UNIVERSITY OF NAIROBI

ECOLOGY OF IMMATURE STAGES OF THE DENGUE FEVER VECTOR *Aedes aegypti* (L.) (DIPTERA: CULICIDAE) IN RURAL AND URBAN SITES OF THE SOUTHERN COAST OF KENYA

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DECLARATION

This thesis is entirely my work and has not been submitted for a degree in any other University.

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DEDICATION

My lovely family, wife Wambui, sons, Ngugi, Rimui, Waweru and daughter Mumbi who was born at the beginning of this studies.

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ABBREVIATIONS AND ACRONYMS

AFC	Animal feeding containers
AFT	Animal feeding troughs
ARC	Africa rainfall climatology
BI	Breteau index
Bs	Bacillus sphaericus
Bti	Bacillus thuringiensis var israelensis
CDC	Centers for Disease Control
CHIKV	Chikungunya virus
CI	Container index
DEET	N, N-diethyl- 3-methylbenzamide (N,N-Diethyl-meta-toluamide)
DENV	Dengue virus
DF	Dengue fever
DHF	Dengue haemorrhagic fever
DSS	Dengue shock syndrome
DVBNTD	Division for Vector Dorne and Neglected Tropical Diseases
GAMM	Generalized additive mixed model
GPS	Global positioning system
HI	House index
HKMO	Hourly temperature data for Mombasa Kenya
IQR	Inter quartile range
IRS	Indoor residual spraying

ITM	Insecticide treated materials
ITU	International toxic units
IVM	Integrated vector management
KEMRI SSC	Kenya Medical Research Institute Scientific Steering Committee
LSM	Larval source management
METEOSAT	Meterological satelite
MoH	Ministry of health
NOAA	National oceanic and atmospheric administration
PAHO	Pan Americal Health Organization
ppm	Parts per million
RIDL	Release of Insects carrying a Dominant Lethal
SDC	Small domestic containers
SE	Standard Error
ULV	Ultra low volume
VBDCU	Vector Borne Disease Control Unit
WG	Wetable granules
WHO	World Health Organization

ABSTRACT.

Aedes aegypti is the most important vector of dengue fever and several other arboviruses of public health such as Zika and Chikungunya. Currently vector management is the only available option for disease control. Efficient vector control and development of meaningful surveillance methods depends on a good understanding of vector ecology of which little is known in Kenya. The objectives of this study were to characterize breeding habitats of *Ae. aegypti*, determine seasonal distribution and abundance of *Aedes aegypti* larvae and pupae in rural and urban sites in coastal Kenya, identify households that are consistently productive for *Ae. aegypti* pupae and to determine susceptibility of *Aedes aegypti* larvae to the biological larvicide *Bacillus thuringiensis var israelensis* (Bti). Entomological, demographic and environmental data was collected from twenty sentinel households once a month for 24 months (June 2014 to May 2016) in the rural and urban sites of southern coast of Kenya. All water holding containers in and around houses were inspected for *Ae aegypti* larvae and pupae and oviposition traps set weekly in the study households. Susceptibility of *Ae aegypti larvae* to a biocontrol agent *Bacillus thuringiensis var israelensis* (*Bti*) was evaluated. Of the 6,566 container visits, only 5.11%, were found positive for *Ae aegypti* immatures in the study sites. In both sites significantly more *Ae aegypti* positive wet containers were found outdoors than indoors. The most important containers were buckets, drums and tyres which produced over 70% of all the immatures in both sites. The median number of months in which pupae were observed in households was 4 and ranged from 0 to 15.The strongest risk factor for pupal abundance was presence of high habitat counts (OR = 1.27, 95% CI 1.00-1.60). Initial efficacy results showed that *Bacillus thuringiensis* AM65-52 WG

formulation eliminated 100% of larvae in 24 hours. The results of this study indicate that breeding habitats of *Ae. aegypti* are abundant outdoors , but, only a few containers are productive. Further, *Ae. aegypti* pupal persistence at the household level in urban and rural sites was observed. High counts of breeding containers was associated with increased risk of pupal abundance.in households. Targeting productive containers and households that exhibit high pupal abundance and persistence in vector control interventions may result into cost-effective management of the dengue vector and arboviral transmission in this region.

CHAPTER ONE

1. Introduction

1.1 Background.

Dengue fever (DF) is an arboviral infection that occurs in tropical and sub-tropical regions of the world. The infection, caused by either one of the four closely related virus serotypes (DENV1-4), is usually self limiting but the severe forms, Dengue hemorrhagic fever (DHF) and Dengue shock syndrome (DSS) can be lethal in some patients (Rigau-Perez et al. 1998). In recent decades, the transmission of dengue virus (DENV) has increased predominantly in urban and semi-urban areas, and with continued expansion of the geographic range of the disease, DENV is a growing international public health problem (Gubler 1998; WHO 2012; Messina et al. 2014; WHO 2015). DENV cases are under-reported in many parts of the world leading to an underestimation of the dengue burden especially in Africa (Nathan and Dayal-Drager 2006; Sang 2006; Messina et al. 2014). However, it is estimated up to 300 – 400 million new cases occur annually (Brady et al. 2012; Bhatt et al. 2013) and approximately 2.5- 4 billion people are at risk of DENV infection globally (WHO 2012; Bhatt et al. 2013). Over half the world's population living in about 128 countries is potentially at risk for DENV transmission; currently the disease is ranked the most important and rapidly spreading human arboviral disease (Gubler 1998; Mairuhu et al. 2004; WHO 2009; Brady et al. 2012).

In Africa, although many dengue cases are underreported due to inadequate surveillance and misdiagnoses (Gubler et al. 1986; Nathan and Dayal-Drager 2006; Sang 2006), epidemic dengue has been documented in several African countries in the last 50 years, with a much higher

frequency in the East African region. During these epidemics, all the four dengue serotypes (DENV -1, DENV-2, DENV- 3 and DENV-4) were involved, but DENV- 2 has been the most frequently reported virus strain (Carey et al. 1971; Metselaar et al. 1980; Johnson et al. 1982; Gonzalez et al. 1985; Gubler et al. 1986; Sang 2006). In Kenya since the first dengue epidemic was reported (Johnson et al. 1982), cases of dengue fever have been on the increase, with more recent outbreaks in the coastal city of Mombasa(Ellis et al. 2015; Lutomiah et al. 2016) and Mandera in the North eastern region (Akhwale 2013). These and the periodic dengue outbreaks primarily along the Kenya coast in recent years (Akhwale 2013; Ellis et al. 2015; Ochieng et al. 2015; Lutomiah et al. 2016) is a worrying trend.

Transmission of DENV is effected by mosquito vectors in the genus *Aedes*. *Aedes aegypti* is the primary vector in most dengue epidemics worldwide , although other mosquitoes in the subgenus *Stegomyia* such as *Aedes albopictus* and *Aedes polynesiensis* are competent as well (Gubler 1998; Mairuhu et al. 2004). The worldwide resurgence of dengue has been associated with several factors, key among these being demographic and societal changes experienced in different parts of the world in recent decades (Gubler 1989; Newton and Reiter 1992; Gubler 1996; Gubler 1998; Rigau-Perez et al. 1998). These coupled with inability to suppress vector densities by many control programmes, have led to increased transmission of dengue especially in urban areas of the tropical world (Gubler 1998). *Ae. aegypti*, the principal vector for dengue, chikungunya (CHIKV), Zika and other emerging arboviruses, has adapted to domestic and peridomestic environment where it exploits the ubiquitous artificial containers and other water receptacles of various characteristics for its breeding (Service 1992; Focks and Chadee 1997; Gubler 1998; Troyo et al. 2008; Armistead et al. 2012; Dom et al. 2013). *Aedes* mosquitoes are

typically daytime feeders with peak biting times early morning and late in the evening before dusk, this renders bed nets un-protective to humans. Thus control strategies are now being directed towards source reduction through environmental management, and killing of larvae using chemical and biological larvicides, and biological control agents (Focks et al. 2000; Martinez- Ibarra et al. 2002; Kroeger et al. 2006; Tun-Lin et al. 2009; WHO 2009).

In Kenya, the two sub specific taxa, *Aedes aegypti aegypti* and *Aedes aegypti formosus* are the primary dengue vectors (Trpis and Hausermann 1986) with the latter being more prevalent in western Kenya (Yalwala et al. 2015). Of the two subspecies, reported to be sympatric in coastal Kenya (Trpis and Hausermann 1986), *Ae.ae. aegypti* is more domestic/peri-domestic, than *Ae.ae.formosus* which breeds mainly in forest tree holes. In the coastal town of Malindi in north coast of Kenya *Ae.aegypti* immatures were found both indoors and outdoors at high numbers (Midega et al. 2006). In a recent study (Saifur et al. 2012), *Ae.aegypti* immatures were found mostly in outdoor habitats in what is thought to be an adaptive strategy by this vector. Human activities within households play a crucial role in determining *Ae. aegypti* breeding, thus productivity and abundance of these mosquitoes tends to fluctuate greatly in individual houses. Various factors may influence productivity of *Ae. aegypti* larval habitats in different container types, these include, the frequency of water replenishment, the availability of food for the larvae (Subra 1983) the degree of sunlight exposure (Maciel-de-Freitas et al. 2007; Paul et al. 2018) and container covering.

Few studies on the ecology of larval *Ae. aegypti* have been conducted in Kenya (Trpis and Hausermann 1986; Midega et al. 2006; Tun-Lin et al. 2009; Yalwala et al. 2015), despite the evidence of dengue virus transmission in the country in recent years (Akhwale 2013; Ellis et al.

2015; Ochieng et al. 2015; Vu et al. 2017). Moreover, data on routine surveillance of *Ae. aegypti* or other potential vectors is lacking. The presence of DENV and other arboviruses in Kenya (LaBeaud et al. 2015), coupled with conditions suitable for the proliferation of the *Aedes* vector, increases the risk for dengue outbreaks in this region. In the absence of a viable vaccine (Halstead 2012; WHO 2018; CDC 2019) , epidemiological surveillance and vector control remain the best practices for preventing dengue outbreaks (Hiscox et al. 2013; Ghosh and Dar 2015; Rather et al. 2017). Effective vector control and development of meaningful surveillance methods depends on a good understanding of larval and adult vector ecology of which little is known in Kenya.

The purpose of this study was to characterize breeding habitats of *Ae. aegypti*, determine seasonal distribution and abundance of *Aedes aegypti* larvae and pupae in rural and urban sites in coastal Kenya, identify households that are consistently productive for *Ae. aegypti* pupae and determined the ecological and socio-demographic factors associated with the persistence and abundance of pupae in households. The study also sought to determine susceptibility of *Aedes aegypti* larvae to *Bacillus thuringiensis var israelensis* (Bti). This study makes a meaningful contribution to the dengue vector surveillance in the region, in addition to providing information that is vital guiding vector control efforts.

1.2. Statement of the problem

Occurrence of DENV, CHIKV and other arboviruses in Kenya, coupled with conditions suitable for the proliferation of the *Aedes* vector, increases the risk for dengue outbreaks. Recent reports of epidemic dengue and the rising number of new cases being reported primarily along the Kenya coast is a worrying trend, given that there are neither viable vaccines nor standard therapeutic procedures for DENV infections. Epidemiological surveillance and vector control remain the primary means for preventing dengue outbreaks. Control programmes targeting adult vector mosquitoes by insecticidal sprays in different parts of the world have achieved limited success, thus control strategies are now being directed towards source reduction through environmental management, and killing of larvae using chemical and biological larvicides, and biological control agents. Effective vector control and development of meaningful surveillance methods depends on a good understanding of larval and adult vector ecology of which little is known in Kenya. The purpose of this study was to characterize breeding habitats and determine seasonal distribution and abundance of *Aedes aegypti* larvae and pupae in rural and urban sites in coastal Kenya. The study also sought to identify households that are consistently productive for *Ae. aegypti* pupae, determined the household risk factors for the persistence and abundance of pupae in households. and evaluate susceptibility of *Aedes aegypti* larvae to *Bacillus thuringiensis var israelensis* (Bti).

1.3 Main objective

To characterize breeding habitats of *Ae. aegypti*, establish container productivity profiles and establish key households for *Ae.aegypti* pupal production in rural and urban sites in southern coast of Kenya

1.4 Specific objectives:

1. To characterize larval habitats for *Ae aegypti* in urban and rural sites in the southern coast of Kenya.

2**.** To determine seasonal distribution and abundance of *Aedes aegypti* larvae and pupae in rural and urban sites of the southern Coast of Kenya.

3. To establish households that are consistently productive for *Ae. aegypti* pupae and determined the ecological and socio-demographic factors associated with the persistence and abundance of pupae in households in rural and urban sites of the southern coast of Kenya

4. To determine susceptibility of *Aedes aegypti* mosquitoes in the southern coast of Kenya to the biolarvicide *Bacillus thuringiensis* var *israelensis* (Bti).

1.5 Hypotheses

1. Larval habitats for *Ae aegypti* are not abundant and diverse in the southern coast of Kenya.

2. The abundance and distribution of *Ae. aegypti* larvae and pupae in the southern coast of Kenyadoes not show any pattern of seasonality

3. There are no specific households that are consistently productive for *Ae.aegypti* pupa in the southern coast of Kenya and that ecological and socio-demographic factors in this region are not associated with the persistence and abundance of pupa

4. Larval *Aedes aegypti* mosquitoes in coastal Kenya do not exhibit significant levels of susceptibility to the biolarvicide *Bacillus thuringiensis var israelensis* (Bti).

1.6 Justification

As dengue continues to emerge as a major public-health problem world-wide, surveillance and control of the dengue vector *Aedes aegypti* is paramount. A thorough understanding of the ecology of vector mosquitoes is a prerequisite for success in vector control and management programs. In Kenya there are no published studies on routine surveillance of dengue vectors and information on the ecology of larval *Ae. aegypti* is limited to only a few studies that have been done mostly at the coast. Identification of Key container habitats and households that are consistently productive for *Ae. aegypti* pupae is particularly important in guding targeted vector control efforts offering a cost-effective way to manage the dengue vector and arboviral transmission in this region.This study therefore, makes meaningful contribution to the surveillance of *Ae. aegypti* in the region, in addition to providing information that will be vital in guiding vector control efforts.

CHAPTER TWO

2. Literature review

2.1 Dengue infections

Dengue is an arboviral infection that occurs in tropical and sub-tropical regions of the world. The infection is caused by the dengue virus (DENV) that exists in four distinct but closely related virus serotypes (DENV1-4) that show extensive genetic variability (Gubler 1989; Rigau-Perez et al. 1998; WHO 2009). The small spherical single-stranded RNA flavivirus belongs to the family Flaviviridae. Of the 2.5 billion people at risk of dengue infections globally over 70% live in South-East Asia and Western Pacific regions (WHO 2009). Dengue may be asymptomatic or may present clinically as a non specific febrile illness, classic dengue fever (DF), and dengue hemorrhagic fever (DHF), in which plasma leakage may lead to dengue shock syndrome (DSS)(WHO 1997; Mairuhu et al. 2004). Symptomatic dengue virus infections are classification based on the severity of clinical manifestations.

2.1.1 Dengue fever

Dengue fever (DF) is an acute febrile illness with an incubation period of 3- 8 days. The febrile period is characterized by a rapid onset of high fever (≥39ºC), accompanied by severe headache, pain behind the eye, nausea and vomiting. Other symptoms that may occur during this period include muscle and bone or joint pain and a rash which initially maculopapular may become erythematous. Although severe bleeding is unusual, minor haemorrhagic manifestations such as petechiae, epistaxis and bleeding gums can occur (WHO 1997; Mairuhu et al. 2004). Most of this signs and symptoms are common in older children and adults contrary to infants and children

where DF presents as an undifferentiated febrile illness accompanied by a maculopapular rash. The infection is well tolerated and recovery usually occurs in 7-10 days of illness (WHO 1997; Mairuhu et al. 2004).

2.1.2 Dengue haemorrhagic fever and dengue shock syndrome

Dengue haemorrhagic fever (DHF) is a severe form of dengue infection which is often characterized by high fever haemorrhagic manifestations, enlargement of the liver and circulatory failure which results from increased vascular permeability and plasma loss (WHO 1997; Mairuhu et al. 2004). Initial symptoms and incubation period of DHF are similar to those of dengue fever. However, 3-4 days after the onset of the disease haemorrhagic manifestations varying from petechiae to bleeding from gastrointestinal tract, nose and gums appear, reaching the critical stage within 3-7 days of the febrile period. This is followed by a rapid fall in temperature and as the disease progresses signs of circulatory failure occasioned by plasma loss may appear (WHO 1997; Mairuhu et al. 2004). Patients experience generalized abdominal pain, persistent vomiting, and change in the level of consciousness and also show signs of plasma leakage which include hypoproteinaemia, thrombocytopenia, elevated haematocrit and serous effusion (Kalayanarooj et al. 1997; WHO 1997; Mairuhu et al. 2004). With continued and excessive loss of plasma hypovolaemic shock may occur as the disease progresses to dengue shock syndrome (DSS). Dengue shock syndrome is preceded by an acute abdominal pain as the signs of circulatory failure become more apparent. Patients develop a rapid weak pulse with narrowing pulse pressure or hypotension with cold clammy skin and become restless. As the condition progresses bleeding from the gastrointestinal tract and other organs become severe leading to metabolic and electrolyte imbalance (WHO 1997). Dengue shock syndrome can be

fatal and patients may succumb to death in 12- 24 hours from the onset of shock. However, with early diagnosis and immediate replacement of fluids patients may recover within 2-3 days (WHO 1997; Mairuhu et al. 2004).

2.2 Dengue vectors

Transmission of DENV is effected by day biting mosquito vectors in the genus *Aedes. Aedes aegypti* is the principal vector in most dengue epidemics worldwide. *Aedes albopictus*, *Ae. polynesiensis* and several other mosquitoes in the subgenus *Stegomyia* are also important vectors of dengue. (Gubler 1998; Perich et al. 2000; Mairuhu et al. 2004; WHO 2009). *Aedes aegypti*, described as a highly domestic species lives in close association with humans where it exploits a variety of artificial and natural water receptacles for breeding, while maintaining strong endophilic and anthropophilic characteristics (Perich et al. 2000; Harrington et al. 2005; Chadee 2013), factors which makes it an efficient vector compared to other species.

Aedes albopictus is an invasive species that is native to Asia but has spread to the Americas, Europe and parts of Africa (Hawley 1988; Gratz 1991; Mairuhu et al. 2004; WHO 2009). In the Western hemisphere where the species is well established it is the second main vector of dengue which is also implicated in yellow fever epidemics and in the transmission of other arboviruses in globally (Knudsen et al. 1996).The species is mainly exophilic, and breeds mainly outdoors in a variety of natural water receptacles such tree holes, leaf axils, and bamboo stumps in the forest and also artificial water-filled containers within the peridomestic environment (Hawley 1988; Gratz 1991; Armistead et al. 2012). However, a shift from outdoor to indoor breeding activity has also been observed in some cases (Dieng et al. 2010) and this may offer the mosquito more breeding and blood feeding opportunities with important epidemiologic implications on its

vectorial capacity. *Aedes. albopictus* readily obtains blood meals from humans and domestic animals as well and unlike *Ae. aegypti*, it has nocturnal blood feeding habits in addition to the usual day biting behavior (Hawley 1988; Gratz 1991; Dieng et al. 2010; Chadee 2013).

2.3 Biology and ecology of *Aedes aegypti*

2.3.1 Occurrence and geographic distribution of *Aedes aegypti*

Aedes aegypti, belongs to the subgenus *Stegomyia* and is thought to have its origins in sub-Saharan Africa, where the ancestral sylvatic strain still exists (Christophers 1960). The mosquito is widely distributed in tropical and sub-tropical regions of the world at altitudes not exceeding 1000 meters above sea level, and within the 35° North and 35° South latitude limits which roughly correspond to the 10° January and 10° July isotherms (Gratz 1991; Gubler 1998; Mairuhu et al. 2004; Nathan and Dayal-Drager 2006; Powel and Tabachnick 2013). However, exceptional cases of occurrence at altitudes above 1000m above sea level and latitudes of up to 45° North have been recorded (Gratz 1991). Dispersal of *Ae. aegypti* in many parts around the globe has been associated mainly with sea transport and trade in used car tyres, while much of the maintenance and spread is greatly influenced by human factors. Of these, rapid urbanization has been cited to be key due to the associated lifestyles that create many potential larval habitats and other ideal conditions necessary for mosquitoes to thrive (Service 1992; Powel and Tabachnick 2013).Unlike other Aedes mosquitoes, *Ae. aegypti* is more domestic and thrives in human dwellings, where it exploits numerous water storage containers and a wide variety of other water receptacles for its breeding. In addition humans offer an easy access to a blood source for the highly anthropophilic species that mainly rests indoors (Service 1992; Harrington

et al. 2001; Chadee 2013; Dzul-Manzanilla et al. 2017). These factors are of immense epidemiological importance by increasing chances vector-human contact thus enhancing the vectorial capacity of *Ae. aegypti.*

In Kenya the two sub specific taxa; *Aedes aegypti aegypti* and *Aedes aegypti formosus* are the primary vectors of dengue. *Aedes.aegypti. aegypti* (subspecies *queenslandensis*) is more common in human dwellings, while *Ae.ae.formosus*, the sylvatic form, breeds in forest tree holes (Trpis and Hausermann 1986; Yalwala et al. 2015).

2.3.2 Blood feeding behavior of *Ae. aegypti*

Female *Ae. aegypti* mosquitoes typically bite during the day. In general biting cycle is diurnal with peak biting activity early in the morning about 2- 3 hours after sunrise and in the evening 1-3 hours before dusk (McClelland 1959; McClelland 1960; Gratz 1991; Chadee and Martinez 2000; Chadee 2013). The species which is an aggressive biter, will bite freely whenever the host is available and may bite more than one host during blood feeding session (Schoof 1967; De Benedictis et al. 2003). In East Africa clear differences in the peak biting times was reported between the domestic (subspecies *queenslandensis*) and the wild (subspecies *formosus*) forms of *Ae. aegypti*. The wild form mainly an outdoor species, had two sharp peaks of biting time (a few hours after sunrise and just before sunset), while the domestic form exhibited a multi-peaked biting cycle which intensified towards late evening hours. In both species biting activity reached the climax 1-2 hours before sunset. (McClelland 1959; McClelland 1960). *Aedes. aegypti* has a preference for human blood although domestic animals mainly vertebrates may also serve a source of a blood meal (Christophers 1960; Gratz 1991; Scott et al. 1993b; Harrington et al. 2001; De Benedictis et al. 2003). This preference to human blood has been associated to its low

isoleucine content and other components which are thought to promote accumulation of energy reserves and fitness advantage (Harrington et al. 2001). Female *Ae. aegypti* have a tendency to blood feed more than once in each gonotrophic cycle (Trpis and Hausermann 1986; Scott et al. 1993a; Scott et al. 1993b; Xue et al. 1995; Scott et al. 2000; Farjana and Tuno 2012; Farjana and Tuno 2013), a behavior that has important epidemiological implications for it increases opportunities for pathogen acquisition and transmission.

2.3.3 Flight range and resting habits of *Ae. aegypti*

Although *Ae. aegypti* mosquitoes have elusive resting habits (Schoof 1967), human dwellings have been shown to provide suitable environment for resting mostly in and around houses (Trpis and Hausermann 1986; Muir and Kay 1998; Perich et al. 2000; Chadee 2013). The preferred resting places especially for the domestic form which is primarily endophilic, include bedrooms, kitchens, sitting rooms, and bathrooms, where the order of preference may vary but in most cases bedrooms are more preferred (Perich et al. 2000; Chadee 2013; Dzul-Manzanilla et al. 2017). Resting of female *Ae. aegypti* soon after taking a blood meal is of great necessity, for it allows the physiological processing of blood and completion of vitellogenesis before the pre-oviposition flight (Chadee 2013). The preferred resting surfaces are usually in dark, shaded and secluded places, and these include walls, under furniture, in cupboards, wardrobes and hanging clothing (Gratz 1991; Reiter 1991; Perich et al. 2000). Chadee (2013) reported that most of the mosquitoes resting on dark walls did so on surfaces close to the floor and rarely at higher levels. This finding on the preferred height of resting is consistent with a study done in Mexico where adult *Ae. aegypti* were found significantly resting below 1.5m (Dzul-Manzanilla et al. 2017). In outdoor environment the mosquitoes will be found resting in shaded and sheltered locations

around the houses such as gardens, flower beds, drains (Perich et al. 2000), inside tyres, drums and cisterns (Schoof 1967). The house environment thus provides ideal conditions for increased vector-human contact and transmission of dengue viruses. The sylvatic form, *Ae. ae. formosus* is predominantly exophilic, resting and breeding outdoors preferably in forest habitats (Trpis and Hausermann 1986; Yalwala et al. 2015). A polymorphic form that occurred either indoors or outdoors was reported in the peridomestic environment in a coastal village of Rabai (Trpis and Hausermann 1986).

Aedes. aegypti rarely disperses very far from the breeding and resting sites (Trpis and Hausermann 1986; Gratz 1991; Muir and Kay 1998; Getis et al. 2003; Harrington et al. 2005). In East Africa, Trpis and Hausermann (1986) reported a mean dispersal rate of 57.0m and 44.3m per day and the maximum dispersal distance of 154m and 113m in 24 hrs for females and males respectively. From this and other observations (McDonald 1977; Muir and Kay 1998), males generally have shorter dispersal range compared to female mosquitoes. In most cases, majority of the mosquitoes rarely disperse far from the houses from where they are released and only a few attaining an average maximum distance of 400 (McDonald 1977; Harrington et al. 2005; Maciel de Freitas et al. 2007; David et al. 2009). It is only in exceptional circumstances that dispersals of over 400m have been reported to occur, such us when gravid females cannot find suitable oviposition sites in a given locality (Christophers 1960; Schoof 1967; McDonald 1977; Gratz 1991; David et al. 2009). Unlike other mosquitoes known to disperse widely, the activities of *Ae. aegypti* are restricted to the domestic environment as long as breeding and resting sites are freely available and an access to a human blood meal is guaranteed. The mosquitoes are capable of rapid and directive flights for considerably long distances, but in most cases flight is

interrupted by searches and stop-over's in houses (Christophers 1960) and is influenced by wind (McDonald 1977; Muir and Kay 1998). Dispersal is an important determinant of vectorial capacity of *Ae. aegypti* mosquitoes. Higher dispersal rates can increase chances of an infected vector to encounter humans and effect transmission.

