below 0.5% after biolarviciding.

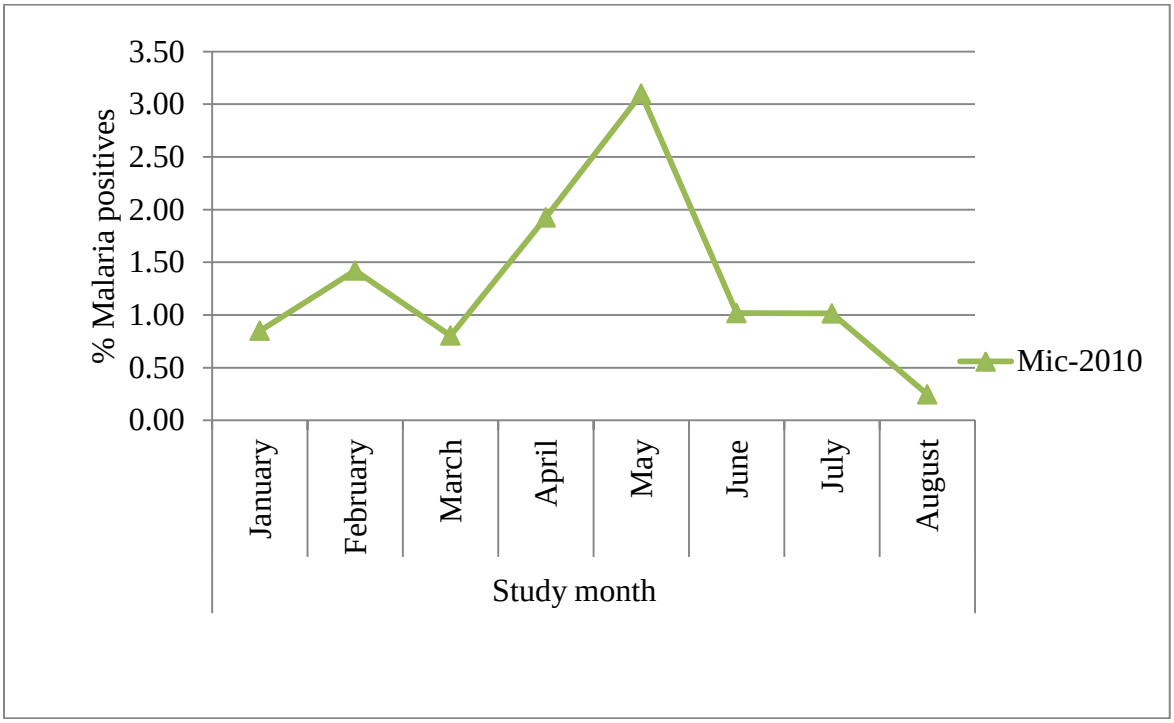

Figure 4.2a Incidences of malaria in Chelstone, January-August 2010.

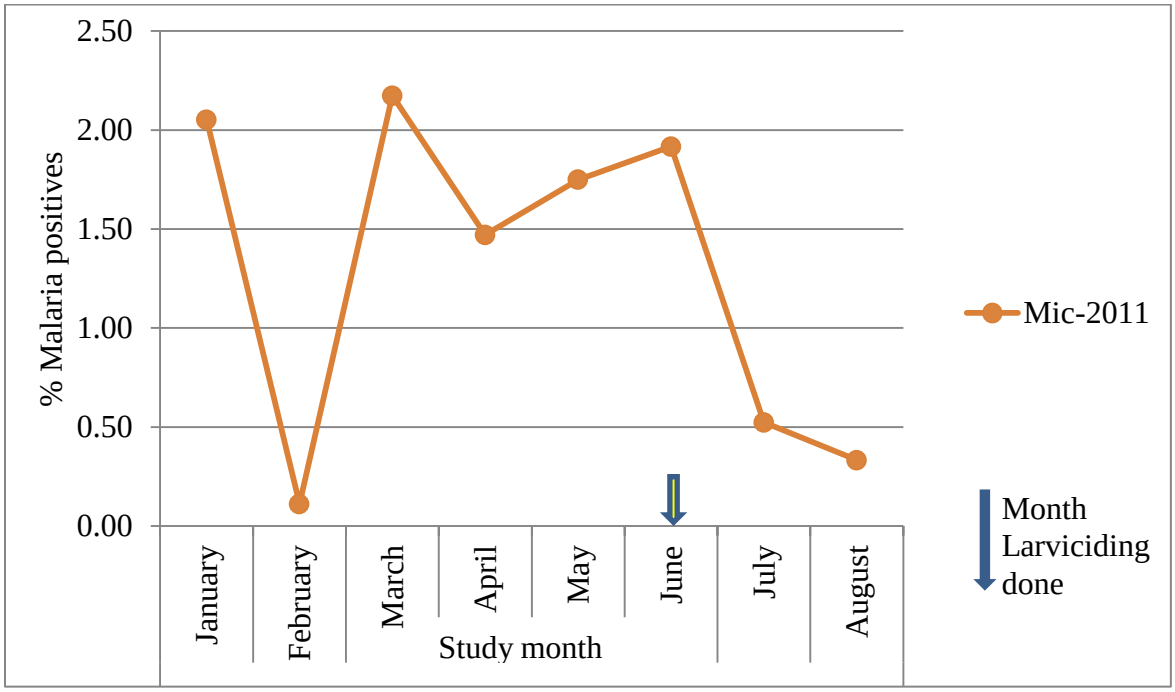

Figure 4.2b Malaria Rates in Chelstone compound, January- August, 2011

In Mtendere compound, the highest peak for malaria was in June, 2010 (Fig 4.3a). This health facility did not have the RDTs in stock during the period, March to August, 2010, and hence malaria diagnosis done by microscopy alone was considered for analysis.

For the period January to August, 2011 malaria rates peaked in May (Fig 4.3a). The microscopy graph shows general downward trend in malaria disease rates after the application of larvicides in June, 2011 to August 2011, compared to the same period in 2010. However line tends upwards from July to August, 2011. This is probably as a result of malaria cases importation from outside Mtendere compound health facility catchment area.

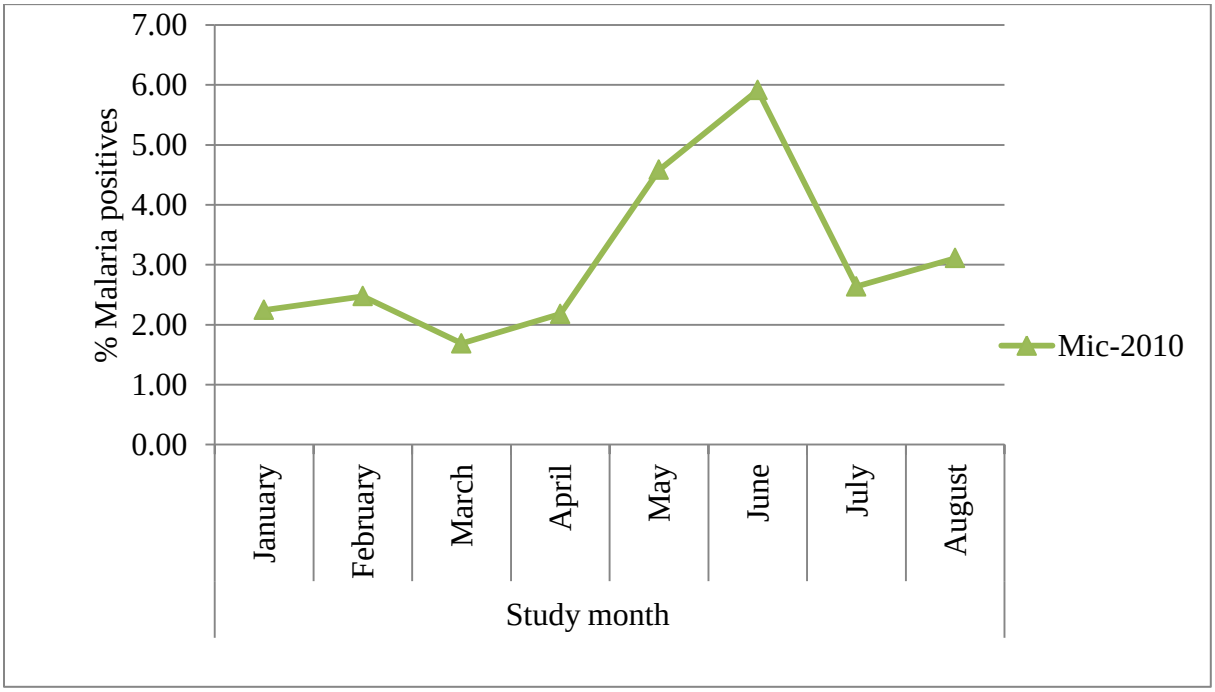

Figure 4.3a Incidences of malaria in Mtendere, January-August, 2010.

