Abundance and control of malaria mosquito larvae in the traditional water management agro-ecosystem of Kasagam, western Kenya

By:

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Award of the Degree of Master of Science (Applied Parasitology) in the School of Biological Sciences of The University of Nairobi.

August, 2009

DECLARATION

I, Collins Kalwale Mweresa, hereby declare that this thesis is my original work and has not been presented for a degree in any other university.

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Acknowledgement

I express my deep and sincere gratitude to all those who assisted me to realize my goals of undertaking this study. I appreciate Prof. Willem Takken for generously accepting to fund my attachment on Wageningen - KEMRI project in Kisumu and for being my second supervisor. You were always concerned about my progress by insisting on results and you provided prompt responses whenever I communicated to you. I am very grateful to my supervisor and mentor, Dr. Wolfgang Richard Mukabana who went extra miles of being very patient with me and who sacrificed a lot of his precious time sitting or frequently calling me to discuss the progress of this study. You were always ready to show me the way. I am grateful to Ms Susan Imbahale for guiding me frequently, closely monitoring all aspects of this study and for being very patient with me for the whole period of this study. I also thank Dr. John Vulule, Dr. Andrew Githeko and Mr. Mulaya from the Centre of Global Health Research (CGHR), Kenya Medical Research Institute (KEMRI) at Kisian for accepting my attachment and allowing me to access their research facilities. My thanks go to Mr. David Madahana, Nick Juma, Tom Ouna, David Obuom, Maurice Ombok, Dr Yaw and Ms Annet Obusula for the team spirit that I enjoyed as I worked with them during data collection. I also thank Mr. Paul Mabuka and Mr. Amos Wawire for collecting data with me in Kasagam during the hard moments of 2007 post election violence. My special thanks go to all my classmates for the regular encouragement and consultations that we shared. Last but not least, I gratefully acknowledge my brothers and sisters, dad Nelson, mum Erica, wife Pamella and daughters Lyn, Ella, Recho and Finny. Your great moral support at times of indecision and endurance while I was away was a blessing to me. Finally I thank the almighty God for having kept me in a sound health condition throughout this study.

Dedication

I dedicate this work to my dad Nelson Mweresa, mum Erica Mweresa, wife Pamella Akure Kalwale and glory be to God for the great things he has done in my life.

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Abstract

This study investigated the abundance and control of malaria mosquito larvae in a traditional water management agro-ecosystem of Kasagam, Kisumu City, western Kenya. The study of larval habitats of malaria vectors is important in determining abundance and fitness of resultant adult mosquito populations and planning for suitable control measures. Dominant types of plants found in the study site were identified, recorded and categorized. Sampling of mosquito larvae was done in particular habitats with different plant cover types, water management practices, growth phases of rice and larval control methods. *Anopheles gambiae* Giles *sensu stricto* larvae were fed on different diet based treatments while others were subjected to different larval control treatments. Out of 2494 L3-L4 *Anopheles* mosquito larvae identified in Kasagam, 3.93% were *An. arabiensis* Patton, 0.76% were *An. funestus* Giles while 95.31% were non-vector species of malaria. Abundance of larval stages of anopheline mosquito larvae was significantly influenced by plant cover types ($P < 0.001$), rice growth phases ($P < 0.001$) and larval control methods ($P < 0.001$). However, the density of anopheline mosquito larvae was not significantly influenced by the types of water management practices ($P = 0.174$) as compared to culicine ($P < 0.003$). Contrastingly, different plant cover types ($P = 0.462$), rice growth phases ($P = 0.104$) and larval control methods ($P = 0.960$) showed no significant difference in the habitat abundance of different types of late instar (L3 and L 4) stages of anopheline mosquitoes identified. Diet based treatments were observed to have a significant effect on larval survival ($P < 0.001$), development period ($P < 0.001$) and body size ($P < 0.001$) of *An. gambiae s.s* mosquitoes. Traditional water management agro-ecosystem practices and larval control methods influence the abundance of malaria mosquito larvae in Kasagam, Kisumu city.

Chapter One: Introduction and Literature Review

1.1. Introduction

Activities of man associated with traditional water management agro-ecosystem practices are important in the epidemiology of malaria because they provide breeding grounds for malaria vectors. The study of breeding habitats resulting from such practices can be vital in predicting the abundance, dynamics and fitness of the resultant adult mosquito populations (Paaijmans *et al.*, 2008). This understanding is useful in timing of vector control programs with the aim of reducing malaria burden (Minakawa *et al.*, 1999)

Human malaria is a protozoan disease caused by five species of *Plasmodium* parasites: *Plasmodium falciparum*, *Plasmodium malariae, Plasmodium ovale, Plasmodium vivax* (Arora and Arora., 2002) and *Plasmodium Knowlesi* (Cox-Singh *et al.*, 2008). *Plasmodium Knowlesi* is a simian parasite reported in Malaysia and is widespread in other parts of South East Asia (Cox-Singh *et al.*, 2008; Cox-Singh and Singh, 2008). These *Plasmodium* parasites have two hosts during their life cycle which involves female *Anopheles* mosquitoes as the definitive host and man as the intermediate host (Service, 2004).

Worldwide an estimated five hundred million malaria infections occur yearly, with two to three million deaths. The World Health Organization estimates that more than 85% of the burden occurs in Sub-Saharan Africa, where approximately three thousand people die every day (WHO, 2005). Children less than five years and pregnant mothers

are most affected. This hinders achievement of millennium development goals pertaining to reduced child mortality, improved maternal health, attainment of universal education, fighting HIV/AIDS and other diseases and eradication of poverty and hunger (Sachs and Malaney, 2002; Kiszewski *et al.*, 2007).

More than 41% of the world population is at a risk of acquiring malaria especially children, pregnant women, travelers, refugees, displaced persons and laborers entering endemic areas. This proportion is increasing yearly due to deteriorating health systems, growing drug and insecticide resistance, lack of effective vaccines, war and climatic change (Shiff, 2002). Poor people in rural areas bear a greater burden of the disease due to lack of access to prompt treatment. As a result infection rates are highest in rural areas especially during rainy seasons because this is a time of intense agricultural activities that provide more breeding grounds for *Anopheles* mosquitoes that transmit malaria (Yaw *et al.*, 2004). Malaria risk is also increasing with the daily growth of city populations that are engaging in irrigated traditional suburban agriculture. Further studies have shown that there is a slow adaptability of anopheline species to existing polluted water in urban habitats (Sattler *et al.*, 2005).

The experiments reported in this thesis were designed to investigate abundance and control of malaria mosquito larvae in the traditional water management agro-ecosystem of Kasagam, Kisumu city, Western Kenya.

1.2. Literature Review

1.2.1. Traditional Agriculture and Water Management Practices

Traditional agriculture is an indigenous form of farming which results from co-evolution of local, social and environmental systems that exhibit a high level of ecological rationale (Altieri, 1987). This rationale is expressed through intensive use of local knowledge and natural resources, including management of agro-biodiversity in form of diversified agricultural systems (Altieri, 1987; Chang, 1977). It may also involve traditional water management practices characterized by primitive forms of irrigation. Such types of irrigation systems are dependent on available raw materials, social structures and indigenous knowledge that lack technical knowhow and financial capacity (Nitya, 2008)

1.2.1.1. Characteristics of Traditional Agriculture

Traditional agriculture involves accumulated experience of indigenous farmers interacting with the environment without access to external inputs, capital or modern scientific knowledge (Chang, 1977). The farming systems used have emerged over centuaries of cultural and biological evolution that has shaped the sophisticated knowledge they represent. Farmers use inventive, self reliance, experimental knowledge, and locally available resources to produce sustained food yields for local consumption (Harwood, 1979). A strategy of high degree of plant biodiversity in form of polyculture is used to minimize risks, maximize yields and diversify diet while preserving the cultural aspects of the society (Altieri, 1981). Consequently, interaction

between crops, animals and trees allows agro-ecosystems to optimize their own soil fertility, pest control and productivity (Chang, 1977). However, traditional agriculture is rapidly disappearing in the face of major social, political and economic changes.

1.2.1.2. Relationship between Agriculture, Water and Malaria Transmission

The ecology of malaria is associated with the availability of water which provides oviposition sites of malaria vectors. Malaria is generally considered as a rural disease because *Anopheles* mosquitoes prefer relatively fresh water for breeding. However, Sattler *et al* (2005) confirmed that in Dar es Salaam malaria vectors and other anophelines are adapting progressively to man-made polluted environments. Proximity of human dwellings to breeding sites enhances propagation of vector populations and increases human vector contact (Koenraadt *et al.*, 2003).

The highest *Anopheles* population in areas with urban agricultural land use mainly occurs in rice paddies, agricultural trenches between vegetable patches and irrigation wells and in other man-made habitats (Barbara *et al.*, 2006). In such areas, habitats of *An. gambiae* are characterized by the presence of algae, absence of floating vegetation and co-occurrences of *Culex* larvae. On the other hand, shade that is provided by emergent plants over the water surface is suitable for breeding of *An. funestus* (Mwangangi *et al.*, 2007). Planting of rice and other crops like coco yams, cassava and sweet potatoes result into areas of shallow surface water for the breeding of malaria vectors (Lock and De Zeeuw, 2001). This forms an important epidemiological parameter of malaria in areas with urban agriculture. Recent studies by Munga *et al* (2006) have shown that larvae of *An. gambiae* sensu lato (s.l.) occur more frequently in

sunlit temporary pools, or slow moving waters in cultivated areas than in forested and natural swamps.

Hand transplanting of rice creates slurry of mud and shallow open pools which are highly suitable for the breeding of *An. gambiae* mosquitoes (Highton and Chandler, 1975). However, larval densities of these vectors reduce with the increase in age of the rice plant. This explains why the construction of irrigation systems and water reservoirs like man made pools in some parts of the world has had a dramatic impact on malaria distribution and intensity (Service, 2004).

1.2.1.3. Maize Pollen and Development of *Anopheles arabiensis* larvae

Anopheles gambiae is the most efficient malaria vector and it develops in transient bodies of turbid water that are generally vegetation free (Gimnig, 2002). The presence of inert particles in larval habitats of these malaria vectors suggests that their feeding strategy may be adapted peculiarly to disturbed environment (Ye-Ebiyo *et al.*, 2000). Ye-Ebiyo *et al* (2000) demonstrated that *An. arabiensis* larvae did not develop into pupae where no maize was grown. More pupae were observed where newly emerged larvae had immediate access to maize pollen than when pollen supplementation was delayed, absent or where they were distant from flowering maize. Consequently, adults obtained from habitats close to flowering maize or where larvae were continuously supplemented with maize pollen developed more rapidly and frequently into more adults than where there was less or no maize pollen. Such adults had longer wings and larger body size (Kebede *et al.*, 2005). Larger mosquitoes live longer and increase the intensity of malaria transmission (Oketch *et al.*, 2007). Enhanced development of *An.*

arabiensis by maize pollen also increases malaria transmission (Ye-Ebiyo *et al.*, 2002). The ability of anopheline mosquitoes to feed on maize pollen in turbid water is enhanced by the release of a water-soluble phagostimulant which may be used to increase ingestion of microbial entomotoxins (Ye-Ebiyo *et al.*, 2003). The rate of malaria transmission in Sub-Saharan Africa might be reduced if pollen of crops like maize would express entomotoxins (Ye-Ebiyo *et al.*, 2003). This is because maize is predominant in many malaria endemic areas of Africa and it also has close proximity to the breeding sites of *An. gambiae* complex and homes. Studies done by Kebede *et al.*, (2005) suggested that the intensity of maize cultivation was positively correlated with malaria incidence in Bure District of northwestern Ethiopia. However, the nutritional relationship between rice pollen and malaria vectors should be explored given the fact that malaria vectors predominantly breed in endemic areas with irrigated rice fields.

1.2.2 Major Malaria Vectors in Africa

Malaria in Africa is mainly transmitted by three mosquito species namely: *An. gambiae*, *An. arabiensis* and *An. funestus*. *Anopheles gambiae* and *An. arabiensis* belong to the *An. gambiae* complex, while *An. funestus* belongs to the *An. funestus* complex.