2.3.4 Survivorship of adult *Ae. aegypti*

Survival of adult vector mosquitoes directly influences their vectorial capacity and hence plays a crucial role in pathogen transmission. A vector should survive long enough to effect pathogen transmission between hosts and this should be longer than the periods of no-feeding and extrinsic incubation (Garret-Jones 1964). A number of factors are critical for the survival of adult *Ae. aegypti* mosquitoes in the environment, key among these being, larval and adult nutrition, climate, predation, and genotype (Christophers 1960; Muir and Kay 1998). Availability of drinking water and high humidity has been found to be particularly important for adult survival in the laboratory (Christophers 1960). Survivorship may be also influenced by the degree of urbanization and the structure of urban dwellings, where survival rates were noted to be higher in slum areas than in the more organized high - income neighborhoods (Maciel de Freitas et al. 2007; David et al. 2009). A possible explanation to this observation is that in the crowded slums female *Ae. aegypti* mosquitoes disperse less to obtain a blood meal and oviposition sites, as such they are less likely to encounter hazardous environmental conditions (David et al. 2009). Probability of daily survivorship of adult *Ae. aegypti* in different ecological settings has been found to range between 0.70 - 0.90 and an average life expectancy range of 2 – 16 days. These values are generally higher in females than in male mosquitoes (McDonald 1977; Service 1992; Muir and Kay 1998; Maciel de Freitas et al. 2007; David et al. 2009). Considering the extrinsic

incubation period of dengue virus, a slight increase in survival rates will have significant epidemiological effect on the transmission cycle. However with the low survival rates observed, generally a higher vector density would be necessary to maintain transmission (Service 1992).

2.3.5 Oviposition behaviour of *Ae aegypti*

Usually, a batch of eggs develops after each blood meal. Under optimum conditions of temperature and humidity oviposition occurs in about 3 days after a blood meal, mostly late in the afternoon under normal daylight hours (Christophers 1960; Chadee 2010). Eggs are deposited singly in a line on the wet surface of water receptacles just above the edge of the water, where the sticky chorionic pads cement them in position upon drying (Christophers 1960). Containers with rough walls providing foothold are mostly preferred. However, presence of predators or pathogens may influence the selection of oviposition sites by gravid *Ae. aegypti* (Pamplona et al. 2009).Generally clean and clear water with some organic matter is often preferred for oviposition (Christophers 1960; Gratz 1991). During oviposition the egg batch may be distributed in several containers during a single laying episode (Brown 1974; Chadee 2010). This oviposition behavior is thought to be an adaptation to increase the chances of larval survival by minimizing risks associated with overcrowding and transient nature of some breeding habitat (Reiter 2007).

Under ideal conditions of warmth and humidity, *Ae. aegypti* eggs become fully embryonated within 48 hours and will soon hatch once flooded (Gratz 1991). Eggs can remain viable for several months in dry containers and will hatch easily on flooding (Christophers 1960; Gratz 1991). The capacity of eggs to withstand desiccation for long periods makes it easy for them to

be disseminated over great distances in dry containers, emphasizing the importance of humans in the dissemination of dengue virus within and between human dwellings, given that *Ae. aegypti* mosquitoes rarely fly far from their breeding sites (Harrington et al. 2005).

2.3.6 Breeding habitats of *Ae. aegypti*

Aedes aegypti has been described as a domestic species due to its close association with human dwellings, where it preferentially breeds in artificial containers and natural water receptacles in and around households (Gratz 1991; Service 1992; Wongkoon et al. 2007; Troyo et al. 2008; Armistead et al. 2012; Saifur et al. 2012). Majority of the artificial containers serving as breeding habitats are for domestic water storage, some find use as animal drinking points or as flower pots. Others include numerous rain-fed habitats such as discarded containers, blocked gutters and used motor vehicle tyres. Natural water receptacles include tree holes, leaf axils of banana and flower plants, coconut shells and hollows in tree stumps. Breeding in unusual habitats such as underground septic tanks has been reported in some cases (Barrera et al. 2008). *Aedes aegypti* larvae are adapted to obtaining food in fairly clean and clear water devoid of natural predators , conditions which are amply provided by container habitats found in and around human dwellings. With few exceptions, the occurrence and breeding activities of *Ae. aegypti* are limited to about 100m radius of human habitations (WHO 2009). However, where suitable breeding sites are not available the gravid mosquitoes have been found to disperse for over 400m further away from human dwellings in search of oviposition sites (Schoof 1967; Gratz 1991; Reiter 2007). The species has become adapted to urban habitats where numerous breeding opportunities are provided by numerous water holding containers and other water receptacles that are ubiquitous,

especially in unplanned residential areas characterized by poor water supply and solid waste management systems. Rural infestation has been attributed to larvae or eggs in containers transported for water storage (Gratz 1991).

2.3.7 Influence of environmental factors on the development of *Ae. aegypti* larvae

Eggs hatch once submerged, this is after 1 – 2 days when embryonic development is complete and the resultant first instar larvae pass through four developmental "instars" each separated by a moult to attain the pupa stage. Under optimum conditions this may take about 5 days. Adult male and female mosquitoes emerges on average within 1– 2 days . However, larval development may take longer periods if unfavorable conditions prevail in their environment.

Several factors influence larval development. Of these, temperature and food have been found to play a major role (Christophers 1960; Tun-Lin et al. 2000; Farjana et al. 2012; Couret et al. 2014; Garcia-Sánchez et al. 2017). Temperature range of 20ºC- 30ºC was reported to be optimum for larval development and survivorship, although adults emerging at higher temperatures tend to be smaller (Tun-Lin et al. 2000; Farjana et al. 2012). On the contrary, at 35ºC temperature had negative effects on larval development despite the availability of adequate nutrition (Farjana et al. 2012), while at low temperatures (<20ºC) *Ae. aegypti* larvae respond by prolonging development period and decreased survivorship (Christophers 1960; Tun-Lin et al. 2000; Farjana et al. 2012; Couret et al. 2014), and temperatures below 16ºC can be lethal (Christophers 1960). Temperature may also impact larval development by influencing the growth of microbial food resources for the larvae (Couret et al. 2014). Other factors such as larval density and presence of congenerics also exert their influence on the duration of larval development in a number of ways (Moore and Fisher 1969; Couret et al. 2014). Water-filled container habitats are prone to crowding and limited food resources, thus

competition among coexisting species is likely to occur (Armistead et al. 2008). Crowded larvae under conditions of suboptimal nutrition levels have been known to produce growth factor retardants, which by acting on a negative feedback mechanism, regulate the population of larvae in response to increasing densities in an environment with limited food resources. Such metabolites produced by *Ae. aegypti* larvae have been shown to act as inhibiters to the population of a competitor species (Moore and Fisher 1969). High larval densities in containers can result in accumulation of chemical toxic wastes such as ammonia which though promoting microbial growth, may act as a stressor for the larvae prolonging development time (Couret et al. 2014),with the overall effect of producing small sized mature larvae, pupae and ultimately adults (Christophers 1960). Artificial containers in the domestic environment rarely harbor predators (Service 1992). As such competition may be of much effect than predation in *Ae. aegypti* domestic larval habitats. When a breeding site has a considerable amount of organic matter, food resource is likely to be adequate to allow coexistence with other Aedes species. Inadequate nutrition usually has the effect of prolonging larval development period (Couret et al. 2014) and adult mosquitoes resulting from such larvae, besides being small in size have other numerous deficiencies (Christophers 1960). Although natural factors may interfere with the growth of *Ae. aegypti* larvae, some human activities related to hygiene and handling of water receptacles may probably account for much of the immature mortality in the domestic environment (Gratz 1991). Some of these include flooding, rinsing or frequent draining and drying of containers.

2.3.8 Behavior of *Ae. aegypti* larvae in their natural habitat

Aedes. aegypti larva characteristically rests hanging almost vertically downwards from the water surface from where it is suspended on the respiratory siphon that establishes contact with the

surface film. Surface tension provides support to the larva while allowing movement in the horizontal plane. All larval instars are observed to spend most of their time resting or moving slowly along the surface(Christophers 1960). The angle at rest which is 20º from the vertical may vary with instar stage, motion status and speed.

Basically swimming in *Ae. aegypti* larvae is effected by side-to- side lashing movements of the whole body, which unlike other mosquitoes produces a characteristic looping motion with the tail leading. Movement generally tends to be lateral, upwards and downwards, in addition action by feeding brushes also produces considerable movement forward in a smooth and effortless manner (Christophers 1960).

Aedes. aegypti larvae feed mainly by pharyngeal filtering of minute food particles from water currents generated by the feeding brushes. This mode of feeding is supplemented by gnawing of solid food particles using mandibles and browsing on particulate matter on the surfaces of the container or on the surface film (Christophers 1960; Merritt et al. 1992). During browsing the feeding brushes aid the larvae to glide along the feeding surface and at same time dislodging food particles. Bacteria and yeasts have been found to be part of the essential components of *Ae. aegypti* larval food (Christophers 1960) though they may also feed on detritus and parts of dead invertebrates on organic surfaces and sediments (Merritt et al. 1992).

Aedes. aegypti larvae are extremely sensitive to light and vibrations (Christophers 1960).The strong negative phototropism is exhibited by larvae at all instars and appears to be characteristic of the species. Larvae quickly move away from the source of light and aggregate in region of lowest light intensity. With respect to sensitivity to mechanical stimuli larvae of *Ae. aegypti* will

instantly dive at the slightest tapping of the container, with the depth of the dive depending on intensity of tapping (Christophers 1960).

2.4 Surveillance of the dengue vector *Ae. aegypti*

In the absence of a vaccine and therapeutic treatment for dengue, epidemiological surveillance and vector control remain the best practices for preventing dengue outbreaks (Chadee 2009; WHO 2009; Hiscox et al. 2013). Effective vector control and development of meaningful surveillance methods depends on a good understanding of larval and adult vector ecology. In dengue endemic developing countries, entomologic surveillance remains an important tool to estimate potential risk of virus transmission in view of limited resources for effective serological and viral surveillance (Getis et al. 2003).

Dengue vector surveillance is based on the detection or monitoring of immature or adult populations of *Ae. aegypti* which according to the World Health Organization (WHO) should be the main target for surveillance and control activities, unless other epidemiologically significant species proven to transmit dengue virus are present in the area of concern (WHO 1997). Although surveillance for vectors of dengue has traditionally focused on immature stages of *Aedes* mosquitoes, surveillance methods targeting adult female population can provide a more direct assessment of the impact of interventions on dengue infection (Achee et al. 2015). Entomologic surveillance is an important component of vector control programmes that forms a basis of appropriate and timely decision making on intervention measures. Not only does it inform on the changes in the presence, abundance and geographic distribution of the target species but also highlights the areas of high density infestation in addition to being an effective

evaluation tool for vector intervention (WHO 1997). Depending on the objectives of the surveillance activity among other factors, vector surveillance methods employed may include larval surveys, human bait collections, collection of resting mosquitoes, ovitraps and insecticide susceptibility bioassays. The *Stegomyia* indices have traditionally been used in surveillance activities to estimate the entomological measure of risk of dengue virus transmission (WHO 1997; Focks 2004). The indices which are based on the presence of immature stages of *Aedes* mosquitoes include house index (HI) (percentage of houses infested with larvae and/or pupae), container index (CI) (the percentage of water-holding containers infested with larvae and/or pupae), and Breteau index (BI) (number of containers infested with larvae and/or pupae per 100 houses inspected) (Tun-Lin et al. 1995b; Tun-Lin et al. 1996; Focks and Chadee 1997; WHO 1997; Morrison et al. 2004; Bowman et al. 2014) . Values obtained from each of these indices are related to the density figure/index (range: 1 to 9) to estimate transmission thresholds for dengue and yellow fever viruses. *Stegomyia* indices corresponding to a density figure of >5 on the scale (HI >29-37; CI >15-20; BI >35-49) is considered to indicate high virus transmission risk and a possible dengue outbreak (Brown 1974; Service 1974; Focks 2004).

The *Stegomyia* indices assume a strong association between immatures and adult female mosquitoes and thus dengue transmission. However, their validity and sensitivity in estimating risk of dengue transmission has been questioned due to a number of shortcomings (Focks 2004). Their failure to account for larval mortality, heterogeneity in container productivity, spatial association of larvae and their development sites, and temporal differences in mosquito life stages, point to a weak correlation of these indices with the abundance of adult mosquitoes (Tun-Lin et al. 1996; Focks and Chadee 1997; Getis et al. 2003; Morrison et al. 2004; Bowman et al.

2014). In addition the indices do not take into account susceptibility of human population to dengue virus infection among other important factors. As such the relationship between these indices and dengue transmission in unclear and despite the fact that transmission thresholds based on these indices may be applicable in some situations, a universal critical threshold for dengue is lacking (Bowman et al. 2014). As a result a weak association between the *Stegomyia* indices and dengue virus transmission (Tun-Lin et al. 1995a; Tun-Lin et al. 1996; Focks and Chadee 1997), the pupal indices (Number of pupae per person, Number of pupae per container) have been proposed as a suitable alternative in the evaluation of dengue risk (Focks and Chadee 1997; Focks et al. 2000; Focks 2004) and in identifying the most productive types of containers (Barrera et al. 2006; Lenhart et al. 2006; Midega et al. 2006; Chadee et al. 2009). Compared to larvae, pupal mortality is low and well characterized and it is possible to have absolute counts of pupae in containers. This coupled with the ease with which *Aedes* pupae can be separated and identified to species on emergence as adults make it possible for pupal densities in containers to be strongly correlated with the number of adult mosquitoes. Thus pupal indices are found to be strongly associated with dengue transmission (Focks and Chadee 1997; Focks 2004; Edillo et al. 2012). However, pupal surveys can be limited by the existence of unknown cryptic or inaccessible breeding sites that may be very productive (Achee et al. 2015).

In vector surveillance methods based on detection of adult mosquitoes landing rate or indoor resting density in a given time period may be used to express the abundance of adult mosquitoes (WHO 1997). Although human landing collection is labour intensive it is a sensitive tool for surveillance of adult mosquitoes especially when the infestation levels are quite low as it is often for *Ae. aegypti* mosquitoes (WHO 1997; Bowman et al. 2014). Resting collection involve

systematic searches for resting mosquitoes in and around households, where each house is taken as a unit for estimating abundance. Oviposition traps are valuable in detecting the presence or absence of vectors in an area (Focks 2004). Aspirators, gravid traps (Chadee and Ritchie 2010) and Biogents Sentinel traps (Krockel et al. 2006) have been proposed as inexpensive means to measure actual adult populations (Achee et al. 2015). During surveillance strategies individual households are considered the appropriate spatial unit for entomological surveys and based on standard sample size calculations and resource limitations every center house in a selected cluster is sampled. This is especially so because immature forms of *Ae. aegypti* lack a spatial structure (Getis et al. 2003).

2.5 Control of the dengue vector *Ae. aegypti*

The main objective of vector control for dengue is to reduce transmission to levels that will result into decreased incidence of disease and eventually prevent dengue outbreaks (WHO 2012). Control activities usually target immature and adult stages of the vector in their habitat within the household environment and other areas of potential vector–human contact (WHO 2009). According to the Ross-Macdonald model (Smith et al. 2012), interventions targeting adult vector mosquitoes are bound to have the greatest impact on reducing virus transmission (Achee et al. 2015). Such interventions are those that are aimed at reducing the abundance of mosquito vectors, their daily survival and vector-human contact. However, under certain circumstances interventions directed against the immature stages of the mosquitoes may also have a significant impact in reducing pathogen transmission (Smith et al. 2013). Traditionally, *Ae. aegypti* control has been done by elimination or management of breeding sites, removal of immatures by

larvicides and biocontrol agents, and the killing of adults especially during epidemics, by use of insecticides (Getis et al. 2003; Erlanger et al. 2008; WHO 2012). The success of these traditional intervention measures has been questionable and has often been judged ineffective (Erlanger et al. 2008) due to several drawbacks, which include insecticide resistance (Ranson et al. 2008; Achee et al. 2015), inadequate coverage, short lived effects of insecticides, and lack of reliable entomologic indices for the evaluation of the impact of control measures (Barrera et al. 2008; Achee et al. 2015). Consequently, development of new vector suppression methods and/or the technological improvement of the existing tools are currently in progress (Achee et al. 2015). Some of these methods include, use of transgenic mosquito strains which affect fertility of *Ae. aegypti* populations (Wise de Valdez et al. 2011), entomopathogenic fungi (Luz et al. 2007; Scholte et al. 2007), and lethal ovitraps (Barrera et al. 2014) that attracts and kill gravid mosquitoes. The goal of eradicating dengue vectors by several control programs in the 1950s-1970s was unattainable (Reiner et al. 2016) and reinvasions were reported in areas where the vectors had been successfully eliminated (Kourí et al. 1998; Ooi et al. 2006). These control programs that were so rigorous and successful but could no longer be sustainable due to a combination of factors such as low herd immunity, virus introduction by immigrants from dengue endemic zones, breakdown in eradication measures and case- reactive approaches to vector control (Kourí et al. 1998; Ooi et al. 2006). Thus contemporary intervention programs have since shifted the strategy and are now focused more on reducing *Ae. aegypti* populations to levels that are below the entomological thresholds required for virus transmission (Getis et al. 2003; WHO 2009). World health Organization (WHO) recommends the adoption of integrated vector management (IVM) strategy to combat dengue. This rational decision making process for

the optimal use of limited resources for vector control, emphasizes among other things, the use of integrated approaches to disease control, in which various methods of disease and vector control are integrated (WHO 2009). Integrated vector management has been found to be an effective strategy in dengue vector control intervention efforts compared to single intervention measures (Erlanger et al. 2008).

2.5.1 Control of immature *Ae.aegypti* mosquitoes

Unlike the control of other major vector-borne diseases, which target adult vectors, the control of *Aedes* mosquitoes is typically directed against the immature stages of the mosquito, principally by eliminating containers that serve as breeding sites (WHO 2009). Elimination of container habitats may entail making them inaccessible for oviposition, management of containers to disrupt development cycle or prevent production of adult mosquitoes by use of biological or chemical agents. These measures can be complemented with social mobilization and legislation to have better outcome and sustainability of larval interventions (Achee et al. 2015). In addition larval source management (LSM) measures can have a greater impact if they are targeted to a sub set of most productive containers in what is commonly referred to as targeted larval control (WHO 2009; Smith et al. 2013). Several studies have shown that identification and treatment of productive larval habitats can also be cost effective, thus making larval control more feasible in a given local setting.(Suaya et al. 2007; Toledo et al. 2007; Tun-Lin et al. 2009)

2.5.1.1. Environmental management for larval control

Aedes mosquitoes appear to thrive well in human dwellings often characterized by inadequate and unreliable piped water system, sanitation and waste disposal services as observed in many sprawling unplanned urban settlements around the world (Maciel-de-Freitas et al. 2007; WHO

2009). These conditions promote traditional water storage practices and proliferation of discarded containers that create potential breeding habitats for *Aedes* mosquitoes. Therefore, sustainable environmental management practices can have a significant impact in reducing *Aedes* mosquito populations. Environmental management for larval control entails changing the environment in such a way that vector propagation is prevented or reduced by modifying and/or manipulating the environment where vector mosquitoes thrive (WHO 2009). In modifying the environment, long term physical infrastructural changes undertaken to reduce larval habitats, such as provision of reliable piped water supply system to households. Reliable tap water supply makes it unnecessary for people to store water in containers that would otherwise serve as potential breeding sites for *Aedes* mosquitoes. Environmental manipulation on the other hand involves temporary measures aimed at changing larval habitats to make them unsuitable or unavailable for mosquito breeding. Some of these measures include management of water storage containers and other water receptacles in households by provision of tight fitting lids and mesh screens (Chadee et al. 2009; Philbert and Ijumba 2013; Phuanukoonnon et al. 2005), frequent emptying and thorough cleaning (Subra 1983; Maciel-de-Freitas et al. 2007; Hiscox et al. 2013).Tight fitting lids are ideal for large containers that keep water for long periods of time while mesh screens are handy in rain harvesting tanks where they allow inflow of rainwater while blocking access to gravid mosquitoes. Weekly emptying and cleaning water storage containers such as drums and buckets can prevent production of adult mosquitoes by interrupting the aquatic phase of *Aedes* mosquito life cycle which lasts for at least 9 days under optimum conditions (Christophers 1960). In a study done in Thailand, covering of water-storage containers with tightly fitting lids and cleaning of containers was reported to have significant

reduction in container infestation by *Ae. aegypti* larvae from a container index of 37.6% to 12.1% (Phuanukoonnon et al. 2005). Other environmental manipulation methods include unblocking roof gutters and drains and recycling or proper disposal of unused containers and tyres, practices that effectively render such water receptacles unavailable for breeding activities of the mosquitoes (WHO 2009). The choice of environmental management options is determined by practicability and appropriateness of the method to the local situation. Environmental management measures can produce better results if they are accompanied by rigorous health education and community involvement in order to effectively influence behaviour change in the local population (Heintze et al. 2007; Erlanger et al. 2008).

2.5.1.2 Chemical control of *Ae.aegyti* larvae

Chemical larvicides are widely used in dengue vector control interventions, however WHO (2009) recommends their use for intervention to augment environmental management practices targeting mainly productive containers that cannot be covered, removed or recycled. An effective larvicide should have among other factors, low mammalian toxicity, little or no impact on non-target organisms and persistence in the environment (Invest and Lucas 2008). Substances recommended for larval control are placed into three categories; organophosphates (temephos and pyrimiphos- methyl), Insect growth regulators (Diflubenzuron, rs-methoprene, Novaluron, and Pyriproxyfen) and Biopesticides (*Bacillus thuringiensis israelensis* and Spinosad) (WHO 2009).

Temephos (Abate®) formulated as granules or emulsifiable concentrate is the most frequently used organophosphate in many dengue intervention programs (Bang and Pant 1972; Suaya et al. 2007; Toledo et al. 2007; Erlanger et al. 2008; Tun-Lin et al. 2009). At a recommended dosage

of 1mg per liter of the active ingredient (1ppm) (WHO 2006) temephos is lethal to *Ae. aegypti* larvae in domestic and potable water containers. This larvicide whose normal use has been found to be safe in drinking water is generally acceptable in households (Bang and Pant 1972; WHO 2006; Suaya et al. 2007; Tun-Lin et al. 2009) and has prolonged toxicity lasting up to 24 weeks (Bang and Pant 1972) making it ideal for long term control of *Ae. aegypti*. Pyrimiphos-methly (Actellic®) is classified under slightly hazardous chemical compounds and may not be suitable in treatment of drinking water (WHO 2006). It is formulated as emulsifiable concentrate that is usually applied at a dosage of 1mg per liter of water (WHO 2009). Development of resistance to organophoshates has been reported in different parts of the world and this has generated interest in use of alternative larvicides such as insect growth regulators and biopesticides (Andrade and Modolo 1991; Lima et al. 2003; Jirakanjanakit et al. 2007; Seccacini et al. 2008).

Insect growth regulators are generally less hazardous to human and other mammalian species at recommended dosages. However, they may affect non-target organisms especially arthropods, thus their use in sites with other arthropod species may be limited and may require some impact assessment prior to their use (WHO 2006; WHO 2009). Fomulations and recommended dosages for some insect growth regulators include Pyriproxyphen (Sumilarv) granules, 0.01ppm, Methoprene emulsifiable concentrate, 1ppm, Diflubenziuron wetable powder, emulsifiable concentrate or tablet, 0.02-0.025ppm (WHO 2009). Insect growth regulators inhibit emergence of adult mosquitoes by inducing larval and pupal deaths at different stages of development (Braga et al. 2005; Seng et al. 2006) and like biopesticides there are no reported cases of resistance hence they can be suitable alternatives to the chemical larvicides (WHO 2006; Invest and Lucas 2008). Pyriproxyphen and methoprene cause mortality at the pupal stage, while

Novaluron and Diflubenzuron induce death of larvae at early and late instar stages respectively (Braga et al. 2005; Achee et al. 2015).

2.5.1.3 Biopesticides in the control *Ae. aegypti* larvae

Various microbial insecticides have been found to be effective against mosquito larvae. *Bacillus thuringiensis var israelensis* (Bti) and spinosad are some of these biopesticides that are currently being used in container treatment for the control *Ae. aegypti* larvae (WHO 2009; Boyce et al. 2013; Achee et al. 2015). Several studies have shown that Bti, at the recommended concentration of 1-5mg per liter is effective in the control of immature stages of dengue vectors in different environmental settings and breeding habitats, eliminating almost all of the larvae in treated containers within 24 hours and remains effective for an average period of 2 to 4 weeks (Haq et al. 2004; Lee and Zairi 2005; Lee and Zairi 2006; Ritchie et al. 2010; Tan et al. 2012). Bti is available in different commercial formulations which include wettable powders, granules, briquettes, slow release tablets and emulsifiable concentrates that allow application in diverse breeding sites possible. In addition some of these formulations allow application of high potency compounds while having little or no effect on the quality of domestic water (Mulla et al. 2004). However, water dispersible granules are preferred in most cases, thanks to the ease of application by conventional methods (Lacey 2007; WHO 2009). The bacteria which is essentially active against mosquito and black fly larvae (Merritt et al. 1989; Mittal 2003; Lacey 2007) has little or no adverse effects on non- target organisms and is safe for application in drinking water containers (Merritt et al. 1989; Hershey et al. 1998; WHO 2006; WHO 2009), thus suitable for community use. Lethal effect of Bti on mosquito larvae is attributed to a variety of endotoxins that are released upon ingestion and when activated damage the cells of the midgut epithelium

causing larval death (Gill et al. 1992). Complexity of the mode of action by these toxic proteins lessens the selection pressure on the target larvae thereby delaying resistance development under natural conditions (Boyce et al. 2013). The impact of Bti particularly is further enhanced by fact that it gradually settles at the bottom of treated containers where *Ae. aegypti* larvae commonly graze (Christophers 1960), thus promoting its uptake by target larvae. Various studies have integrated Bti with other larval intervention measures with reports of improved efficacy in suppressing larval populations and reducing entomological indices (Lee and Zairi 2005; Lee et al. 2005; Ocampo et al. 2009; Marcombe et al. 2011; Boyce et al. 2013). Some of the integration strategies have been found to be effective at prolonging the residual activity of Bti (Lee et al. 2005).