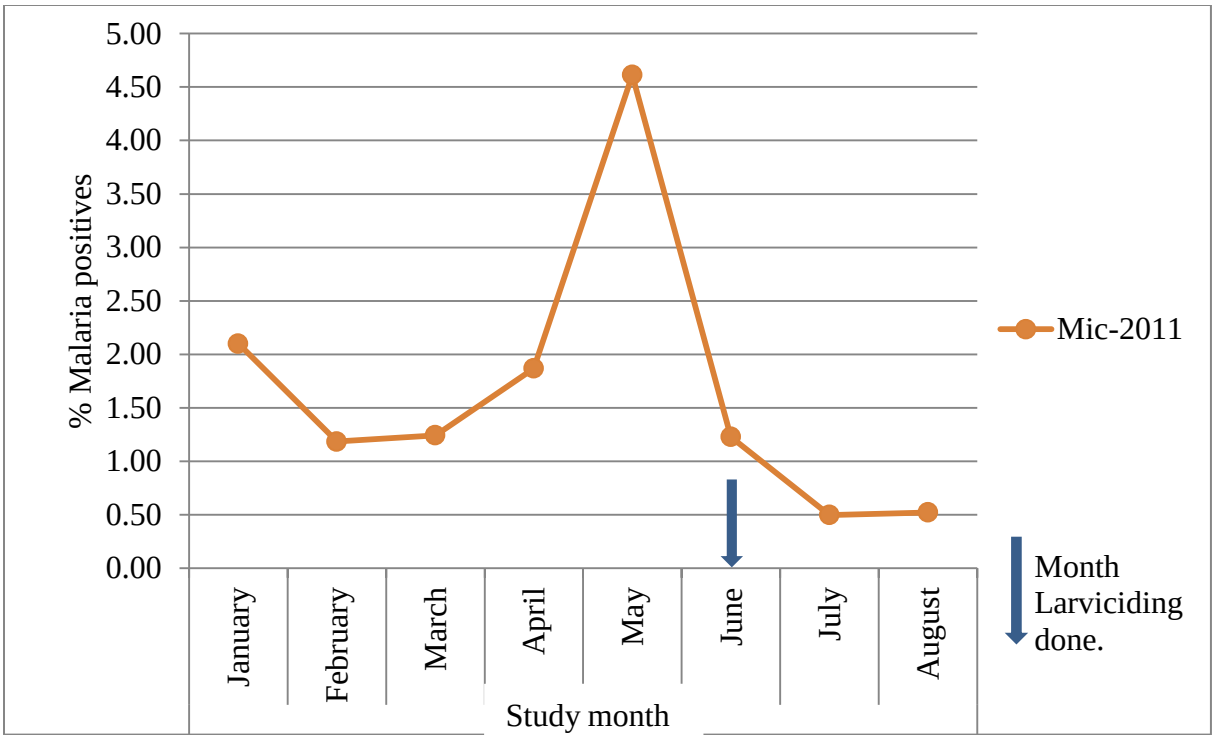

Figure 4.3b Malaria rates in Mtendere compound, January- August, 2011.

Finally, the incidence of malaria in Ng'ombe compound from January to August, 2010 before the implementation of the larviciding programme in the study areas peaked in May, 2010 (Fig 4.4a). This health facility unlike the Mtendere one did not have microscopy services during the period, January to August, 2010, and hence malaria diagnosis was done by RDT alone. For the period, January to August, 2011, before and after the implementation of the larviciding programme in the study areas peaked in May, 2011 (Fig 4.4b). The health facility had RDT stock outs in February and May to August, 2011. The 2011 trend in malaria rates showed a continued reduction from May, through the breeding site treatment in June, 2011 to August 2011 where as the 2010 graph showed an increase in the same period (Fig. 4.4b).

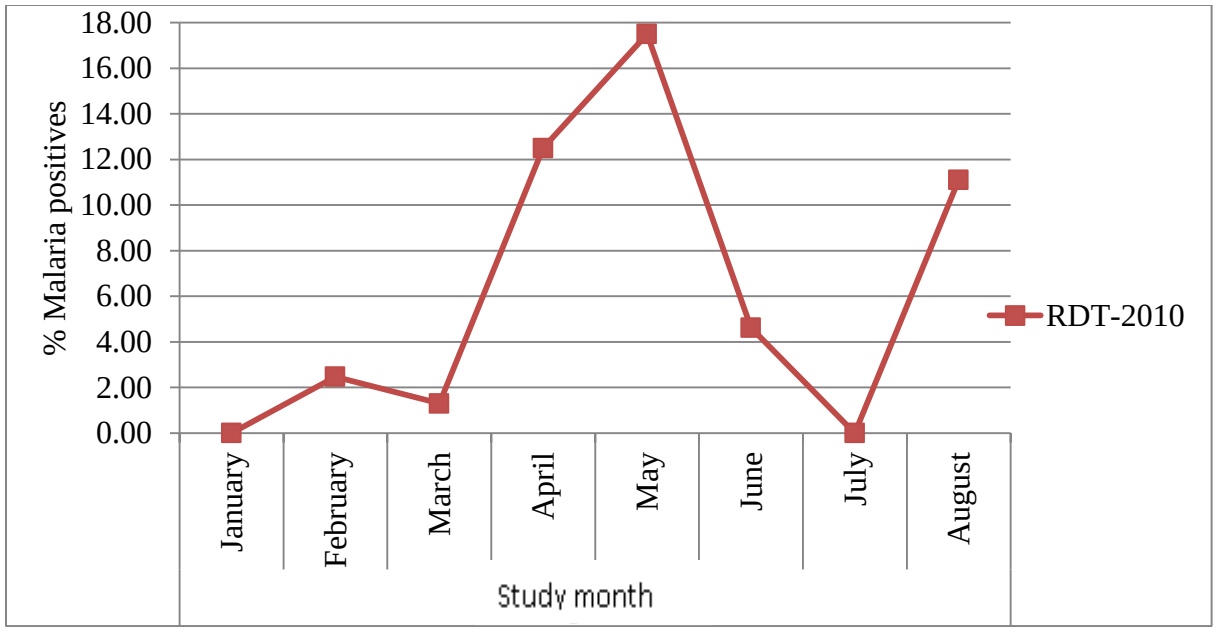

Figure 4.4a Incidences of malaria in Ng'ombe compound, January- August, 2010.