The *Anopheles gambiae* complex comprises of the world's most effective malaria vectors (Coetzee *et al.*, 2004). For a long time this complex was considered to have seven morphologically indistinguishable species (Service, 2004). The seven sibling species are *An. gambiae*, *An. arabiensis*, *An. melas*, *An. merus*, *An. bwambae*, *An. quadriannulatus* species A and *An. quadriannulatus* species B are most responsible for transmitting malaria in Africa

Anopheles gambiae s. s is an efficient vector because it has very high endophilic and anthropophilic characteristics (Githeko *et al.*, 1996; Service, 2004). The vectorial capacity of *An. arabiensis* is slightly lower than that of *An. gambiae* because of its ability to feed on other animals when humans are not available (Githeko *et al.*, 1996). After a blood meal, majority of *An. gambiae* females rest indoors (endophily) while *An. arabiensis* rests in outdoor shelters. These two species are sympatric although *An. arabiensis* is more widely distributed in the drier low altitude areas unlike *An. gambiae s.s* that prefers humid, higher altitude areas (Onyabe and Conn, 2001; Koenraadt *et al.*, 2003). However, Mahande *et al* (2007) observed that in Northern Tanzania, *An. arabiensis* displayed a higher exophilic tendency compared to *An. gambiae s.s* and *Culex spp.* The predominant zoophily by both *An. arabiensis* and *An. gambiae s.s* in Madagascar (Duchemin *et al.*, 2001) is a contrast to their greater anthropohily in continental Africa.

Anopheles quadriannulatus species A occurs in South Africa while species B is restricted to the Ethiopian highlands. *An. bwambae* is uniquely distributed in Uganda, while *An. melas* is commonly found in central and west Africa (Bigoga *et al.*, 2007). *An. merus* occurs in Southern and Eastern Africa (Coetzee *et al*, 2004; White *et al.*, 1974). Apart from these major vectors, *An. nili* has also been reported to transmit malaria (Dia *et al.*, 2003). In general, the distribution of malaria vectors is dependant on the prevailing temperature, rainfall, humidity, vegetation, human population density, type and condition of the larval habitats and distance from the breeding sites (Koenraadt *et al.*, 2003; Smith, 1995).

1.2.3.0. Location and Selection of Oviposition Sites

Oviposition is an important component of mosquito borne diseases because pathogen acquisition requires the adult to take in at least one blood meal for egg formation (Michael and Jonathan, 1989). Oviposition attractants and responses are also important because they can be used in mosquito trapping for identification, surveillance and control purposes (Geetha *et al.*, 2003). The factors involved in location and selection of oviposition sites are classified as visual, olfactory and tactile behavioral responses.

1.2.3.1. Visual Cues

Visual attributes of oviposition sites include brightness (relative to the surrounding) and color. The background color of an oviposition site influences both the preference and manner in which eggs are laid. For instance, sites with dark colored backgrounds are preferred over those with light color (McCrae, 1984). Above a dark surface *An. gambiae* oviposits while in flight, but above a bright surface oviposition occurs from a settled posture, which suggests a response to sub-optimal stimuli (McCrae, 1984). *Anopheles gambiae* mosquitoes have also been associated positively with increasing levels of turbidity (McCrae, 1984; Gimnig *et al.*, 2001). The size of breeding sites also play a vital role because *An. gambiae* prefers temporary, small and shallow pools of water while *An. funestus* prefers large, more permanent water bodies (McCrae, 1984; Gimnig *et al.*, 2001; Mwangangi *et al.*, 2007).

Some mosquito species associate positively with the presence of specific aquatic vegetation (Gass *et al.*, 1983). *Mansonia* species prefer sites where plants like *Pistia*

and *Eichormia* species are found. Once a mosquito has identified a potential oviposition site water vapor stimulates it to proceed with the actual oviposition (Kennedy, 1942).

1.2.3.2. Physicochemical Factors

Microbial populations in breeding sites produce volatiles that serve as semiochemicals for gravid *An. gambiae* (Munga *et al.*, 2005). These signals along with other chemical and physical cues may be used by the females to assess the suitability of potential larval habitats in order to maximize the fitness of her offspring (Sumba *et al.*, 2004). In nature, chemical compounds do not act independently in attracting or stimulating gravid female mosquitoes. They interact with other biotic and abiotic factors at the oviposition site, thus presenting an integrated picture of the attractiveness of the oviposition site (Beehler *et al.*, 1993). Maybe this is why mosquitoes get attracted towards a given oviposition site, but relatively few of them may actually oviposit (Sunish *et al.*, 2003). This could also explain why gravid *Aedes aegypti* settles on the water surface to oviposit when a suitable oviposition site is encountered (Kennedy, 1942). However, eggs are distributed in more than one oviposition site when an unsuitable breeding site is encountered (Chua *et al.*, 2004)

Gravid female mosquitoes might have acquired an evolutionary adaptation of avoiding ovipositing in unsuitable breeding sites in which toxic compounds might be detrimental to the survival and development of their offspring (Yih-shen *et al.*, 1980). Mosquitoes may also leave behind conspecific chemical cues that inform others about the status of the water. For instance, oviposition responses to larval rearing water by *Aedes albopictus* was greater than to normal water (Yap *et al.*, 1995). They may also

overcome immediate competition by avoiding habitats containing their immature stages. For instance, presence of larvae was repellent to both *An. gambiae* and *Ae. aegypti* mosquitoes seeking to oviposit their eggs (McCrae, 1984). Mosquitoes are also able to avoid sites likely to be inhabited by potential predators like *Gambusia affinis* (Blaustein *et al.*, 2004). Memory of a rearing environment also influences oviposition site preferences (McCall and Eaton, 2002). This is why mosquitoes reared in water containing a compound that deters them from oviposition will prefer such waters during their adulthood.

Chemical factors also affect oviposition site preferences. *Anopheles arabiensis* prefers sites with elevated concentrations of carbonates and hydrogen carbonates, but low concentrations of nitrates and sodium chloride. On the other hand anophelines are highly deterred by the presence of nitrates in breeding sites. However, culicines are attracted by sites that are highly polluted with organic matter and urea (Sunish *et al.*, 2003).

1.2.4. Ecology of Oviposition Sites of Anopheline Mosquitoes

The ability to discriminate between different breeding sites is more or less genetically determined by natural selection (Yih-shen *et al.*, 1980). Oviposition sites chosen by mosquito species may differ in size, appearance, presence or absence of vegetation, types of aquatic vegetation, whether the water is still or flowing, salinity of the water, degree of pollution, degree of turbidity and presence or absence of shade. For instance gravid female *An. gambiae* mosquitoes prefer to breed in sunlit pools of fresh water that are small, shallow and turbid (Gimnig *et al.*, 2001). These pools are usually temporary;

they persist for about 4-5 weeks and are often associated with the presence of algae and absence of aquatic vegetation. *Anopheles gambiae* may have evolved ecologically to exploit such types of habitats because they are r-strategists and therefore they exploit increased resources of warmer open habitats that tend to produce food in the form of algae than shaded habitats (Gimnig *et al.*, 2002; Munga *et al.*, 2006). Predation is also less prevalent in temporary habitats than in large, permanent ones (Sunahara *et al.*, 2002). These small sunlit habitats with higher water temperatures have shorter time of mosquito larval to pupal development (Munga *et al.*, 2006). Lower bacterial populations in such habitats reduce the level of organic pollution and lowers mortalities associated with organic pollution. However, slow adaptability of anopheline species to existing polluted water in urban habitats has been observed (Sattler *et al.*, 2005; Vincent *et al.*, 2003). Larval mortality due to desiccation or poor nutrient conditions may be reduced until water temperature rises above 30^0C. Consequently, enhanced larval habitat conditions may increase productivity of adult mosquitoes, which in turn increases the risk of malaria transmission (Munga *et al.*, 2006).

1.2.5. Control of Malaria Mosquito Larvae

Malaria continues to be a plaque to mankind at the advent of the new millennium. This is caused by increased resistance to the antimalarial drugs, insecticides resistance, lack of effective vaccines, reduced immunity, loss of infrastructure for mounting systematic attack on the parasites and their transmission in countries with endemic infections (Shiff, 2002).

1.2.5.1. Environmental Management

Environmental management refers to the planning, organization, implementation and evaluation of deliberate changes of environmental factors with the aim of preventing the propagation of vectors and reducing human-vector-pathogen contact (Shiff, 2002; Service, 2004). Environmental management has been successfully applied in African cities, notably Dar es Salaam in Tanzania (Castro *et al.*, 2004) and may have an important role to play in protecting the rapidly growing urban population of Africa from malaria (Keiser *et al.*, 2004).

In Zambia environmental management measures that were launched consisted of vegetation clearing, modification of river boundaries, drainage of swamps and house screening (Utzinger *et al.*, 2001). However, in India and Malaysia sluice gates are periodically opened to flush out larvae from small isolated pools of water. In rice fields, intermittent flooding is done to allow drying out every 3-5 days to reduce vector populations. Impoundment transforms marshy areas into relatively deep permanent waters with defined vertical banks so that mosquitoes do not lay their eggs on wet muddy edges of pools (Service, 2004). Such impounded waters are commonly stocked with fish to reduce mosquito breeding. Realignment of watercourses has been done to increase water flow and prevent build up of static water pockets for the breeding of mosquitoes.

However, modification of larval habitats may also create suitable conditions for different types of mosquito species that were previously either absent or uncommon (Service, 2004). Since *Anopheles* is opportunistic, their populations expand during rainy

spells and they breed in a variety of habitats. This means that, any attempts to limit the extent of suitable habitats will not be very successful (Shiff, 2002). The situation is worsened by the fact that environmental management for malaria control requires specialist skills that are currently lacking in sub-Saharan Africa where they are needed most (Mukabana *et al.*, 2006). Nevertheless, the length of malaria vector survival contributes more significantly to efficient transmission of malaria than the population (Shiff, 2002).

1.2.5.2. Larvivorous Fish

The most commonly used larvivorous fish is *Gambusia affinis* and it is traditionally called mosquito fish. This is a warm water fish, originally a native of Southern USA. The use of fish significantly decreased the incidences of mosquito borne diseases especially in Iran, Afghanistan, Somali, Ethiopia, Greece, Russia and China (Service, 2004). This is why Bay (1967) considered this fish to be the most generally suited for mosquito control. It is small in size; relatively tolerant to polluted water, extreme salinity and temperature; feeds at the water surface; is a prolific life bearer producing several broads each season in warm areas thus making it highly adaptable to many different mosquito larval habitats. Surface feeding enhances the efficiency of *G. affinis* in controlling *Anopheles* mosquito larvae because they lie horizontally and parallel to the water surface and rarely dropping to the bottom as compared to *Culex* mosquito larvae (Blaustein, 1992). The long lifespan and population dynamics of *G. affinis* makes it to be a more successful control agent of mosquitoes than other seasonal predators like tadpoles (Bence, 1988).

The major environmental problem is its adaptability and aggressive behavior. This has resulted in displacement or extermination of several indigenous species where it has become established (Garcia, 1983). The role of *G. affinis* for mosquito control occurs under local conditions where vegetation is abundant and micro fauna is rich enough to supply their needs. Studies done by Homski *et al* (1994) suggested that different sizes of *Alphanius dispar* and *G. affinis* can complement each other as effective mosquito control agents when the two predators are used in different habitat conditions. *Gambusia affinis* yield better results in deep canals and pools having limited food supply.

Studies conducted by Garcia (1983) concluded that reasonable mosquito control can be achieved by early season stocking rate of about 300 adult females per acre. These are the suitable features that constituted the selection criteria of *G. affinis* in larval control experiments that are reported in this study.

1.2.5.3. Microbial Larvicides

The use of *Bacillus thuringiensis* var *israelensis* (*Bti*) and *Bacillus sphaericus* (*Bs*) is undoubtedly the most useful biological agent in tropical water against mosquito larvae. These microbial larvicides can be mass produced, are toxicologically safe to humans and wildlife, they are more or less specific in killing mosquito larvae and are easily applied (Service, 2004). They also kill *Simulium* species thus reducing transmission of onchocerciasis. The formulation of *Bti* as release granules extends the insecticidal activity for many days. When *Bti* is ingested mortality is caused by endotoxins acting as a stomach poisons. Genetic engineering has improved the larvicidal activity of *Bacillus*

larvicides and has also transferred the genes responsible for the production of the poison endotoxin into other bacteria (Service, 2004).

Unlike *Bti*, *B. sphaericus* can be recycled in larval habitats and it is more effective in organically polluted waters thus being suitable against *Culex* mosquito larvae. Both microbial larvicides recycle and persist in the environment for at least seven days (Fillinger *et al.*, 2006). The use of these two microbial larvicides in Mbita, Western Kenya has proved that the main vectors of malaria have a high susceptibility to these control agents. Low dosages are required and environmental impacts are negligible (Fillinger *et al.*, 2003).