Bacillus sphaericus (Bs) whose mode of action against mosquito larvae is similar to that of Bti has limited application in the control of *Ae. aegypti* larvae owing to its inability to effectively suppress larval populations in treated containers (Monnerat et al. 2004).

Spinosad is another contemporary biolarvicide that is active against *Ae. aegypti* larvae with a residual effect lasting up to 15 weeks (Bond et al. 2004; Marcombe et al. 2011). The larvicide which is a combination of two potent neurotoxins (spinosyn A and D) is produced by a soil fungus *Saccharopolyspora spinosa* and is active against various insects in the order Diptera, Lepidoptera and Coleoptera. The mode of action is based on hyperexcitation of central nervous system leading to paralysis and death of target insects (Salgado et al. 1998; Watson 2001). At the recommended dosage of 0.1-0.5 ppm, spinosad is available in three formulations; tablet for direct application, granules and suspension concentrate (WHO 2009). Despite the fact that spinosad has minimal risks to human health, its broad spectrum activity can be toxic to non target organisms,

especially aquatic invertebrates thus an impact assessment may be required before its use in some cases (Bond et al. 2004).

2.5.1.4. Biocontrol organisms for the control of mosquito larvae

Biological control targeting mosquito vectors is mainly based on a variety of organisms that predate, parasitize or compete with mosquito larvae. This intervention method is considered to be among the practical alternatives to synthetic chemical larvicides whose widespread use has often been associated with environmental damage and development of resistance among the target mosquito species (Rozendaal 1997; WHO 2009). Organisms that have been investigated for the control of dengue vectors include predatory arthropods such as copepods (*Mesocyclops* spp.), dragon fly larvae (*Crocothermis* spp.), mosquito larvae in the genus *Toxorhynchites* and larvivorous fish (Gerberg and Visser 1978 ; Sebastian et al. 1990; Martinez- Ibarra et al. 2002; Nam et al. 2005; Erlanger et al. 2008; Nam et al. 2012). Some of these organisms are known to occur naturally in water storage containers especially in large drums and water tanks harbouring *Aedes* mosquito larvae (Nam et al. 2005) and this can therefore make their application in vector control activities more practical in local ecological settings. Although success in the use of predatory organisms to control larval mosquito populations has been reported in some regions of the world, evidence-based control programs that link introduction of predaceous organisms to actual reduction in disease burden, are still limited (Huang et al. 2017). Moreover, some of the organisms such *Toxorhynchites* spp. are sylvatic thus limiting their application in urban environment where most arboviral vectors thrive, while other such organisms may be exotic to local ecological settings where their introduction may require critical cost-benefit analysis to avert any possible ecological risks (Huang et al. 2017). Other novel biocontrol agents such as

entomopathogenic fungi are currently being investigated for the control larval mosquito vectors of arboviruses (Achee et al. 2015; Huang et al. 2017).

Predatory cyclopoid copepods

Different species of predaceous copepods are popular in biological control of mosquito larvae. In several studies they have been reported to effectively reduce the population of *Ae. aegypti* in large water storage containers both as single control agents and when integrated with other source reduction measures in diverse ecological settings (Marten et al. 1994; Jennings et al. 1995 ; Gorrochotegui-Escalante et al. 1998; Nam et al. 2005; Nam et al. 2012; Suárez-Rubio and Suárez 2004). However, better results were often obtained where treatment with *Mesocyclops* was integrated with other source reduction measures such as environmental management and biopesticides with community involvement. For instance, in northern Vietnam a community-based dengue control program that involved treatment of large concrete tanks and wells with local *Mesocyclops* species achieved complete control of *Ae. aegypti* immatures in two villages with control efficacy of over 95% in three other villages (Kay et al. 2002). Similar results were reported in central (Nam et al. 2005) and southern Vietnam (Nam et al. 2012). In these and other similar studies *Mesocyclops* were always augmented with recycling of discarded containers where appropriate and clean up campaigns, to remove small domestic containers unsuitable for *Mesocylcops* treatment. Integration with Bti was also found to yield good results especially if containers had late instar larvae, because *Mesocyclops* mainly predate on first and second instar larvae (Marten et al. 1994). In Queensland Australia, introduction of *Mesocyclops* and *Metacyclops* effectively reduced densities of *Aedes* larvae in subterranean habitats with extended control effects to other habitats as a result of flooding (Kay et al. 2002). Suitability of cyclopoid

copepods in the control of *Aedes* larvae is attributed to their high reproductive potential and ability to survive best in containers thus capable of maintaining long-term population in containers where they are appropriately maintained (Marten et al. 1994). In addition their small size makes it economical and convenient for mass production and distribution (Suarez et al. 1992).

Predatory *Toxorhynchites* spp

Toxorhynchites species reported to be predaceous on *Ae. aegypti* larvae include *Toxorhynchites splendens, Tx. brevipalpis, Tx. moctezuma, Tx. amboinensis* and *Tx. rutilus* (Yasuno and Tonn 1970; Trpis 1973; Padgett and Focks 1980; Sherratt and Tikasingh 1989). Of these, only *Tx. amboinensis* has been found to adapt well to urban environments and successfully reduce *Ae. aegypti* populations (Focks et al. 1985; Focks et al. 2014). Most of the other species are sylvatic where they have been shown to effectively control *Aedes* species breeding in natural breeding habitats including treeholes, thus limiting their application in the control of the highly domestic *Ae. aegypti* mosquitoes. Predatory hehaviour of *Toxoryhnchites* on larvae of other important vectors of the genus *Aedes* such as *Ae. polynesiensis* and *Ae. albopictus* has been investigated with reports of significant reductions in the population of larval mosquitoes (Mercer et al. 2005; Nyamah et al. 2011). Since *Toxoryhnchites* do not feed on blood their release in to the environment is unlikely to cause any health hazard.

Larvivorous fish

Traditionally indigenous larvivorous fish species have been exploited in the control of larvae of mosquitoes of medical importance (Bay 1967; Neng et al. 1987; Erlanger et al. 2008). Unlike other biological control agents, larvivorous fish do not discriminate between the different instars

of immature mosquitoes in their predatory behaviour; hence they may efficiently impact populations of immature mosquitoes. Most of these fish species tend to feed on other aquatic organisms a behaviour that is likely to have off-target effects on other arthropod species (Linden and Cech 1990; Van Dam and Walton 2007), and as such use of indigenous species is recommended for vector control programs (Huang et al. 2017). Moreover, a thorough environmental impact assessment is of necessity when introductions are made in new ecological settings, thus the nature of mosquito infested water and interaction with local fauna need careful consideration (Bay 1967). However, since majority of dengue vectors breed in domestic and peridomestic container habitats which are closed water systems, introduction of larvivorous fish is much less likely to have a negative impact on the environment. Examples of fish species that have been used in control of arboviruses include *Gambusia spp., Poecilia spp., Ictalurus spp*., *Astyanax* spp., *Lepisostus* spp., *Brycon* spp and *Tilapia mosambicus* (Erlanger et al. 2008; Huang et al. 2017). A study done in Taiwan showed the potential of simultaneous application of four different species of larvivorous fish: *Gambusia affinis, Poecilia reticulata, Tilapia mossambicus, and Sarotherodon niloticus* in the control of dengue vectors, *Ae. aegypti* and *Ae. albopictus* larvae in domestic water storage containers (Wang et al. 1990). In this study the container index reduced from 97.32 in containers without fish to 18.00 within four months of treatment with predatory fish. A similar study evaluated five indigenous fish species, *Lepisoteus tropicus, Astynax fasciatus, Brycon guatemalensis, Ictalurus meridionalis* and *Poecilia sphenops* in southern Mexico and found them to be valuable in the control of *Ae. aegypti* (Martinez- Ibarra et al. 2002). Larvivorous fish species have also been integrated with other larval abatement

measures with encouraging results being reported from different parts of the world (Kramer et al. 1988; Lardeux et al. 2002; Phuanukoonnon et al. 2005).

2.5.2 Control of adult *Ae. aegypti* mosquitoes

Existing vector intervention methods for adult mosquitoes are mainly based on chemical insecticides. These methods can be broadly categorized into, space spraying, indoor residual spraying(IRS) and personal protection (Achee et al. 2015). Insecticide space spraying strategies are recommended for control only in emergency situations to mitigate an epidemic or when source reduction is less effective, whereas IRS and personal protection can be used for sustained management of vector populations. The various methods adopted for the control of adult vectors of dengue work by reducing vector abundance, shortening of their lifespan and preventing vector-human contact.

Chemical control methods targeting adult vectors of dengue have been shown to have limited success in most cases despite their continued use (Reiter and Nathan 2001; Erlanger et al. 2008; Reiner et al. 2016), hence the increased interest in development of novel intervention tools and/or improvement of the existing ones through modern technologies (Achee et al. 2015). Some of these new tools include, toxic sugar baits, lethal ovitraps (Perich et al. 2003; Ritchie et al. 2004), insecticide treated materials (Kroeger et al. 2006) and those that involve behaviour modifications to influence the vectorial capacity of mosquito. Others involve release of *Wolbachia* infected *Ae. aegypti* mosquitoes (Hoffmann et al. 2011; Lambrechts et al. 2015), and molecular techniques such as para-transgenesis and introduction of antimicrobial genes (RIDL) that impact female adult populations and vectorial capacity (Wilke and Marrelli 2015).

These genetic control methods are aimed at producing mosquitoes that are either refractory to dengue viruses with a potential to replace natural populations (Moreira et al. 2000; Achee et al. 2015) or carry lethal genes that can suppress target mosquito population (Heinrich and Scott 2000). *Wolbachia spp,* an intracellular bacterium limits replication of the virus in the infected mosquitoes thus reducing transmission potential in addition to shortening the lifespan and decreasing susceptibility of mosquitoes to infection with arboviruses. Since the agent is maternally inherited it has a potential for area- wide implementation (Hoffmann et al. 2011; Ye et al. 2015).

2.5.2.1 Insecticide space sprays

Space spraying has been the main approach in the chemical control of adult dengue vectors in many parts of the world. A space spray is an aerosol that disperses extremely numerous tiny droplets of an insecticide into the air where they remain suspended for an extended period sufficient to achieve a large scale and rapid destruction of adult mosquitoes (Reiter and Nathan 2001; WHO 2009). Two methods are used to deliver the insecticide, dense thermal fogs and cold fogs. Thermal fog is produced by diluting the insecticide in large volumes of fuel oil or water and blasting it with hot air to vaporize and once it hits cool air it condenses to form a dense white cloud of fog. In cold fogs a mixture of ultra low volume (ULV) of insecticide formulations and cold water is passed through high pressure nozzles or high speed rotary nozzles used generate spray droplets without external heat (Reiter and Nathan 2001; WHO 2003; WHO 2009). For both methods vehicle- mounted and portable equipment are used to deliver the insecticide.

Application of the insecticide by vehicle-mounted equipment is ideal for use in urban areas and surrounding suburbs with good infrastructural network that can permit easy access between

buildings. While in the sprawling unplanned settlements, storeyed buildings and other areas not accessible by vehicle-mounted equipment, portable fog spraying equipment can be used. Where ground access is not possible due to infrastructural constraints and size of the target area (> 1000ha), or when rapid intervention is required, low-flying aircraft are recommended for release of space sprays (WHO 2009).

Some of the insecticides used for cold aerosols or thermal fogs space sprays include organophosphates: fenitrothion (250-300g/ha active ingredient), malathion (116-600g/ha), pyrimiphos-methyl (250g/ha) and pyrethroids: cyfluthrin (1-6g/ha), Deltamethrin (0.5-1.0g/ha, lambda-cyhalothrin (1g/ha) and permethrin (5-10g/ml)(WHO 2003; Erlanger et al. 2008). Since these insecticides are either slightly hazardous or moderately hazardous, choice of a given insecticide should be guided by environmental impact and community compliance (WHO 2009). For instance, oil based thermal fogs produce a thick dense smoke which is smelly and leaves an oily residue these properties may cause some community members to resent application of these insecticides and may even decline to the request leave their doors and windows open for indoor penetration of the space sprays.

Space sprays have been extensively used in various parts of the world to control dengue vectors with variable reports of successes and challenges (Espinoza-Gómez et al. 2002 ; Chadee et al. 2005; Erlanger et al. 2008; Boubidi et al. 2016). Most of the interventions involving space spraying has been executed in south east Asia (Erlanger et al. 2008). In Thailand a 99% reduction in *Ae. aegypti* density and landing rate was achieved by ground ULV applications of fenitrothion and malathion however repeated treatments at suitable intervals are recommended to maintain control for longer periods (Pant et al. 1971; Pant et al. 1974). In Vietnam insecticidal

aerosol cans were found to be more effective and feasible in reducing the number of dengue haemorrhagic fever cases compared to ULV fogging (Osaka et al. 1999).

Effectiveness of space sprays in the control of *Ae. aegypti* is influenced by a number of crucial factors that include droplet size, frequency of application, insecticide susceptibility and indoor penetration. Indoor penetration by the insecticide is particularly critical because it dependent on the structure of buildings, whether windows and doors are open or closed and when motorized applications are involved, weather conditions and route of spray vehicle (WHO 2009). Indoor application by portable equipment may be adopted in places where droplet penetration is likely to be inadequate. Frequency of application will depend on the susceptibility of target species and environmental considerations. To be effective space spraying should be done early in the morning or evening when ground temperatures are low and at wind speed of about 1-4 meters per second which is ideal for optimal drift of droplets of the insecticide downwind to the direction of travel. This timing also happens to correspond to the peak flight activity of dengue vectors which increases the likelihood of mosquitoes to be impinged by droplets of insecticide (Reiter and Nathan 2001; WHO 2003; WHO 2009). Space sprays do not remain effective for long periods of time and so spraying should be done repeatedly to effectively sustain suppression of the adult mosquito population (Reiter and Nathan 2001). World health organization recommends treatment cycles of 2-3 days for 10 ten days then once or twice a week (WHO 2009)

Although the efficacy of this approach to interrupt an ongoing epidemic is unclear, particularly due to limited indoor penetration in some situations, application just at the beginning of an

epidemic and on large scale coverage may reduce the intensity of transmission giving room to other intervention strategies (Newton and Reiter 1992; WHO 2009; Boubidi et al. 2016). The highly cryptic nature of *Ae. aegypti* mosquitoes presents a major challenge to the success of space sprays. The mosquitoes usually rest in secluded and sheltered indoor sites that are devoid of air currents thus hardly reached by aerosol droplets.

2.5.2.2. Residual surface treatment

Residual surface treatment involves indoor spraying and perifocal spraying of residual insecticides on potential resting surfaces for mosquitoes before or after taking a blood meal. The primary objective is to kill mosquitoes up on contact with the treated surface as well as to repel them from houses while retaining potency for relatively longer periods of time. This method has been recommended for dengue mitigation and sustained management of dengue vectors (Achee et al. 2015). Residual surface treatment can be done with hand-operated pressure sprayers but in case of perifocal treatment of large scale accumulation of containers, power operators can be considered for delivery of the insecticide (WHO 2009). Indoor residual spraying (IRS) entails application of the insecticide on walls and roofs or the ceiling inside of buildings while in perifocal treatment the residual insecticide is applied both on the inside and outside surfaces of non-potable water containers and on peripheral surfaces (WHO 2009). Although IRS is widely used in the control of malaria vectors (Lengeler and Sharp 2003; WHO 2008) limited application in many dengue vector control programs is evident (Gubler 1989) probably due sequestered resting habits of *Ae. aegypti* mosquitoes which in most cases are found resting on non- sprayable surfaces (Reiter and Gubler 1997). Since IRS primarily targets mosquitoes resting on walls, to be more effective in the control of dengue vectors other indoor resting surfaces preferred by *Aedes*

mosquitoes should be considered during application (Ritchie et al. 2004). Some of these surfaces include under furniture, in closets such as cupboards and wardrobes, hanging clothing, bed nets and curtains (Reiter 1991; Perich et al. 2000). Targeted IRS carried promptly and at an appropriate coverage (≥60%) of houses around a premise with a dengue case can significantly reduce the risk of DENV transmission (Vazquez-Prokopec et al. 2010) and in some instances IRS has been used successfully to control dengue vectors during epidemics (Roberts et al. 2010). Some of the challenges facing successful implementation of IRS include early recognition of increased risk of dengue transmission and speed of response (Achee et al. 2015), reduced coverage due to inadequate personnel, closed premises, and restricted entry to houses for control operations (Vazquez-Prokopec et al. 2010). Perifocal spraying with residual insecticides targeting discarded water receptacles that serve as potential breeding and resting sites can efficiently suppress *Aedes* mosquitoes by killing both larvae and resting adults (Bay 1967; Pettit et al. 2010). Several studies have reported the potential of residual treatment of potential larval habitats in suppressing the population of dengue vectors. In Australia residual treatment with bifenthrin, alpha cypermethrin and lambda cyhalothrin was found to prevent breeding of *Aedes* mosquitoes in discarded tyres for periods ranging from 2- 20 weeks (Nguyen et al. 2009; Pettit et al. 2010). In Cayman Islands a combination of perifocal treatment residual spraying of indoor and outdoor walls with temephos and larval source reduction efforts resulted in the eradication of *Ae. aegypti* (Nathan and Giglioli 1982). World Health Organization recommends up to 12 insecticide compounds for use in residual treatment for malaria control (WHO 2006). These insecticides are available in formulations such as wettable powders and emulsifiable concentrates most of which remain effective on applied surfaces for periods ranging from 2-6 months.

Currently new classes of insecticides that are effective at suppressing dengue transmission with a long lasting residual efficacy (6 months or more) and low off-target effects are being developed (Achee et al. 2015).

2.5.2.3 Personal and household protection

Individual and household protection measures are aimed at preventing or controlling biting activity of the dengue vectors. Personal repellents and protective clothing provide some protection from bites by dengue vectors and are therefore recommended especially during epidemics to control dengue transmission (WHO 2009). Protective clothing covers most of the skin surface and should be worn during daylight hours when dengue vectors are most active. Active ingredients in personal repellents used against *Ae. aegypti* mosquitoes include DEET (N, N-diethyl- 3-methylbenzamide), IR3535 (3-[N-acetyl-N-butyl]-aminopropionic acid ethyl ester) and Icaridin (1-piperidinecarboxylic acid, 2-(2-hydroxyethyl)-1-methylpropylester) (WHO 2009). Evaluation of various types of mosquito repellents have shown that DEET based repellents are not only safe under normal use but also provide long-lasting repellent effect compared to other synthetic and plant- based mosquito repellents (Mark et al. 2002). In this study a formulation containing 23.8% DEET provided complete protection against *Ae. aegypti* bites for an average time period of 5 hours after a single application. However, complete protection periods lasting up to 12 hours after single application have been achieved at higher concentrations (Gupta and Rutledge 1991). Repellents are usually applied to the exposed skin surfaces but may be also applied to clothing.

Household protection entails measures that help to reduce indoor biting by mosquito vectors. The methods range from screening of windows and doors, insecticide aerosols, vaporizers, mosquito

coils to use of insecticide treated materials (Bed nets, window curtains and water jar covers) (Kroeger et al. 2006; WHO 2009; Achee et al. 2015). Insecticide treated bed nets are widely used in the control of nocturnally transmitted vector borne diseases (Pedersen and Mukoko 2002; Kroeger et al. 2003; Nahlen et al. 2003; Mutuku et al. 2011) but have not found much application in dengue vector control efforts thanks to the day biting behaviour of *Ae aegypti* the primary vector of dengue. However, insecticide treated bed nets and treated lining can be effective against indoor resting *Ae. aegypti* mosquitoes (Lenhart et al. 2008), moreover infants and children sleeping under insecticide treated bet nets during the day may be protected from the day biting *Aedes* mosquitoes. Insecticide treated curtains and water container covers have been evaluated in the recent past for the control of dengue vectors with reports of significant success in the reduction of mosquito indices (Kroeger et al. 2006; Seng et al. 2008; Vanlerberghe et al. 2011). The Insecticide treated materials (ITMs) inside homes and personal repellents may complement indoor residual sprays for epidemic mitigation owing to their potential repellency effects or in the direct killing of resting *Aedes* mosquitoes that come into contact with the ITMs (Achee et al. 2015). In addition insecticidal jar covers were also found to reduce the parity rates of indoor resting adult females besides killing of the newly emerged nulliparous *Ae. aegypti* (Seng et al. 2008). Although ITMs have potential for reducing dengue vector densities, their impact as vector control tools is heavily dependent on acceptance and adequate coverage of the target population (Vanlerberghe et al. 2011).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Study areas

The study was conducted in the southern coastal region of Kenya, where two sites, each covering approximately 25 km², both in Kwale County, were selected: Ukunda, an urban site (4° 17' 59.9994" and 39° 31" 59.8794"), and Msambweni, a rural site (4° 28' 0.0114" and 39° 28’ 0.12"), located approximately 30 and 60 kilometers south of the port city of Mombasa, respectively (Figure 1). Selection of rural and urban sites was done in order to capture a range of possible human and environmental factors that influence vector ecology. The coastal climate is tropical: hot and humid throughout the year with annual mean temperatures of 23 – 34 °C and average relative humidity of 60 - 80%. Ukunda is a rapidly growing urban center located about a kilometer from the Indian Ocean with population density of about 2,000 people/ square kilometer. The area is characterized by a proliferation of unplanned residential houses, with unreliable water, sewer and waste management systems. Most residents engage in small scale trade, fishing and casual labour in the tourist industry along the Indian Ocean coast. Msambweni is a rural area located about 2 kilometers from the Indian Ocean. It has a population density of about 460 people/ square kilometer, where most of the residents are fishermen and subsistence farmers. Residents rely mainly on wells and rainfall for their water for domestic use, since the piped water system is inadequate and unreliable. The coastal region of Kenya has been experiencing periodic outbreaks of dengue fever in the recent years (Akhwale 2013; Ellis et al. 2015; Ochieng et al. 2015)

Figure 1 A map of Kenya showing the location of study areas: Ukunda (urban) and Msambweni (rural) both in Kwale county, southern coast of Kenya.

Residents in the two study sites store water for domestic use in diverse containers because the water supply system is unreliable. Water supply in the rural study sites is mainly from harvested rainwater, wells and boreholes, and these also supplement the irregular piped water supply

system characteristic of the urban study site. The study areas both located at an elevation of 24 meters above sea level are characterized by four seasons: long dry (January – March), long rainy (April – June), short dry season (July – September), and short rainy (October – December) (Mutuku et al. 2011; Onyango et al. 2013)

3.2 Mapping and selection of households for data collection

All households in the study sites were mapped using global positioning system (GPS) and each house was assigned a unique identification number. Forty sentinel houses in each of the two study sites were then selected from the mapped houses by simple random sampling; twenty houses for immature mosquito (larvae and pupae) sampling and the other 20 for oviposition surveys. Practicability of repeated mosquito surveys in sentinel houses in each site over an extended period (2 years) guided the choice of 40 houses. Each household chosen was contacted in order to obtain consent from the head of the household. If consent was obtained, the household was visited monthly throughout the duration of the study. If a sampled household did not consent to participate in the study, the most immediate neighbor was contacted. If a household that had been sampled and included in the study was unavailable for data collection at a given point in the study, the closest adjacent household was sampled in its place as a substitute.

3.3 Data collection

Demographic, environmental and entomological data was collected from the 40 households in each of the two study sites for a period of 24 months (from June 2014 to May 2016). To enable

determination of pupae persistence in households data collection in the 20 houses selected for immature mosquito survey was extended for a further 24 months (up to June 2018).

3.3.1 Collection of demographic and household information

Demographic and household information was collected monthly throughout the data collection period from households using paper forms (Appendix IV). Survey questions were designed based on known risk factors and control measures for vector persistence and abundance (e.g. use of mosquito repellent coils), as well as to collect general household and demographic information. Variables surveyed include type of housing (wall and roofing materials), use of bed nets, use of mosquito repellent coils, presence of open eaves, presence of grass or bushes in the peri-domestic environment and number of household occupants on the night prior to sampling. Where applicable, an attempt was made to observe the relevant variable as opposed to recording a reported response.

3.3.2 Entomological surveys

Entomological surveillance protocols (Appendix V) were used during mosquito surveys. The selected 20 sentinel houses were assessed for immature mosquito infestation once a month for a period of approximately two years (June 2014 - May 2016). To enable determination of pupae persistence or continued pupal presence in a household (> three months of pupal presence in a year) in study households pupal survey was extended for a further 24 months (up to June 2018). For the mosquito surveys, a household was defined as a single residential building, and its surrounding area within approximately a 10-meter radius. The distance between selected

households ranged from 100 to 200 meters. Informed consent was obtained from all heads of households, and when a house was inaccessible, it was replaced by the nearest house.

3.3.2.1 Larval and pupal sampling

All natural and artificial water-holding containers in and around each household were inspected for mosquito larvae and pupae. A spot light was used to inspect containers located in dark or shaded areas of the households. All pupae and larvae (3^{rd} and 4^{th} instars) from positive containers were collected with the aid of pipettes and ladles (Chadee et al. 2007), counted and recorded on field-data forms (Appendix IV). Water from large containers was first sieved and mosquito samples were placed in a white plastic tray with some water from which the immatures were pipetted. All pupae and a sample of at least ten larvae or all the larvae in case a container had less than ten larvae from each breeding site were placed in 10ml falcon tubes and/or Whirl- pak® plastic bags (Nasco, Fort Atkinson, WI), labeled, and taken to the Vector Borne Disease Control Unit (VBDCU) laboratory in Msambweni hospital. The larval samples were used to confirm field identification of *Ae aegypti*. Immature mosquitoes were reared in 200ml plastic cups under laboratory conditions at an average temperature of 28.15±1.8ºC and relative humidity of 80.9±6.3%, and larvae were fed on TetraMinbaby® fish food. Emerged adults were identified to species using standard taxonomic keys . *Aedes. aegypti* (L) subspecies were distinguished morphologically following keys as provided by Huang (Huang 2004).