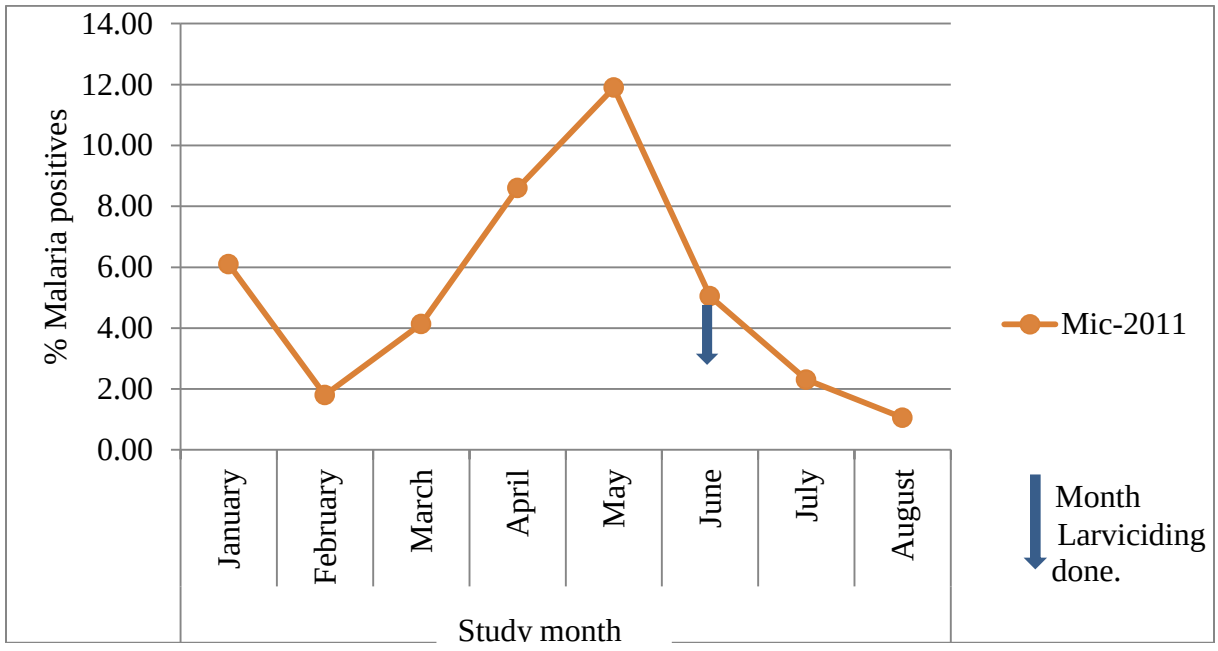

Figure 4.4b Malaria rates in Ng'ombe compound, January- August, 2011.

CHAPTER 5: DISCUSSION

The programme employed aerial spraying by aero plane in larger and inaccessible breeding sites and hand spraying with Hudson expert pumps in smaller water bodies. On post-larviciding sampling, some parts of the aerial sprayed sites remained positive for mosquito larvae while the hand sprayed ones were not. The retreatment of positive parts of the breeding sites by hand spraying method resulted in the clearance of the culicine mosquito larvae. Similar observations were also made during the programme monitoring and evaluation sampling in aerial and manual sprayed breeding sites in Kafue District where the programme was also implemented (Unpublished Larviciding Programme M&E report). Whereas the aerial treated study sites only had culicine mosquito larvae in Lusaka urban, the Kafue sites remained anopheline larvae positive. This was thought to be due to the presence of a banana plantation that provided canopy cover for the mosquito larvae from aerial sprays at one site and due to thick vegetation cover at the banks of the Kafue River at another site which also prevented the larvicide spray from reaching the water surface.

5.1 Mosquito species identified from the study areas.

No primary vectors of malaria reported for Zambia in the literature from, the *Anopheles gambiae* and *Anopheles funestus* groups were identified from collections of mosquitoes made in this study. Chanda (2007a) reported the occurrence of malaria vector mosquitoes, *An. gambiae* s.s and *An. arabiensis* in Kalikiliki and Kabanana parts of Lusaka urban where these species are said to have accounted for 10 percent of his indoor mosquito collections. The present study intersected with Chanda's study in the Kalikiliki study area. The absence of

these vectors in the collections made in this study could be as a result of the introduction of Indoor Residual House Spraying in 2003 in Lusaka district covering 16,000 house structures, expanding in each subsequent year and reaching 300,000 structures by 2007 and 336,000 houses in 2010 (MoH, 2011a-unpublished report). The other likely reason for the absence of the *An. gambiae* sibling species in the study areas could be the disappearance of breeding sites due to rapid housing and construction activities in the areas. As an observation, for example, a lot of construction work had taken place around the Kalikiliki dam, greatly reducing it in size and the deposition of crushed stone material in the Chelstone-Zambia airways ponds where construction was underway. It is possible that parts of the water bodies that provided suitable breeding grounds for the anopheline species in the study areas had been modified or had been completely destroyed (Service, 1989; Robert et al., 2003).

The three mosquito species found to occur in the Lusaka urban district in this study (*Anopheles coustani* s.l., *An. squamosus* and *An. rufipes)* are classified as secondary or incidental vectors of malaria in Zambia and other parts of the world e.g. Tanzania, Congo and Zimbabwe (Gillies and De Meillon, 1968; Fornadel et al., 2011). Secondary vectors of malaria are mostly responsible for maintaining the transmission of malaria at low epidemiological levels in urban areas (Okara et al., 2010).

The finding in this study of only secondary vectors of malaria occurring in the study areas supports an earlier observation made of low epidemiological status of the disease for Lusaka urban (WHO/RBM, 2011). However, it is too early to be conclusive about this. More surveys are required to be conducted in the same and other parts of Lusaka urban to ascertain this

statement. Further, the absence of malaria vector species of the *Anopheles gambiae* s.l and *Anopheles funestus* s.l from the collections made from the study areas does not warrant that these mosquito species are not present in Lusaka Urban District, in general and the study area in particular.

The distinction of these individual species is important for vector control as the species have diverse ecological behaviours and vectorial capacities (Service, 1963, 1989).

5.2 Mosquito Larval Habitat Colonisation Rates in the Study Areas.

Mosquito larval habitat colonisation rates ranged 33- 50 % in the study areas. These findings suggest that at least 30 and up to 50 percent of potential breeding sites identified in the study areas were suitable for mosquito breeding. Colonisation rates serve as indicators of the availability of breeding ponds suitable to for the development of vector mosquito species in the area (Opoku et al., 2009).

The Chelstone and Chamba valley study areas had the highest number of available breeding sites compared to the Ibex hills and Venta dams, but the Ibex hills and Chamba valley were relatively higher in colonisation rates than the other two areas.

The implications of these high numbers of available mosquito breeding grounds provide opportunities for mosquito vectors of malaria and other mosquito-borne diseases, e.g. filariasis and yellow fever to propagate in high densities and transmit diseases (Opoku, et al.,

2009). The colonised habitats provide a reference in terms of water body quality, i.e. ecological requirements for a particular anopheline mosquito where and when collected, making future breeding site identification efforts and control area targeting for larval source management easier.

Though not presented in this paper due to its crudeness and bulky nature, monthly data, showed seasonal variations in availability and colonisation rates of breeding. As expected, the rain season had higher numbers of potential breeding sites than the drier months of the year, but lower larval habitat colonisation rates, these findings were similar to those made by Opoku and co-workers in Accra Ghana (2009) and Majambere et al., (2010) in the Gambia with colonisation rates of 12-40% before larviciding and rates of 43% were reported by Ndenga et al., (2011).

These findings are also similar to those of Ahmad et al., (2011) in malaria endemic areas in Malaysia where wet months presented more numbers of potential mosquito breeding sites than the drier ones. Culicine mosquito species were less selective in habitat types than Anopheles species and were found to breed in all positive water bodies. Resetarits et al., (2009) demonstrated that habitat colonization rates were important factors in determining the abundance and diversity in spatially distinct populations (Opoku et al., 2009). The identification of mosquito larvae positive breeding sites remains of primary importance as shown by a study by Fillinger et al., (2009) in the Gambia which showed that even water bodies with similar characteristics produced different mosquito species and that vector species thrived in confined habitats i.e. small isolated water bodies.

In the post-larviciding sampling time, mosquito larvae habitat colonisation rates reduced to zero from the 30-40 % observed in the pre-larviciding periods. This was similar to findings by Majambere et al., (2010) in Gambia and Fillinger and Lindsay (2006), i.e. all mosquito larvae were cleared from the breeding sites. This suggested that the biolarvicides used in the treatment of mosquito breeding sites were efficacious against all mosquito larval species previously present in the study areas. These findings are supported by studies on the efficacy of Bti and Bs against mosquito larvae in both laboratory and field settings in which both products were efficacious (Fillinger et al., 2003, 2006; Gu et al., 2006; Chanda et al., 2007b; Ali et al., 2007).