Larviciding and source reduction are important because they have a major advantage of controlling mosquitoes before they disperse and transmit diseases (Killeen *et al.*, 2002). Further studies in Western Kenya have proved that, appropriately applied, microbial larvicides can substantially and cost effectively reduce human exposure to malaria in rural Sub-Saharan Africa (Fillinger *et al.*, 2006). Recent studies in The Gambia have shown that environmentally safe microbial larvicides can significantly reduce larval abundance in natural habitats (Fillinger *et al.*, 2007).

However, the cost of intervention in these settings could be reduced with formulations that provide a greater residual effect (Fillinger *et al.*, 2006). Results from field trials in Western Kenya with *Bti* water dispersible granules (WDG), showed that only a very low dosage of 200g/ha (2700 International toxic units/milligram (ITU/mg)) is required to effectively suppress late instars and resulting pupae of malaria vectors despite low residual effect (Fillinger *et al.*, 2003). *Bacillus thuringiensis* var *israelensis* is used

exclusively during periods of heavy rains that dilute it and it also has an advantage of reducing the risk of resistance development. On the other hand *B. sphaericus* provides a greater residual larvicidal activity. This is because its spores persist longer in the environment and thus providing a recycling effect that leads to the control of several mosquito generations (Fillinger *et al.*, 2003). This is why *Bti* was used in the laboratory and field experiments reported in this thesis.

1.3. Justification and Significance of the Research

Sub-Sahara Africa has very high malaria endemicity with transmission occurring throughout the whole year. This creates a vicious circle of disease and stagnant socioeconomic development (Sachs and Malaney, 2002; Kiszewski *et al.*, 2007). Increasing urban populations have also contributed to this vicious cycle by engaging in subsistence perennial irrigated urban farming of different types of crops. This is aimed at alleviating poverty, providing food security and improvement of nutrition (Yaw *et al.*, 2004). The whole situation sustains a vicious cycle of subsistence irrigation farming, breeding of malaria vectors, infection, and re-infection of people with malaria, poverty and inadequate food supply.

Larval control is neither part of the strategy of Roll Back Malaria, nor is it a central theme supported by major institutions engaged in malaria control (Shiff, 2002). However, malaria transmission depends on the presence of a competent female *Anopheles* mosquito to act as a vector and whose density is determined by the number, distribution and productivity of larval habitats that are highly diversified and generally closer to human habitats (Koenraadt *et al.*, 2003). Most larval control may work best and may be most cost effective in sites where larval habitats are seasonal, relatively fewer, well defined, accessible and where human population density is high enough to justify repeated treatment of all breeding sites (Fillinger *et al.*, 2006). This controls mosquitoes before they disperse and transmit diseases (Killeen *et al.*, 2002). It is for this reason that more studies of this type should be done in areas associated with traditional water management agro-ecosystems because they provide breeding grounds for malaria vectors.

The information gathered from this study on mosquito larval ecology and control will be used to: (1) identify traditional agricultural and water management practices that affect the breeding of malaria vectors, (2) provide mosquito larval control strategies that are environmentally, technically and financially feasible for routine implementation in the African context (Fillinger *et al.*, 2003; 2006), and (3) form part and parcel of integrated vector control strategy.

1.4. Objective

1.4.1. Overall Objective

To evaluate the influences of traditional water management agro-ecosystem practices and larval control methods on abundance of malaria vector larvae in Kasagam, Kisumu city, western Kenya.

1.4.2. Specific Objectives

a) To identify traditional agricultural practices that influences the breeding and abundance of malaria mosquito larvae in the suburban agro-ecosystem of Kasagam.
b) To investigate the effect of traditional water management practices on mosquito larval density in the suburban agro-ecosystem of Kasagam.
c) To determine the effect of different growth phases of rice (*Oryza sativa*) in paddies on the productivity and larval abundance of malaria vectors in the suburban agro-ecosystem of Kasagam.
d) To investigate the best larval control method that would reduce the abundance of malaria vector larvae in a suburban agro-ecosystem of Kasagam.

1.5. Hypothesis

Traditional water management agro-ecosystem practices and larval control methods have a significant impact on the abundance of malaria vector larvae in Kasagam.

Chapter Two: Materials and Methods

Studies reported in this thesis were conducted in the suburban agro-ecosystem of Kasagam located two kilometers on the eastern part of Kisumu city centre (0° 5', 23''N, 34°, 45', 0''E) along the Kisumu-Nairobi road in Western Kenya. Complimentary laboratory experiments were conducted in a screenhouse at the Centre of Global Health Research (CGHR), Kenya Medical Research Institute (KEMRI), Kisian which lies 13 km on the north western part of Kisumu city.

2.1. Study Area

The Kasagam study site covered an area of about 0.900 km² in the suburbs of Kisumu city, western Kenya. It is located in Nyalenda A sub-location, West Kolua location, Winam division of Kisumu district, Nyanza province, Western Kenya. It lies at an altitude of approximately 1135 meters above sea level with an average yearly rainfall of about 1,127mm characterized by two peak seasons. Long rains occurred between late March and June, while short rains were experienced between September and November. The average annual temperature was about 23.1°C. Kasagam is a transformed swampland; is fairly flat and has black volcanic soil.

Land in Kasagam was sub-divided into small plots (225m²) on which diversified subsistence farming was done using locally available knowledge and resources. The plots were irrigated through unplanned water channels which originated from poorly defined and unreliable water sources. Such water sources included ground seepage,

leakages from urban water pipes, surface run-off, sewage and waste water from adjacent residential estates.

The small water channels that irrigated the plots contained slow moving or stagnant water which branched off from the larger water channels. Both types of man-made water channels were either used for irrigation during the dry seasons or for drainage of the plots during rainy seasons. Small scale subsistence agriculture was practiced in the area. Crops grown included maize (*Zea mays*), beans (*Phaseolus spp*), pigweed (*Amaranthus spp*), finger millet (*Eleusine coracana*), bananas (*Musa paradisiaca*), green grams (*Phaseolus aureas*), cow pea (*Vigna unguiculata*), pumpkins (*Cucurbita spp*), *Brassica spp* (e.g. cabbages and kales), rice (*Oryza sativa*), coco yams also called taro (*Colocasia esculenta*), Sorghum (*Sorghum spp*), cassava (*Manihot esculenta*), sweet potatoes (*Ipomea batatas*) and tomatoes (*Lycopersicon lycopersicum).*

Decline in crop farming had transformed once existing rice paddies into grasslands and swamplands (dominated by *Cyperus rotundus*). This created more room for grazing, harvesting of grass for dairy farming and expansion of commercial nurseries for trees and ornamental plants. Growing of plants in nurseries and car wash activities thrived along the Nairobi – Kisumu highway where they utilized lots of water supplied through large man-made water channels. Some of the water was stored in man-made pools and used during dry seasons while the surplus was released for irrigation of crops through small water channels. The non-crop plants growing in Kasagam included water fern *(Azolla filiculoides)*, napier grass (*Pennisetum purpureum*), African star grass (*Cynodon nlemfuensis)*, African couch grass (*Digitaria scalarum*), papyrus (*Cyperus rotundus*),

red algae (*Rhodophyte spp*), brown algae (*Phaeophyte spp*), castor oil plant (*Ricinus communis*) and wondering Jew (*Commelina hybridus*).

The southern and eastern parts of the site were surrounded by slums located 30m to 1.5km away. The slums had a rapidly expanding human population of different tribes with ethnic Luos being the most dominant. Majority of the people were mainly employed in the informal sector within Kisumu City and its neighborhoods. Most people lived in mud-walled houses with single or double rooms, rusty roofs and open eaves which allowed mosquitoes to enter and leave. This proximity of increasing human settlements to the Kasagam study site created a unique ecological set up which greatly contributed to the local and perennial transmission of malaria.

2.2. Plant Cover Types and Abundance of Mosquito Larvae

One objective of this study was to identify traditional agricultural practices that influence the breeding and abundance of malaria mosquito larvae in the suburban agro-ecosystem of Kasagam. This involved identification of dominant plants, sampling of mosquito larvae in habitats with different plant cover types and estimation of percentage shade. Daily amount of rainfall (mm) was measured (November 2007 to January 2008) using a rain gauge installed at the Kisumu Redeemed Gospel church located 300 meters away from the study site.

2.2.1. Identification of Dominant Plant Cover Types

Plants that grew in the study area were identified and categorized into two broad groups: those which grew along banks of water channels and those which grew inside the water channels. Plants which grew along the banks of the water channels were identified and grouped as food and non food crops. Those which grew inside water channels with roots anchored in the soil were identified and classified as rooted emergent plants while plants suspended on the water surface were grouped as floating.

2.2.2. Abundance of Anopheline Larvae under Different Plant Cover Types

Six artificial habitats with different plant cover types were established inside or along the banks of man-made water channels (Figure 1). The habitats consisted of coco yams (*Colocasia esculenta*) growing inside or along banks of water channels, sweet potatoes (*Ipomea batatas*) growing along banks of the water channels, African couch grass

(*Digitaria scalarum*) growing inside water channels, water ferns *(Azolla filiculoides)* growing on the water surface and open sites with no plant cover (control).

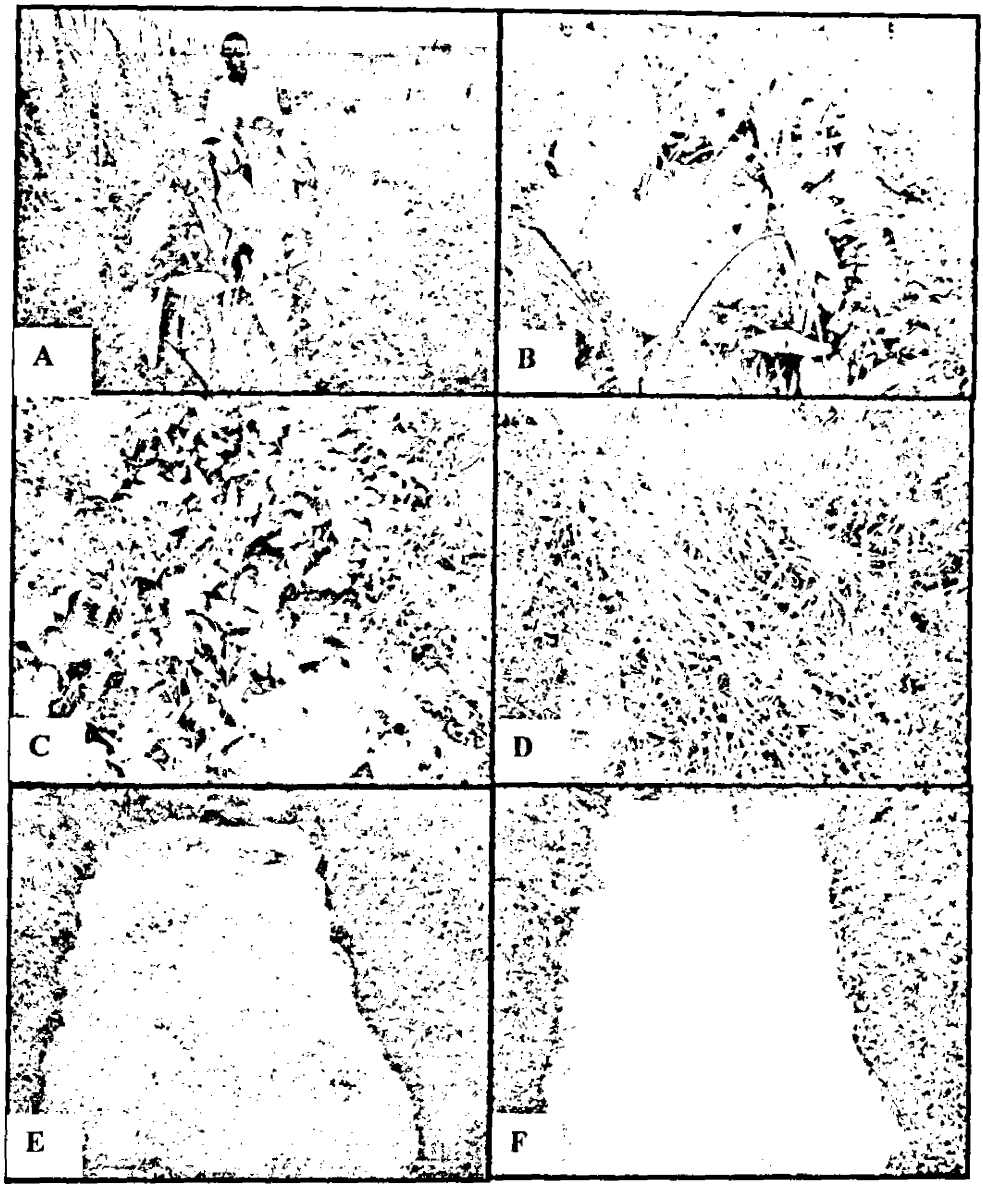

Figure 1. Mosquito breeding habitats with different plant cover types. **A** represents coco yams growing inside water, **B** coco yams growing along banks of water channels, **C** sweet potatoes growing along banks of water channels, **D** African couch grass growing inside water, **E** water ferns growing on the water surface and **F** is an open site (control).

