DEVELOPMENT OF A PRACTICAL TECHNIQUE FOR SAMPLING THE AFROTROPICAL MALARIA VECTORS *Anopheles gambiae* S.L. AND *An. funestus*

By

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This document is dedicated to my parents, Kent and Judy Harbison.

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Malaria, transmitted by Anopheline mosquitoes, is not only the most important insect-borne disease but one of the top three infectious diseases in the world. Resting boxes are a well-established method for sampling Anopheline mosquitoes. Few resting box designs, however, are used indoors. A practical method, utilizing cloth resting boxes and "resting nets" for sampling indoor malaria vectors of Africa, was developed in semi-field (a modified greenhouse) and field conditions. In semi-field conditions, a cloth resting box recaptured 36.1 ± 9.9% (mean ± SE) of three different densities of *An. gambiae s.s.* (Giles) females of varying gonotrophic status. The accuracy method then was compared to the collection of mosquitoes using an oral aspirator in a rural Kenyan village. The developed method caught a significantly higher percentage (P=0.05) of resting mosquitoes than hand catches with an oral aspirator and required slightly less time to complete (8.0 ± 3.9 minutes versus 15.0 ± 0.0 minutes). A "resting basket" was also developed as an even more practical alternative to the cloth resting box. The resting

basket was tested under the same semi-field conditions as the resting box. No significant difference (P=0.47) was found in recapture rates between the resting basket and box, suggesting that it could be used as an alternative to the cloth resting box. The materials used in the method, which requires little training to implement, can be easily obtained in rural settings where malaria is of great concern.

CHAPTER 1
INTRODUCTION

Approaches to Malaria Control.

Historically, there are two general approaches (vertical and horizontal) to control of diseases such as malaria. Vertical approaches call for centralized national programs that act as an independent entity in national health care systems. Horizontal approaches involve broad based local programs emphasizing basic needs such as health education, safe water, and adequate food supply (Tan et al. 2003). Positive aspects to vertical approaches are a government commitment to standardized health care and attention given to the control of all aspects of the disease (such as prevention and treatment). Also, due to clear hierarchical structures typically found in such programs, there are fewer organizational complications compared to those observed in decentralized systems (Kroeger et al. 2002). Vertical programs, however, are criticized for focusing solely on one health problem and failing to build local capacities with wider health benefits (Tan et al. 2003). In some cases vertical programs must be initiated by larger international organizations when national governments do not have the needed resources.

Horizontal control programs are strongly based in decentralized health systems and in community mobilization. They are geared to become more focused to the needs of each specific region. Problems that arise from horizontal approaches are that often decentralized health systems do not have the resources and support held in vertical programs, and in many rural regions people cannot access basic health facilities (Deressa et al. 2003, Garfield 1999, Killeen et al. 2002).

History of Malaria Control

In the 1950s, the World Health Organization (WHO) realized the severity of malaria in terms of human morbidity and mortality and the resulting reduction of agriculture and industry on a global scale. In 1956, the Global Malaria Eradication campaign was launched. The campaign was a relatively worldwide vertical effort based on interrupting transmission through indoor residual spraying (and other mosquito control measures), management of marshes, creation of agricultural land, and modification of human lifestyle (i.e., window screens, air conditioning, and television). The success of the National Malaria Eradication Program in the United States was, in part, due to vertical disease programs from the Tennessee Valley Authority and the Communicable Disease Center (previously the Office of Malaria Control in War Areas) (CDC 2004a, CDC 2004b). Malaria eradication was realized in much of the world's temperate areas, but failed or was not attempted in many tropical areas. In Africa, eradication was only attempted in Zimbabwe, South Africa, and Ethiopia since malaria was considered too great a problem in the other countries (Kager 2002). In response to the anticipation that national primary health care systems would take over comprehensive malaria information systems, aiding in management of malaria, the vertical approach programs of the WHO were dismantled in the 1980s. International interest and funding in malaria research waned during this time. Since then malaria transmission in many of the affected regions has increased dramatically (Gilles 2002).

Future of Malaria Control

In the past 15 years, global efforts have been made to combat the increasing morbidity and mortality associated with the disease. Initiatives such as Roll Back Malaria (RBM), Multilateral Initiatives on Malaria (MIM), and the African Initiative on

Malaria (AIM) have been created to reestablish malaria as a global priority, to increase support from wealthier nations, and to develop sector-wide approaches to malaria control. This global push to create regional programs involves not only peripheral health care systems, but educating and empowering community members of the affected areas (Conteh et al. 2004, Deressa et al. 2003, Magnussen et al. 2001). Because such horizontal approaches still have shortcomings, it is likely that large scale malaria control will only be achieved through the integration of aspects from both horizontal and vertical programs (Kroeger et al. 2002).