The total number of pupae during a household visit in a given month is used as the pupae count for the household, while the number of containers sampled is a proxy for habitat availability and the sampling effort applied. The number of pupa counted was used as a measure of adult

productivity because of low mortality in pupa and emerging adults and also because it is possible to make absolute pupal counts (Focks and Chadee, 1977).

3.3.2.2 Oviposition survey

Oviposition traps (ovitraps) which are mostly used for surveillance of dengue vectors (Favaro et al. 2008) were used in this study to complement larval surveys in the investigation of the preferred location for *Ae aegypti* breeding activity within the domestic environment (indoor vs outdoor). Two modified ovitraps were placed in each of the 20 households that were randomly selected as fixed sampling points in each of the two study sites. Each ovitrap consisted of a black plastic cup and filled with about 350 ml of tap, borehole or rainwater. The inside of the cup was lined by a brown filter paper that was partially submerged (Figure 2). Eggs were laid on the damp filter paper just above the water line. The indoor trap was placed on ground level in a dark sheltered location of the living or bed room. Outdoor ovitraps were placed within a 10-meter radius in suitably sheltered locations. Ovitraps were set once a week every month for a period of 24 months (June 2014 – May 2016). The ovitraps were inspected and serviced (Chadee 2009) after 5 days. During servicing of the ovitraps the filter paper in each trap was removed, wrapped in white paper towels and placed in a plastic Ziploc bag that was labeled appropriately for storage and transportation to VBDCU laboratory at Msambweni county referral hospital.The ovitraps were then cleaned, refilled with clean water and a clean filter paper put in position and the trap set for another round of ovitrapping. In the laboratoryeach filter paper was examined under the dissecting microscope (X40) (Nikon® -SMZ Japan) for identification and counting of *Ae.aegypti* eggs. To confirm the species, the filter papers with eggs were submerged in seasoned

tap water for eggs to hatch and larvae reared to adults which were identified using standard taxonomic key (Huang 2004).

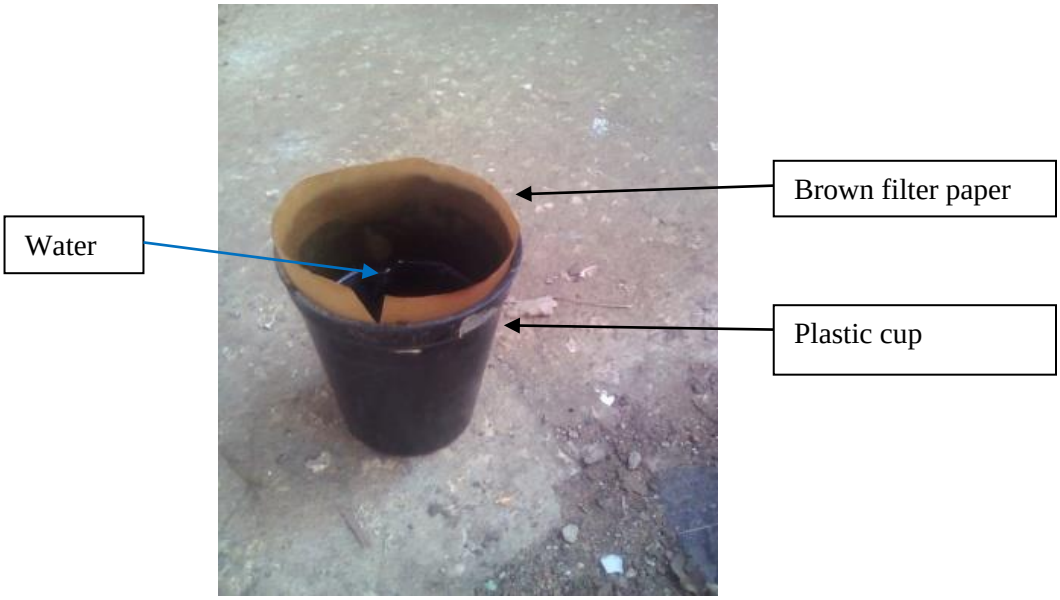
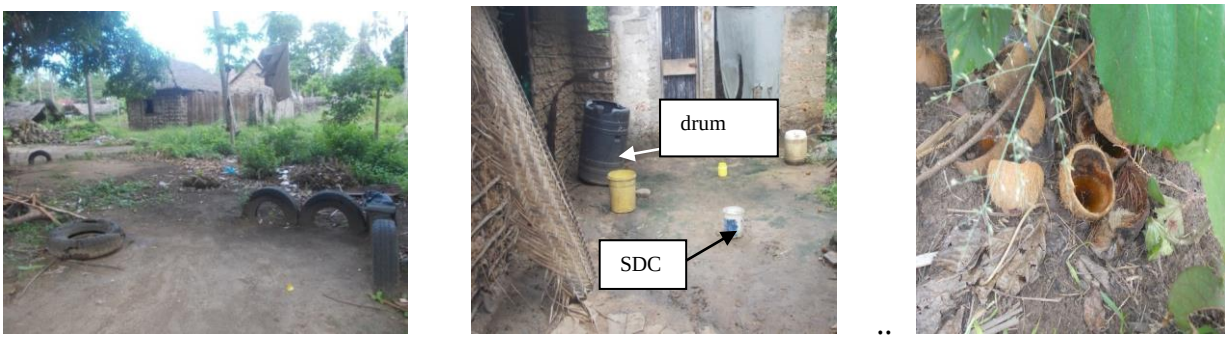


Figure 2. A modified oviposition trap for *Ae aegypti* mosquitoes.

3.3.3 Classification and characterization of container breeding habitats

Figure 3 shows some of the typical breeding sites for *Ae aegypti* in the study sites. A total of ten container types were identified and classified based on their use and material: drums, animal feeding containers (AFC), tires, pots, small domestic containers (SDC), treeholes, wells, buckets, jerrycans and others Drums were defined as 100 – 500 L capacity plastic or metal water storage containers. Animal feeding containers, ranged from small 1L bird watering and feeding containers made of plastic or cut tires, to large 300L concrete cattle watering containers. Pots included flower vases and water storage vessels made of clay. Small domestic containers included small plastic food containers, tins, bottles, plates, cans, cooking pots (sufuria) and jars. Others included; polythene bags, fallen leaves, coconut shells, hoof prints, drains, gutters, septic

tanks, shoes, cisterns and sinks. Wells were open dugout pits used for water provision in homesteads. For each breeding habitat, records were made on the container type (small domestic container, bucket, jerrycan, tire, drums, animal feeding trough, pot, tree hole, well and other), container capacity (small; < 2liters; medium, 2-7 liters; large, >7 liters and location in the household (indoor or outdoor)



a) Discarded tyers b) Plastic drums and SDCs c) Coconut shells

e). Animal feeding/watering containers d) Claypot ,jerrycan, buckets

Figure 3. Typical *Ae. aegypti* breeding habitats in coastal Kenya. *Abbreviation* SDCs: small domestic containers.

3.3.4 Climate data collection

Daily temperature and relative humidity data was collected hourly using HOBO data loggers (HOBO, Onset Computer Corporation, Bourne, MA, USA). One data logger was installed on sheltered location (under the eaves) of a selected house in each of the two sites. Rainfall data were collected daily using event data logger rain gauges (HOBO, Onset Computer Corporation, Bourne, MA, USA) that were placed in each of the two study sites. The rain gauges were fixed at 2 meters above the ground level on a metallic pole. Data from the data loggers was downloaded monthly using the boxcar software. All weather data were exported to excel sheets for management. Supplementary temperature data was obtained from publicly available data from Weather Underground (www.wunderground.com; weather station code for the coast is HKMO) and for rainfall from the National Oceanic and Atmospheric Administration (NOAA) Africa Rainfall Climatology (ARC) data at $0.1° \times 0.1°$ spatial resolution (Shah et al. 2019). The ARC dataset is produced using a combination of rainfall gauge measurements and METEOSAT satellite data to provide gridded rainfall estimates.

3.3.5 Susceptibility of larval *Ae. aegypti* to *Bacillus thuringiensis var israelensis* (Bti)

3.3.5.1 Bti Strain and dosages used

Susceptibility of *Ae. aegypti larvae* to a biocontrol agent Bti was investigated under semifield conditions during the rainy and dry seasons. A water dispersible granule formulation of the biopesticide VectoBac® WG (AM65-52) at the recommended concentration of 8mg/L was used in the bioassays. VectoBac® WG delivers 3000 international toxic units (ITU) per mg against *Ae.*

aegypti larvae. In this study three concentrations of VectoBac®: 1×, 10× and 20× the manufacturer's recommended dose (8mg/L, 80mg/L and 160mg/L respectively = 16mg, 160mg and 320mg in each 2-L plastic tray) were tested with untreated water as control. The formulation was weighed on a weighing balance and sprinkled on the water surface of the test containers where it dispersed uniformly over the water surface and gradually settled at the bottom of the containers.

3.3.5.2 Mosquito colony for the Bti susceptibility bioassays

Aedes aegypti eggs collected from the field study sites using ovitraps were used to raise a mosquito colony for the susceptibility bioassays. The eggs were submerged in seasoned tap water for hatching and larvae reared in 2 liter rectangular plastic trays under laboratory conditions at an average temperature of 28.15±1.8ºC and relative humidity of 80.9±6.3%. Larvae were fed on TetraMinbaby® fish food (Tetra Werke, Melle, Germany) ad libitum. Third and early fourth instar larvae (L3/L4) were used in the bioassays.

3.3.5.3 Test design

The experiment was conducted in 2 liter rectangular white plastic trays. The plastic trays were filled with tap water that was allowed to season for at least 24 hours prior to the experiment.

VectoBac® WG in the three concentrations was introduced into the test containers. Initial evaluation was done 24 hours after treatment. Ten laboratory reared *Ae. aegypti* larvae (3^{rd} and 4^{th} instar) from a field colony were introduced into the test containers at 24 hours post treatment followed by weekly introductions until mortality was less that 50% for two consecutive weeks.

The experiment was done in five replicates per treatment and run for 17 weeks from 7th January, 2016 to 24th April, 2016 period which covered both dry and rainy seasons. Rainfall and temperature at the site were recorded as described in the weather data. Larval mortality was determined at 24 hour and recorded daily for up to six days at each introductory period. Each day dead larvae were removed from the test containers and all pupae collected within 3 to 5 days post exposure. Pupae were placed in 200ml plastic emergence cups containing water from the respective test containers and emerging adults recorded appropriately. Test containers were placed suitably outside the insectary on a raised platform about 3 feet from the ground level under a partial shade but exposed to rainfall. The containers were covered by mosquito netting cloth to prevent oviposition by wild mosquitoes. Larvae were fed daily on TetraMinbaby® fish food (Tetra Werke, Melle, Germany) ad libitum.

3.4 Data Analyses

All data were entered and managed in Microsoft Excel 2007 and data analysis was performed using SAS 9.1 statistical software (SAS Institute, Gary, NC). For pupae persistence data all analyses were conducted in the R programming language (R Core Team 2018). *Moran's I* tests were conducted using the *spdep* R package (Bivand and Wong 2018). Preparation and visualization of spatial data was conducted in ArcGIS (Version 10.8). *BayesX* software package, via its interface *R2BayesX*, was used to build Generalized mixed models (GAMMs) and examine their output (Umlauf et al. 2015). Positive containers were considered to be those with one or more *Ae. aegypti* larvae or pupae. Proportion of wet containers that were positive in each site was determined and a Chi- square test was used to compare the distribution of positive

containers between indoor and outdoor locations. Key larval habitats were defined as containers that are most productive for *Ae aegypti* pupae. Kruskal Wallis test was used to compare the distribution of *Ae. aegypti* infestation in the four study sites, and productivity between seasons.

For pupal persistence, a spatial analysis and determination of risk factors for pupal persistence and abundance were done. Households were defined as persistent within a year if any pupae were found in the household in at least three of the 12 months surveyed within that year. Pupae persistence data was collected over four years, 2014 – 2015 (July 2014 – June 2015), 2015 – 2016 (July 2015 – June 2016), 2016 – 2017 (July 2016 – June 2017) and 2017 – 2018 (July 2017 – June 2018). This resulted in persistence data corresponding to 160 house-years, i.e. four years per household for 40 households.

Aedes. aegypti mosquitoes' average dispersal range has been found to be approximately50m to 100m per day (Trpis and Hausermann 1986; Getis et al. 2003; Harrington et al. 2005). The Moran's I statistic was used to test for spatial autocorrelation of pupae counts due to possible movement of adult *Ae. aegypti* between households (Waller and Gotway 2004). This allows for evaluation of spatial correlation of pupae counts between neighboring households. The Moran's I was conducted using household latitude and longitude values and a distance-based neighbor approach. Households were defined as neighbors if they were within a 150m radius of one another. Spatial autocorrelation amongst houses in overall pupae counts (pupae collected over the entire data collection period) was tested as well as seasonal pupae counts (long-dry season 2014, short-rainy season 2014, etc. for a total of 16 seasons). A sensitivity analysis of this test was conducted by testing a range of radii around the house of 50m to 500m (Appendix I).

A generalized additive mixed model (GAMM) framework was used to model the risk factors of pupal persistence and abundance in the study households (Wood 2017). Generalized additive mixed models allow modeling of linear and non-linear effects using penalized regression splines. Generalized additive mixed models were used to investigate the possible non-linear effect of seasons, as well as other factors such as habitat counts, temperature and rainfall. Separate GAMMs were built to evaluate pupal persistence and pupal abundance. In the abundance model a proportional-odds model framework (Antoine and Frank 2000; Harrell 2001) was used. The outcome was monthly pupae count categorized into one of four groups; zero (0 count), low (0 – 15 count), intermediate (15 – 30 count) or high (>30 count).

A separate regression model to evaluate the risk factors for pupal persistence was built. The persistence model evaluates the risk factors for continued pupal presence in a household (> three months of pupal presence in a year). Generalized mixed models were used to perform logistic regression to model the risk factors for within-year pupal persistence. House and site were included in all models as nested random effects. Spatial autocorrelation was accounted for by including a spatial term modeled via geosplines on the latitude and longitude of the households (Brezger et al. 2005; Kneib and Fahrmeir 2011; Umlauf et al. 2015). Average habitat count, and other demographic risk factors were included as terms in all models. Details on model building are shown in Appendix II.

The initial efficacy of *Bacillus thuringiensis* (*Bti*) was estimated through mortality of L3and L4 larvae within 24 hours after treatment. *Bti* persistence in test water was defined as the period in which larval mortality was 80%, estimated by the number of live pupae.

3.5 Ethical approval

Ethical approval and oversight for overall data collection for this study were obtained from the the Kenya Medical Research Institute (KEMRI SSC 2611)(Appendix III) Written and verbal, informed consent was obtained from relevant individuals in the households (heads of households) for each household included in the study at the beginning of the data-collection period.

CHAPTER FOUR

4. RESULTS

4.1 Climate factors at the study sites

During the study period, a mean annual temperature of 23–32 °C and annual rainfall of 1300 mm were recorded for the rural site at Msambweni (Figure 4) and a mean annual temperature of 23–34 °C; average annual rainfall of 1188 mm for the urban site at Ukunda (Figure 5). Seasonal variations in rainfall and temperature recorded for the rural and urban study sites were similar though a slightly higher amount of average temperature and rainfall was recorded in the rural site.

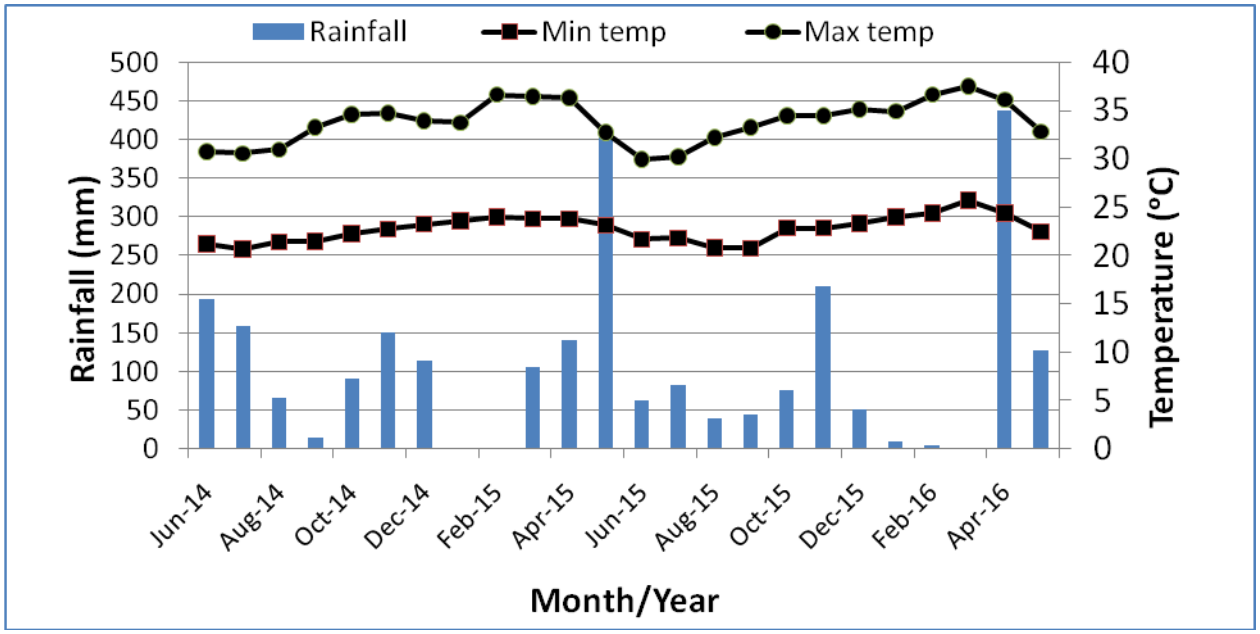


Figure 4. Monthly distribution of rainfall, minimum and maximum temperatures for rural site (Msambweni) from June 2014 to May 2016. Seasons: long rainy (April-June), short dry (July-September), short rainy (October-December); long dry (January-March).

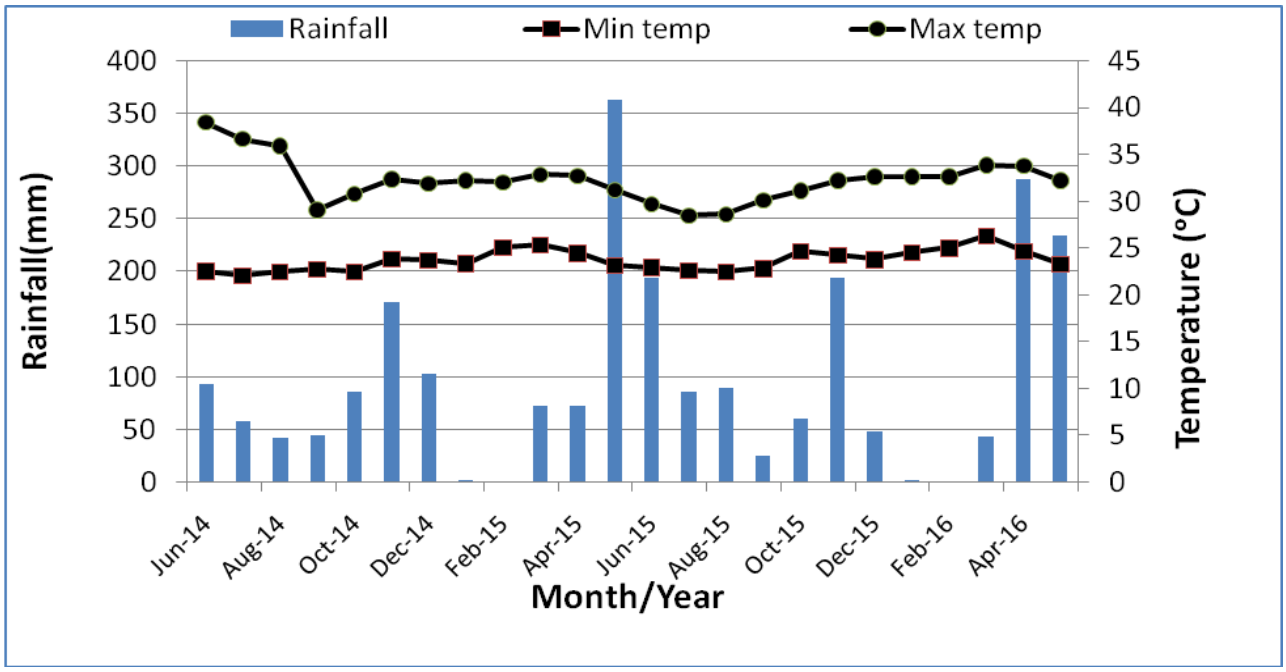


Figure 5. Monthly distribution of rainfall, minimum and maximum temperatures for urban site (Ukunda) from June 2014 to May 2016. Seasons: long rainy (April-June), short dry (July-September), short rainy (October-December); long dry (January-March).

4.2 Frequency and presence of *Ae. aegypti* immatures in container habitats

Collections were made from 6,566 wet container visits: Msambweni (3,199), and Ukunda (3,367). During the visits, some of the containers were repeatedly sampled. Of these, only 5.56%, and 4.54%, respectively, were positive for *Ae.aegypti* immatures. In both sites more positive containers were located outdoors (84.02 and 90.00%) than indoors (15.98 and 10%) $\left(\chi^2 = 112.4, \mathrm{DF} = 1, \mathrm{P} < 0.0001\right)$ $\left(\chi^2 = 895.1, \mathrm{DF} = 1, \mathrm{P} < 0.0001\right)$ for Ukunda and Msambweni respectively (Table1). There was a significant variation in the proportion of *Ae. aegypti* positive containers in outdoor locations in the two sites $\left(\chi^2 = 8.98, \mathrm{DF} = 3, \mathrm{P} < 0.05\right)$ (Msambweni

15.5%, and Ukunda 12.1%). Despite the low presence of *Ae. aegypti* positive containers indoors, the proportion of wet containers indoors was higher (52.8%) than outdoors (47.2%) in both study sites $\left(\chi^2 = 67.7, \mathrm{DF} = 1, \mathrm{P} < .0001\right)$. However, for the indoor container habitats, a much higher proportion $\left(\chi^2 = 50.4, \mathrm{DF} = 3, \mathrm{P} < 0.0001\right)$ of *Ae.aegypti* positive containers was observed in the urban site (43.1%) than in the rural site (31.0%) (Table1)

4.3 Container productivity profiles

A total of 15,817 immatures were collected from the two study site. Of these, 84.1% were identified as *Ae. aegypti*. All the *Ae. aegypti* (L) in this study were identified as *Ae. aegypti aegypti* (Aaa) and none as *Aedes aegypti formosus* (Aaf). Other mosquito species included: *Culex spp.* (12.5%), *Ae. simpsoni* (1.9%), and *Toxorhynchites* (1.5%). **A total of** 13,303 immature *Ae. aegypti* were collected from 331 positive container visits in rural and urban sites. Buckets, drums, and tires, produced 82.0% (2,250/2,744) of *Ae.aegypti* pupae in rural and urban study sites combined (Figure 6).

Table 1 Container types with *Ae. aegypti* larvae and pupae for indoor and outdoor locations in Msambweni [rural site (R)] and Ukunda [urban site (U)], southern coast, Kenya.