A completely randomized replication design was used in this study. Each of the six habitats (2m × 0.75m × 0.3m) was replicated five times. All plants were planted and allowed to establish for five weeks except water ferns which were suspended on the water surface. The five water channels with different plant cover habitats were located 20m away from large water channels that irrigated the tree nurseries and about 90m away from adjacent houses. Silt was removed; edges of the habitats were slashed while weeds growing between the plants were uprooted on weekly basis. This prevented any unforeseen effects of siltation and unwanted plant cover during the study period. Each habitat type was separated from each other by a10cm thick wall made up of soil/mud with a narrow outlet on the upper part which allowed water to flow out.

After five weeks (of plant establishment) sampling of mosquito larvae was done twice a week (on every Monday and Friday morning) for twelve weeks (November 2007 to January 2008). This was done using a standard (350ml) white plastic larval dipper (Clark®) having a wooden handle. Ten dips were randomly made at the water edges, surface and close to plants in each habitat. All individual mosquito larvae including L1 and L2 (early instar), L3 and L4 (late instar) stages and pupae of culicine and anopheline mosquitoes were counted and recorded. All culicine and early stages of anopheline mosquitoes were returned in habitats where they had been sampled. All L3 and L4 anopheline larvae sampled were handpicked using a rubber pipette and put into specimen bottles. Collected L3 and L4 anopheline larvae were killed in hot water (about 60-90^{0}C) then preserved in 99% ethanol before they were stored at -20^{0}C. All L3 and L4 anopheline larvae were thereafter identified, categorized, counted and recorded as *An. gambiae s.l* or *An. funestus*, or *An. coustani* or other *Anopheles* mosquito species (non vector species of malaria). Distinction between species was done using

morphological keys published by Gillies and Coetzee (1987). Additional morphological identification of anopheline mosquitoes was done by allowing adults to emerge. Newly emerged adults were killed by storing them at -20^{0}C for ten minutes and then put in vials containing silica gel. Microscopically identified samples of *An. gambiae s.l* larvae and adults were preserved for rDNA-PCR (ribosomal Deoxyribonucleic acid-Polymerase Chain Reaction) analysis (Scot *et al.*, 1993).

2.2.3. Shade and Abundance of Malaria Vector Larvae

The percentage of shade provided by each plant cover type (section 2.2.2) was determined at 07.30 hours, 12.00 hours and at 16.00 hours once a week for twelve weeks. This was done using a spherical densitometer D-MODEL (Ben Meadows Company). It contained a spherical reflector mirror engraved with 24 quarter inch square shaped grids and a leveling bubble for accurate positioning (Figure 2).

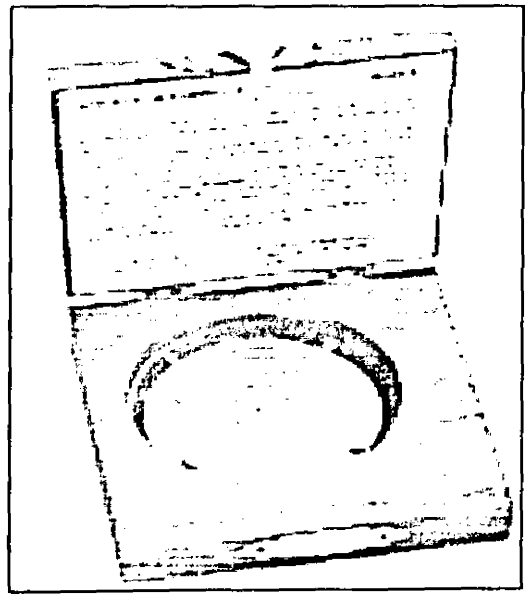

Figure 2. Spherical Densiometer (D-model).

The densiometer was held away from the body at an unobstructed sighting position and below the area shaded by the plants in each habitat. Given that each square of the grid had equally spaced dots, the number of dots equivalent to quarter-square openings were systematically counted and recorded. The total count was multiplied by 1.04 (constant value) to obtain the percentage of overhead area not occupied by shade as shown below.

Percentage area not covered by shade = Total number of quarter square opening × 1.04

Percentage shade provide by plant cover = 100 – percentage area not covered by shade

The average percentage of shade provided by each plant cover was calculated and compared with the average number of malaria vector larvae sampled from each habitat type per week. All the data was entered on data sheets and then transferred into Microsoft Excel (2003) spread sheet.

2.3. Traditional Water Management and Mosquito Larval Density

This study was aimed at investigating the effect of traditional water management practices on the density of mosquito larvae. A completely randomized replication design was used in this investigation. Four types of habitats associated with different traditional water management practices were identified and used in this study (Figure 3). These habitats were: pools (averagely 0.7m deep × 2.1m in diameter), small water channels (15m × 1m × 0.3m deep), paddies (15m ×15m ×0.5m deep) and swamps (control) measuring 15m × 15m ×0.3m deep.

All these habitats were man-made except swamps (control). Sampling of mosquito larvae was done on Tuesday and Friday morning for thirteen weeks (February to May 2008). This was done using a standard (350ml) white plastic larval dipper (Clark®) having a wooden handle. Ten dips were randomly made from each habitat type. Dipping was done at the water edges, surface and close to plants found within the habitat. All individual mosquito larvae including L1 and L2, L3 and L4 stages and pupae of culicine and anopheline mosquitoes were counted, recorded and returned back to the water. The dominant vegetation cover of each sampling site was also recorded. Daily amount of rainfall was measured in millimeters at 07.00 hours. After data collection mosquito density was expressed as number of larvae per dip. This was determined by dividing the total number of each type of larvae collected from each type of habitat by the total number of dips made in that habitat.

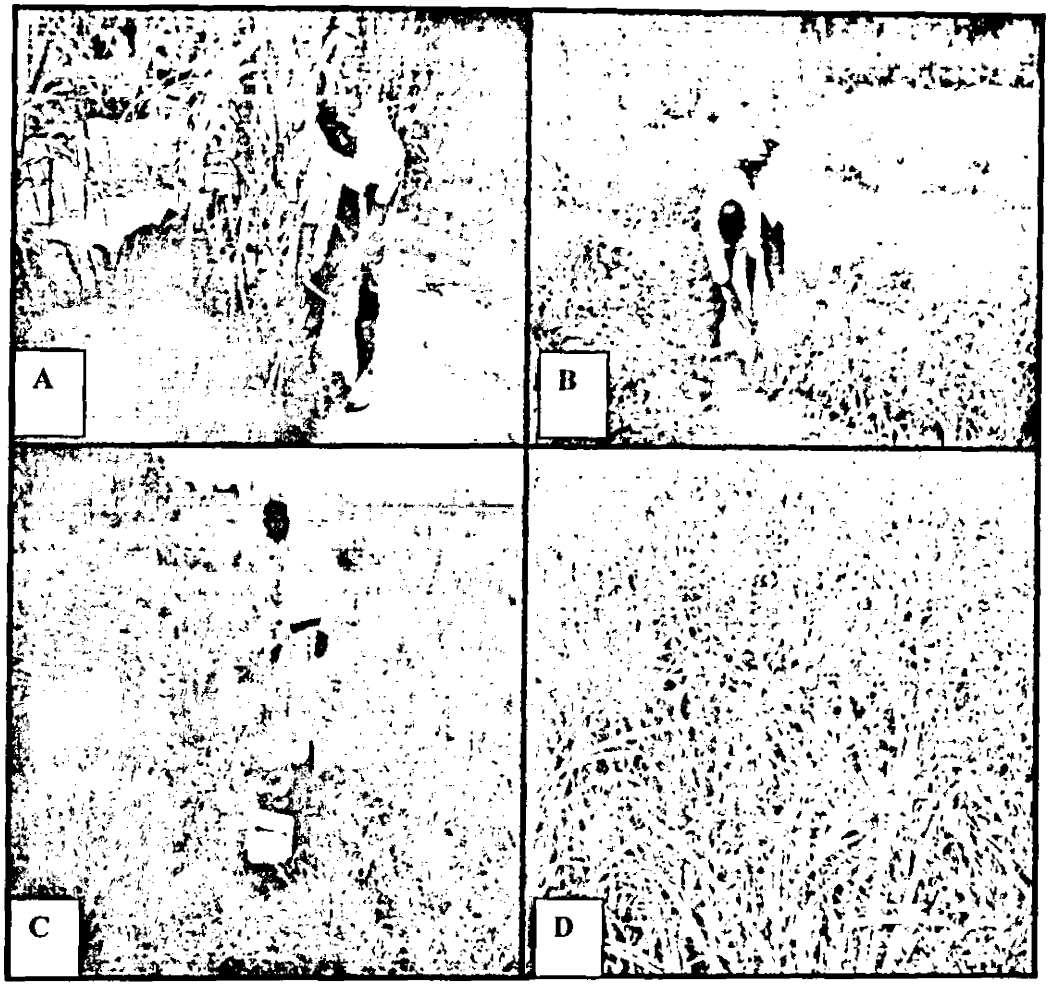

Figure 3. Habitats associated with traditional water management practices. **A** is a man-made pool for temporal storage of water, **B** is a small man-made water channel used for irrigation and drainage, **C** is a man-made paddy that has been abandoned after cultivation of rice and **D** is a swamp (control).

2.4. Productivity of Anopheline Mosquito larvae in A traditional Rice Agro-ecosystem

The aim of this study was to determine the association between growth phases of rice (*Oryza sativa*) and productivity of anopheline mosquito larvae in a traditional water management agro-ecosystem. Productivity was expressed as the number of anopheline mosquito larvae/1.2 m^2/week. This agro-ecosystem was typified by subdivision of land into small scale subsistence paddies (measuring about 225m^2) irrigated through unplanned man-made channels fed from poorly defined and unreliable water sources. Growth stages of rice were divided into three phases namely: vegetative (1-8 weeks after transplanting), reproductive (9-15 weeks old) and mature (16-21 weeks old). However, the duration of each phase depended on the variety of rice and ecological conditions. Since previous studies had explicitly demonstrated the effect of maize pollen on the productivity of malaria mosquitoes (Ye-Ebiyo *et al.*, 2000; 2003) preliminary investigations were carried out to test if rice pollen had similar effects.

2. 4.1. Preliminary Investigations

Newly hatched unfed larvae of *An. gambiae* Giles *sensu stricto* (*Iguhu* strain) obtained from a laboratory reared colony at the CGHR-KEMRI in Kisian were subjected to different diets. Details of the experiment are summarized in Table 1.

Table 1. Diet based treatments that were administered to larvae of *An. gambiae s.s.* Number of replicates, numbers of larvae in each replicate and total number of larvae involved in each type of treatment are shown.

Treatments based on diet	Number of replicates	Larvae per replicate	Total number of larvae
a) Rain water plus rice pollen	4	200	800
b) Rain water plus brewers yeast	4	200	800
c) Rain water plus maize pollen	4	200	800
d) Kasagam water plus maize pollen	4	200	800
e) Kasagam water plus rice pollen	4	200	800
f). Kasagam water only (control)	4	200	800

A completely randomized replication design was used. Six types of treatments based on four types of larval diets (maize pollen, rice pollen, brewers yeast and substrates in Kasagam water) were used. Each type of treatment was replicated four times. A total of 200 newly emerged larvae of *An. gambiae* Giles *s.s* were dispensed in each larval rearing tray (measuring 35cm × 25cm × 5cm) for every replicate. The trays contained 200cm^3 of water filled to a depth of 2.5cm. These experiments were performed in a screen house at the CGHR-KEMRI in Kisian. Each larva was fed on 0.5mg of brewer's yeast, maize pollen or rice pollen per day; except in one case where water from Kasagam study site was offered without additional food (i.e. it acted as the control). The pollen used was obtained from rice and maize plants that were traditionally grown in Kasagam study site.