Role of Rural Housing in Malaria Transmission

The physical condition of rural houses plays an important part in the epidemiology of malaria, especially when transmitted by endophagic and endophilic vectors like *Anopheles gambiae* (Giles). High levels of malaria transmission are usually associated with vectors that prefer to feed on humans indoors (Harwood and James 1979). Successful eradication of malaria in many regions of the world is due in part to mosquito-proofing houses (i.e., screened windows and air conditioning). Many people living in regions where malaria causes the highest morbidity and mortality do not have access to such luxuries.

Rural houses and villages (especially those of poor construction) are considered the main foci for malaria transmission in these regions (Muirhead-Thomson 1982, Konradsen et al. 2003, Fullerton and Bishop 1933, Gamage-Mendis et al. 1991). In Kenya, Githeko et al. (1994) and Bogh et al. (1998) found that 74% and 99%, respectively, of *An. gambiae* sensu lato (Giles) collected resting indoors had fed on human blood. Open eaves provide a ready entry point into rural houses and increase the chance of finding higher densities of mosquitoes resting indoors (Lindsay et al. 2003, Schofield and White 1983).

Houses built without ceilings also have been shown to increase human exposure to malaria vectors. (Lindsay et al. 1995, Schofield and White 1983). Houses also provide suitable habitats for vectors of human filariasis (*Culex spp.)*, yellow fever, and dengue (*Aedes aegypti* L.).

Sampling Methods for Malaria Vectors Indoors

Although there are various methods for sampling the eggs and larvae of mosquitoes, the most commonly used approach is to sample adults (Service 1993). There exist numerous methods to sample adults, especially host-seeking females. Many of the techniques used to sample mosquitoes seeking human blood are conducted indoors or in artificial shelters. Such methods include bednet traps, hand net collections, drop net collections, and aspirating mosquitoes attempting to bite a sleeping human subject (Service 1993). However, ethical issues arise when studies attempt to quantify the numbers of mosquitoes actively trying to bite humans. Studies involving methods such as counting the number of mosquitoes landing on a human (human landing catches) and human-baited traps can potentially expose human subjects to malaria through a bite from an infected mosquito. These studies are often conducted during the time of day when many female mosquitoes are actively seeking blood. Ways to circumvent using human subjects include the use of carbon dioxide and light traps, as well as animal baited traps. The accuracy of these methods varies, but their cost and practicality are often beyond the means of community level control programs in developing countries. Also, mosquitoes may be attracted to mechanical devices for reasons other than host-seeking and mosquitoes attempting to feed on animals may not behave in the same manner as anthropophagic species.

Collecting adult resting mosquitoes, however, is a method that is considered to be more representative of mosquito populations than collections made by trapping adults in flight. This is because a wider range of mosquitoes, in terms of bloodmeal status, age, sex, and gonotrophic cycle, are taken in resting collection (Service 1993). Resting collections are often made from outdoor natural shelters, such as vegetation and tree-holes, but a few important vector species can be found in man-made structures. Because of this, sampling a population of vectors resting indoors can be valuable to surveillance and control programs (WHO 1992, Lindblade et al. 2000).

Indoor Sampling of Resting Mosquitoes

Hand catches with a plastic tube or mechanical aspirator and Pyrethrum Spray Catches (PSC) are two commonly used methods for collecting mosquitoes resting indoors (WHO 1992). However, depending on the ability and willingness of the collector, human error can reduce the accuracy of counts made by hand catches. Pyrethrum spray catches are not only expensive but can cause unnecessary exposure to chemicals. A wide variety of artificial resting places have been tested to sample outdoor resting mosquitoes, but relatively few artificial resting places have been used indoors. Those tested indoors have seen limited success (Service 1993, Komar et al.1995, Smith 1942). Yasuno et al. (1977) tested the use of plywood boxes as a method to sample indoor mosquitoes, but found the boxes worked only in high densities of mosquitoes and low humidities. Resting boxes made of cardboard and black muslin cloth caught 30-60% of all *Aedes aegypti* collected indoors by hand catches (Edman et al. 1997). Das et al. (1997) developed an insecticide-impregnated fabric (IIF) trap for use indoors, but this is an impractical tool in much of the rural settings in Africa where many of the materials needed for construction are not readily available. In Kenya, Sexton et al. (1990) used a 5 x 6 ft reed ceiling mat from

which to make weekly hand catches of indoor resting densities of Anophelines but did not test this method against other kinds of indoor resting collections