5.3 Mosquito Abundance.

5.3.1 Mosquito Larvae Abundance.

Mosquito species abundance is the frequency of individual mosquito species found at each of the breeding sites in the study areas. High densities of mosquito larvae species in breeding sites will eventually lead to the production of adult mosquitoes in great abundances. High densities of primary vector species correlate positively with epidemic outbreaks of vector borne diseases including malaria (Service, 1989; Robert et al., 1998; Geisbühler et al., 2009).

In the pre-larviciding sampling period of this study, culicine mosquito species were the most abundant of all collected mosquitoes at larval and pupal stages whilst no mosquito

larvae were found after biolarviciding. The finding of culicine mosquitoes as moat abundant mosquitoes is similar with findings by Chanda (2007a) in Kabana, Kalilikiki and Chipata compounds of Lusaka urban. *Anopheles coustani* was the most abundant anopheline mosquito, followed by *Anopheles squamosus* and *Anopheles rufipes* being the least in abundance and was only collected from the Chamba Valley study area breeding sites. The three species have also been reported to occur in other parts of the country; Chongwe, Kafue, Mazabuka, Monze, Mumbwa and Lusaka Districts by routine National Malaria Control Centre entomological surveys.

The abundance of collected mosquitoes correlated positively with the order of habitat colonisation rates observed in the study areas, the Ibex hills and Chamba valley areas combined accounted for 70 % of all collected mosquito larvae. The study's findings agree with those made by Fillinger et al., (2009) and Majambere et al., (2010) in the Gambia, Basseri et al., (2010) in Iran, and studies carried out in Macha Zambia (Fornadel et al., 2011).

The identification of most productive sites in terms of mosquito larval abundances is an essential requirement for appropriate prioritising and temporal implementation of larviciding intervention for effective vector control (Ahmad et al., 2011, Fillinger et al., 2004, 2009). Fillinger's study showed that larval control interventions can be better implemented effectively in drier months of the year when potential breeding sites are fewer. This study thus supports the roll-out use of the biolarvicides; Bti and Bs in integration with the already existing adult mosquito targeting strategies of IRS and LLINs

as supportive vector control interventions and/or as sole control interventions in non IRS/ LLIN areas in the country. Similar calls for the incorporation of larviciding in malaria vector control efforts by programmes have been made by many studies including; Majambere et al., (2010) in the Gambia and Devine and Killeen (2010) and elsewhere (Fillinger et al., 2003, 2009, Fillinger and Lindsay, 2006; Shaukat et al., 2010).

Larval abundance comparisons among the four sites either in the pre- or the post-larviciding times respectively showed no significant differences ($p > 0.05$). There was however a significant difference ($p < 0.05$) in the mosquito larval abundance between the pre- and post-larviciding periods in the study areas, implying that the biolarvicides Bti and Bs affected the abundance of mosquito larvae in the study areas. This finding proves one of the hypotheses set for this study, that LSM with the biolarvicides Bti and Bs reduces the abundance of anopheline mosquito larvae in breeding sites and is similar to results reported by Castrol et al., (2009) in Dar es Salaam, Tanzania, and Yakob and Yan (2009).

5.3.2 Indoor Adult Mosquito Abundance.

The Indoor sampling of adult endophagic and/or endophilic mosquito species only produced culicine mosquitoes in all the 120 selected houses in both the pre-larviciding and post-larviciding periods in the study areas. Comparatively, the Ibex Hills and Venta sampling compounds showed higher abundances of mosquitoes than the N'gombe and Chelstone sites. Similar sampling exercises by Chanda in 2003-2004, in Kabanana, Chazanga, Chipata and Kalikiliki parts of Lusaka yielded anopheline mosquitoes, members of the *An. gambiae* complex from two of the four areas namely Kalikiliki and Chazanga (Chanda, 2007a). However, further sampling efforts of both larvae and adults

by the Malaria Control Programme in different parts of Lusaka urban including Kaunda Square, Northmead, Bauleni and Olympia, in recent years have not produced any primary malaria vector species (unpublished data NMCC).

Despite being found in the breeding sites in the study areas, anopheline mosquito species were not collected from indoor pyrethrum spray sheet collections; this was because all the mosquito species found at larval stages were exophilic species (Gillies and De Meillon, 1968; Service, 1989; Geisbühler et al., 2007; Fornadel, 2011). These observations are similar to those from other studies where *An. arabiensis,* an exophilic and to a large proportion, zoophilic (Cattle) species was found as dominant species of the *An. gambiae* s.l species at larval stages while *An. gambiae* s.s was the dominant species in indoor collections in the same area (Fillinger et al., 2009). Despite being documented as outdoor resting and biting species, *An. coustani* and *An. squamosus* were found to exhibit unusual anthropophily in Macha of the southern part of Zambia (Fornadel, 2011). That is preference to feed on humans as opposed to animals and feeding and resting indoors.

The study hypothesized that *An. gambiae* s.s was the dominant malaria vectoring mosquito species in the Lusaka urban district and opted to utilize indoor sampling methods. However, with the collected anopheline species being classified as exophilic, the study proposes the use of outdoor adult sampling techniques to assess the abundances of these collected anopheline species. For example Fillinger et al., (2009) used light emergence traps to access the densities of these exophilic mosquito species and found *An.*

coustani s.l. as the most abundant anopheline species. Interestingly, the application of the two larvicides into the study breeding sites in the study areas had no significant effects ($p>0.05$) on indoor feeding and resting densities of adult culicine mosquitoes. This finding suggests that there existed other sources of culicine mosquitoes in which breeding was unaffected by the application of biolarvicides in the monitored breeding sites during the study period. These post-larviciding productive sites could not have been the study breeding sites from which both anopheline and culicine larvae were cleared by the application of larvicides. The possible unidentified breeding grounds would be; pit latrines and household bathroom drainages, which harboured water in which culicine mosquitoes were breeding (Service, 1989).

5.4 Mosquito Species Diversity.

5.4.1 Mosquito Larvae Species Diversity.

Whereas no mosquito larvae of any species were collected after larviciding, culicine mosquitoes and three anopheline mosquito species; *Anopheles coustani, Anopheles squamosus* and *Anopheles rufipes* were collected from the breeding sites prior to larviciding. Compared to the other areas, the Chamba valley had one mosquito species (*Anopheles rufipes*) not found in any other. The area thus has one extra potential secondary vector mosquito species. All mosquito larvae were completely cleared from the breeding sites by the action of the bio-control agents applied. This implied that the larvicides were efficacious against mosquito larvae, and hence reduced the diversity of

anopheline mosquito larvae in the breeding sites, validating one of the study hypotheses (Fillinger et al., 2003, 2009, Fillinger and Lindsay, 2006; Shaukat et al., 2010).

The diagnosis of the number of species occurring in an area is important for vector control planning. This would help the targeting of control interventions to breeding sites that produce important vector species and provide information about their biting behaviour, ecology, abundance and spatial distribution (Ranson et al., 2011). Studies in other countries like Benin have demonstrated that *An. funestus* species become primary vectors of malaria in dry-hot months in areas where *An. gambiae* s.s is normally the principal vector species in wet months (Moiroux et al., 2011). *An. arabiensis* is a documented exophilic/ exophagic and zoophilic vector species; however the communities of the study areas in Lusaka Urban district are non livestock keeping hence the mosquito species lack the non-human alternative host if it occurs in the district. Control of this species by adult targeting ITN/LLINs and IRS (WHO, 2006b; Moiroux et al., 2011) would prove ineffective against this vector mosquito enabling it to continuously drive malaria transmission in the country.

5.4.2 Indoor Adult Mosquito Species Diversity.

No adult anopheline mosquitoes were collected during indoor sampling from the study areas. The spraying of biolarvicides in the study area did not affect the indoor collected adult culicine mosquito species found in human habitats. The possible explanation for presence of these adult mosquitoes indoors a month after the control of larvae could be

that; other water bodies were available for breeding and continued producing adult culicine mosquitoes. Another likely reason for their presence would be that adult mosquitoes emerged from the breeding sites before the treatment of the breeding sites with larvicides in the study areas.