The number of larvae present in each tray were counted and recorded daily. This was used to determine mortality rates and the amount of food to be dispensed per tray. Water was changed in each tray after every two days in order to allow oxygenation, prevent accumulation of metabolic wastes and eliminate dead larvae. During water replacement, larvae were sieved using a piece of cloth, counted, recorded and transferred into clean trays containing water whose composition was based on the type of treatment. This process was repeated until all larvae pupated. Trays containing larvae were covered with a mosquito net secured with a rubber band. This barred stray gravid female mosquitoes from ovipositing in the tray. Pupae were collected using rubber pipettes and transferred into cups containing clean rain water or Kasagam water depending on the type of treatment. The cups were then put in standard mosquito holding cages that were separated according to the type of diet based treatment used. Each mosquito holding cage (measuring 30 × 30 × 30 cm) was covered with a mosquito net secured with a rubber band.

The cups were left in the cage until adult mosquitoes emerged. Newly emerged mosquitoes were counted and recorded depending on the type of treatment. Adult mosquitoes were held for two days then killed by keeping them at -20°C for ten minutes before transferring them into vials containing silica gel pending measurement of wing lengths. Twenty adult female mosquitoes were randomly selected from every treatment to avoid bias that could have arisen and affected the fitness outcome involving determination of body size. One wing was removed from each adult female mosquito using a pair of fine tip forceps.

The wing was spread on a clean microscope slide and covered with a cover slip. Each wing was measured using an ocular micrometer mounted in the eye piece of a dissecting microscope. Magnifications of the eye piece and objective lenses used were × 10. The wings were measured from the alula to the posterior tip and recorded to the nearest 0.01mm. The average duration taken by the larvae to develop into adult mosquitoes, number of larvae that survived to adulthood and wing lengths of the mosquitoes in each treatment was recorded. As a follow up field experiments were carried out to determine the growth phase of rice that is associated with the highest density of malaria mosquito larvae. The hypothesis tested was that pollinating rice crops are associated with a higher larval productivity.

2.4.2: Field Experiments

The IR-64 variety of rice was grown in Kasagam using local production practices. Daily amount of rainfall (mm) was measured at 07.00 hours. A completely randomized replication design was used to determine the association between rice (*Oryza sativa*) growth phases and mosquito larval density. Transplanting of rice was done in five paddies while five open paddies were left empty (control) and this was repeated three times at an interval of three weeks. Each paddy measured 1.2m × 1.2m × 0.6m and was located at a distance of one meter away from the next. The whole experiment was done for six months (January to June 2008). The IR-64 seed variety of rice used was bought from the National irrigation board in Ahero town.

A nursery was set up in Kasagam to raise seedlings of uniform age and size. Transplanting into the experimental paddies was done when each seedling had

developed three leaves (28 days old). The spacing used was 10cm × 10cm × 20 cm while gapping was done after seven days. The paddies were left intact for one week for the seedlings to establish and to allow gravid female mosquitoes to oviposit. Growth stages of rice plants were subdivided into vegetative, reproductive (produces rice pollen) and mature phase, while the empty paddies were used to act as a control (Figure 4).

Larval sampling was done every Monday, Wednesday and Friday mornings in all paddies for twenty weeks based on the procedures described in section 2.2.2. Edges of all paddies (up to 30cm away) were kept clean on weekly basis by cutting grass. Weeds and other foreign materials were removed between the plants and from the boundaries of each paddy to prevent unforeseen effects on the experiment. Sulphate of ammonia fertilizer was applied (72 grams in every rice plot) 14 days and 49 days after transplanting to promote tillering and panicle formation, respectively.

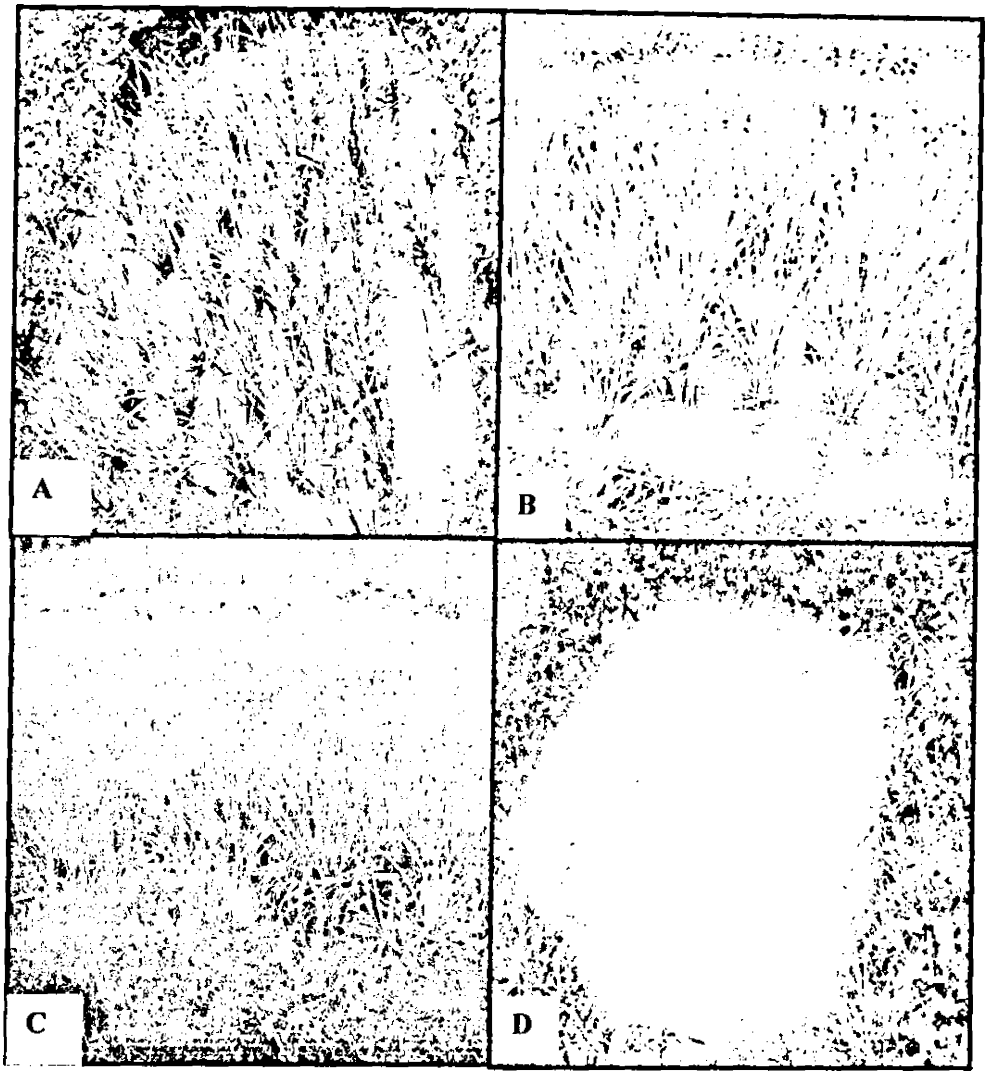

Figure 4. Growth phases of rice plants. **A** is a vegetative phase characterized by tillering, stem elongation, a lot of wide open spaces between the young green plants and along the edges, **B** is the reproductive phase depicted by more tillering, reduced open spaces between the plants and edges, occurrence of heading, flowering and pollination, **C** is a mature phase shown by crowded plants that are brown, have dry leaves, very few open surfaces along the edges, grains are changing from milky dough stage to hard stage ready for harvesting while **D** is an empty open paddy (control).

2.5. Larval Control in A traditional Water Management Agro-ecosystem

This study was designed to investigate the best options for the control of malaria mosquito larvae in man-made breeding sites resulting from traditional water management agro-ecosystem practices. Laboratory investigations were carried out in a screen house at the CGHR-KEMRI in Kisian. Field studies were conducted at Kasagam for four months (February to May 2008). The daily amount of rainfall (mm) was measured at 07.00 hours using a rain gauge.

2.5.1. Laboratory Investigations

The efficacy of mosquito fish (*G. affinis*) and *B. thuringiensis* var *israelensis* (*Bti*) for controlling malaria mosquito larvae was evaluated. The underlying goal was to estimate the number of mosquito fish and quantity of *Bti* needed to control mosquito larvae effectively. Randomized replication design was used. Two types of larval control methods involving four types of larval control treatments were investigated. Each treatment option was replicated twenty five times (Table 2). Each replicate contained sixty mosquito larvae consisting of thirty early and thirty late instar larvae. The sixty larvae were randomly dispensed using a rubber pipette into a plastic basin filled with two liters of Kasagam water to a depth of three centimeters. A total of one hundred plastic basins and 6000 laboratory reared larvae of *An. gambiae s.s* were used. Equal numbers of treatments for each larval control option were randomly allocated into the different plastic basins containing larvae.

Table 2. Types of treatments used for larval control of malaria vectors. The numbers of replicates, larvae per replicate and total number of larvae used in each treatment are shown.

Types of treatments used for larval control	Number of replicates	Larvae per replicate	Total number of larvae
(1) *B. thuringiensis* var *israelensis* (*Bti*)	25	60	1500
(2) Mosquito fish (*G. affinis*) only	25	60	1500
(3) *Bti* and *G. affinis*	25	60	1500
(4) No treatment (Control)	25	60	1500

The optimum dosage and concentration of 5mg/liters of water for *B. thuringiensis* var *israelensis* corn granule (CG) formulation (VectoBac®, potency 200 ITU/mg) was determined based on the existing literature (Fillinger *et al.*, 2003; 2006). The number of larvae present after treatments were introduced in different basins was recorded three times (07.00 hours, 13.00 hours and 18.00 hours) per day. This was done until all larvae could be accounted for. Different sizes of mosquito fish ranging from 1.2cm to 3.4cm in length were used. The fish were collected from water channels in Manyatta estate of Kisumu city and reared in the screen house. Thereafter the efficacy of each larval control treatment (Table 2) was tested in the Kasagam field site.

2.5.2. Field Experiments: Mosquito Larval Control in Man-made Water Channels

Completely randomized replication design was used in this investigation. Each of the four types of larval control treatments (Table 2) was randomly assigned to six habitats with different plant cover types for thirteen weeks. The six habitats consisted of open man-made water channels, *A. filiculoides* species growing on the water surface, sweet potatoes and coco yams growing along the banks of water channels, African couch grass and coco yams growing inside the water channels (Figure 2). Granule formulation of *Bti* (VectoBac® CG, [potency 200 ITU/mg]) was broadcasted into each sampling site at the rate of 5mg/liter of water. The average quantity of *Bti* applied was determined by calculating the volume of water present in the site before treatment was administered. The total number of mosquito fish used was based on results obtained from laboratory investigations (section 2.5.1). This entailed estimation of the average population density of mosquito larvae prior to the treatment. The average number of mosquito larvae per dip was multiplied by the surface area of the sampling site (1.5m²) and divided by the upper surface area of the dipper (95cm²) to obtain the average density of mosquito larvae in each habitat type.

Treatments were repeatedly carried out fortnightly on every Monday morning. Larval sampling of mosquitoes was done twenty four hours after treatment. Regular sampling of mosquito larvae was conducted on Monday and Friday mornings for thirteen weeks (February to May 2008) based on procedures described in section 2.2.2. A hole measuring 30 × 30 × 30cm was dug in sampling sites where mosquito fish were used as larval control agents in order to provide a hiding place whenever water levels reduced.

2.5.3. Field experiments: Mosquito Larval Control in Man-made Ponds

Five types of larval control treatments (*Bti*, mosquito fish, *Bti* and mosquito fish introduced once after two weeks, control and mosquito fish introduced only once for the whole study period) were involved. Each treatment option was replicated five times. A total of twenty five man-made ponds each measuring 1m × 1m × 1m) were used (Figure 5).