Habitat type	Indoor						Outdoor							
	Msambweni(R)			Ukunda(U)			Msambweni(R)			Ukunda(U)			Total larvae and pupae	
	Number of container visit (positive)	No. of Larvae	No. of Pupae	Number of container visit (positive)	No. of Larvae	No. of Pupae	Number of container visit (positive)	No. of Larvae	No. of Pupae	Number of container visit (positive)	No. of Larvae	No. of Pupae	Larvae	Pupae
Tires	0	0	0	0	0	0	18(6)	541	127	64(28)	1365	206	1906	333
AFC	0	0	0	2	0	0	33(3)	12	5	53(11)	891	99	903	104
Tree holes	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tanks	201(7)	195	91	294(12)	147	28	113(38)	2398	714	137(35)	728	104	3468	937
Wells	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pots	58(0)	0	0	27(2)	45	45	20(5)	236	32	21(8)	482	33	763	110
SDC	15(1)	0	1	85(1)	11	0	145(18)	547	134	178(7)	191	36	749	171
Buckets	1159(9)	73	26	666 (5)	19	0	766(80)	1523	468	559(19)	545	486	2160	980
Jerrycan	400(1)	0	1	785(5)	18	1	164(10)	155	16	368(9)	100	18	273	36
Others	26(0)	0	0	12(0)	0	0	81(5)	163	24	116(11)	175	49	338	73

AFC-Animal feeding container; SDC-Small domestic container

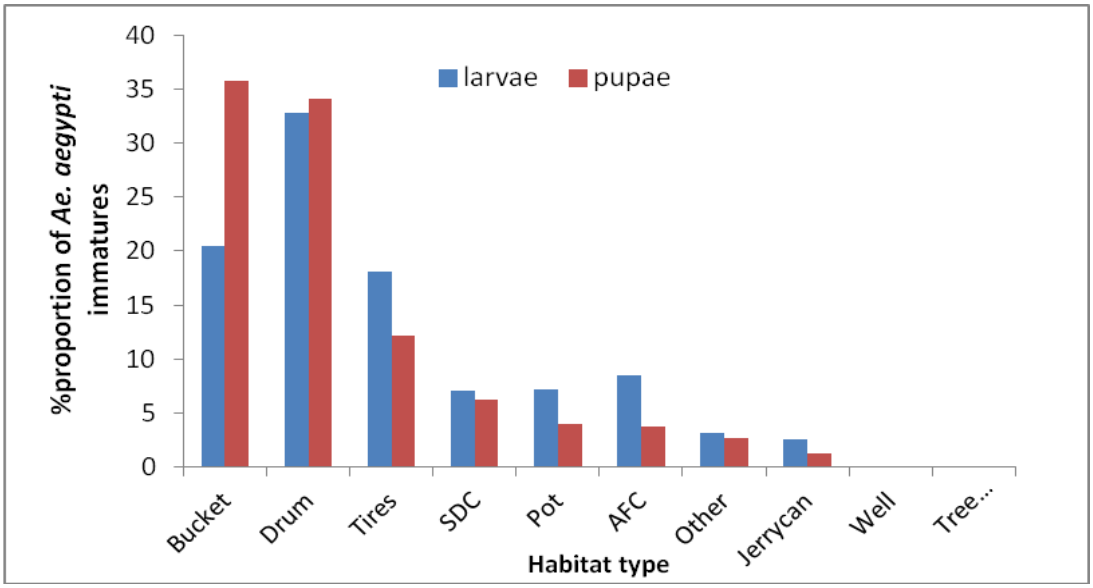


Figure 6**.** Overal container/habitat productivity profiles (%) for *Ae. aegypti* immatures in both rural (Msambweni) and urban (Ukunda) sites in the southern coast of Kenya from June 2014 to May 2016. *Abbreviations*: AFC-Animal feeding container; SDC-Small domestic container

Drums and buckets were the key *Ae. aegypti* containers indoors in the rural site, while in the urban site the most productive habitat types were pots and drums (Figure 7a), producing nearly all (117/119 and 73/74) collected *Ae.aegypti* pupae in the rural and urban sites respectively. Important outdoor containers in the rural site were drums and buckets which contained 78% of all pupae collected in the rural site, while in the urban site buckets and tyres were the main producers yielding 67.1% of total pupae from the urban site (Figure 7b). When productivity among sites was compared, rural site produced more *Ae. aegypti* pupae (16,39) than the urban site (11,05) however this difference was not significant (Kruskal-Wallis χ^2 = 3.3, DF=1, P< 0.0682)

a b

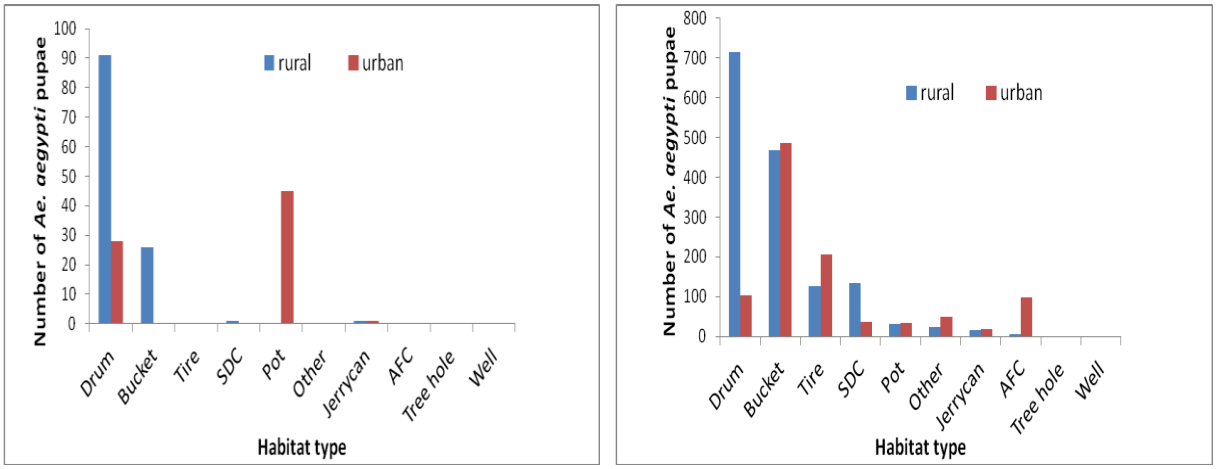


Figure 7. Productivity of *Ae. aegypti* container habitats located indoor (a) and outdoor (b) in rural and urban sites in the southern coast of Kenya during the study period. *Abbreviations*: AFC-Animal feeding container; SDC-Small domestic container

Over 80% of the containers inspected in both rural and urban sites were for water storage (drums, pots, buckets and jerrycans) and were important producers especially indoors (Table 1). They contributed 78.4% and 83.2% of larvae and pupae, respectively in the rural site, while in the urban site they contributed 44.2% and 65.1% of larvae and pupae, respectively. Other container types, such as tires, small domestic containers, animal food troughs, tree holes, and wells produced the rest of the larvae and pupae collected. Of these, tires contributed most of the larvae and pupae (Table 2).

Table 2**.** Productivity of water storage containers in comparison with other container types in rural (R) and urban (U) sites in the southern coast, Kenya

Container type	Msambweni (R)			Ukunda(U)		
Container type	Container count (+ve)	Larvae	Pupae	Container count (+ve)	Larvae	Pupae
Jerrycan	564(11)	155	17	1153(14)	118	19
Bucket	1925(89)	1596	494	1225(24)	564	486
Pot	78(5)	236	32	48(10)	527	78
Drum	314(40)	2593	805	431(47)	875	132
Total	2863(145)	4580	1348	2857(95)	2084	715
Other container types						
Other	107(5)	163	24	128(11)	175	49
SDC	160(19)	547	135	263(8)	202	36
AFC	33(3)	12	5	55(11)	891	99
Tire	18(6)	541	127	64(28)	1365	206
Treehole	0	0	0	0	0	0
Well	0	0	0	0	0	0
Total	318(33)	1263	291	510(58)	2633	390

AFC-Animal feeding container; SDC-Small domestic container

Analysis of relative pupal productivity by different container categories showed that buckets, tires and drums were the most productive in the urban site overall with 44.0%, 18.6%, and 12.0% pupae per container type, respectively (Table 3). While in the rural site drums and buckets were the two most productive container types with 49.1% and 30.1% pupae per container respectively (Table 4). Overall pupal productivity in the two sites varied significantly between different container categories (Kruskal-Wallis χ^2 = 276.5, DF=7, P< 0.0001). Pupal index was higher (0.47) in the urban site at Ukunda than in the rural site at Msambweni (0.35).

Table 3. Relative importance of container categories in the urban site (Ukunda**)**

Container category	Number of container Visits(N)	Total larvae in Each container category	Proportion of Larvae in each Container category (%)	Containers with Pupae (N)	Total number of Pupae(N)	Mean±SD (pupae per container type)	Frequency of containers with pupae (%)	Proportion of pupa in each container category (%)
Buckets	1225	564	12.0	7	486	0.4±9.12	0.20	44.0
Tires	64	1365	28.9	14	206	3.67±12.16	0.40	18.6
drums	431	875	18.5	16	132	0.33±2.80	0.46	11.9
SDC	263	202	4.2	2	36	0.13±2.10	0.06	3.3
Pots	48	527	11.2	6	78	1.5±5.68	0.17	7.1
AFC	55	891	18.1	4	99	1.18±6.74	0.11	9.0
"Others"	128	175	3.7	6	49	0.42±2.60	0.17	4.4
Jerrycans	1153	118	2.5	4	19	0.02±0.34	0.11	1.7
Wells	0	0	0	0	0	0	0.00	0.00
Treeholes	0	0	0	0	0	0	0.00	0
Total	3367	4727	100	59	1105			100

AFC-Animal feeding container; SDC-Small domestic container

Table 4. Relative importance of container categories in the rural site (Msambweni)

Container category	Number of container Visits(N)	Total larvae in Each container category	Proportion of Larvae in each Container category (%)	Containers with Pupae (N)	Total number of Pupae(N)	Mean±SD (pupae per container type)	Frequency of containers with pupae (%)	Proportion of pupa in each container category (%)
Drums	314	2593	44.4	25	805	2.50±17.62	0.78	49.1
Buckets	1925	1596	27.3	24	494	0.26±3.50	0.75	30.1
SDC	160	547	9.4	11	135	0.85±5.65	0.34	8.2
Tires	18	541	9.3	5	127	7.06±11.51	0.16	7.7
Pots	78	236	4.0	2	32	0.41±2.55	0.06	2.0
Others	107	163	2.8	1	24	0.31±2.42	0.03	1.5
Jerrycans	564	155	2.7	5	17	0.03±0.36	0.16	1.0
AFC	33	12	0.2	1	110	0.15±0.87	0.03	0.3
Wells	0	0	0	0	0	0	0.00	0.00
Treeholes	0	0	0	0	0	0	0.00	0.00
Total	3199	5843	100	74	1639			100

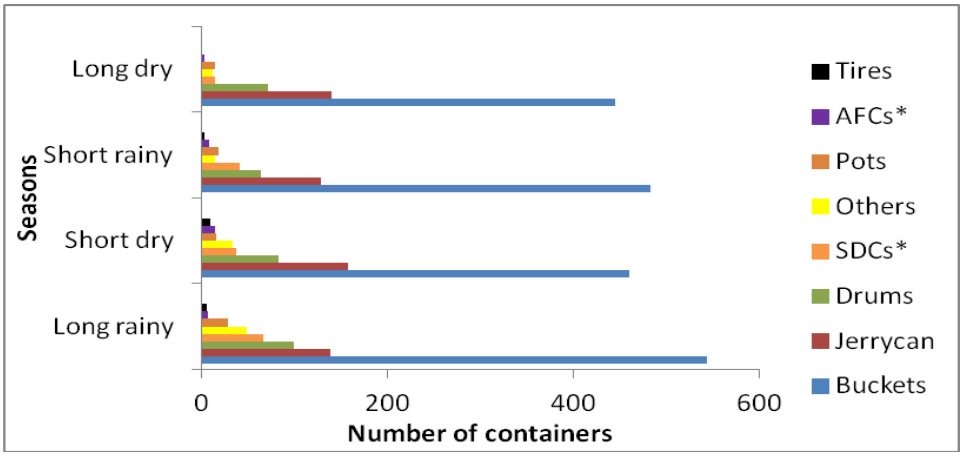
AFC-Animal feeding container; SDC-Small domestic container

4.4 Seasonal distribution of *Ae aegypti* in wet containers

The abundance and distribution of different types of container habitats followed a consistent pattern over the dry and rainy seasons in rural and urban sites (Figure 8a and b). In all the four seasons, buckets were notably the more abundant habitat type in the rural site while buckets and jerrycans were the abundant habitats in the urban site with over 400 and 200 container visits respectively in the four seasons.

Immature *Ae. aegypti* were found in more container types during the three seasons, i.e. long rainy (April- June), short dry (July-September) and short rainy (October-December) than in the long dry (January-March) season especially in the urban site (Figure 9a), In the rural site, during the long rainy season except for animal feeding containers (AFCs) and others, nearly all container types were important producers of *Ae. aegypti* immatures with drums having a highest productivity. In contrast, in the urban site tires were the main producers of *Ae. aegypti* immatures in the short dry season (Figure 9b). Productivity among seasons in both sites varied significantly (Kruskal-Wallis χ^2= 270.1, DF<0.0001) with more immatures in long rainy and short dry seasons and the least in long dry seasons.

a)



b)

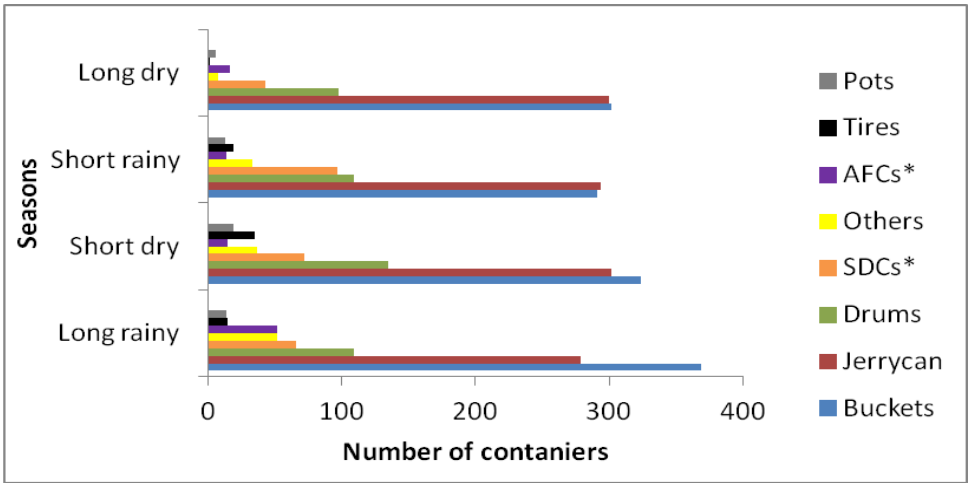
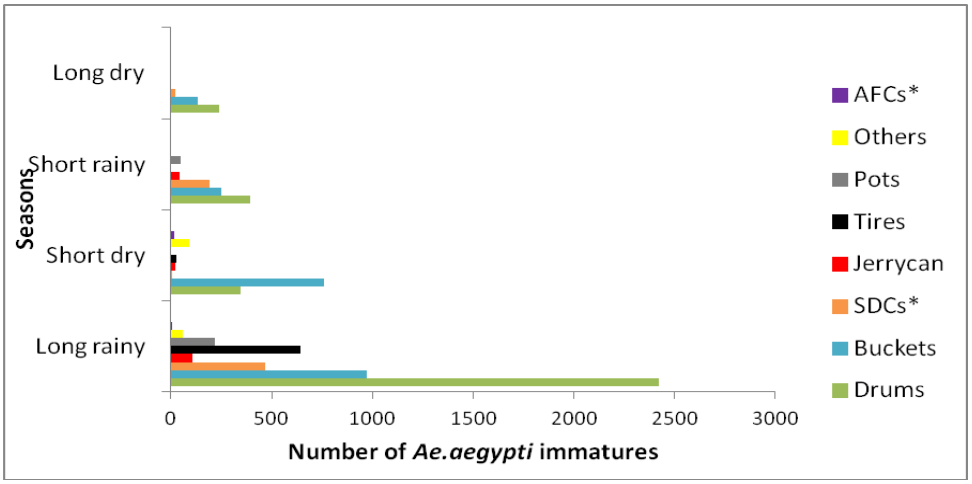


Figure 8. Seasonal abundance of *Ae. aegypti* larval habitats **a** rural (Msambweni) and **b** urban (Ukunda)] sites, between May 2014 and June 2016. Seasons: long rainy (April-June), short dry (July-September), short rainy (October-December); long dry (January-March). *Abbreviations*: AFC, animal feeding troughs; SDC, small domestic containers

a)



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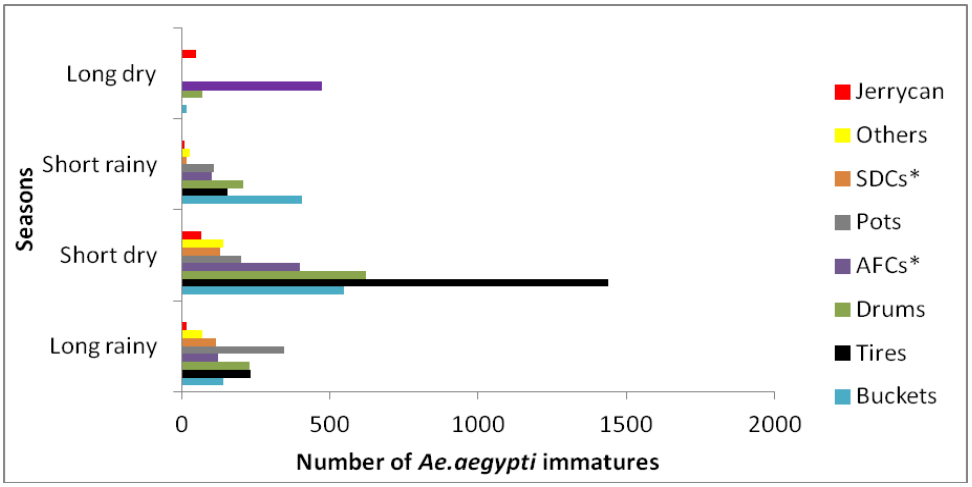


Figure 9. Seasonal abundance of *Ae. aegypti* immatures in different container types in **a** rural (Msambweni) and **b** urban (Ukunda)] sites, between May 2014 and June 2016. Seasons: long rainy (April-June), short dry (July-September), short rainy (October-December); long dry (January-March). *Abbreviations*: AFC, animal feeding troughs; SDC, small domestic containers

4.5.Oviposition activity

A total of 39,782 eggs were collected from 1,922 ovitraps during the 24-month period in the rural (Msambweni) and urban (Ukunda) sites. Of these, 95.0% were of *Ae. aegypti*, 4.5% *Ae. simpsoni* and 0.5% *Culex spp*. Significantly more ovitraps were positive for *Ae. aegypti* eggs outdoors than indoors ($x^2 = 203.2$, DF =1, P<0.0001) and contained more eggs both in the rural site (215 (44.80%) containing 14,484eggs) vs. (65(13.54%) containing 2,714 eggs) and in the urban site (270 (56.13%) containing 16,858 eggs, vs. (122 (25.36%) containing 5,726eggs). Mean number of eggs per trap (Mean egg index) was significantly higher (Kruskal – Wallis $x^2 =$ 106.68, DF =3, P<0.0001) in the three seasons, i.e. long rainy (April- June), short dry (July-September) and short rainy (October-December) than in the dry season (January-March) for both sites. However, the percentage of positive ovitraps (Ovitrap positivity index) and mean egg index were notably higher $(P < 0.0001)$ indoors in the urban site (Table 5).

Table 5. Ovitraps positive for *Ae. aegypti* eggs (Ovitrap positivty index) and the mean number of eggs per trap (Mean egg index) for theMsambweni (Rural) and Ukunda (Urban) sites during the four seasons.

Site	Trap location	Ovitrap positivity index				Mean egg index			
		Long dry	Long rainy	Short dry	Short rainy	Long dry	Long rainy	Short dry	Short rainy
Msambweni(R)	Indoor	5.8	15.0	13.3	20.0	0.8	8.3	5.7	8.5
	Outdoor	15.0	52.5	53.3	59.2	8.1	46.8	33.4	36.0
Ukunda(U)	Indoor	14.17	32.5	33.1	20.83	4.4	20.1	17.2	5.4
	Outdoor	30.0	61.3	61.2	67.5	10.2	48.0	43.3	34.8

4.6 Temporal patterns in the abundance and distribution of *Ae. aegypti* immatures

Year round, *Ae. aegypti* breeding activity was recorded during the study period except for one month in the year 2014 (October) and two months in 2015 (February and March). However, monthly and seasonal variation in the abundance of *Ae. aegypti* immatures was evident in both rural and urban study sites. Overall, there was an increasing trend in the abundance of *Ae. aegypti* immatures over the two years of the study in both sites (Figure 10). A notable pattern of seasonality in *Ae. aegypti* immature production was observed across the 24 month study period, where a steady increase in the abundance of *Ae. aegypti* immatures was recorded especially from the month of April to August (Figure 10), that was followed by a decline in immature productivity in succeeding months (September-March).

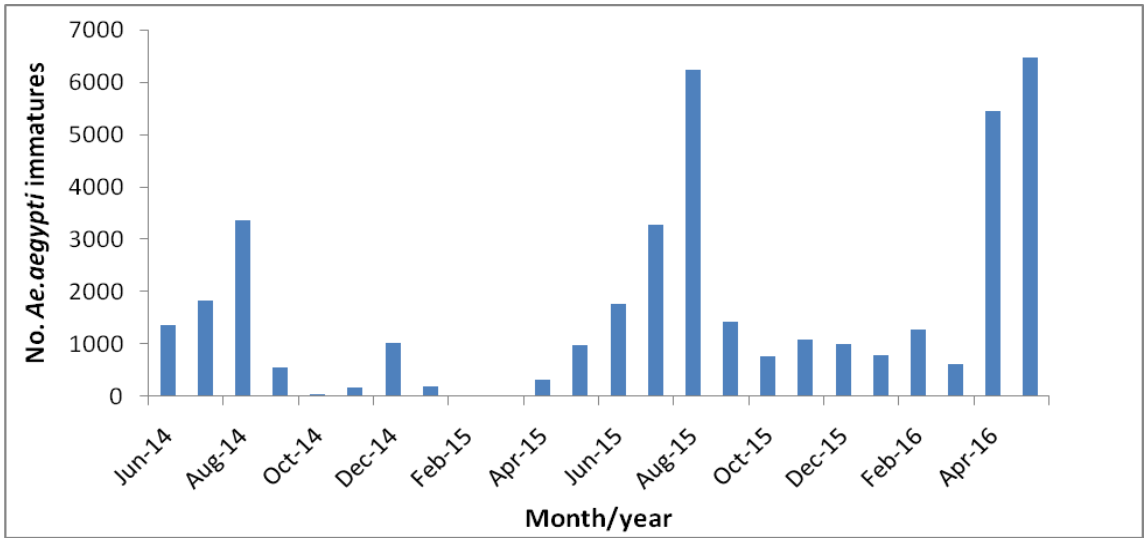


Figure 10. Temporal abundance of *Ae. Aegypti* immatures for both rural and urban sites from June 2014 - May 2016

Pupal abundance in the rural and urban sites also varied over the months of the study period with more pupae being observed in the months of June – September 2014 and May – December 2015

and low productivity between January- March for both 2015 and 2016 except in the month of February 2016 in the urban site where a slight increase in abundance was noted (Figure 11). In the month of April 2016 a sharp increase in pupal productivity was observed in the rural site compared to the previous year, an increase that was also notably higher than that of the urban site (Figure 11).

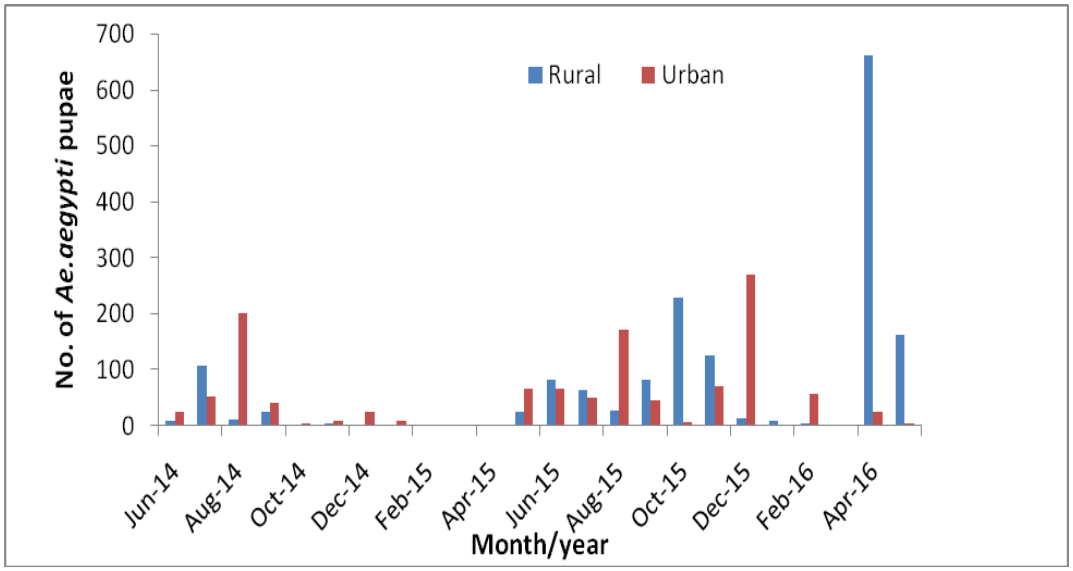


Figure 11. Temporal abundance of *Ae. aegypti* pupae in the rural and urban sites of coastal Kenya from June 2014 to May 2016

Abundance patterns in *Ae. aegypti* immatures appeared to be seasonal with peak productivity in the long rainy and short dry seasons across the twenty four months of the study and low abundance being observed in the long dry and short rainy seasons (Figure 12).

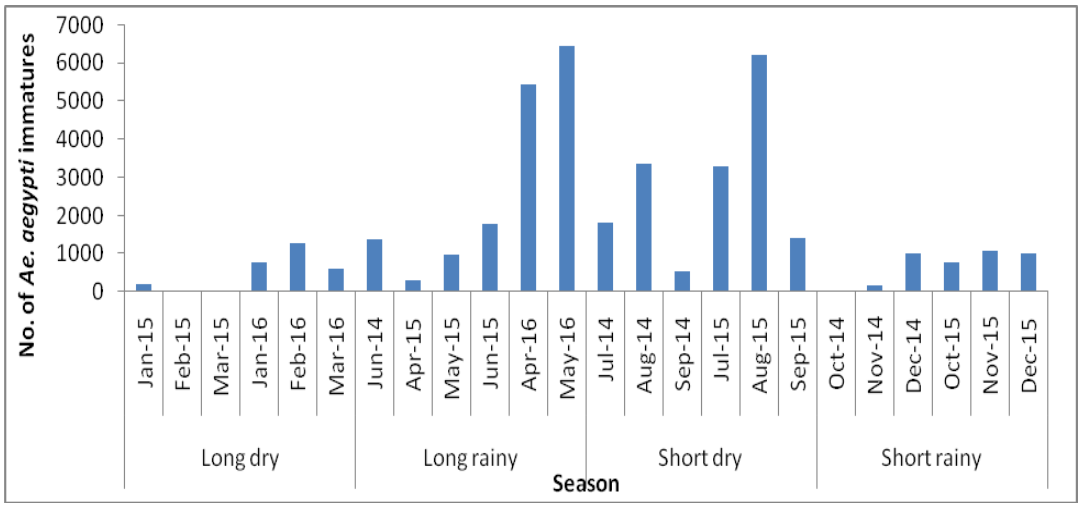


Figure 12. Seasonal abundance in the total number of *Ae. aegypti* immatures for both rural and urban sites for 24 months (June 2014-May 2016).

Pupal productivity also followed a similar pattern but with a third peak in the short rainy season of the year 2015 (Figure 13). However, in the rural site peak pupal productivity was observed in the long and part of the short rainy seasons, while in urban site peak pupal productivity occurred in the short dry and part of the short rainy seasons. Notably low pupal abundance was observed in the long dry season for both rural and urban sites. Part of the short rainy season also recorded low pupal production (Figure12) mainly in the year 2014.