The anopheline mosquitoes collected at larval sampling occasions are reported to exhibit exophagic and exophilic behaviours (Gillies and De Meillon, 1968; Fornadel et al., 2011), suggesting a possible explanation for their absence during indoor sampling using the pyrethrum spray sheet methods as found by a study in the Gambia (Fillinger et al., 2009). This observation suggests that this group of mosquito species may require outdoor collection methods for the estimation of their densities. These anopheline species however, were unexpectedly found exhibiting endophilic and anthropophilic behaviour in Macha rural area of Choma District (Fornadel et al., 2011). This unusual endophilic behaviour necessitates for vector incrimination determination studies on the species to ascertain their position as secondary vectors of malaria. These species have historically been detected with malaria parasites in parts of Africa including; Congo D.R, Tanzania, Cameroon and Zimbabwe (Fornadel et al., 2011).

These are potential secondary vector species of malaria but their role in the transmission of disease needs more investigations. Literature from Senegal suggests that the importance of exophilic and exophagic mosquito species in malaria transmission is inflamed by environmental changes e.g. control strategies targeting endophilic mosquitoes that have abilities to alter species compositions (Geisbühler et al., 2007).

5.5 Mosquito Species Distribution in the Study Areas.

Information on the distribution of mosquito species globally, especially in Africa is still fragmented and most efforts have been concentrated on the major vectors of malaria of the An. *gambiae* and *An. funestus* complexes (Gillies and De Meillon, 1968). Information on the distribution of disease vector species in the study areas and country at large is a key requirement for evidence-based and targeted vector control and subsequent monitoring of impacts of control interventions (Service, 1963; Okara et al., 2010).

5.5.1 Mosquito Larvae Species Distribution.

The study found that prior to the application of biolarvicides Bti and Bs in the breeding sites, the mosquito larvae were contagiously distributed i.e. the mosquito larvae population tended to be clumped or aggregated in some parts of the breeding habitats in the study areas as described by Southwood and Henderson (2000). The mosquito species distribution index (ID) could not be calculated as described by Southwood and Henderson (2000) since mosquito larvae were cleared from all study breeding sites after larviciding. Suggesting that larviciding affected the mosquito larvae distribution pattern which was contagious distribution (over dispersed) prior to the larviciding. The findings of the study proved the hypothesis that, the use of biolarvicides Bti and Bs affects the distribution patterns of anopheline mosquitoes. The documenting of the occurrence of the three anopheline mosquito species in the study areas of Lusaka urban district by this study contributes to the finer knowledge of their distribution.

5.5.2 Indoor Adult Mosquito Species Distribution.

At both pre- and post-larviciding periods, the species distribution of indoor collected anopheline mosquitoes could not be determined as, no *Anopheles* species were found during the sampling exercises in the study areas where only culicine adult mosquitoes were found and determined to be contagiously distributed (over dispersion) (Southwood and Henderson, 2000).

It is shown here that larviciding did not affect the indoor adult mosquito species distribution pattern in the study areas, which remained contiguous. If malaria vector species had been found indoors during collections, was their distribution going to be affected by the application of larvicides unlike was the case with culicine mosquitoes?

Compared to the pre-larviciding periods, the mean number of mosquitoes and distribution index (ID) reduced in value. This suggested that other breeding grounds for culicine mosquito species other than the treated sites existed, which continued producing adult mosquitoes or these adult mosquitoes emerged from these sites before the larviciding took place. These breeding grounds for culicine mosquitoes which are less selective in breeding habitat water quality could be home bathroom drainage and water logged pit latrines in the compounds of the study area.

5.6 Mosquito Species Dominance in the Study Areas.

5.6.1 Mosquito Larvae Species Dominance.

The study hypothesised that An. gambiae s.s was the dominant vector of malaria in Lusaka urban district, a statement which was disproved by the findings. No mosquito larvae (Anopheles or culicine species) were found in the post- larviciding sampling efforts in the study areas as mosquito larvae were completely cleared from the breeding sites; hence mosquito species dominance index (d) could not be calculated. This showed that larviciding affected the dominant mosquito species, which was the culicine mosquito overall and Anopheles coustani as the dominant species of Anopheles mosquitoes.

The application of these biological control agents resulted in complete elimination of mosquito larvae from the study breeding sites, hence affected the mosquito larvae species dominance in the study areas.

The finding of this study that, in the pre-larviciding sampling period, Culicine species were the dominant species of all mosquitoes, while *Anopheles coustani* was the dominant species of *Anopheles* mosquitoes in the breeding sites of the study areas concurs with those of Chanda (2007a) in parts of Lusaka urban and indoor sampling in Macha rural of Zambia (Fornadel et al., 2011). Like this study, Chanda's study conducted in Chazanga, Chipata, Kabanana and Kalikiliki found and Fornadel et al (2011) working in Macha both found *An. coustani* as the dominant anopheline mosquito species behind culicines (Chanda, 2007a). It is possible that *Anopheles coustani* was the dominant species contributing over 50% of anopheline larval numbers at all sampling times and sites in the

study areas but were not found indoors due to their outdoor feeding and resting behaviour (Gillies and De Meillon, 1968, Githeko et al., 1996). For these secondary vectors of malaria, control by indoor and adult targeted interventions involving ITNs and IRS is rather ineffective (WHO, 2006a).

5.6.2 Indoor Adult Mosquito Species Dominance.

The normally exophilic and zoophilic mosquito species collected at larval stages prior to biolarviciding in the study areas were not found in indoor sampling exercises. The same mosquito species were reported to exhibit unusual anthropophilic/ endophilic behavior in Macha (Fornadel et al., 2011).

5.7 Incidences of Malaria in the study areas

Generally, reductions in malaria cases were observed in all four study compounds in the post-larviciding periods, though their correlation to the application of biolarvicides in these areas could not be simply inferred because;

(i) Almost all cases were reported to be imported from outside the Lusaka Urban District which has rates below two percent (WHO/RBM, 2011) and,

(ii) No malaria vector mosquito species were collected in the sampling efforts in the study areas despite the finding of potential secondary vectors.

Similar sentiments are shared by studies from elsewhere over the complexity of relating gained reductions in malaria incidence in control areas to LSM interventions (Service, 1989; Majambere et al., 2010). A great deal of these malaria positive cases were found to be imported cases from outside of Lusaka e.g. from Chadiza, Nyimba, Katete, Rufunsa, Solwezi, Kaoma. Two cases of suspected local transmission i.e. people who did not travel outside Lusaka urban in the study period tested positive for malaria, were reported in Ng'ombe and Mtendere compounds. These findings are in agreement with reports of the World Health Organisation and Roll Back Malaria (2011) malaria prevalence rates of Two percent in Lusaka urban district, and is in the same ranges as reported from other urban areas of sub-Saharan Africa Countries (Robert et al., 2003). Is malaria transmission in the Lusaka urban district being perpetuated by secondary vectors or principal vectors do occur in the study areas?

The malaria control programme and its partners have since embarked on an active case detection and surveillance system to monitor malaria cases in all health facilities in urban Lusaka in which all positive testing cases are followed up and travel history ascertained.

5.7.1 Malaria Rates in Chainda Compound

The observed slight increase in the malaria rates from June (larviciding month) to July in the compound was probably attributable to imported cases of disease from other parts of the country outside the health facility's catchment area. Imported cases were reported to have accounted for 80% of malaria positives in Lusaka urban in 2003, the time at which the

disease was endemic in the urban district (Chanda, 2007a). Cases of malaria positive patients who have not travelled out of Lusaka were reported, should these malaria cases be local in origin and transmission in the urban settings, this would imply that malaria was slowly being re-introduced into the city (Service, 1989; Robert et al., 2003).

Recently, malaria incidence in the Lusaka urban district has been reported to be below 2 percent i.e. in 2010 (WHO/RBM, 2011). Like other studies elsewhere (Shaukat et al., 2010; Majambere et al., 2010), this study reported reductions in malaria incidence after larviciding with Bti and Bs. However, it is further concluded that realising the impacts of larviciding on incidences of malaria cannot be over simplified into linear relationships (Service, 1989; Majambere et al., 2010).