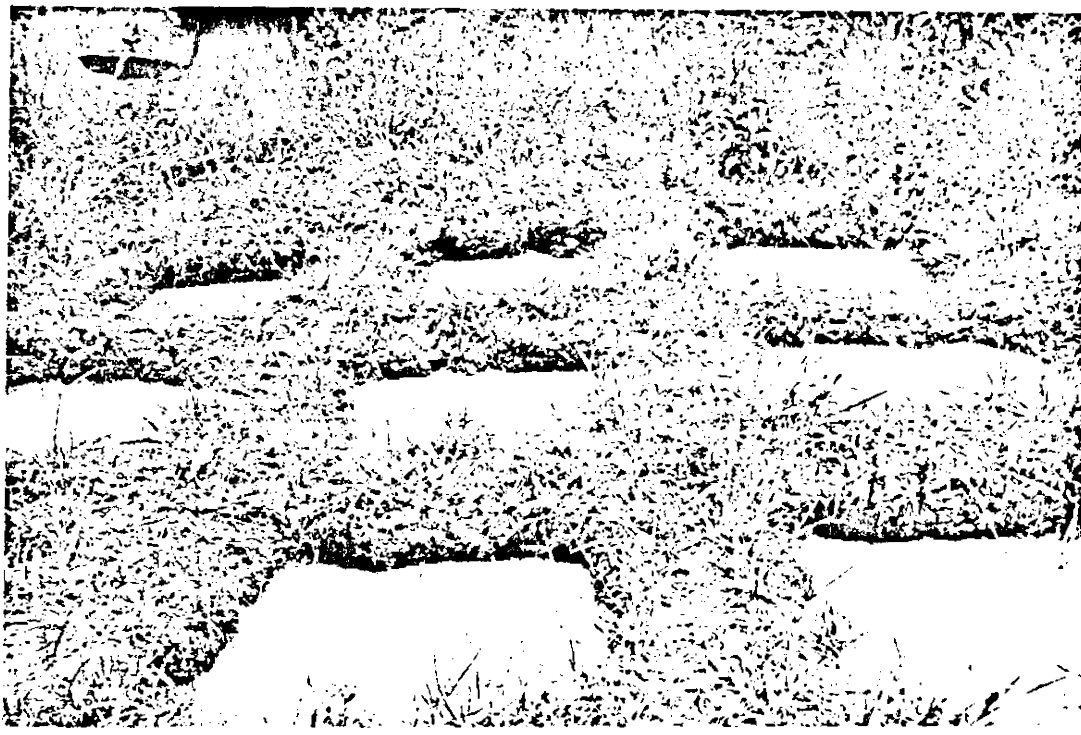

Figure 5. Layout of man-made ponds used for larval control experiments.

The ponds were sixty centimeters apart and were arranged in five rows and five columns. Completely randomized replication design was used. The average quantity of *Bti* (VectoBac® CG, [potency 200 ITU/mg]) and number of mosquito fish applied in each man-made pond along with the sampling procedure of mosquito larvae was similar to that of section 2.5.2.

2.6. Data Management and Analysis

All collected data were recorded on data sheets labelled depending on the type of study before being entered in Microsoft Office Excel (2003) spread sheets. Variations in the abundance of larval stages of anopheline mosquitoes due to the effect of plant cover habitat types and different water management practices were analyzed using one way ANOVA. Two way ANOVA was used to calculate variation in the numbers of malaria vector larvae resulting from the effect of percentage shade in the plant cover habitat types. All these analyses were done using the Statistical Package for Social Scientists (SPSS for windows version 15.0).

Variations in the densities of larval stages of anopheline mosquitoes due to different growth phases of rice and effect of larval control options were analyzed by using SAS System (NPAR1WAY procedure). Poisson regression model using generalized estimation equations (GEE) was involved. All count data of late instar species of anophelines collected and identified from various sampling sites along with laboratory experiment results were analyzed by Microsoft Office Excel (2003) spread sheet. One way ANOVA was used to determine variations in the abundance of different species of late instar larvae of anopheline mosquitoes collected and identified from various plant cover habitats, growth phases of rice and larval control options. Summary statistics were used to determine the mean numbers of larvae obtained from larval feeding and larval control experiments.

Chapter Three: Results

Studies reported in this thesis were conducted within a period of ten months (October 2007 to July 2008). *Anopheles arabiensis* was the only sibling species of *An. gambiae* complex identified from the field and it was more predominant than *An. funestus*. Water used in Kasagam agro-ecosystem was polluted and it had diversified origins

3.1. Plant Cover Types and Abundance of Mosquito Larvae

Plants which formed habitats of different plant cover types were either grown inside or on the banks of man-made water channels (section 2.2.2). An average of 214mm of rainfall was recorded per month during the study period.

3.1.1. Identification of Dominant Plants

A survey involving identification and classification of dominant plants which grew in the study site was done for two weeks. The identified plants are shown in Table 3.

Table 3. Dominant plants in Kasagam

Category of plants	Plant species
a) Plants grown along the water banks	
(i) Food crops	*Zea mays, Phaseolus vulgaris, Phaseolus aureas, Elucine coracana, Sorghum sativum, Musa paradisiaca, Brassica spp (eg Kales), Oryza sativa, Colocasia esculenta, Manihot esculenta, Ipomea batatas, Lycopersicon sp, Saccharum officinarum, Cucurbita spp.*
(ii) Non food crops	*Pennisetum purpureum, Digitaria scalarum, Cynodon nlemfuensis, Cyperus rotundus, Commelina spp, Ricinus communis.*
b) Plants growing in water	
(i) Emergent plants	*Colocasia esculenta, Digitaria scalarum, Cynodon nlemfuensis, Cyperus rotundus*
(ii) Floating plants	*Azolla filiculoides, Spirogyra spp, Rhodophyte spp, Phaeophyte spp*

3.1.2. Abundance of Anopheline Larvae under Different Plant Cover Habitats

Sampling of mosquito larvae was done in six habitats covered by different types of plants for three months. The abundance of anopheline mosquito larvae was significantly affected by plant cover type ($F = 10.31$, d.f = 1, 5, $P < 0.001$). However, Table 4 shows that abundance of late instar larvae (L3 and L4) of anopheline mosquitoes was not significantly influenced by plant cover type ($F = 0.59$, d.f = 1, 5, $P = 0.710$).

From a total of 722 late instar larvae of anopheline mosquitoes identified, habitats with open sites recorded the highest percentage 31.16% (n = 224) while those with coco yams growing in water had 22.58% (n = 163), coco yams growing along water banks recorded 14.82% (n = 107), African couch grass growing inside water had 14.54% (n = 105), sweet potatoes growing along the water banks noted 13.85% (n = 100), while habitats with water surface covered by water ferns (*A. filiculoides*) recorded the lowest percentage (3.05%, n = 23).

Anopheles arabiensis and *An. funestus* constituted 3.04% (n = 22) and 1.8% (n = 13), respectively, of all late instar larvae of anopheline mosquitoes identified from all habitats while non vector species of malaria were 95.16% (n = 687). Diversity of anopheline mosquito species was more dependent on season than on the type of plant cover.

Table 4. Weekly mean numbers (±S.E.M) of late instar larvae of anopheline mosquito species from plant cover habitats. Percentage of shade provided by each plant cover type is also shown.

Habitat type	Percentage shade	Weekly mean numbers (±S.E.M) of anopheline mosquitoes			
		An. arabiensis	*An. funestus*	*An. coustani*	Others
(a) Coco yams in water	56	0.3± 0.14^{a}	0.1±0.08^{a}	2.8±0.44^{a}	10.3±1.02^{a}
(b) Coco yams on water banks	64	0.3± 0.14^{a}	0.1±0.08^{a}	3.7±0.48^{a}	4.8±0.55^{b}
(c) Sweet potatoes on water banks	76	0.3±0.18^{a}	0.2±0.11^{a}	2.8±0.56^{a}	5.1±0.63^{b}
(d) African couch grass in water	84	0.2±0.11^{a}	0.6±0.29^{b}	3.5±0.83^{a}	4.6±1.00^{b}
(e) *Azolla* on water surface	100	0.1±0.08^{b}	0.1±0.08^{a}	0.9±0.34^{b}	0.8±0.28^{c}
(f) Open site (Control)	0	0.7±0.26^{c}	0.1±0.08^{a}	3.7±670^{a}	14.3±2.0^{d}

±S.E.M is a positive or negative standard error of the mean number of late instar larvae of anopheline mosquitoes sampled per week. Weekly mean numbers in the same column with same superscript letters represent no significant difference ($P > 0.05$) while those with different superscript letters are significantly different ($P < 0.05$).

3.1.3. Shade and Abundance of Malaria Vector Larvae

The abundance of late instar larvae of *An. funestus* and *An. arabiensis* mosquitoes was significantly influenced by the percentage of shade provided by different plant cover types ($F = 19.83$, d.f = 1, 3, $P < 0.001$) and ($F = 15.57$, d.f = 1, 3, $P < 0.011$), respectively (Table 4). There were more *An. arabiensis* mosquito larvae (36.36%, n = 8) in the open habitats while water surfaces covered with *A. filiculoides* recorded the lowest number (4.55%, n = 1). However, habitats where African couch grass was grown in the water channels recorded the highest percentage (53.85%, n = 7) of late instar larvae of *An. funestus* mosquitoes as opposed to other habitats.

3.2. Traditional Water Management and Mosquito Larval Density

An average of 1065mm of rainfall per month was recorded during the thirteen weeks of this study. Swamps had more grass and tall papyrus reeds (*Cyperus rotundus*) than paddies. Edges of pools and small water channels were occasionally covered by short grass (*Digitaria scalarum* and *Cynodon nlemfuensis*).

The densities of culicine larvae varied significantly (Figure 6) with the different types of traditional water management practices ($F = 4.81$, d.f = 1, 3, $P < 0.003$). Swamps had the highest density of culicine mosquito larvae (3.54) while man-made pools which temporarily stored water for watering seedlings of rice, trees and ornamental plants had the lowest (1.77).

However, the various types of water management practices caused no significant difference in the densities of anopheline mosquito larvae ($F = 1.66$, d.f = 1, 3, $P = 0.174$). Small water channels recorded a relatively higher density (0.79) of anopheline larvae as compared to swamps which recorded the lowest (0.25).

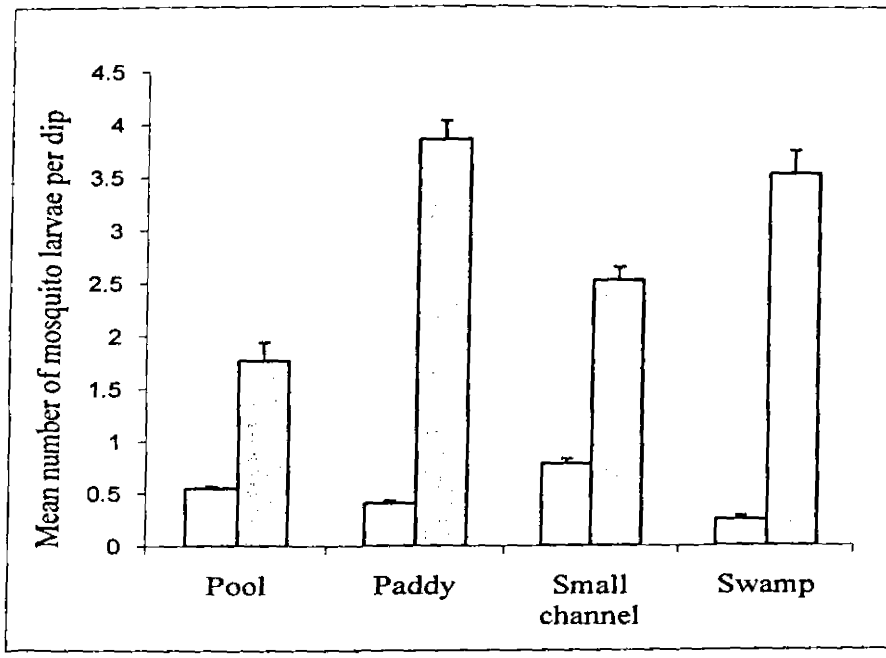

Figure 6. Density of culicine and anopheline mosquito larvae in different types of water management habitats. Open bars (▭) represent anophelines while shaded ones (▭) represent culicine mosquito larvae. Standard error bars of the mean numbers of larvae sampled per dip are shown.

3.3. Productivity of Anopheline Mosquito Larvae in A traditional Rice Agro-ecosystem

Laboratory reared colony of *An. gambiae s.s* was fed on different diet treatments followed by investigation of the association between rice growth phases and productivity of anopheline mosquito larvae.

3.3.1. Preliminary Investigations

Six types of treatments based on diet were replicated four times (Table 1). The experiment was conducted at a temperature of 21°C to 25°C. Treatments based on the type of diet caused a significant difference in larval development period ($F = 74.50$, d.f = 1, 5, $P < 0.001$), number of larvae that survived to adulthood ($F = 70.72$, d.f = 1, 5, $P < 0.001$) and wing length ($F = 16.13$, d.f = 1, 5, $P < 0.001$) of *An. gambiae* s.s mosquitoes (Table 5). The highest proportion of larvae that survived and developed into adults was recorded from a diet of Kasagam water supplemented with maize pollen (0.735) and Kasagam water plus rice pollen (0.723). Larval development into adulthood was faster in a diet of maize pollen plus rain water (14.63±0.24 days) and rice pollen plus rain water (13±0.82 days) although survival was very lower.

Table 5. Effects of diet based treatments on developmental aspects of *An. gambiae s.s* mosquitoes. Proportion of larvae that survived to adulthood and average values (±S.E.M) of development period to adulthood and wing length are shown.