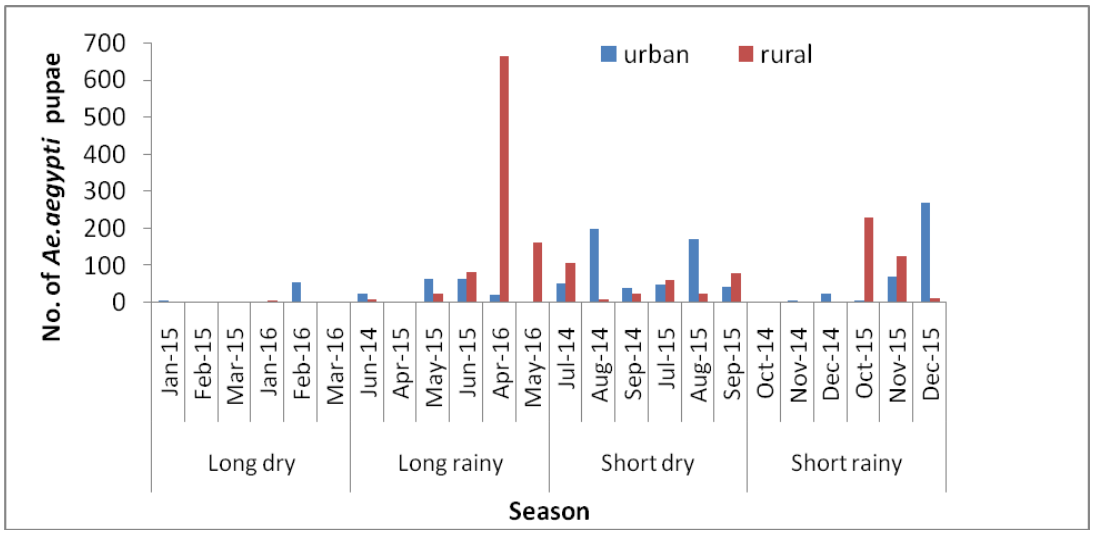


Figure 13. Seasonal abundance in the total number of *Ae. aegypti* pupae in rural and urban sites for 24 months (June 2014-May 2016).

4.7 Temporal patterns in the abundance and distribution of *Ae. aegypti* larval habitats

Productive *Aedes aegypti* larval habitats were present across the two year survey period except for two months (February and March, 2015) in urban site and five months (December 2014-April 2015) in the rural site (Figure 14). Monthly variations in the abundance of pupae positive habitats was found to be significant (χ^2 = 85.6431, DF =24, P<0.0001) in both the rural and urban sites with more pupae positive habitats being recorded in the year 2015 and in part of the rainy season in 2016. Overall, the rural site recorded significantly more pupae positive habitats (χ^2 = 6.54, DF =1, P<0.0105) than the urban site (Figure 14).

Seasonal variation in the abundance of pupae positive habitats was also observed during the study, with significantly more pupae positive habitats (χ^2 = 38.91, DF =3, P<0.0001) being recorded in the rainy and short dry seasons (3.18% and 4.17%) respectively than in the dry and short rainy seasons (0.81% and 0.51%) respectively.

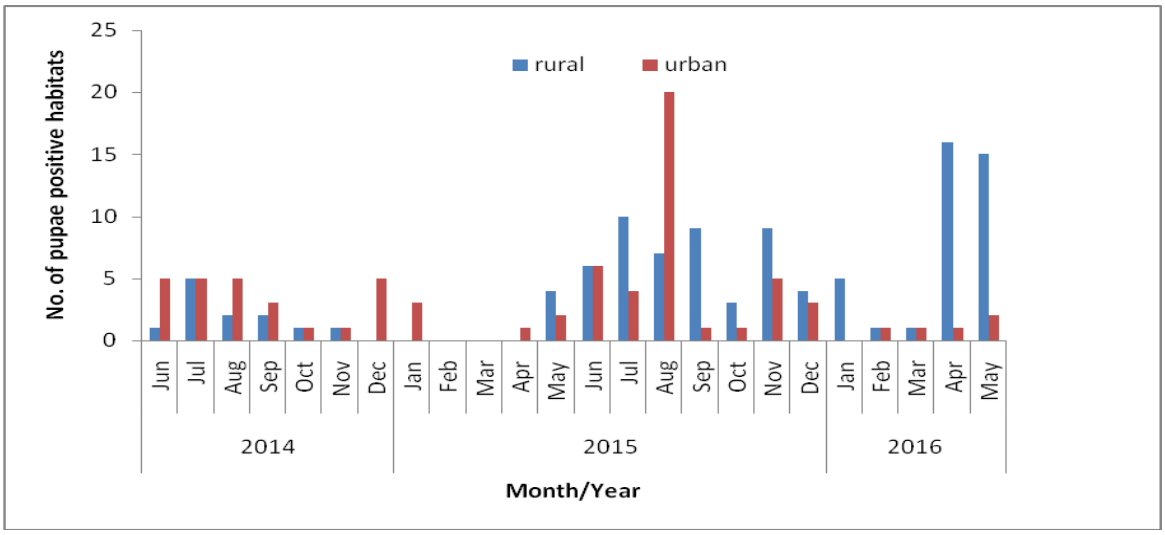


Figure 14. Number of pupae positive habitats in the rural and urban sites from June 2014 to May 2016.

Out of a total of ten container types, eight were found to be positive for *Ae. aegypti* pupae across the two year study period. However, notable monthly variations in the abundance of these container habitat types was observed in year 2014 -2015 and 2015 -2016 for the urban and rural sites respectively. In the rural site an increasing trend in the abundance of different pupae positive containers was observed over the two year study period (Figure15). On the contrary in the urban site the results indicate a decreasing trend in the abundance of these container types (Figure16).

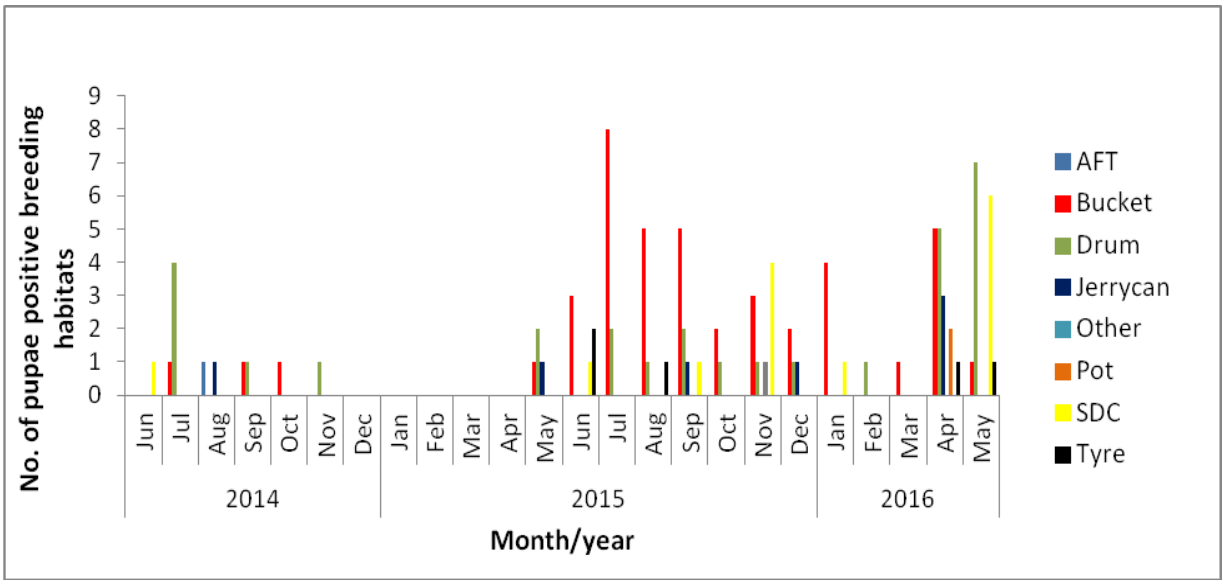


Figure 15. Monthly dynamics in different pupae positive breeding habitats for the rural site from June 2014 to May 2016**.** *Abbreviations*: AFC, animal feeding troughs; SDC, small domestic containers.

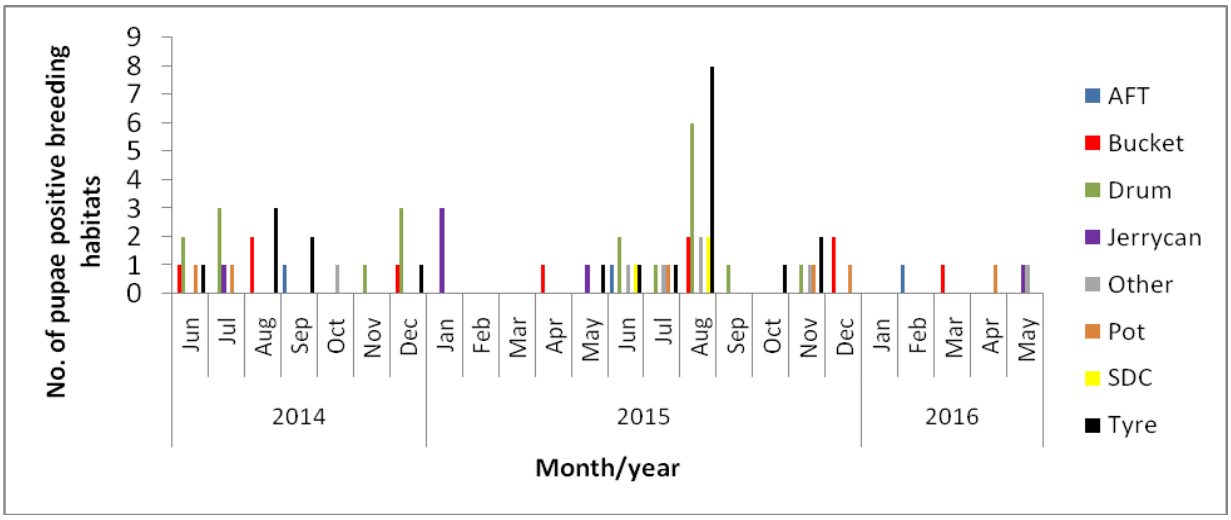
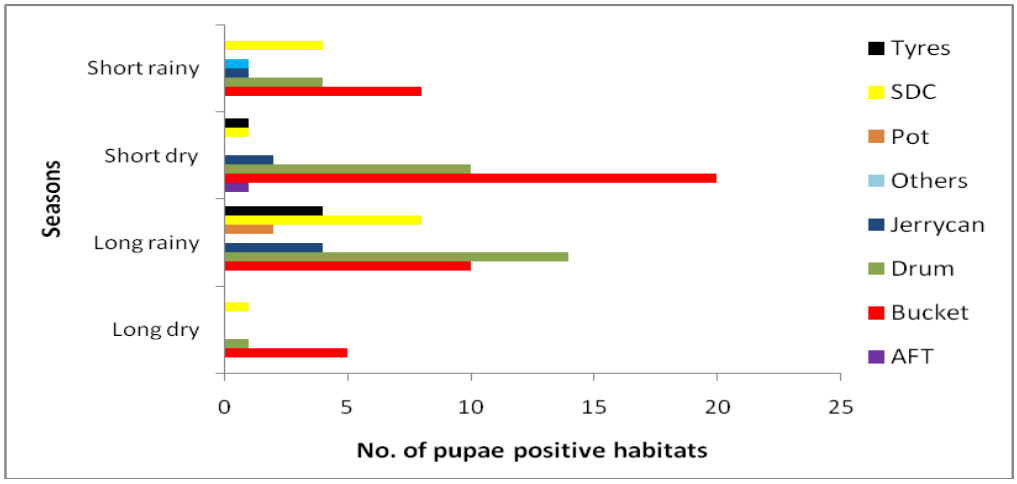


Figure 16. Monthly dynamics in pupae positive breeding habitats for the urban site from June 2014 to May 2016. *Abbreviations*: AFT, animal feeding troughs; SDC, small domestic containers

Seasonal variations in the abundance of the different pupae positive container habitats was also observed and was more pronounced in the rural site than the urban site. In the rural site buckets and drums were the predominant pupae positive container types across all the four seasons. With more of this container categories being recorded in the long rainy and short dry seasons than in the short rainy and long dry seasons (Figure 17a). In the urban site, tyres and drums were the predominant pupae positive container habitats, with more of this containers being recorded in the short dry season (Figure 17b)

a)



b).

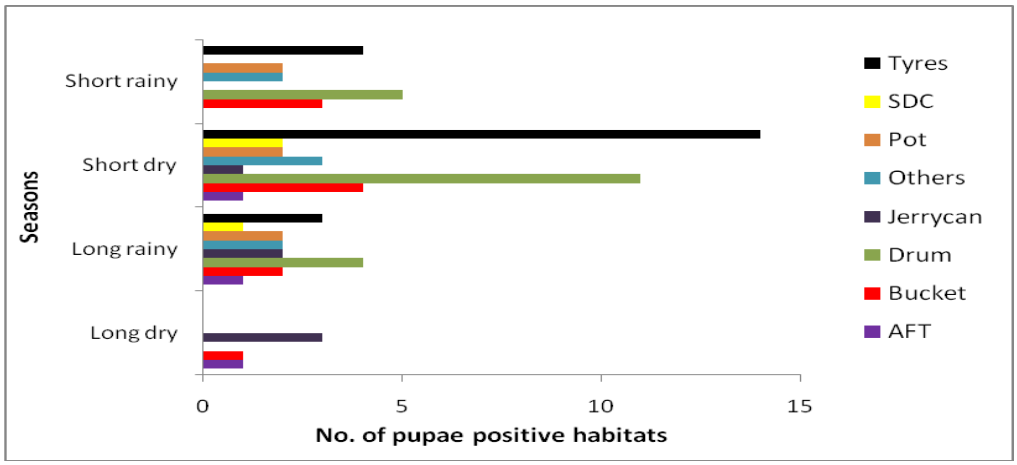


Figure 17. Seasonal variation in the abundance in pupae positive habitat types in a) rural and b) urban sites from June 2014 to May 2016. *Abbreviations*: AFC, animal feeding troughs; SDC, small domestic containers.

4.8 Pupal persistence in study households

Demographic, environmental and entomological data for the determination of continued occurrence of pupae in households (pupal persistence) was collected from 40 households in the two study sites from July 2014 to October 2018.

4.8.1 Demographic and environmental characteristics of households in rural and urban sites of coastal Kenya

Table 6 shows the characteristic of households at base line for the study sites. Some differences in household characteristics between the rural and urban sites were observed. Equal proportions of households in both study sites indicated the use of bed nets, firewood and had open eaves. Houses that had less than three rooms and more than seven occupants were also similar in both locations. Eleven out of twenty households in the urban site and 4/20 houses in the rural site had vegetation in the peridomicile area. Majority of households in the urban site (Ukunda) had iron sheet roofing (15/20) and cement walls (16/20). Insecticide and mosquito repellent coil use were relatively low in both locations.

Table 6. Characteristics of households in urban and rural sites of coastal Kenya

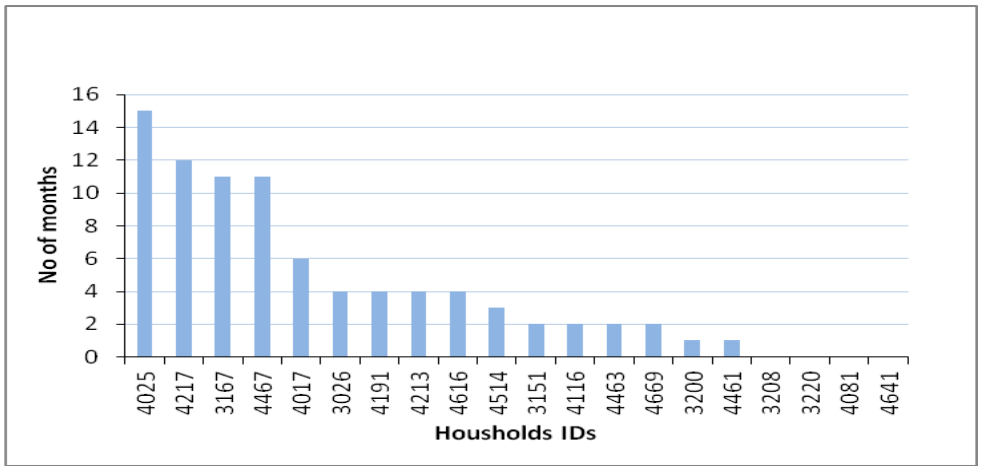
Household Characteristic	Overall (N = 40) *n* (%)	Urban (N = 20) *n* (%)	Rural (N = 20) *n* (%)
House Wall Material			
Mud	17 (43)	4 (20)	13(65)
Cement	23 (58)	16 (80)	7(35)
House Roof Material			
Iron Sheet	18 (45)	15(75)	3(15)
Grass	21 (55)	5(25)	17(85)
No. of Rooms			
Less than 3	12(30)	6(30)	6(30)
3 – 4	17 (43)	6(30)	11(55)
5 or more	11 (27)	8(40)	3(15)
No. of Sleepers			
Less than 4	9 (23)	6(30)	3(15)
4 to 6	15 (38)	6(30)	9(45)
7 or more	16(40)	8(40)	8(40)
Firewood Use	24 (60)	12(60)	12(60)
Insecticide/Coil use	11 (28)	9(45)	2(10)
Bed Net Use	38 (95)	19(95)	19(95)
Eaves Open	36 (90)	18(90)	18(90)
Room Ceilings	9 (23)	7(35)	2(10)
Bushes/Tall Grass	15 (38)	11(55)	4(20)

4.8.2 Abundance and persistence of pupae in the rural and urban households

The total number of *Ae. aegypti* pupae collected in the study sites was 5,439 during the entire study period; 3,292 pupae from Msambweni (20 households, rural site) with a median of 43 [IQR 153] and 2,147 from Ukunda (20 households, urban site) with a median of 54 [IQR 124]

total pupae per household.The median number of months in which pupae were observed in households was 4 and ranged from 0 to 15 (Figure 18).

a)



b)

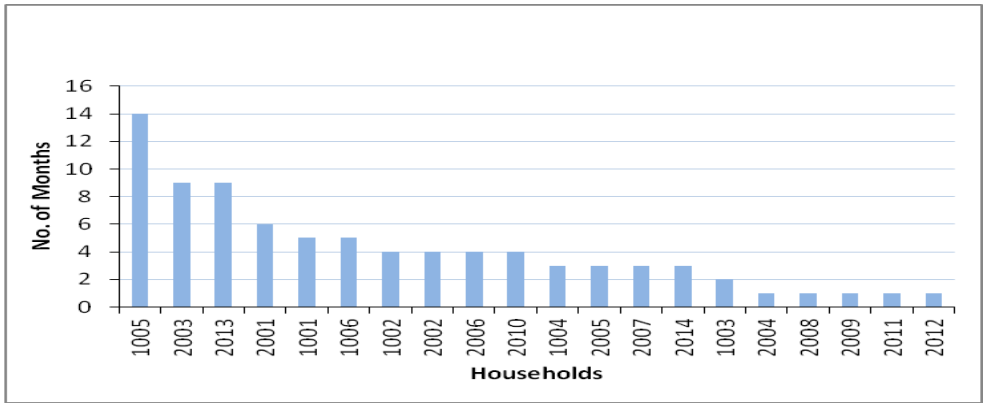


Figure 18. Total number of months of pupae presence over 4 years for **a** Ukunda and **b** Msambweni sites.

Of the 40 households sampled 4/40 (10%) had no pupae observed throughout the entire study period. Within-year pupal persistence was defined as the presence of any pupae within a household for at least 3 months in the year. Pupal persistence was observed from 13 unique households; 6 from Msambweni and 7 from Ukunda (Figure 19).

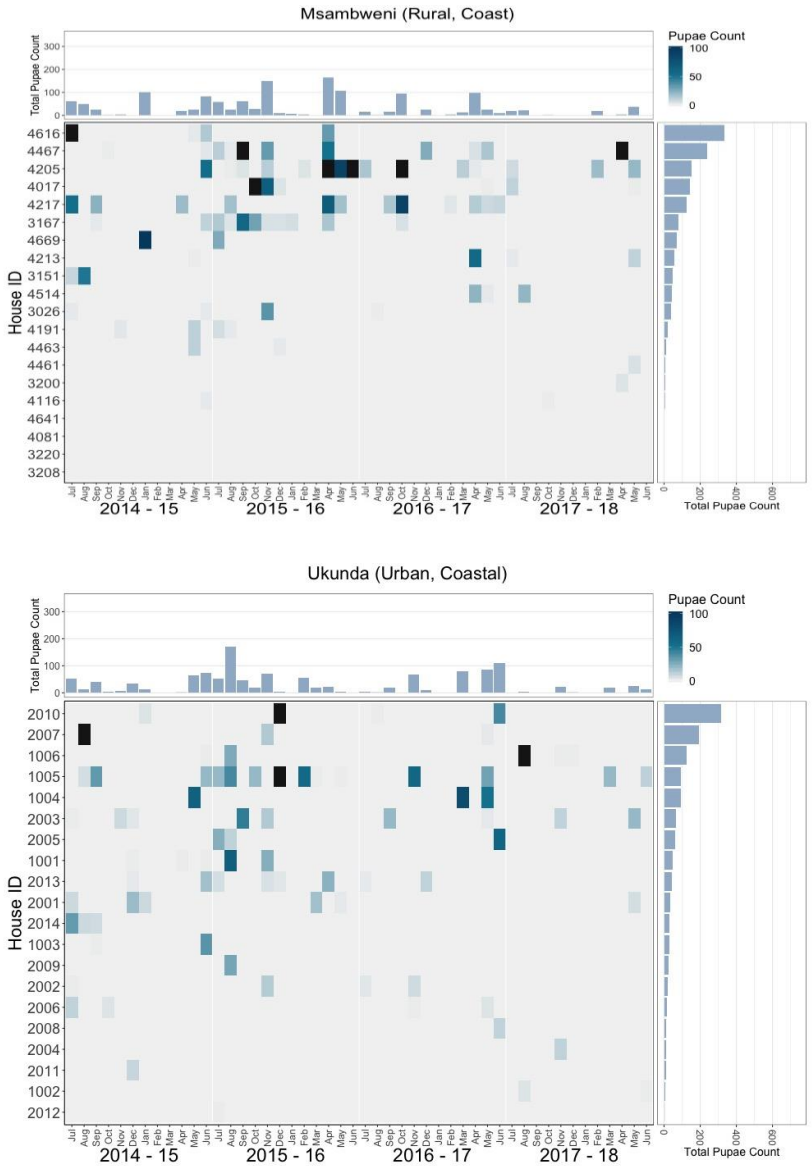


Figure 19. Total number of pupae collected in each household in Msambweni, and Ukunda over 4- year data collection period.The main figure (bottom left) shows the number of pupae counted in that month for the given household (darker colors indicate more pupae). The right marginal figure shows the total number of pupae collected from each household in each site over the 4-year data collection period. The top marginal figure shows the total number of pupae collected in each month.

4.8.3 Spatial autocorrelation of household pupae counts in the study sites

There was no evidence of spatial autocorrelation from the Moran's I test on total pupae counts (Table 7) or seasonal pupae counts (Table 8).

Table 7. Spatial Autocorrelation of Household Pupae Count across different distance thresholds using Moran's I**.**

Site	d = 100m		d = 150m		d = 250m		d = 500m	
	Statistic	p-value	Statistic	p-value	Statistic	p-value	Statistic	p-value
Ukunda	-0.04	0.61	-0.1	0.68	0.1	0.17	-0.06	0.49
Msambweni	-0.05	0.77	-0.13	0.86	-0.14	0.8	0.08	0.12

Table 8. Spatial autocorrelation of household pupae count in the four seasons using Moran's I.

Season	Ukunda		Msambweni	
	Moran's I	p-value	Moran's I	p-value
Cool, Dry	-0.06	0.67	-0.09	0.66
Long, Dry	0.03	0.28	-0.12	0.88
Long, Rain	-0.04	0.53	-0.02	0.52
Short, Rain	-0.06	0.59	-0.1	0.75

4.8.4 Demographic and environmental household risk factors for pupal abundance and persistence

Increasing habitat counts (breeding containers), presence of eaves (gap between the wall and roof), and houses with 3-4 rooms were associated with increasing odds of pupal abundance (OR: 1.27, 95% CI: 1.00-1.60; OR: 2.19, 95% CI: 0.80-6.02; OR: 1.71, 95% CI: 0.78-3.75;), respectively (Table 9). Of the risk factors assessed the presence of bushes/tall grass in the peri-domicile (OR: 2.67, 95% CI: 0.52-13.8) and high habitat counts (OR: 2.30, 95% CI: 0.6-8.82) were found to increase the odds for pupal persistence. However except for the increasing habitat counts, the increasing odds of pupal abundance and persistence were not strongly associated with the risk factors assessed. (Table 9). House roof made of grass and firewood use in a household were associated with a 43% and 33% decrease in the odds of pupal abundance (OR: 0.57 95% CI: 0.22-1.47 and. OR: 0.67 95% CI: 0.40-1.13) respectively.

Results on the influence of month on pupal abundance suggest that the risk of pupal abundance due to seasonality was highest during the July-September season (short dry season) (Figure 20). However, the effect of seasonality was not very strong.

Increasing rainfall and decreasing temperature were associated with increasing risk of pupal abundance (Figure 21

Table 9. Risk factors for pupal persistence and increased pupal abundance in both urban and rural households of coastal Kenya.