CHAPTER 2
LITERATURE REVIEW

Historical Overview

Malaria has long been a plague of mankind. Evidence of this is seen in the high host specificity of the four malaria species that infect humans, suggesting a long association between humans and the parasites (Gilles 2002). Archeological evidence suggests that human malaria existed in the eastern Mediterranean region as early as the beginning of the Neolithic Period (9,500 BC) (Harwood and James 1979). In the fifth century, Hippocrates was the first to describe the clinical picture and some complications of malaria. The English word "malaria" is derived from the Italian *mal aria* meaning "bad air." The French word "paludisme" and the Spanish "paludismo" also refer to malaria and come from the Latin *palus* meaing "swamp." Malaria, paludisme, and paludismo have all originated from the idea that malaria was the result of inhaling "bad air" from swamps (Foster and Walker 2002, Gilles 2002, Harwood and James 1979). The first description of the malaria parasites in human red blood cells was from the French army surgeon Laveran in 1880. Seventeen years later Ronald Ross, a physician in India, discovered a developing form of malaria parasite in the body of an infected mosquito, greatly aiding the understanding of malaria transmission. In the late 1950s the WHO launched a global campaign that successfully eradicated malaria in much of the world's temperate zones including parts of North America, Europe, and Australia. Control of malaria was not as successful in many tropical areas. In fact, in the past twenty-five years there has been a significant increase of malaria incidence in tropical

areas such as southeastern Asia and tropical Africa. In 1998, the WHO stated malaria to be returned to its list of top priorities and introduced a new initiative called, "Roll Back Malaria." The success of this program has yet to be evaluated and reported.

Importance of the Disease Worldwide

Today, malaria is the most important insect-borne disease in public health (Durden and Mullen 2002, Foster and Walker 2002). Malaria, along with AIDS and tuberculosis, are the three most important infectious diseases in the world. Malaria affects around 40% of the world's population, much of which live some of the poorest countries in the world (WHO 2004, WHO 1995). In 1995, countries with intense malaria transmission had income levels equal to roughly a third of those in countries without malaria (Gallup and Sachs 2001). In Africa alone, it is estimated that the annual costs of malaria exceed US $2 billion (WHO 2000). Malaria not only affects poor people, but it keeps them poor.

The disease is found mainly in subtropical and tropical climates. In the past, malaria was more ubiquitous and was found in temperate regions including areas in the United States (Gilles 2002, Honigsbaum 2001). In the early 1900s, 6 to 7 million cases of malaria were reported in the continental United States annually (Harwood and James 1979). Now malaria is responsible for more than 300-500 million illnesses and a least one million deaths each year (WHO 2004, WHO 1995).

Description of Disease Transmission

Human malaria is caused by one of four species of parasite in the genus *Plasmodium,* family Plasmodiidae, suborder Haemosporiidae, order Coccidae (Sinden and Gilles 2002, Marquadt et al 2000). The life cycle of *Plasmodium* species is complex, including an exogenous phase (a cycle in mosquitoes) and an endogenous phase (a cycle in humans) (Foster and Walker 2002). The four species of *Plasmodium* infecting humans

are *P. falciparum, P. vivax, P. ovale,* and *P. malariae*. These parasites are transmitted to humans by mosquitoes of the genus *Anopheles.* The first sexual developmental stage of the parasites, the gametocytes, are ingested along with asexual stages by the mosquito in the bloodmeal of an infected human. After fertilization occurs a diploid zygote is formed. The zygote (or ookinete) moves through the peritrophic membrane of the mosquito gut. Depending on the species of parasite, 8 to 16 days later the ookinete ruptures, releasing sporozoites that make their way to the mosquito's salivary glands where they can be passed on to another human upon a subsequent bloodmeal (Sinden and Gilles 2002, Foster and Walker 2002).

The severity of disease is dependent on the species of parasite and the general health and immune status of the infected person (WHO 1995). Cases of mixed infections of two or more *Plasmodium* species are not uncommon (Sinden and Gilles 2002). Nonfatal infections that are left untreated can last more than five months depending on the immune system of the individual (Foster and Walker 2002). Common symptoms are febrile paroxysm (violent attack of fever), myalgia (muscle pain), headache, nausea, diarrhea, and vomiting.