Treatments based on diet	Average development period ±S.E.M (days)	Proportion of larvae that survived to adulthood	Average wing length ±S.E.M (mm)
a) Rice pollen plus rain water	13 ± 0.82^{a}	0.089^{a}	3.26 ± 0.04^{a}
b) Yeast plus rain water	21 ± 0.41^{b}	0.139^{a}	3.21 ± 0.03^{a}
c) Maize pollen plus rain water	14.6 ± 0.24^{a}	0.055^{a}	3.21 ± 0.02^{a}
d) Maize pollen plus Kasagam water	15 ± 0.41^{a}	0.735^{b}	3.42 ± 0.02^{b}
e) Rice pollen plus Kasagam water	15 ± 0.42^{a}	0.723^{b}	3.16 ± 0.02^{a}
f) Kasagam water (control)	25.8 ± 0.85^{b}	0.0613^{a}	3.07 ± 0.04^{c}

±S.E.M is a positive or negative standard error of the mean number of days and length of wings. Values in the same column with different superscript letters are significantly different ($P < 0.05$).

3.3.2. Field experiments

Productivity was expressed in terms of number of anopheline mosquito larvae sampled/1.2m^2/week. The productivity of all larval stages of anopheline mosquitoes was significantly influenced by growth phases of rice ($F = 11.21$, d.f = 1, 5, $P < 0.001$).

However, the productivity of late instar larvae of different species of anopheline mosquitoes was not significantly influenced by the rice growth phases ($F = 0.49$, d.f = 1, 5, $P = 0.780$) (Table 6). From a total of 1396 late instar larvae of anopheline mosquitoes sampled, 56% (n = 782) were from the rice fields while 44% (n = 614) were collected from empty open (control) paddies. The vegetative stage of rice recorded the highest percentage (39.5%, n = 558) of all anopheline and all *An. arabiensis* (51.2%, n = 21) mosquito larvae that were sampled from all the rice and empty paddies. Vegetative phase of rice was the most suitable for the proliferation and survival of *An. arabiensis* mosquitoes. Anopheline mosquitoes preferred to oviposit in adjacent empty open paddies (control) than in the paddies containing rice plants in reproductive and mature growth phase.

There were more *An. arabiensis* than *An. funestus* mosquito larvae in all the growth phases of rice and empty open paddies except in reproductive phase. *An. funestus* larvae were only found in the reproductive phase of rice while *An. arabiensis* were sampled in all paddies with an exception of mature phase. *Anopheles arabiensis* larvae reduced drastically from vegetative to reproductive phase with none of them being recorded in the mature phase.

Table 6. Productivity of anopheline mosquito larvae in different growth phases of rice and empty open (control) paddies. Duration and mean number of anopheline mosquito larvae sampled/1.2m^2/week for each growth phase of rice are shown.

Growth phases of rice and their controls	Duration (weeks)	Mean number of anopheline mosquito larvae sampled /1.2m^2 / week			
		An. arabiensis	*An. funestus*	*An. coustani*	Others
1 (a) Vegetative	7	0.6^a	0^a	0.4^a	15^a
(b) Control	7	0.4^b	0^a	0.4^a	8^b
2 (a) Reproductive	7	0.02^c	0.06^b	0.4^a	3^b
(b) Control	7	0.06^c	0^a	0.6^b	4^b
3 (a) Mature	6	0^d	0^a	1^c	3^b
(b) Control	6	0.06^c	0^a	1^c	5^b

Mean numbers in the same column with same superscript letters represent no significant difference ($P > 0.05$) while those with different superscript letters are significantly different ($P < 0.05$).

3.4. Larval Control in A traditional Water Management Agro-ecosystem

3.4.1. Laboratory Investigations

Proportions of *An. gambiae s.s* mosquito larvae were significantly influenced by larval control options ($F = 838$, d.f = 1, 3, $P < 0.001$) after 24 hours of introducing treatments (Table 7). All larvae died while three live pupae were observed twenty four and forty eight hours after introduction of *Bti* as the only agent of larval control. Proportions of larvae reduced drastically after twenty four hours of introducing mosquito fish which fed on all of them within 48 hours. There were no live larvae or pupae observed after 24 hours of intervention using both mosquito fish and *Bti*.

Table 7. Proportions of live larvae and or pupae observed amidst different intervention treatments during laboratory experiments. Total number of larvae used is shown.

Types of treatments used for larval control	Number of larvae	Proportions of live larvae or pupae observed after each control option	
		24 hours later	48 hours later
(1) *Bti*	1500	0.1^{a}	0.1^{a}
(2) Mosquito fish (*G. affinis*)	1500	0.1^{a}	0^{a}
(3) Both *Bti* and mosquito fish	1500	0^{a}	0^{a}
(4) Control	1500	0.94^{b}	0.78^{b}

Proportions with different superscript letters in the same column are significantly different ($P < 0.05$).

3.4.2. Field experiment: Larval Control in Man-made Water Channels

The abundance of all larval stages of anopheline mosquitoes was significantly influenced by larval control treatments ($F = 33.12$, d.f = 1, 3, $P < 0.001$) in man-made water channels (Figure 1). All larval control treatments effected an overall reduction of 73.03% in the populations of all larval stages of anopheline mosquitoes. Abundance of late instar larvae of different species of *Anopheles* mosquitoes was not significantly affected by larval control treatments ($F = 0.697$, d.f = 1, 3, $P = 0.571$). All types of late instar larvae of anopheline mosquitoes reduced by 87.03% (n = 173) due to *Bti* only, 59.50% (n = 117) due to mosquito fish only and 92% (n = 183) due to application of both mosquito fish and *Bti* (Figure 7). The population of *An. arabiensis* was reduced by 83.33% due to *Bti* only, 50% by mosquito fish, while both mosquito fish and *Bti* caused a reduction of 100%. However, there were no *An. funestus* recorded in habitats where larval intervention treatments had been administered except in water channels without control agents (control).

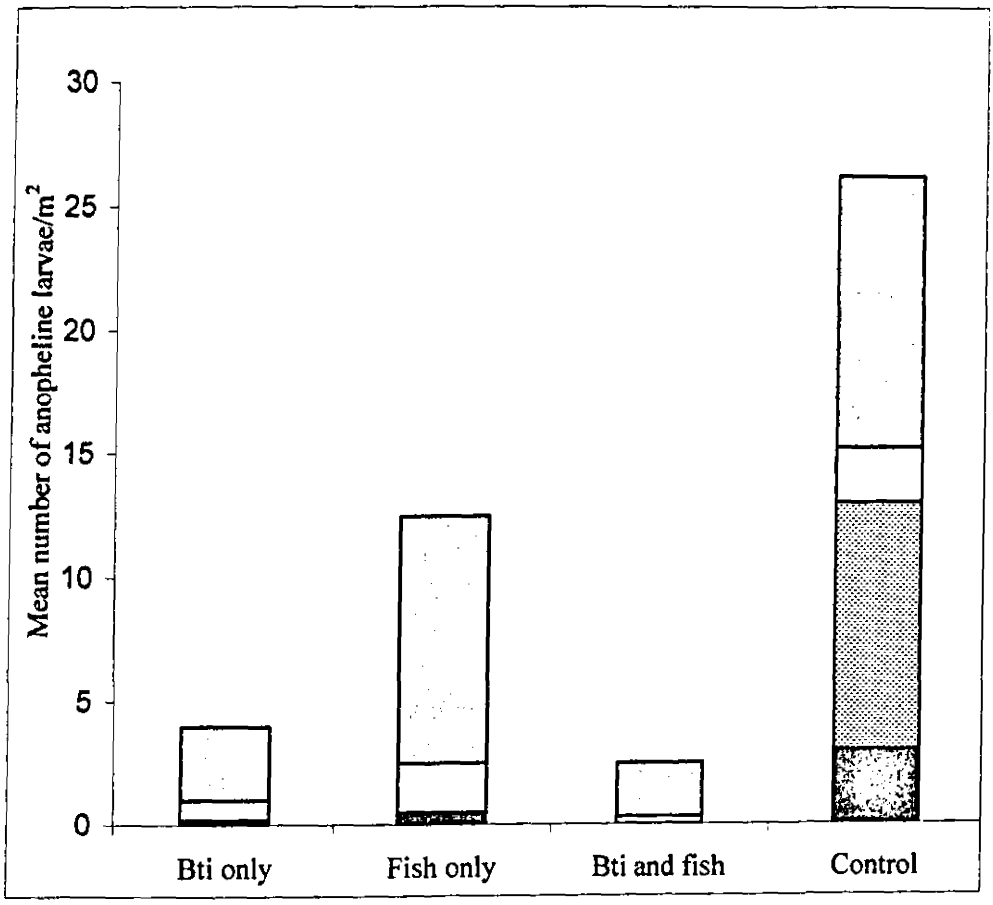

Figure 7. Effects of control treatments on the density of late instar larvae of *Anopheles* mosquitoes in man-made water channels. Bars shaded black (▓) represent *An. arabiensis*, open ones (□) are for *An. coustani*, a bar with white and black dotted pattern depicts *An. funestus* while grey shaded bars (▒) represent other anophelines.

3.4.3. Field experiments: Larval Control in Man-made Ponds

The abundance of all larval stages of anopheline mosquitoes was significantly influenced by larval control treatments ($F = 23.35$, d.f = 1, 4, $P < 0.001$) in man-made ponds (Figure 5). Contrastingly, there was no significant effect of larval control treatments ($F = 0.280$, d.f = 1, 4, $P=0.906$) on the abundance of late instar larvae of anopheline mosquitoes species (Figure 8).

Late instar larvae of anopheline mosquitoes were reduced by 25.46% (n = 97) where *Bti* had been applied, 27.49% (n = 105) by mosquito fish introduced once in two weeks and 74.54% (n = 291) by use of both *Bti* and mosquito fish. However, a single introduction of mosquito fish for the whole study period resulted into 23.61% (n = 89) increase in the total number of anopheline mosquito larvae. *Anopheles arabiensis* was the only malaria vector sampled during the study.

Anopheles arabiensis reduced by 71.42% where both *Bti* and mosquito fish were introduced, 57.14% by *Bti* only and 14.29% by fortnight introduction of mosquito fish. Single introduction of mosquito fish for the whole study period resulted into 28.57% increase in the population of *An. Arabiensis*. *Anopheles funestus* mosquito larvae were not sampled in all habitats during the whole intervention period. All control treatments reduced the population density of anopheline mosquito larvae by 14%.

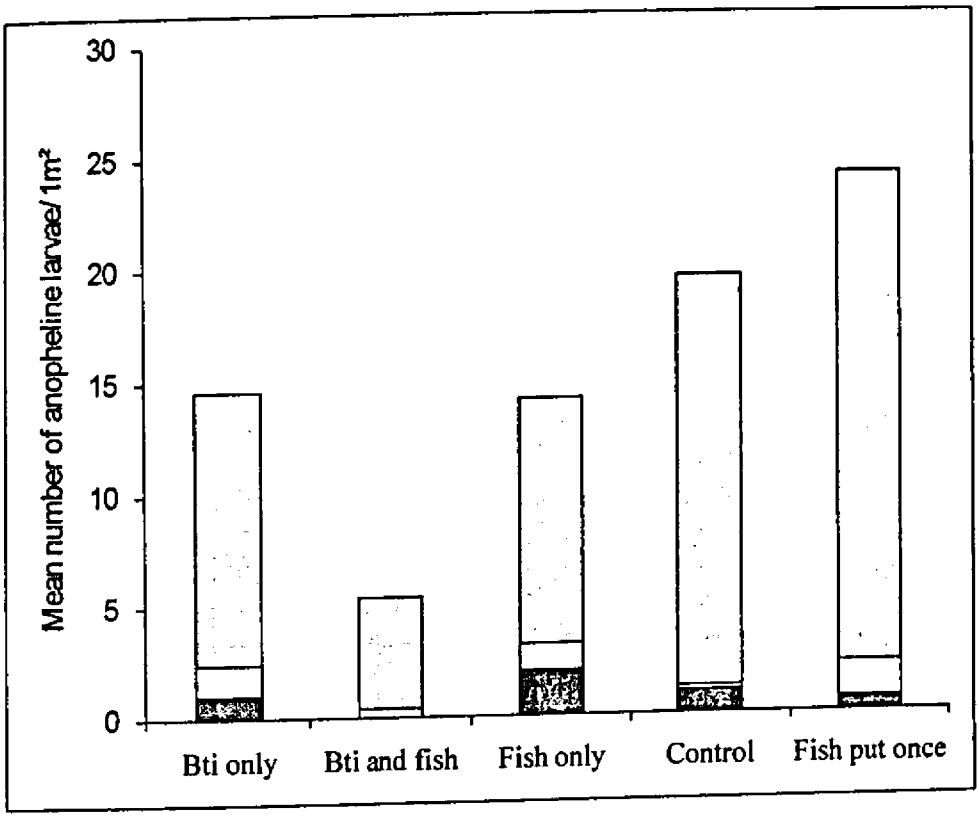

Figure 8. Influences of control treatments on the density of late instar larvae of *Anopheles* mosquitoes in man-made ponds. Bars shaded black ( ) represent *An. arabiensis*, open ones ( ) are for *An. coustani* while grey shaded bars ( ) represent other anophelines.