House Characteristic	Abundance Model			Persistence Model		
	OR	95% CI	p-value	OR	95% CI	p-value
Rooms						
Less than 3	Ref					
3 to 4	1.71	[0.78, 3.75]	0.17	1.44	[0.21, 9.97]	0.71
4 or more	0.76	[0.31, 1.89]	0.56	0.34	[0.021, 5.32]	0.44
No. of Sleepers						
Less than 4	Ref					
4 to 6	0.74	[0.34, 1.64]	0.46	0.96	[0.08, 10.79]	0.97
7 or more	0.89	[0.41, 1.96]	0.77	0.65	[0.05, 8.88]	0.74
House Wall						
Mud	Ref					
Cement	1.08	[0.48, 2.43]	0.85	2.25	[0.26, 19.22]	0.46
House Roof						
Iron Sheet	Ref					
Grass	0.57	[0.22, 1.47]	0.24	1.86	[0.094, 37.1]	0.68
Room Ceilings	1.07	[0.45, 2.53]	0.88	0.81	[0.05, 13.04]	0.88
Bushes/Tall Grass	1.19	[0.71, 2.00]	0.51	2.67	[0.52, 13.8]	0.24
Firewood Use	0.67	[0.40, 1.13]	0.13	1.13	[0.22, 5.79]	0.88
Eaves Open	2.19	[0.80, 6.02]	0.12	1.45	[0.07, 28.9]	0.81
Habitat Count	1.27*	[1.00, 1.60]	0.045	2.30	[0.6, 8.82]	0.22
Urban	0.87	[0.25, 2.97]	0.82	1.22	[0.11, 0.87]	0.87
Insecticide/Coil[a] Location						

a) The Insecticide or Coil use variable was excluded from the location specific models due to small sample sizes.

$*P < 0.05$,

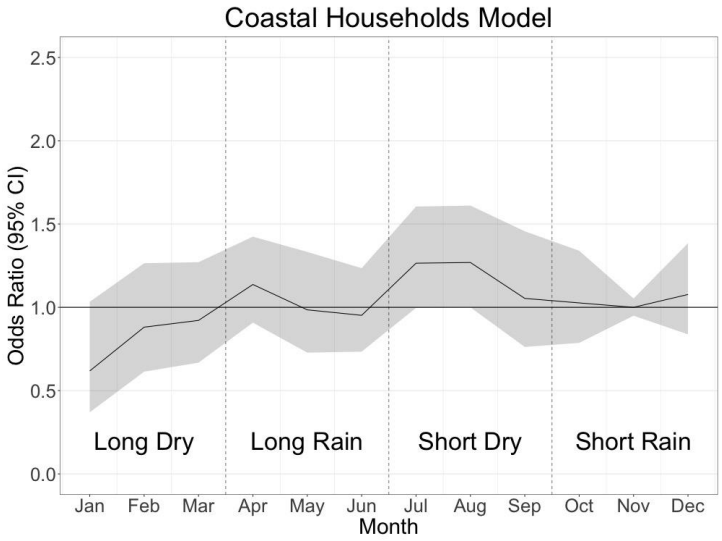


Figure 20. Variation in the risk of increasing pupal abundance by month The four main seasons are shown by dotted lines. The shaded region is the 95% Confidence Interval.

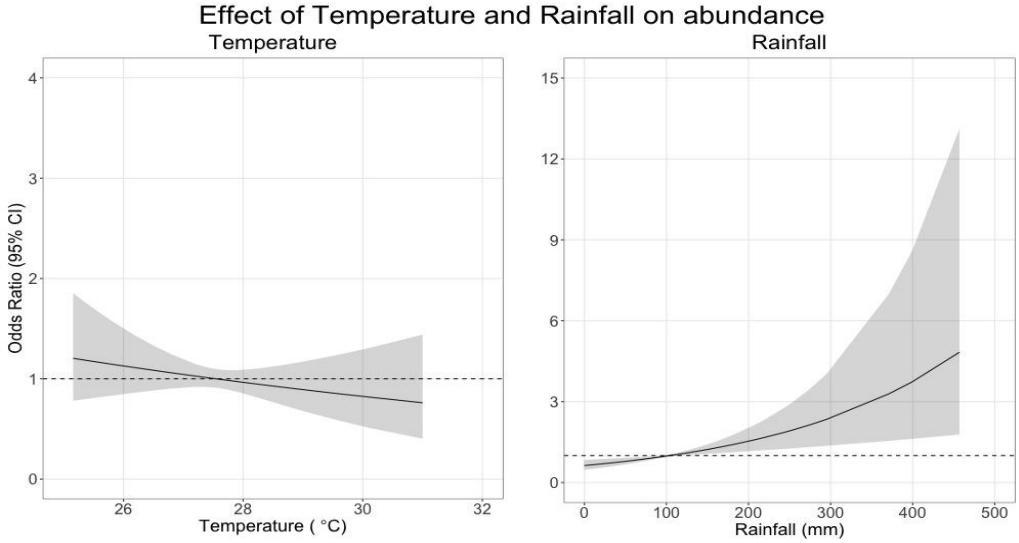


Figure 21. Effect of temperature and rainfall on risk of increasing pupal abundance.The shaded region is the 95% Confidence interval.

4.9 Susceptibility of *Ae. aegypti* larvae to biolarvicidal agent Bti

Initial efficacy results showed that *Bacillus thuringiensis* AM65-52 WG formulation eliminated 100% of larvae in 24 hours and this efficiency persisted for 12- 24 and 12- 18 days in the rainy and dry seasons respectively across the three treatment regimes (Table 10 and 11). *Bacillus thuringiensis* AM65-52 WG formulation was effective against *Ae. aegypti* larvae up to day 24 with larval mortality of 100% at 160mg/L, while mortality of 87% was recorded on day 30 during the rainy season (Table 10). However during the dry season, *Bacillus thuringiensis* AM65-52 formulation at 160mg/L performed effectively up to day 18 with a mortality of 100%. Low larval mortality (<40%) was realized after day 30 (Table11). *Bacillus thuringiensis* AM65-52 WG formulation at 80mg/L was effective against *Ae. aegypti* larvae up to day 18 with larval mortalities of 100%, while mortalities of 80% were recorded at day 24 after which mortality declined sharply to 40% during the rainy season. However during the dry season larval mortalities of 100% were recorded up to day 12, while mortalities of 80% were realized on day 18 (Table11). *Bacillus thuringiensis* AM65-52 WG formulation at 8mg/L was found to be effective up to day 12 yielding 100% mortality in both the rainy and dry seasons (Table 10). A gradual decline in mortality was observed after day 12 during the rainy season; however during the dry season a sharp decline in larval mortality was noted from day 18 onwards (Table 11). There was no mortality observed at day 36 across all the three concentrations during the dry season, but during the rainy season low larval mortality (<18%) was recorded (Table 10 and 11). All pupae collected during the experiments (within 3 to 6 days of exposure) emerged to adult mosquitoes. In general *Bacillus thuringiensis* AM65-52 WG formulation efficacy lasted longer (> 60% mortality up to day 24) during the rainy season than in dry season across the three

concentrations and the residual effect of the boilarvicide was notably higher at concentrations 80 and 160mg/L both during the dry and rainy seasons (Table 10 and 11).

Table 10. Mean mortality (± SE) of *Ae. aegypti* larvae (3rd and 4th instars)in tap water treated with *Bacillus thuringiensis* AM65-52 WG during the rainy season.

	Mean mortality (%) of *Ae.aegytpi* ±SE			
Post treatment (days)	Control	8mg/L	80mg/L	160mg/L
1-6	0 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
7-12	0 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
13-18	0 ± 0.00	70 ± 0.09	100 ± 0.00	100 ± 0.00
19-24	0 ± 0.00	63 ± 0.10	80 ± 0.07	100 ± 0.00
25-30	0 ± 0.00	23±0.09	40± 0.10	87± 0.07
31-36	0 ± 0.00	10± 0.6	18± 0.08	14±0.07

Table 11. Mean mortality (± SE) of *Ae. aegypti* larvae (3rd and 4th instars)in tap water treated with *Bacillus thuringiensis* AM65-52 WG during the dry season.

	Mean mortality (%) of *Ae.aegytpi* ±SE			
Post treatment (days)	Control	8mg/L	80mg/L	160mg/L
1-6	0 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
7-12	0 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
13-18	0 ± 0.00	75 ± 0.09	80 ± 0.07	100 ± 0.00
19-24	5 ± 0.09	11±0.06	60±0.10	85 ± 0.07
25-30	7 ± 0.10	1±0.02	7±0.09	45±.010
31-36	0 ± 0.00	0 ± 0.00	0±.00	0±.00

Chapter five

5 Discussion

This study reports a notable preference for outdoor breeding by *Ae. aegypti*, contrary to earlier findings from the region (Yalwala et al 2015), but consistent with other recent findings elsewhere (Saifur et al 2012; Maciel-de-Freitas,2007; Garcia-Rejon, 2008; Morrison, 2004; Dhimal, 2015). This is clearly indicated by significantly more *Ae. aegypti* immatures found in containers located outdoors, despite a higher number of containers with water indoors. Further in support of this finding, oviposition surveys in both study sites show significantly higher oviposition activity by *Ae. aegypti* outdoors than indoors. Drums, buckets, and discarded tires are the key containers for *Ae. aegypti* development in both study sites, consistent with other studies (Lenhart et al. 2006; Midega et al. 2006; Hiscox et al. 2013; Islam et al. 2019). In addition, water storage containers produced most of the immatures recorded, underscoring the importance of such category of containers in these regions. Significantly higher *Ae. aegypti* breeding activity was observed in the rural site than in the urban, even though the urban site had a notably higher number of containers that were positive for *Ae. aegypti* immatures. Container habitats of *Ae. aegypti* are consistently available throughout the year in both study sites; however, *Ae. aegypti* was found to breed in more container types during the wet and short dry seasons. The observed increased outdoor breeding activity by *Ae. aegypti* suggests an adaptation to outdoor and peri-domestic habitats (Saifur, 2012), a trend that is most likely to have epidemiologically important implications for vector control practices and prevention of virus

transmission. This calls for more emphasis to be placed on the outdoor larval habitats in targeted source reduction measures in the study areas. Widespread use of insecticide impregnated bed nets in the study sites (Mutuku et al., 2011; National Malaria Control Programme, 2016; Bayoh et a ., 2014) could be a factor accounting for the preference of outdoor breeding by *Ae. aegypti*. Traditionally *Ae. aegypti,* has been demonstrated to be a domestic species that prefers indoor resting sites mostly in bedrooms, kitchens, bathrooms and living rooms, where it rests on walls, hanging cloths, bed nets and under furniture (Gratz 1991; Chadee 2013). This may bring resting females into contact with insecticide treated materials that are likely to kill or at least repel them from indoor resting sites. In an ongoing study (unpublished data) *Aedes* mosquitoes have been found to be susceptible to insecticides that are commonly used in bed nets. However, further studies are required to confirm and possibly establish factors that can lead to this observed trend toward outdoor and peridomestic breeding habitats.

Low indoor productivity observed in the study sites can also be attributed to human activities related to the use of domestic water receptacles. Most indoor containers are commonly used for hygiene, cooking and drinking and are subject to frequent emptying and cleaning which can effectively interrupt mosquito development. They are therefore much less likely to harbor *Ae. aegypti* immatures (Maciel-de-Freitas et al. 2007; Hiscox et al. 2013). Moreover, most of the indoor containers for water storage were often covered; this could have possibly contributed to many of them being unproductive. Containers with covers have been found to have a lower probability of infestation by *Aedes* mosquitoes (Hiscox et al. 2013) by preventing gravid females from accessing oviposition sites (Chadee et al. 2009; Philbert and Ijumba 2013). Tires provided good breeding sites for *Aedes* mosquitoes and are responsible for producing

>30% of immatures collected from all larval habitats in outdoor locations of the urban area. The importance of tires as breeding sites of *Ae. aegypti*, has been highlighted before (Midega et al. 2006; Philbert and Ijumba 2013; Getachew et al. 2015; Getachew et al. 2018), and recycling as a means to manage used tires in dengue control has gained popularity around the globe (WHO 2009). In the urban area of the study sites, recycling of tires is limited to small scale use for making sandals and soles of shoes. Therefore, intensifying other recycling options (WHO 2009) is highly recommended. In addition, storage of tires in a dry environment and proper disposal of used tires should be encouraged in instances where recycling may not be feasible. Buckets and jerrycans were found in large numbers in the study sites, but their importance as breeding sites for *Ae.aegypti* mosquitoes was limited to the outdoor locations of both rural and urban sites. Unlike buckets, many jerrycans were consistently present indoors in the study sites, but they were not equally productive. Some studies have identified jerrycans as among the preferred outdoor breeding habitats (Midega et al. 2006; Getachew et al. 2015)), contrary to the situation in this study. Low productivity by this container type can possibly be attributed to their popular usage in short-term storage of water and therefore being subject to frequent emptying and cleaning, which effectively interrupt the breeding cycle of *Ae. aegypti* immatures (Christophers 1960; WHO 2009). Water-holding containers that are in frequent use within the domestic environment were observed to be less likely to harbor *Aedes* immatures (Hiscox et al. 2013), and this can make water storage possible without necessarily creating breeding sites for *Aedes* mosquitoes.

Animal feeding containers were only found outdoor in both sites, and their importance was particularly noticed in urban site where concrete troughs and cut tires, popular as watering points

for cattle and goats in backyards of some homesteads, provided good larval sites for *Ae. aegypti* mosquitoes. The importance of water storage containers is due to the fact that they can hold sufficiently large volumes of water for considerably longer periods that are adequate for complete larval development. However, breeding in these containers can be eliminated by provision of tight fitting covers and mesh screens to prevent access by gravid mosquitoes (Chadee et al. 2009; WHO 2009; Philbert and Ijumba 2013).

In this study, removal or proper management of key containers could result in over a 73.5% reduction in *Ae. aegypti* immature population. This would translate into a corresponding reduction in *Ae. aegypti* population in the study areas, since pupae can be used as proxy estimates of adult *Ae. aegypti* mosquitoes (Focks and Chadee 1997).

Active breeding by *Ae. aegypti* mosquitoes observed in the two study sites can be attributed to many factors. The urban site is characterized by unplanned settlements and a large population of low income earners in the informal business sector, where poor hygiene coupled with inadequate water, sewer and waste management systems are common. These conditions have been found to contribute significantly to the proliferation of breeding sites for *Ae. aegypti* (Gubler 1998; Gubler 2002). In addition the practice of keeping domestic animals in backyards of these neighborhoods, probably to boost the low income levels, provides more breeding opportunities for the highly adaptive *Ae. aegypti* mosquito (Powel and Tabachnick 2013) which readily exploits the water receptacles meant for animal drinking and feeding. Similarly in the rural site absence of a reliable water supply system promotes storage of water in various container types thereby creating ample breeding grounds for *Ae. Aegypti* mosquitoes. Increased breeding activity by *Ae.*

aegypti observed in the coastal sites could possibly account for the dengue outbreaks reported mostly in the region as observed in the recent past (Ochieng et al. 2015).

The year-round activity of *Ae aegypti* can be associated with the lack of a reliable water supply system, and reliance on borehole water, well and rain water collection, necessitating water storage in households. During the long dry season, in particular, except for animal feeding troughs, drums, buckets and jerrycans become important producers of *Ae. aegypti* immatures in both sites. *Aedes. aegypti* breeding in the two study sites is closely related to the rainfall patterns in the region, with higher production of immatures during the rainy and the short dry season seasons, when environmental conditions are also optimal for adult activity, which translates into higher productivity of containers.

Of the high number of water-holding containers inspected, only a few were productive for *Ae.aegypti,* and most of them were located outdoors. Identification of key larval habitats for *Ae aegypti* as part of vector control programs (Chadee et al. 2009; Marylene 2014) will help target larval source reduction measures. Dengue is primarily a problem of human activities that create breeding opportunities for potential vectors, the control of which can be achieved by physical means (PAHO 1994; WHO 2009). In this study, good management practices for water storage containers (PAHO 1994; Hiscox et al. 2013), recycling and proper disposal of discarded tires and small domestic containers(WHO 2009) are recommended in order to achieve a significant reduction in *Aedes* population outdoors, especially during the rainy season when the latter type of containers become important breeding habitats. In addition, provision of reliable piped water supply to households in the study sites throughout the year, would reduce storage of water in containers and thus control *Ae. aegypti* development sites.

Aedes aegypti (L) subspecies were distinguished morphologically following keys as provided by Huang (Huang 2004). While morphological identification of the subspecies is not optimal, the more sensitivity molecular techniques are yet to be fully developed (McBride et al. 2014), and are not readily available in field conditions. *Aedes. aegypti aegypti* has been reported to be predominantly domestic and peridomestic, while *Ae. aegypti formosus* (Aaf) mostly sylvatic (Trpis and Hausermann 1975; Lounibos 2003; Powel and Tabachnick 2013; McBride et al. 2014). All the *Ae. aegypti* (L) in this study were identified as *Ae. aegypti aegypti* (Aaa). Given that all samples in this study were from the domestic or the immediate peridomestic environment, (within a range 10 metres around sampling houses), the presence of Aaf did not have a significant impact on the findings of this study.

Knowledge of *Ae. aegypti* production patterns is important in the design and implementation of targeted vector control strategies (Morrison et al. 2004). This study provides a clear evidence of monthly and seasonal variation in the abundance of *Ae. aegypti* immatures, results that are consistent with findings from similar studies done in different parts of the world (Morrison et al. 2004; Maciel-de-Freitas et al. 2007). The results of this study suggest that *Ae. aegypti* breeding activity in the study area appears to be gradually increasing over the years a trend that could possibly have public health implications in the region as observed in the recent dengue and chikungunya outbreaks in the region (Ellis et al. 2015; Lutomiah et al. 2016; WHO 2018). This is evidenced by a steady increase in the number *Ae. aegypti* immatures recorded over the study period. Marked seasonality in immature production in which there is increased productivity in the months of May to August both in the rural and urban sites, indicates a period of increased breeding activity. This finding could be of importance to vector control agencies which can cost-

effectively focus intervention efforts during seasons of increased immature productivity. Seasonality in *Ae. aegypti* breeding activity appears to be under the influence of rainfall pattern in the study sites as indicated by peak productivity that corresponds to periods of abundant rainfall. Climatic variables such as rainfall, humidity and temperature are strongly associated with transmission of arboviruses due to their effect on vector abundance (Goulda and Higgs 2009; Barrera et al. 2011; Dhimal et al. 2015). Heavy rainfall appears to favor a rapid increase in the abundance of mosquito vectors and may also extend the transmission period of arboviruses (Roiz et al. 2015; Agha et al. 2017). In addition to providing ideal climatic conditions for vector breeding, heavy rainfall is associated with proliferation of rain fed artificial and natural breeding containers (Agha et al. 2017; Ngugi et al. 2017). Populations of *Ae. aegypti* and *Ae. albopictus* have been to found to be on the rise during the rainy season and local rainfall can partly predict the density of *Ae. aegypti* vectors (Tantowijoyo et al. 2015). Influence of rainfall may be partly due to its contribution to the creation of breeding habitats for mosquitoes and since *Ae. aegypti* occur in containers that are primarily filled and maintained through human activity (Tun-Lin et al. 2009), other factors prevailing during the rainy season may operate in favor of their overall activity including increased breeding. Rain water has the effect of stimulating microbial activity thereby increasing the availability of microbial food resources. An increased microbial food resource is one of the important factors necessary for survival and development of *Ae. aegypti* larval stages . Influx of rain water in container habitats may also have a potential effect of diluting organic waste products whose accumulation in water may impact larval survival. A combination of rainfall and temperature have been found to have significant effect on spatial and temporal abundance patterns of *Aedes* mosquitoes (Reinhold et al. 2018). Productivity also

peaked in the short dry season which follows soon after the long rain season. The short dry season is characterized by cool environmental temperature which appears to favor adult breeding activity and probably promote larval development and survival. In addition, during this season breeding sites are still abundant partly due to the rain-fed containers from the just ended rainy season or possibly increased water storage activity on the onset of the short dry season.

Aedes aegypti has been described as an urban vector associated with proliferation of wet containers that serve as potential breeding habitats (WHO 2009). However on the contrary in this study the urban site indicated a decreasing trend in *Ae. aegypti* productivity when compared to the rural study site. A corresponding decline in the number of pupae positive breeding habitats was also noted in this site. Increased public awareness on the breeding habits of vector mosquitoes and improved measures in domestic waste management as evidenced by comparatively fewer small domestic water containers are possible reasons for the observed decrease in abundance of breeding habitats. In addition, existence of boreholes and piped water supply in some of the urban households may have reduced the need for water storage, a practice that has been commonly associated with decreased *Ae. aegypti* breeding (Philbert and Ijumba 2013; Getachew et al. 2015). In the rural site water storage appeared to be a more common practice as shown by increased abundance of water storage containers across all the seasons and a rising trend in the abundance of pupae positive containers over the study period. Absence of a reliable water supply system and domestic waste management measures in the rural site thus could increase potential *Ae aegypti* breeding sites (Maciel-de-Freitas et al. 2007) .

Understanding the influence of human factors on vector abundance is crucial to planning effective vector control interventions. Demographic, environmental and entomological data were

used to evaluate the hypothesis that pupal productivity is driven by a small subset of households that exhibit repeated infestation or pupal persistence, and also evaluate the risk factors for pupal persistence and abundance. This study reports the existence of households that exhibit pupal persistence and identifies high counts of breeding containers as potential risk factors for pupal abundance.

The use of *Stegomyia* indices and surveillance of immatures, as proxies for adult abundance in *Ae. aegypti* vector ecology is well established (Troyo et al. 2008; Chadee et al. 2009; Getachew et al. 2018). While these measures can be useful, they do not account for other epidemiologically important factors and fail to accurately correlate with disease risk (Focks and Chadee 1997; Chadee 2004; Focks and Alexander 2006). This study establishes the existence of specific household premises that are repeatedly infested with pupae, i.e. persistent households. While pupal persistence or repeated infestation has received little attention in the literature, it may provide a more precise measure of vector abundance. Further, numerous studies have evaluated the influence of environmental and human risk factors on pupal presence and abundance (Hiscox et al. 2013; Stewart-Ibarra et al. 2013. ; Islam et al. 2019).While persistence and abundance were found to be two different measures, their potential risk factors are largely similar. Houses that consistently have high pupal counts can be considered key premises and possible super spreader premises and they may play an important role in maintaining vector populations in the study areas. Identifying households with pupal persistence can inform precise and targeted vector control efforts, which would maximize efficiency with limited resources compared to blanket interventions.

High counts of breeding containers, presence of open eaves and houses with 3-4 rooms were potentially associated with risk of pupal abundance. Majority of households (90%) in the study sites had open eaves. A recent study in this region and western Kenya significantly associated vegetation in the peri-domestic area, open eaves and high habitat counts with high pupal abundance and persistence (Ngugi et al. 2020).House designs with open eaves are preferred as a means to regulate house temperature by promoting air circulation, since the region is characterized by hot and humid conditions. Moreover, use of wood fuel in some households makes it necessary to have open eaves for adequate ventilation. However, open eaves can be exploited by female mosquitoes to access human hosts and oviposition sites in and around the houses. Since *Ae. aegypti* is a known endophilic and anthropophilic species that can also be found resting around human dwellings (Perich et al. 2000; Scott et al. 2000; Chadee 2013; Ndenga et al. 2017) and remains close to breeding sites (Trpis and Hausermann 1986; Harrington et al. 2005; Bergero et al. 2013), houses with open eaves and suitable breeding sites are ideal for their development and survival. Although improvement in house designs to include closed eaves plays a critical role in preventing house entry by malaria vectors (Ogoma et al. 2010; Menger et al. 2016; Jatta et al. 2018; Mburu et al. 2018), the role of open eaves on house entry and exit behavior by *Ae. aegypti* which is principally a day biting mosquito has received little attention. The results of this study suggest that houses with closed eaves may play an important role in regulating *Ae. aegypti* house entry and exit behavior particularly in houses with screened doors and windows that are recognized as main entry points for the culicine mosquitoes (Njie et al. 2009; Che-Mendoza et al. 2018). Closed eaves may have an impact on *Ae. aegypti* breeding activity by limiting access to potential breeding sites and human hosts. *Ae. aegypti* obtain blood

meals mostly from people inside a given household (De Benedictis et al. 2003) where they may also find resting places after blood feeding (Chadee 2013). Gravid mosquitoes exploit indoor and outdoor wet containers for oviposition, hence the need for house entry and exit routes which may include open eaves (Njie et al. 2009).

The presence of vegetation such as bushes or tall grass in the peri-domestic environment is also a potential risk factor for pupal persistence (Ngugi et al. 2020). One of the study sites is located in a rural setting where presence of vegetation in the area around households is common due to small scale farming practices that promote vegetation growth in the proximity of houses. This coupled with inadequate environmental hygiene practices in some of the urban households especially those located in the low income-unplanned settlements contribute to the occurrence of vegetation around households. In this region unplanned urban settlements are characterized by poor hygiene, inadequate water, and sewer and waste management systems. Investigations have shown that micro-environmental conditions such as those provided by locations sheltered from sunlight affect the suitability of wet containers as breeding sites for *Ae. aegypti* (Vezzani and Schweigmann 2002; Islam et al. 2019). Vegetation in proximity to potential breeding containers increases the suitability of breeding containers for infestation by providing shade and decreasing rates of evaporation. Although temperature has been considered as the primary driver of development and survival of mosquito immatures (Couret et al. 2014), water in containers under direct exposure to sunlight may reach temperatures that are lethal to *Ae aegypti* immatures. Water temperatures above 35°C have significant impact on larval development (Tun-Lin et al. 2001; Farjana et al. 2012). In addition, vegetation contributes organic nutrients for aquatic organisms such as larvae which feed on aquatic micro-organisms and provides resting sites for

adult mosquitoes. Previous work has found that presence of trees or organic matter results in better survival and faster development of larvae and pupae (Tun-Lin et al. 2000; Barrera et al. 2006).

Houses with 3 to 4 rooms were found to be a potential risk factor for increasing pupal abundance. Most households in the study sites had this range of the number of rooms. Such households tend to be fairly congested providing ample resting places for the cryptic *Aedes* mosquitoes. In the presence of water holding containers such environments may become attractive and ideal for breeding given that these mosquitoes tend to remain close to their breeding sites.