5.7.2 Malaria Rates in Chelstone Compound

Like in Chainda, the malaria cases in the catchment of this health facility were imported. In 2011 after the implementation of the larviciding in the study areas (June, 2011) the downward slope in malaria rates was steeper than the 2010 one. Again, the positive malaria cases observed following larviciding, during the month of August were due to imported cases of malaria from outside Lusaka urban district. Malaria has been reported not to be a major problem in most urban areas, and historically been imported into the urban area by migrating populations e.g. refugees in Choluteca town, Honduras and unplanned urbanisation (Service, 1989) or anthropogenic activities like farming and domestic animal rearing in many developing sub Sahara African cities (Service, 1989; Robert et al., 2003).

5.7.3 Malaria Rates in Mtendere Compound

In Mtendere compound, the malaria rates before the implementation of the larviciding in the study areas were in the low ranges reported by WHO/RBM (2011). The rates show a slight drop in June to July and rise to August. Again, malaria cases in Lusaka urban were reported to be imported from outside the catchment areas. Malaria has been regarded as a minor problem in most urban areas though it occurs in some sub-Saharan Africa countries (Service, 1989). Following the application of biolarvicides in June, 2011, a steep fall in malaria rates to July was observed i.e. the trends as compared to the same period in 2010. These post-larviciding reductions of malaria positive rates were probably due to reductions in disease transmission caused by non-recruitment in the vector population as a result of the clearance of the larval stage mosquitoes in the study areas. A similar observation was made by Majambere et al., (2010) in Gambia in a four-site-wide study where malaria incidence reductions incidences were observed following applications of Bti products in breeding sites. Similarly, Fillinger et al., (2009) reported a 73% reduction in new malaria infections in a population where Larviciding was integrated with ITNs.

5.7.4 Malaria Rates in Ng'ombe Compound

Like in Chainda, a rise in malaria positive rates from June to August was affected by a missing data set in July. Also, the disease rates were low as expected in the Lusaka urban (WHO/RBM 2011). The malaria prevalence rates are similar to those reported in a study in Western Kenya of 3.2-6.5% (Imbahale et al., 2010). The application of biolarvicides in June,

2011 was subsequently followed by a drop in the rates of malaria up to August, while the pre-intervention trend in 2010 went upwards in the same period despite a data gap in July due to RDT stock-out. Despite the recorded malaria cases in Lusaka urban being imported in both years, this observed fall in malaria cases in the Ng'ombe compound may suggest a reduction in local transmission of malaria due to bio-larviciding in the study area.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Findings of this study showed that; Pre-larviciding mosquito larvae habitat colonisation rates of identified breeding sites ranged from 30 to 50 percent, but these rates reduced to zero in all study areas after bio-larviciding with Bti and Bs indicating the high efficacy of the bio-control agents against mosquito larvae.

Three species of *Anopheles* mosquitoes alongside culicine mosquitoes were identified from the study area. *Anopheles coustani*, and *Anopheles squamosus* were collected from all the four study areas while *Anopheles rufipes* were only found in the Chamba Valley study area. Mosquito vectors of malaria i.e. *Anopheles gambiae* sensu lato and *Anopheles funestus* group were not found both in mosquito breeding sites (larvae) and indoors (adults) in the study areas. These findings supported existing belief that little to no local transmission of malaria by the major vectors *Anopheles gambiae* s.l and *Anopheles funestus* group currently occur in urban Lusaka. The absence of these vector species however does not suggest that they do not occur at all in Lusaka Urban District. They may not have just been collected in the district. More sampling for the vectors is needed to ascertain this as this study only sampled a part and not the whole of Lusaka Urban District.

Further, the application of biolarvicides in the study areas resulted in significant reduction ($p<0.05$) in mosquito larvae abundance and species diversity, and altered the distribution

($p<0.05$) and species dominance, but had no significant impacts on indoor collected adult culicine mosquito parameters.

Overall culicine species were the dominant mosquitoes in the study areas *while Anopheles coustani* was the dominant anopheline mosquito species contributing over 50% of anopheline larval numbers collected from all sampling points.

Despite most or all of the positive recorded cases of malaria based on review of health facility records in the study areas having been reported as imported from other districts of the country; malaria positive rates at all the four study compounds were less and showed a downward trend in the post-larviciding assessment period. A rise in positive cases was however observed in one health facility in the second month post-larviciding.

6.2 Recommendations

This study recommends that;

a) The mosquiticidal bacterial products Bactivec® (*Bacillus thuriengiensis* var. *israelensis)* and Griselesf® (*Bacillus sphaericus*) which have proved very efficacious against both anopheline and culicine mosquito larvae in Lusaka district in this study should have their applications rolled out to other control sites in the country. Their use could be in combination with other interventions such as Long Lasting Insecticide

treated Nets (LLINs) and IRS in Integrated Vector Management settings or separately.

b) Similar studies on LSM should be embarked upon in other parts of Zambia to provide the much needed information on malaria vector control efforts needed for policy decision making.

c) If local transmission of malaria occurs, the secondary vectors *Anopheles coustani*, *Anopheles rufipes*, and *Anopheles squamosus* may be responsible transmitters in Lusaka urban. Therefore, it is recommended that vector incrimination studies be done to ascertain the status of these mosquito species with regards to malaria transmission in the Lusaka Urban District.

d) Since the collected anopheline mosquitoes were exophilic and exophagic, it is recommended that other, more appropriate adult mosquito sampling techniques be used to study their ecology and biology in the district.

e) Future studies aiming to evaluate the impacts of larviciding on malaria incidences should consider setting up research specific malaria diagnosis rather than depend entirely on retrospective review of routine health facility data which tends to be limited at times due to e.g. diagnostics stock-outs and missing data.

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APPENDICES

Appendix A: Household Questionnaire

Study Title: The Effects of Habitat Management on Mosquito Larvae Abundance, Diversity and Distribution in selected areas of Lusaka District, Zambia.

Name of Investigator: Alister Kandyata

Institution: The University of Zambia

Date:_____ Village/Community:_____

District:_____ Interviewer: _____

Household number/Study number: _____

Informed Consent form for householder.

Dear Sir/Madam,

I am a Master of Science Student at the University of Zambia, conducting a Study on The Effects of Applied Mosquito Habitat Management on Mosquito Abundance, Diversity and Distribution in Selected Areas of Lusaka Urban District, Zambia.

The study aims to evaluate the impacts of the Larval Source Management Programme for Malaria Control currently running in Lusaka urban District on Mosquito Abundance, Diversity

and Distribution in the study area. The study will involve collection of mosquitoes from peoples' homes in the mornings between 04:30 Hrs and 08;30 Hrs using standardized methods.

Your house was selected to be sampled for mosquitoes by the study. I would be grateful if you would permit me to sample for mosquitoes in your household.

Please note that participation in the study by you is totally voluntary; therefore refusal of your household to take part in the study will not have any consequences. All information and data obtained from you during this interview and the rest of the study shall be treated as confidential. If you agree to participate in the study, please sign on the space provided below and provide the requested information.

A. Your name and Signature

Householder' Name (in Block letters): _____

Householder' signature: _____Date_____

Witness' Name (in Block letters): _____

Witness's signature: _____Date_____

B. Provide the following information about yourself.

1. Householder's Sex: Male [ ] Female [ ]
2. Householder's Age :____(Years).
3. What is the number of members in your household? _____

How many of these are under five years of age? ____

4. Householder's Education status;

a. Never been to School [ ]

b. Primary School level [ ]

c. Secondary School [ ]

d. Tertiary Level(College/University) [ ]

5. Householder's Employment status;

a. Unemployed [ ]

b. Self/ Informal sector [ ]

c. Formal employment [ ]

d. Student [ ]

6. Was your house sprayed in the last Indoor Residual Spraying(IRS)

Season or Privately? Yes [ ] No [ ].

If yes, approximately how many months ago? _____ months.

7. Does your household own ITNs? Yes [ ] No [ ].

If yes, how many?_____. If No, skip to question 10.