Chapter Four: Discussion

In this study, *An. arabiensis* was the most predominant malaria vector than *An. funestus* in Kasagam in spite of very high populations of non vector species of *Anopheles* mosquitoes. Plant cover type significantly influenced the population abundance of all larval stages of anopheline mosquitoes. Contrastingly, larval abundance of late instar larvae of malaria vectors and other anopheline mosquitoes was not significantly affected by the type of plant cover.

This study confirms that co-existing larvae of malaria vectors and other anopheline mosquitoes (Mwangangi *et al.*, 2007, Vincent *et al.*, 2003) may be adapting progressively to man-made polluted habitats in Kasagam just like in Dar es Salaam (Sattler *et al.*, 2005). Memory of the environment could have also influenced oviposition site preference of the observed mosquito species (Yih-shen *et al.*, 1980; McCall and Eaton, 2002; Sunish *et al.*, 2003). This contrasts the suggestion that *An. gambiae* often develop in fresh, sunlit, transient pools, which are not organically polluted (Gimnig *et al.*, 2001). Maybe this could also be a reason as to why malaria vectors formed the lowest proportion as compared to other mosquito species (Chua *et al.*, 2004, Sunish *et al.*, 2003). Cultivation of rice and other crops like sweet potatoes, yams and cassava on ridges also present a very high risk of malaria transmission by promoting the formation of areas of shallow water surfaces that are suitable for the breeding of malaria vectors (Lock and De Zeeuw, 2001; Barbara *et al.*, 2006). Most proliferation of anopheline mosquitoes occurred in open sites or those with less shade because they allowed passage of more light to the water surface (Barbara *et al.*, 2006, Fillinger *et al.*, 2004). This may explain why the weekly mean numbers of *An.*

arabiensis declined in all sites as the percentage of shade provided by emergent plants over the water surface increased, as opposed to *An. funestus* (Mwangangi *et al.*, 2007). However, shaded areas could have also provided a hiding habitat for adult mosquitoes and predators for mosquito larvae (Blaustein, 1992; Blaustein *et al.*, 2004).

Population densities of anopheline mosquito larvae were not significantly affected by the types of water management practices as compared to culicines. Population densities of all larval stages of culicine mosquitoes increased as vegetation cover increased progressively from man-made ponds, small water channels, paddies to the swamps. This was the reverse for the population densities of all larval stages of *Anopheles* mosquitoes in the same habitats. Presence of less canopy cover and clean water with less organic deposits (Yamagata, 1996) favored oviposition of anopheline mosquitoes as opposed to culicine mosquitoes. These results show consistence with the general description for the breeding sites of *An. gambiae s.l* (Gimnig *et al.*, 2001) and *An. funestus* (Mwangangi *et al.*, 2007). These types of habitat that are associated with water management practices can be used conveniently to manage interventions in the dry seasons when alternative, suitable breeding sites for the malaria vectors are few (Shililu *et al.*, 2003, Yamagata, 1996). *Anopheles gambiae s.l* larvae have the ability to survive for a few days in dry habitats given sufficient humidity in the soil (Koenraadt *et al.*, 2003). A situation of this type may occur in the man-made water channels or man-made ponds when water flow is diverted thus increasing malaria risk. Such habitats can provide alternative breeding sites of malaria vectors if minimal rainfall or water flow is resumed within a few days.

Different types of treatments based on diet significantly influenced larval development period to adulthood, survival rate and adult body size of *An. gambiae s.s.* The highest survival rates were associated with water supplemented with maize and rice pollen. Larval feeding experiments confirmed the findings of Shafiei *et al* (2001) on the dung beetle. They suggested that availability and type of food regulates larval development and timing of subsequent developmental events. Rice like maize pollen has proved to be an important source of larval nutriment for *An. gambiae s.l.* This type of malaria vector thrives in small, sunlit, temporal and turbid water collections (Gimnig *et al.*, 2001) that are usually found close to villages or human settlements where these crops are grown (Ye-Ebiyo *et al.*, 2000). Pollen increased and diversified the nutriment status of the water. This reduced development and emergence periods. Larger adults were also produced. Larger female adults of *An. gambiae s. s* mosquito have been observed to live longer and to have a higher probability of being infected with *Plasmodium* parasites (Oketch *et al.*, 2007; Kebede *et al.*, 2005) than their smaller counterparts. Consequently, larger male adults are also more competitive during mating than their smaller counterparts (Oketch *et al.*, 2007). This may explain why production of pollen from both types of crops coincides with periods of intense malaria transmission as documented by Ye-Ebiyo *et al* (2002). Proximity of rice and maize fields to human dwellings further promotes malaria outbreaks because it falls within the flight range of *An. gambiae* dispersal (Chandler and Highton, 1975). However, it is not clear whether rice pollen also contains a water soluble phago-stimulatory component like maize pollen. Such a component would accelerate ingestion of other materials in the water by *An. gambiae s.s* larvae in order to enhance their growth, survival and longevity (Ye-Ebiyo *et al.*, 2003). Existence of such type of compound would be vital in increasing

ingestion of microbial entomotoxin like *Bacillus* larvicides thus reducing the larvae of malaria vectors.

Growth phases of rice showed a significant effect on the productivity of all larval stages of anopheline mosquitoes as opposed to late larval stages. Nevertheless, reproductive growth phase of rice which is characterized by pollination was not associated with high abundance of mosquito larvae in the field. Proliferation and abundance of *An. arabiensis* and other anopheline mosquito species with an exception of *An. funestus* was short lived and intense during the vegetative growth phase of rice plant and its control paddies. Chandler and Highton (1975) observed that the density of mosquito larvae decreases as rice grows tall and old. This is because shade, water depth, decomposition and predators increase, while water temperature and algal growth reduce thus decreasing nutrients required for larval growth. This observation could explain why more *Anopheles* mosquito larvae were collected during the vegetative growth phase of rice plants. This implies that reproductive phase of rice has no direct association with increased production of malaria vectors. Effect of shade and predation could be explained by restriction of anopheline mosquito larvae to the narrow exposed edges of rice plots during the reproductive and mature stages. This may also account for the existence of more anopheline larvae in empty open paddies (control) than in adjacent rice paddies during reproductive and mature growth phases. A higher population of anopheline mosquito larvae was also seen in fields where rice plants were distantly spaced or in paddies used for control. Apparently it was not clear whether there is an established field relationship between body size of malaria vectors and growth phases of rice plants. Such information would shade more light on the intensity of malaria transmission.

Intervention options or treatments in plant cover habitats and man-made ponds had a great impact on abundance of all larval stages of anopheline mosquitoes. However, a greater efficacy of each control treatment was higher in the laboratory and habitats with clear running water than in stagnant turbid water found in man-made ponds. Results from larval control options suggest that mosquito fish and *B. thuringiensis* var *israelensis* are more capable of reducing transmission of malaria when both of them are used in the right quantities to complement each other in the same habitat. This study also confirmed the outcome of experiments conducted by Blaustein (1992) where mosquito fish alone failed to control mosquitoes in experimental rice plots. Predatory effectiveness of mosquito fish on mosquito larvae diminishes when introduced in the natural breeding habitats of mosquitoes unlike in laboratory experiments. Bence (1988) reported that in nature mosquito fish feeds on other mosquito predators, other alternative prey and external food or invertebrate sources. Such feeding habits tend to divert the feeding ability of mosquito fish on mosquito larvae thus causing resurgence in mosquito larval density. This observation seems to account for the higher population densities of larvae in field sites where fish was the only control agent. However, this could be a transitional stage to effective larval control of mosquitoes especially if the mosquito fish reverts to feeding on larvae in the absence of the preferred prey (Bence, 1988). Effective predation of mosquito larvae may be enhanced by their long life span, high rate of proliferation and population dynamics over other seasonal predators like tadpoles. Blaustein (1992) suggested that this control strategy would be enhanced by repeated introduction of mosquito fish so that it can cope with complex multivariate factors of the specific larval habitats. This concurs with higher larval densities of anopheline mosquitoes in man-made ponds where mosquito fish were introduced only

once than in the control ponds and where introduction of mosquito fish was done fortnightly. However, this contrasts previous recommendations made by Garcia (1982) on specific optimum numbers of mosquito fish stocked in a given area (300 female adults per acre).

Higher effectiveness of mosquito fish in habitats with different types of plant covers than in man-made ponds with stagnant silted water conformed to a comparative study done by Homski *et al* (1994). Homski *et al* (1994) reported that higher turbidity in man-made ponds may have favored a higher abundance of invertebrates and reduced the visibility of mosquito fish for anopheline mosquito larvae than in plant cover sites. They observed that mosquito fish were a more successful larval control agent in fresh open water with less vegetation than in polluted water bodies where mosquito problems were often associated.

Results obtained from lower larviciding efficacy of *Bti* only, disagree with the findings of Fillinger *et al* (2006) and Majambere *et al* (2008) where larviciding reduced anopheline larval density by 95%. Nevertheless, efficacy of *Bti* would have been greater if optimum quantities of *Bti* would have been applied once a week as suggested by Fillinger and Lindsay (2006). In this study *Bti* application was done after larval density of mosquitoes had started to increase and this is why it was only applied after every two weeks. The fortnightly applications of *Bti* in this study matched with larviciding studies carried out by Shililu *et al* (2003) in Eritrea. Nonetheless, occasional presence of heavy downpours caused a lot of runoff and drifting of *Bti* granules from sampling sites and therefore a full dose could not have been achieved in all instances. Presence of dense growth of algae like spirogyra especially in the plant cover habitats may have also

compromised the dissolution of *Bti* and feeding activity of anopheline larvae. As a result formulations that can penetrate such algal masses would be a better option. Fillinger *et al*, (2003 and 2006) suggested that *Bti* is a convenient larvicide due to its ability to withstand dilution even when it rained heavily, easy application, and non toxic to non target organisms. It is the non toxic property of *Bti* to other organisms that served as a basis of integrating it with *G. affinis* for increased efficacy of larval control. This study suggests that integrated larval control measures should be adopted in order to reduce the larvae of malaria vectors in areas with traditional water management agro-ecosystems.

4.1: Conclusion and Recommendations

This study has shown that *An. arabiensis* was the predominant malaria vector in Kasagam. Plant cover type influenced the breeding and abundance of all larval stages of anopheline mosquitoes but had no effect on abundance of late instar larvae of malaria vectors and other anopheline mosquito species. Water management practices significantly affected the population density of culicine mosquito larvae as opposed to anopheline mosquito larvae. Growth phases of rice showed a significant impact on the abundance of all larval stages of anopheline mosquitoes unlike on late larval stages. Nevertheless, field studies showed that the reproductive growth phase of rice was characterized by pollination but it was not associated with high productivity of anopheline mosquito larvae. Larval control treatments influenced larval abundance of all larval stages of anopheline mosquitoes and not the late instar stages. However, greater efficacy of each control treatment or option was higher in laboratory experiments and in habitats which contained clear running water within the man-made water channels than in stagnant turbid water found in man-made ponds.

Recommendations:

1. Plant cover types can provide a basis for consistent planning on the type of crops to be grown and time of initiating suitable larval control methods.
2. Habitats resulting from water management practices can be targeted conveniently for larval control of mosquitoes especially during dry seasons.
3. Rice and maize pollen could form a major component of controlling larvae of malaria vectors if these crops were genetically designed to synthesize and secrete ant mosquito larval entomotoxins.

4. The vegetative growth phase of rice and adjacent open water (control) provides the most ideal opportunity of reducing malaria burden by use of predator fishes and larvicides.
5. Introduction of high yielding and fast maturing varieties of rice that can withstand close spacing with fast and uniform tillering would increase shade over the water surface thus providing an alternative measure of larval control. This can be supplemented by adequately stocking rice fields with mosquito fish
6. Covering of water surfaces with *A. filiculoides* would also fix nitrogen for the rice plants while preventing malaria vectors and other anopheline mosquitoes from ovipositing.

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