Temporal variation in pupal abundance within households across months was consistent with several other studies (Midega et al. 2006; Hiscox et al. 2013; Paul et al. 2018; Islam et al. 2019). Peak pupal abundance was observed in the months of July-September, coinciding with the Short dry season. Climatic variables such as rainfall, humidity and temperature are strongly associated with transmission of arboviruses due to their effect on vector abundance (Goulda and Higgs 2009; Barrera et al. 2011; Dhimal et al. 2015).The short dry season in this region is characterized by cool atmospheric temperatures and occasional light showers. This together with numerous rain-fed artificial and natural containers that persist from the previous heavy rains season may favor a rapid increase in the abundance of mosquito vectors. A gradual decline in pupa abundance from 2016- 2018 was observed, a trend that suggests a likelihood of some intervention measures being undertaken at household level by residents in the study sites. This may have been prompted by increased awareness of mosquito vectors as a result of the

prolonged study period in the region. Further work is required to identify what factors could be attributed to the observed trend.

The presence of large numbers of potential breeding containers is a strong risk factor for pupal abundance and persistence. This relationship is well documented for both *Aedes* and *Anopheles* mosquitoes (Nguyen et al. 2011; Garelli et al. 2013; Li et al. 2014; Zahouli et al. 2017). Potential breeding containers accumulate as a result of human activities within the domestic environment such as water storage and management of solid waste (Barrera et al. 2011). Due to inadequate water supply system in most households in our study sites, storage of water in diverse containers is common. This coupled with poor management of solid waste promote the proliferation of potential breeding containers for *Ae. aegypti*. Management of containers has been found to be highly variable in time and space, depending on their function and several interventions focus on reducing the number of containers available for breeding. Factors contributing to the infestation of containers with *Aedes* immatures, such as shade and water temperature, have also been examined (Tun-Lin et al. 2001; Overgaard et al. 2017; Islam et al. 2019). Reducing breeding containers remains one of the most important general vector control interventions available.

Houses with grass roofing and firewood use were found to have potentially protective effect on pupal abundance. Roofing of houses with grass and the use of wood fuel is common in poor households in rural areas and low income-urban settlements as observed in some households in our study sites. Smoke from domestic fuels may have repellent effect on mosquitoes however, it may not provide effective protection against mosquitoes and has been linked to some health concerns due to indoor air pollution (Biran et al. 2007). Unlike iron sheet roofed houses, grass roofing material does not provide for rain water harvesting thus such houses with this kind of

roofs are less likely to have containers associated with rain water harvesting that may serve as *Aedes* breeding habitats. Coupled with the effect of smoke such households may be unsuitable for mosquito breeding. Grass thatched roof houses have been associated with a higher density of anopheles mosquitoes than those with iron sheet roofs (Ondiba et al. 2018). However, knowledge on the microclimate effect of roofing material on *Ae aegypti* pupal abundance and persistence is lacking thus a need for further investigation.

Pupal abundance and persistence did not exhibit any spatial correlation. Previous work has shown that pupal productivity in premises has highly focal spatial correlation within approximately 30m (LaCon et al. 2014). This corresponds to the known *Ae. aegypti* range of about 50 – 100m. Households included in the study were relatively sparsely distributed (more than 100m apart), and this may be why no spatial effect on total pupae counts or seasonal pupae counts was observed To identify spatial correlation of productivity and potential productivity hotspots, a more precise study including large numbers of adjacent households (within 30 – 50m of one another) would need to be conducted.

This study shows that larvae of *Ae. Aegypti* are fully susceptible to the bio-larvicidal agent *Bacillus thuringiensis* under semi-field conditions, findings that are consistent with reports from other parts of the globe (Lee and Zairi 2006; Ritchie et al. 2010; Boyce et al. 2013; Farajollahi et al. 2013). For a *Bti* formulation to be considered efficacious, World health organization (WHO 2016) recommends larval mortalities above 90% within 24 hours. In this study a single application of *Bti* across all the tested concentrations performed effectively against *Ae. aegypti* for a minimum of 12 days during the rainy and dry seasons. These periods of residual persistence

were prolonged to 24 and 18 days for the respective seasons at the highest concentration tested (160mg/L). Under field conditions *Bti* shows a short residual activity (2 - 4weeks) against *Aedes* mosquitoes (Lima et al. 2005; Boyce et al. 2013) but in the laboratory and simulated field conditions longer residual activity lasting from 3 – 6 months can be realized (Lee et al. 2005; Ritchie et al. 2010; Marcombe et al. 2011). Low residual activity creates a need for frequent reapplication of the boilarvicide agent and this comes with additional treatment cost. Thus *Bti* formulations with longer residual activity are necessary for mosquito control programs. Several studies have evaluated the applicability of higher concentrations (mega doses) of *Bti* as one of the ways to prolong field residual activity (Ritchie et al. 2010; Boyce et al. 2013; Farajollahi et al. 2013). Apart from extending residual persistence of *Bti* in natural habitats, "mega doses" have an advantage of being easier to measure than the maximum recommended dose of 8mg/L, thus more practical in field applications (Ritchie et al. 2010). This is especially so where small size discarded containers such as tyres, buckets and flower pots rather than the large water storage containers are the important producers of *Ae. aegypti*. Persistence of *Bti* in the environment has also been associated with recycling of toxins probably as cadavers are ingested (Melo-Santos et al. 2009). However, in the present study residual effect of *Bti* may not be linked to recycling bacteria toxins since all dead larvae were removed daily from the test containers on a daily basis.

In this study *Bti* remained effective for only 18 days during the dry season indicating the potential impact of elevated environmental temperatures on the efficacy of *Bti*. An increase in residual efficacy (up to 24 days) observed during the rainy season can partly be attributed to an influx of fresh water into the test containers which could have possibly minimized the potential impact of elevated environmental temperatures stimulated microbial activity as well as the

dilution of organic waste products accumulating in the test water. Some study designs, have shown that replenishing of test water during experiments had the effect of significantly extending residual efficacy of *Bti* both in the field and laboratory settings (Lee et al. 2005; Lee and Zairi 2006). Environmental factors such exposure to sunlight, high temperature and organic content may influence potency of *Bti* (Ignoffo et al. 1981; Kramer 1990; Nayar et al. 1999; Lee et al. 2005) and this may affect its residual persistence in natural habitats. Other biotic and abiotic factors that may influence Bti efficacy have been reviewed (Nayar et al. 1999). For instance, high temperature and exposure to higher intensity of sunlight can adversely affect potency of Bti formulations (Nayar et al. 1999). In the tropics and subtropics water temperature and light intensity tend to be high especially during the dry season and this could be one of the factors that can be attributed to reduced persistence of *Bti* observed in this study during dry the season. This suggests that field applications done late in the afternoon (after 4.00 pm) can be ideal for maximum potency in these regions; however for extended persistence better formulations are still much needed.

5.2 Limitations of the study

The results of this study should be interpreted with caution since households that were randomly selected for larval and ovitrap surveys were systematically and repeatedly sampled. Though this sampling design enabled surveys to be conducted over an extended period, it is important to note that the study population may change its behavior over time, which in turn, may impact findings of the study. In addition, although the sample size of 20 sentinel houses in each of the two study sites may have been statistically inadequate, it was not possible to inspect other larval sites

beyond the large number in the individual sentinel households, given limited resources and larval surveys being labor intensive.

Several important risk factors for pupal productivity examined in this study did not reach statistical significance. Due to the documented effects of these variables this may be a result of the low power of the study to detect these effects especially on the sample size. In addition, while attempts were made to always include the same house during data collection in the surveys certain houses were only available at certain time points. Missing houses were replaced with houses in close proximity (Appendix II).

Households included in the study sample were randomly selected from a census enumeration list. While this reduced bias in inclusion of households in the study the sample may still not be representative. This is particularly true if urban areas have a larger number of households than rural areas. Future work should sample households based on the underlying population distributions. The larger study sites are themselves not representative of rural/urban areas in Kenya but were chosen to include a wide range of environmental and socio-demographic localities (urban/rural, etc). This allows for the control of any underlying influence these factors have on abundance or persistence. To specifically examine the influence of these larger geographic factors a wider range of areas would need to be sampled.

5.3 Conclusions

The results of this study indicate that *Ae. aegypti* breeding habitats are abundant outdoors and are diverse both in the rural and urban landscapes of the coastal region of Kenya. However, only a few containers are productive. In this region *Aedes aegypti* exhibits year round breeding activity with peak productivity in the rainy and short dry seasons.

This study shows the existence of pupal persistence in a subset of households in rural and urban Kenya. High counts of potential breeding containers, vegetation in the peri-domicile area and presence of open eaves are potentially associated with increased risk of pupal abundance and persistence. These results suggest that targeting source reduction efforts toward productive container types particularly during peak productivity may be a cost-effective way to manage the dengue vector and arboviral transmission in this region. Moreover households that exhibit pupal persistence and the risk factors for pupal abundance and persistence such as vegetation in the peri-domicile area should also be targeted in vector control efforts. Further studies are required to confirm and possibly establish factors that can lead to the observed increased outdoor breeding activity by *Ae. aegypti* and its epidemiological implications to vector control.

Efficient vector management of *Ae. aegypti* is vital to the control and management of arboviruses in endemic areas. In this study Larvae of *Ae. Aegypti* were found to be fully susceptible to the bio-larvicidal agent *Bacillus thuringiensis* under semi-field conditions, and field evaluation is recommended for potential use as component in vector control interventions in this region.

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APPENDICES

Appendix I Moran's I Statistic

The Global Moran's I statistic was used to evaluate spatial autocorrelation of the total number of pupae observed in the households throughout the data collection period (Waller and Gotway 2004). The Moran's I allows the evaluation of correlation of pupae counts by comparing the counts of each household with counts in neighboring households. A significant result from the Moran's I would suggest that not only are there specific households that produce more pupae than others, but also that these households cluster together in space, forming pupal productivity hotspots. Neighboring households were defined based on distance (distance-based neighbors) (Bivand and W. S. Wong 2018).

A radii of *150m* was used to define the distance band for a household's neighbors (i.e. all households within 150m of the current household are defined as the current household's neighbor) (Trpis and Hausermann 1986). All derived neighbors were evenly weighted, as opposed to assigning farther neighbors smaller weights. Since the outcome is a count, the significance of the resulting statistic was evaluated using permutation tests (Bivand and W. S. Wong 2018). In the permutation tests, the set of outcome values were randomly reassigned to the sample of households and a new Moran's I is calculated. This was repeated 500 times. The random permutations of the outcome represent the distribution of the outcome under the null hypothesis. A p-value is derived by comparing the actual Moran's I to the Monte-Carlo permutations at the $p = 0.05$ level.

The presence of spatial autocorrelation was tested among each of the 20 houses within the 2 sites (Table 7). The sensitivity of the outcome to the distance threshold (*d = 50m – 500m,* Table 7) was also tested Finally, an evaluation if spatial autocorrelation varied by seasons was done. This was done by summing up the total pupae counts within a season for the households. These seasonal pupae count sums were then tested for spatial autocorrelation using the process described above (Table 8). The results suggest that for the houses sampled, pupae counts did not exhibit spatial correlation

Appendix II Models and Sensitivity Analyses.

Proportional Odds Pupal Abundance Models

The risk factors for pupal abundance in households were modeled using a spatially explicit, longitudinal generalized additive model, with a proportional odds model framework (Antoine and Frank 2000; Harrell 2001; Wood 2017). The outcome of interest is pupal abundance category, defined as zero (no pupae observed), low (0 – 15 pupae observed), intermediate (15 – 30 pupae observed) or high (> 30 pupae observed). Given a set of *N* households, each sampled at *T* time points, the observed outcome for a household at a given time point is y_{it},where$i = 1, \ldots, N$(N = 40), $t = 1, \ldots, T$(T = 48 months),and takes 1 of *J* values (J = zero, low, intermediate or high). The probability of being in a given abundance category *j* or higher at each time point is modeled using a proportional odds model as follows;

$$logit(P(y_{it} \geq j)) = \alpha_j + h_i + f_1(x_1) + \cdots + f_p(x_p) + \boldsymbol{X_t\beta} + f_{spat}(lat_i, long_i) + \varepsilon_{it}$$

α represents the intercept corresponding to each of the *J* outcomes and *h*represents the household intercept (i.e. random effect for households). The terms$f_p(x_p)$ represent one of*P* non-linear effects, which are modeled using smoothing splines in additive models(Wood 2017). The design matrix$\boldsymbol{X}$ and vector$\boldsymbol{\beta}$represent the linear terms of interest and their corresponding coefficients. The models include a spatial term $f_{spat}(lat_i, long_i)$,to account for any spatial correlation(Umlauf et al. 2015). Geo-splines and their corded longitude and latitude values of each household were used to account for spatial correlation. The ε_{it}term represents the independent and identically distributed errors. Household characteristics (use of firewood, presence of eaves, number of occupants, etc.) were modeled as linear terms, while seasonal and temporal effects (month, year, temperature, rainfall) were included in the model as non-linear terms to account for seasonality and any shifts in abundance patterns by year. Models were fit using Restricted Maximum Likelihood estimation in BayesX via its R language interface R2BayesX (Brezger et al. 2005; Umlauf et al. 2015).

Logistic Regression Pupal Persistence Models

The risk factors for pupal persistence in households were modeled using a spatially explicit, longitudinal generalized additive model with a binomial outcome. Pupal persistence within a household was defined as presence of pupae within that household three months or more within a year. 40 Households were followed for 48 months (four years) and this resulted in 160 house-years of data. The pupal persistence logistic regression is structured as follows:

$$logit(P(y_{it} = 1)) = \alpha + h_i + f_1(x_1) + \cdots + f_p(x_p) + \boldsymbol{X_t\beta} + f_{spat}(lat_i, long_i) + \varepsilon_{it}$$

The outcome y_{it} represents pupal persistence in a household *i, i = 1 . . ., N,* at timepoint *t, t = 1, . . .,4*(4 years). The model included a non-linear term for year, as well as a spatial term to account for spatial correlation. The spatial effect was modeled using geo-splines. The ε_{it} term represents the independent and identically distributed errors. A single model for persistence that included both rural and urban households due to small sample sizes. Seasonality was not accounted for in the persistence model because persistence is defined by year as opposed to month.

Sensitivity analyses of household replacements

Households included in the study at the beginning of the data collection period were sampled randomly from a 2014 census enumeration list. In the subsequent months of data collection, during a routine data collection period, if an originally sampled household was unavailable for data collection an additional house was sampled in its place as a substitute household. In some instances, a household was unavailable for sampling at multiple time points. If this occurred, effort were made to resample the same substitute household, but this was not always possible. All original households and household replacements were recorded and stored. In the main analysis, in instances where household replacements were conducted, data from the original and substituted houses was merged and the result treated as a single household.

In order to evaluate the influence of household replacements on the results the regression analyses were repeated with only households that had complete data for the entire data collection period and excluded household replacements. Both the persistence and abundance models were

re ran excluding the replacement households, and it was found that the house replacements did not have a significant effect on the final results.

Appendix III

KENYA MEDICAL RESEARCH INSTITUTE

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KEMRI/RES/7/3/1 **October 1, 2013**

TO: **DR. DESIREE LABEAUD**
PRINCIPAL INVESTIGATOR

THRO': **DR. STEPHEN MUNGA**
ACTING DIRECTOR, CGHR,
KISUMU

RE: **SSC PROTOCOL NO. 2611 (*RESUBMISSION 2*): THE BURDEN OF CHIKUNGUNYA AND DENGUE TRANSMISSION, INFECTION AND DISEASE IN KENYA - *VERSION 1.2, 21 SEP 2013*.**

Reference is made to your letter dated September 20, 2013. The ERC Secretariat acknowledges receipt of the revised proposal on September 27, 2013.

This is to inform you that the Committee determined that the issues raised at the 218th ERC meeting of 20th August, 2013 are adequately addressed. Consequently, the study is granted approval for continuation effective this **1st day of October 2013.** Please note that authorization to conduct this study will automatically expire on **September 30, 2014**. If you plan to continue with data collection or analysis beyond this date, please submit an application for continuing approval to the ERC Secretariat by **August 19, 2014**.

You are required to submit any proposed changes to this study to the SSC and ERC for review and the changes should not be initiated until written approval from the ERC is received. Please note that any unanticipated problems resulting from the conduct of this study should be brought to the attention of the ERC and you should advise the ERC when the study is completed or discontinued.

Work on this project may begin.

Yours faithfully,

EAB

DR. ELIZABETH BUKUSI,
ACTING SECRETARY,
KEMRI/ETHICS REVIEW COMMITTEE

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KEMRI/RES/7/3/1 **September 05, 2018**

TO: **ANGELLE DESIREE LABEAUD,**
PRINCIPAL INVESTIGATOR,

THROUGH: **THE DIRECTOR, CGHR,**
KISUMU

Dear Madam,

RE: **PROTOCOL No. SSC 2611 (*REQUEST FOR ANNUAL RENEWAL*): THE BURDEN OF CHIKUNGUNYA AND DENGUE TRANSMISSION, INFECTION AND DISEASE IN KENYA**

Thank you for the Continuing Review Report for the period dated **August 01, 2017** to **June 30, 2018.**

This is to inform you that the expedited review team of the KEMRI Scientific and Ethics Review Unit (SERU) was of the informed opinion that the progress made during the reported period is satisfactory. The study has therefore been granted **approval**.

This approval is valid from **September 16, 2018** through to **September 15, 2019**. Please note that authorization to conduct this study will automatically expire on **September 15, 2019**. If you plan to continue with data collection or analysis beyond this date please submit an application for continuing approval to the SERU by **August 04, 2019.**

You are required to submit any amendments to this protocol and any other information pertinent to human participation in this study to the SERU for review prior to initiation.

You may continue with the study.

Yours faithfully,

ENOCK KEBENEI
THE ACTING HEAD,
KEMRI SCIENTIFIC AND ETHICS REVIEW UNIT

Appendix IV. Data collection sheets

a).Demographic and household information.

DEMOGRAPHIC/ HOUSEHOLD INFORMATION DATA SHEET

Study site : **Person ID:** **Study ID:** **Household No……..**
Interviewer Name:: _____ Interview Date (DD/MM/YYYY): / /

1	**The nearest common point to residence**		
2	**Collect GPS coordinates of the house (***GPS coordinates can only be collected when outside***)**	Latitude=	Latitude=
3	**What is the name of the household head?**		
4	**What are the walls of the house made of**	□ Dirt/earth/mud	□ Bricks/cement
5	**What is the roof made out of?**	□ Natural material □ Roofing Tiles	□ Corrugated iron □ Other _____
6	**What is the type of flooring in your dwelling?**	□ Dirt/earth □ Wood/plank □ Cement	□ Tile □ Other _____
7	**Does the family house have eaves**	□ Eaves present	□Eaves absent
8	**Does family house have any of the following?**	□ Windows with screens □ Air conditioning	□ Swamp cooler □ None of the above
9	**How many rooms are there in your house?**	__ __ Rooms	
10	**What is the principal household source of drinking water?**	□ Piped water in house □ Piped water in public tap □ Public Well	□ Borehole well □ Other_____
11	**How many people live in this house?**	__ __ People	
12	**How many people slept here last night?**	__ __ People	
13	**Do you sleep under a mosquito net?**	□ Yes	□ No
14	**Do you use mosquito coils to avoid mosquitoes?**	□ Yes	□ No
15	**Do you use firewood inside the house?**	□ Yes	□ No
16	**Presence of vegetation around the house.**	□ Bushes or tall grass	□ None

b) Larval Mosquito Sampling Data Sheet

Date: Day|__|__| Month|__|__| Year|__|__|__|__| Time …….Team Leader …….

Site……... Site #……|__| House ID ……..

INDOORS

Room	Habitat type	Habitat count	Species	Early instars	Late instars	Pupae

OUTDOORS

Place	Habitat type	Habitat count	Species	Early instars	Late instars	Pupae

Remarks: _____

Key: 1. Jerry can, 2. Small plastic food container/tin, 3. Bucket, 4. tire, 5.drum, 6.water tanks, 7.domestic animal drinking container, 8.flower pot, 9.vase, 10.cistern, 11.coconut, 12.bottle, 13.sufuria, 14.basin,15 pot, 16, other

Size/capacity; 1.small: <2 liters, 2. Medium: 2 to 7 liters, 3. large: >7 liters

Place: 1. Front yard (FY), 2.bushes around house (BH), 3.dump site (DS) 4.backyard (BY), 5.garden (G)

Room: Bedroom (BD), Sitting room (SR), Corridor (C), Kitchen (K), Bathroom (BR), Toilet (T), Store (S)

c). Ovitrap Sampling Data Sheet

Date set: Day|__|__| Month|__|__| Year|__|__|__|__| Team Leader …….
Date collected: Day|__|__| Month|__|__| Year|__|__|__|__| Site……... Site #……|__| House ID …….. House type: Wall …… Roof: ……...

INDOORS

Room	Mosquito type	Egg count	Early instars	Late instars	Males	Females

OUTDOORS

Place	Mosquito type	Egg count	Early instars	Late instars	Males	Females

Remarks: _____
_____

Appendix V. Entomological surveillance protocols

a). larval surveillance SOP

Larval sampling SOP

1. Purpose/applicability

1.1 Purpose: To provide guidelines for the procedures to be followed when sampling *Aedes* mosquito larvae and pupae.

1.2 Applicability: Entomology and all larval survey field staff.

2. Summary

This SOP describes the sampling of immature stages of container breeding mosquitoes of the genus *Aedes.* The survey involves identifying all natural and artificial wet containers in and around houses and examining each of them for larvae and pupae. Larval surveys are traditional methods used for monitoring mosquito populations. They can also be used to monitor the presence, distribution, and density and to determine the efficacy of treatment procedures.

3. Abbreviations and terms

3.1 SOP	Standard Operating Procedure.
3.2 Q/A	Quality Assurance
3.3 DVBNTD	Division of Vector Born and Neglected Tropical Diseases

4. Resposible personel

4.1 Field staff. Ensure adherence to this SOP

4.2 Field supervisor. Ensures that all the field staff adhere to the SOP

5. Equipment/materials

5.1 Pipettes

5.2 Ladles and plastic larvae rearing trays

5.3 Vials/specimen bottles

5.4 Field data entry forms

5.5 Masking tape

5.6 Pupae emergence paper cups

5.7 Marker pen

5.8 Torches/flashlights

5.9 Cool box

6. Procedure

6.1 Field team will carry out larval survey from 0700 to 0100 hours.

6.2 Verbal consent to inspect a house is sought from each house hold head. When consent is given the field team inspects all the natural and artificial containers in and around the house, including habitats such as tree holes and leaf axils that might harbor *Ae. aegypti* and other mosquitoes to determine whether the containers are wet or dry and whether they contain larvae and/or pupae. Containers located in dark or shaded areas will be inspected using flashlights.

6.3 When the field team is denied access into a household, they move and seek consent in the immediate neighboring household.

6.4 All pupae in wet containers are counted, and together with a sample of larvae are collected using ladles and pipettes, placed in vials, labeled and recorded on standard forms.

6.5 Samples are placed in a cool box and taken to DVBNTD laboratory, where they are counted and identified. Pupae are held in paper cups and allowed to emerge and the adults are identified using appropriate taxonomic keys.

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b). Ovitrap sampling SOP

1. Purpose/applicability

1.1 Purpose: To provide guidelines for the procedures to be followed in ovitrapping of *Aedes* eggs.

1.2 Applicability: Entomology and all ovitrapping field staff.

2. Summary

This SOP describes ovitrapping method for *Aedes* mosquitoes. The method involves setting ovitraps in households, identifying, counting and recording the eggs in each ovitrap. To confirm the identity of *Aedes*, the eggs are hatched and resultant larvae reared to adults. Egg densities in ovitraps have been used as a surrogate measure of the abundance and local activity of adult mosquitoes.

3. Abbreviations and terms

3.1 SOP Standard Operating Procedure.

3.2 Q/A Quality Assurance

3.3 DVBNTD Division of Vector Born and Neglected tropical Diseases

4. Resposible personel

4.1 Field staff. Ensure adherence to this SOP

4.2 Field supervisor. Ensures that all the field staff adhere to the SOP

5. Equipmnt/materials

5.1 Modified ovitraps* 5.9 Counter.

5.2 Plastic bags and plastic rearing trays

5.3 Masking tape

5.4 Marker pen

5.5 Field Data forms

5.6 Cool box

5.7 Stereomicroscope

5.8 Filter papers/paper towels

*Ovitraps modified by replacing the wooden paddles(oviposition substrate) with brown filter papers that lined the inner surface of the cup.

6. Procedure

6.1 Ovitraps will be set in selected households by being placed at ground level. The indoor ovitraps are placed in dark room corners, near racks with cloths, and at least 2 meters from water containers, while outdoor traps are placed suitably around the house (under vegetation).

6.2 The ovitraps will be exposed for oviposition for five days, after which paddles (or paper towels) are collected and placed in plastic bags which are labeled, placed in a cool box and transported to the DVBNTD laboratory.

6.3 In the laboratory, each paddle (or paper towel) will removed from its protective bag, superficially dried by being placed between sheets of white paper tissue. Paddle and tissues are examined under a stereomicroscope (x 40); eggs observed are counted and recorded.

6.4 Immediately after counting, the eggs are submerged in seasoned tap water (in plastic rearing trays) to hatch. The resultant larvae are reared to adults that will be checked for identification.

References.

1. Chadee, D.D. (2009). Oviposition strategies adopted by gravid Aeries aegypti (L.) (Diptera" Culicidae) as detected by ovitraps in Trinidad, West Indies (2002-2006) *Acta Tropical*. 11: 279-283.

2**.** Chadee, D.D, Corbet, P. S . and Talbot. (1995). Proportions of eggs laid by *Aedes aegypti* on different substrates within an ovitrap in Trinidad, West lndies. *Medicul and Veterinary Entomology* 9. 66-70

3**.** Mogi, M., C. Khamboonruang, W. Choochote, and P. Suwanpanit.(1988). Ovitrap surveys of dengue vector mosquitoes in Chaing Mai, northern Thailand: seasonal shifts in relative abundance of *Aedes albopictus* and *Aedes aegypti*. Med. Vet. Entomol. 2: 319-324.

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