8. Have the nets been treated or retreated? Yes [ ] No [ ].

If yes, when was the last treatment date?_____(months).

9. In your household, who sleeps under these mosquito nets? _____

_____

10. How can you describe the mosquito problem in the past___ months (LSM Period) compared to months before that? _____

_____

End of Interview and Thank you for your time.

Appendix B: Study Areas Characteristics

Figure B1. Dam/Stream systems of the Ibex Hills/ Kalikiliki study area

A = wall fence foundation trench.

B = Macro habitat covered by tall grass.

C = Main dam surrounded by reeds.

Figure B2. Dam and pond in the Venta study area

A = Main water dam with emergent vegetation at the edges.

B = Smaller, sunlit pond with emergent vegetation.

Figure B3. Chelstone marsh ponds study area

A = Water reservoir.

B = Heaps of building sand and crashed stones.

C = House construction foundation/ furrow.

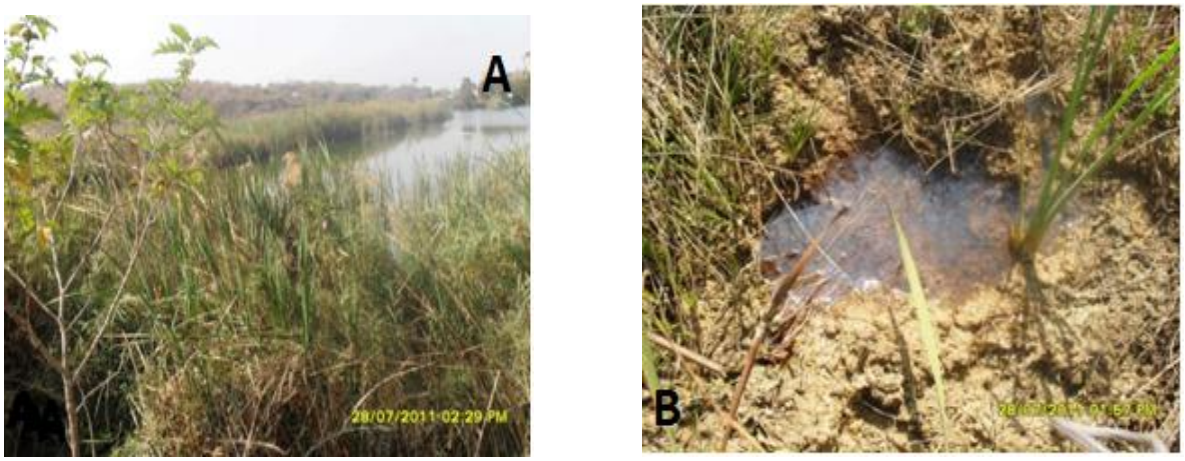

Figure B4. Chamba valley study area.

A = Main dam surrounded by reeds

B = Sunlit micro habitat

Figure B5. Vegetable gardening in the study area

A = Rape crop,
B = Tomato crop.

Figure B6. Land use in the Chelstone and Chamba valley study areas

A= Fish pond,

B and C = Portable water abstraction points

Figure B7. House structure in the study areas

A and B = Chelstone and Ng'ombe study area

C and D = Ibex Hills study area

E and F = Venta area (Outside (E) and Inside house (F)).

Figure B8. Biolarvicide spraying equipment

A. Hudson X-pert pressure spray pump.

B. Fixed-wing Aeroplane.

FIGURE B9. Breeding site larval sampling by dipper method.

A = Taking a water scoop at the edge of water body.

B = inspecting the scooped water for mosquito larvae/ pupae.

Figure B10. Adult mosquito collection

A = Non residual pyrethroid sprays for knock down of adult mosquitoes

B = Technician applying pyrethroid in a sampling house.

C = Knockdown mosquito on collection sheet.

D = Technician collecting knockdown mosquitoes.

E = Knocked down mosquito preserved in a vial containing silica gel.

Appendix C: Study Area Locations

Figure C1. Chelstone and Chamba valley Study areas.

Estimates of location of study areas; 1= Chamba valley study area and 2= Chelstone Zambia airways ponds study area. (Source: Google Earth, 2012).

Figure C2. Ibex hills/ Kalikiliki study area.

Estimates of location of study areas; 4.1= Ibex hills dam/stream study area and 4.2= Kalikiliki dam study area. (Source: Google Earth, 2012).

Figure C3. Venta/ Manzi valley study area
Estimates of location of study areas; 3= Venta/ Manzi valley dam study area. (Source: Google Earth, 2012).

Figure C4. Chelstone study area
Estimates of location of study areas; 2= Chelstone-Zambia airways ponds study area. (Source: Google Earth, 2012).

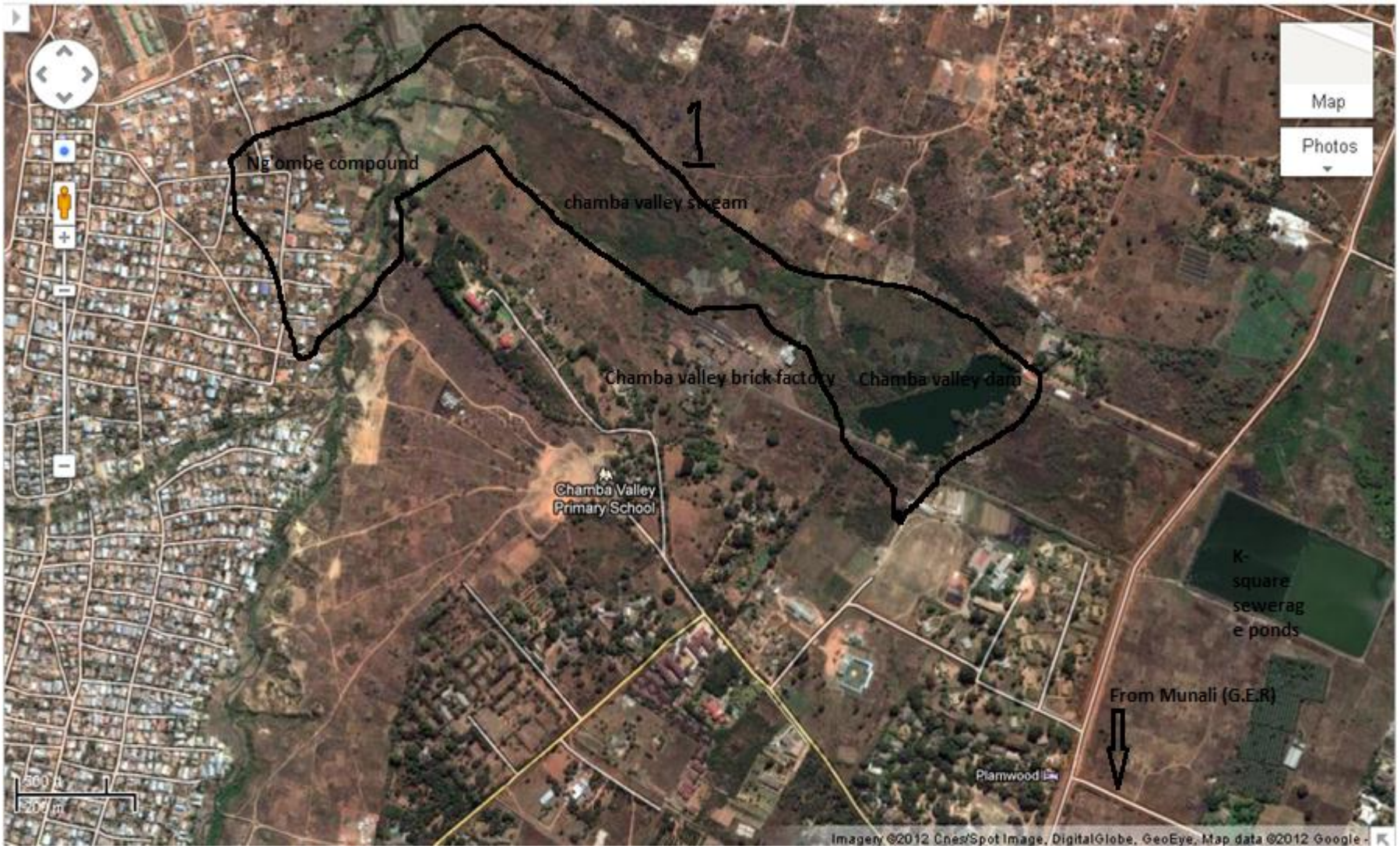

Figure C5. Chamba valley study area.

Estimates of location of study areas; 1= Chamba valley dam/ stream study area. (Source: Google Earth, 2012).

Appendix D: Geographical Positioning System (GPS) Way Points

Table D1. GPS way point readings from the Study Areas for Ibex Hills and Venta Compound Study Areas

House	Ibex Hills/ Kalikiliki			Venta Compound		
	Latitude; S	Longitude; E	Elevation; m	Latitude; S	Longitude; E	Elevation; m
1	15^0 24.750'	028^0 22.267'	1245	15^0 22.592'	028^0 24.170'	1216
2	15^0 24.717'	028^0 22.300'	1250	15^0 22.600'	028^0 24.163'	1216
3	15^0 24.707'	028^0 22.296'	1255	15^0 22.540'	028^0 24.120'	1217
4	15^0 24.640'	028^0 22.310'	1274	15^0 22.600'	028^0 24.118'	1217
5	15^0 24.613'	028^0 22.275'	1272	15^0 22.570'	028^0 24.115'	1217
6	15^0 24.600'	028^0 22.217'	1247	15^0 22.500'	028^0 24.050'	1218
7	15^0 24.600'	028^0 22.183'	1271	15^0 22.530'	028^0 24.090'	1218
8	15^0 24.601'	028^0 22.150'	1250	15^0 22.580'	028^0 24.91'	1218
9	15^0 24.583'	028^0 22.217'	1272	15^0 22.577'	028^0 24.073'	1218
10	15^0 24.588'	028^0 22.067'	1270	15^0 22.578'	028^0 24.146'	1216
11	15^0 24.610'	028^0 22.133'	1273	15^0 22.505'	028^0 24.080'	1218
12	15^0 24.785'	028^0 22.274'	1266	15^0 22.565'	028^0 24.130'	1217
13	15^0 24.650'	028^0 21.760'	1254	15^0 22.497'	028^0 24.048'	1218
14	15^0 24.645'	028^0 21.582'	1271	15^0 22.600'	028^0 24.051'	1218
15	15^0 24.615'	028^0 21.520'	1258	15^0 22.595'	028^0 24.049'	1218
16	15^0 24.652'	028^0 21.906'	1267	15^0 22.575'	028^0 24.048'	1218
17	15^0 24.784'	028^0 21.899'	1260	15^0 22.700'	028^0 24.010'	1218
18	15^0 24.598'	028^0 21.948'	1266	15^0 22.503'	028^0 24.002'	1218
19	15^0 24.694'	028^0 21.698'	1272	15^0 22.513'	028^0 24.028'	1218
20	15^0 24.597'	028^0 21.898'	1268	15^0 22.600'	028^0 24.033'	1218
21	15^0 24.545'	028^0 21.871'	1270	15^0 22.500'	028^0 24.100'	1218
22	15^0 24.794'	028^0 21.598'	1263	15^0 22.566'	028^0 24.038'	1218
23	15^0 24.536'	028^0 21.967'	1272	15^0 22.572'	028^0 24.024'	1218
24	15^0 24.530'	028^0 21.980'	1263	15^0 22.630'	028^0 24.150'	1216
25	15^0 24.512'	028^0 21.904'	1273	150 22.660'	028^0 24.091'	1217
26	15^0 24.796'	028^0 21.493'	1271	150 22.696'	028^0 24.050'	1217
27	15^0 24.702'	028^0 21.488'	1256	150 22.692'	028^0 24.050'	1217
28	15^0 24.460'	028^0 21.915'	1259	150 22.693'	028^0 24.088'	1217
29	15^0 24.458'	028^0 21.937'	1267	150 22.694'	028^0 24.072'	1217
30	15^0 24.408'	028^0 21.945'	1266	150 22.400'	028^0 24.030'	1218
31				15^0 22.438'	028^0 24.021'	1218
32				15^0 22.564'	028^0 24.148'	1216
Total			**30**			**32**

Table D2. GPS way point readings from the Study Areas for Chelstone and Ng'ombe Compound Study Areas

House	Chelstone Airways			Ng'ombe Compound (Chamba Valley Brick Factory)		
	Latitude; S	Longitude; E	Elevation; m	Latitude; S	Longitude; E	Elevation; m
1	15^0 22.080'	028^0 23.850'	1207	15^0 21.220'	028^0 20.110'	1210
2	15^0 22.082'	028^0 23.831'	1210	15^0 21.320'	028^0 20.140'	1213
3	15^0 22.070'	028^0 23.838'	1205	15^0 21.562'	028^0 20.019'	1255
4	15^0 22.040'	028^0 23.820'	1213	15^0 21.552'	028^0 20.142'	1218
5	15^0 22.000'	028^0 23.995'	1223	15^0 21.525'	028^0 20.156'	1221
6	15^0 21.940'	028^0 23.900'	1206	15^0 21.533'	028^0 20.155'	1220
7	15^0 22.032'	028^0 23.724'	1217	15^0 21.526'	028^0 20.085'	1225
8	15^0 22.009'	028^0 23.827'	1212	15^0 21.530'	028^0 20.060'	1228
9	15^0 22.050'	028^0 23.503'	1214	15^0 21.350'	028^0 20.120'	1208
10	15^0 22.050'	028^0 23.500'	1216	15^0 21.200'	028^0 20.100'	1213
11	15^0 21.998'	028^0 23.918'	1209	15^0 21.528'	028^0 20.149'	1219
12	15^0 21.702'	028^0 23.488'	1211	15^0 21.594'	028^0 20.174'	1213
13	15^0 21.989'	028^0 23.900'	1208	15^0 21.527'	028^0 20.153'	1220
14	15^0 21.560'	028^0 23.510'	1221	15^0 21.547'	028^0 20.066'	1234
15	15^0 21.545'	028^0 23.871'	1212	15^0 21.350'	028^0 20.018'	1252
16	15^0 21.520'	028^0 23.520'	1222	15^0 21.420'	028^0 20.030'	1216
17	15^0 21.408'	028^0 23.945'	1212	15^0 21.330'	028^0 20.115'	1254
18	15^0 21.520'	028^0 23.460'	1210	15^0 21.561'	028^0 20.020'	1254
19	15^0 21.296'	028^0 23.953'	1215	15^0 21.096'	028^0 20.098'	1263
20	15^0 21.620'	028^0 23.818'	1223	15^0 21.105'	028^0 20.093'	1265
21	15^0 21.976'	028^0 23.532'	1214	15^0 21. '	028^0 20. '	No GPS unit (Faulty)
22	15^0 21.965'	028^0 23.689'	1220	15^0 21. '	028^0 20. '	
23	15^0 21.860'	028^0 23.878'	1219	15^0 21. '	028^0 20. '	
24	15^0 22.698'	028^0 23.698'	1209	15^0 21. '	028^0 20. '	
25	15^0 22.010'	028^0 23.860'	1217	15^0 21. '	028^0 20. '	
26	15^0 21.610'	028^0 23.900'	1218	15^0 21. '	028^0 20. '	
27	15^0 21.702'	028^0 23.488'	1211	15^0 21. '	028^0 20. '	
28	15^0 21.530'	028^0 23.980'	1213	15^0 21. '	028^0 20. '	
29	15^0 21.865'	028^0 23.880'	1221	15^0 21. '	028^0 20. '	
30						
Total			**29**			**29**

EFFECTS OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* AND *BACILLUS SPHAERICUS* LARVICIDES, ON MOSQUITO ABUNDANCE, DIVERSITY AND DISTRIBUTION IN SELECTED AREAS OF LUSAKA URBAN DISTRICT, ZAMBIA

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