In areas of stable malaria transmission (little variation in transmission over several years) some degree of acquired immunity is common in adults who survived bouts of malaria as children. This means that infected adults may be asymptomatic or exhibit only slight clinical symptoms. Effects of immunity to malaria on individual are; 1) prevention of infection with the same species of parasite, 2) reduction in parasite multiplication, 3) destruction of parasite, and 4) aid in tissue repair (Marquadt et al. 2000). This immunity is no doubt beneficial to the individual, but typically in such cases proper treatment is not

sought and the infected immune person can serve as a suitable host for the disease. In areas of unstable malaria transmission (much variation in transmission over several years), epidemics become a problem since people in these regions aren't exposed to the disease long enough to develop immunity.

Children under the age of five and pregnant women are the most vulnerable to malaria infection. Mortality in children results from three main presentations of malaria. One being an acute infection (often presented as seizures or coma) killing the infected child quickly. The second is the development of severe anemia from repeated infections of malaria parasites. The final is a low birth weight often the consequence of the mother being infected with malaria during her pregnancy. This last presentation is a major risk factor during the child's first month of life. Malaria can also cause children to become more susceptible to other infections including respiratory illnesses, diarrhea, and other common childhood illnesses. In regions of unstable malaria transmission, pregnant women are at extremely high risk of maternal and perinatal death. In regions of stable malaria transmission, infection is usually asymptomatic due to some acquired immunity, but commonly causes severe maternal anemia and babies with a low birth weight. A curious occurrence that results from malaria during pregnancy is that there is often a heavy infection of parasites concentrated in the placenta, impairing fetal nutrition. The reason for this is poorly understood (Shulman and Dorman 2002).

Importance of Malaria in Africa

Of at least one million annual deaths attributed to malaria, 90% occur in sub-Saharan Africa with the majority (90%) of these deaths occurring in children (WHO 2003). For many of the sub-Saharan countries more than a quarter of hospital admissions are due to malaria. Data suggests that the number of malaria cases have increased over

the last decade (WHO 2004). *Plasmodium falciparum* is most common in Africa and is responsible for the majority of malaria deaths worldwide (WHO 2003).

The appearance of a *P. falciparum* infection can range from almost asymptomatic, to an acute febrile, illness, to severe life-threatening cerebral malaria sometimes resulting in coma. Cerebral malaria can also cause anemia (lower than normal amount of hemoglobin or red blood cells), hemoglobinuria (presence of red blood cells in the urine), jaundice, hypoglycemia (low blood sugar), renal dysfunction (improperly functioning kidneys), psychosis, shock, and pulmonary edema (fluid in the lungs) (Warrell 2002).

Important Vector Species in Africa

The most important and well-studied vectors of malaria are found in the *An. gambiae* complex. The complex consists of seven described species: *An. gambiae* sensu strictu (s.s.)*, An. arabiensis, An. merus, An. melas, An. quadriannulatus, An. quadriannulatus* species B*, and An. bwambae* (Service 2002). Their behavior, vector status, and distribution differ in various aspects. *Anopheles quadriannulatus* and *An. quadriannulatus* species B feed mainly on cattle and are not vectors of malaria. *Anopheles bwambae* is a rare mosquito found only to breed in the Semiliki forest in Uganda and is not considered an important malaria vector. *Anopheles merus* is a malaria vector found in lagoons and mangrove swamps along the coast of West Africa. This species breeds only in salt-water. *Anopheles melas* is considered to be the East African equivalent to *An. merus* breeding in salt-water lagoons and swamps.

The two most important species in the complex are *An. arabiensis* and *An. gambiae* s.s. The first is found in regions of dry savannah. The second is found in more humid climates and readily becomes anthropophilic and endophilic (preferring to feed on humans both outdoors and indoors and to rest indoors). Because most of its time is spent

indoors close to people, it is considered a highly efficient vector for malaria. *Anopheles arabiensis* also exhibits some endophily but will also rest and feed outdoors, sometimes feeding on domestic animals. Often *An. gambiae* s.s. will feed at least two times before beginning its gonotrophic cyle (Foster and Walker 2002). The extra blood meals act as a substitute for sugar (Foster and Walker 2002). *Anopheles gambiae* s.s. is found in almost all sub-Saharan countries. Collectively all the species in the *An. gambiae* complex are referred to as *An. gambiae* sensu lato (s.l).

The most important vector in Africa after *An. gambiae s.s.* and *An. arabiensis* is *An. funestus*. It has a widespread distribution south of the Sahara and feeds predominately indoors. The larvae are found in more permanent waters associated with vegetation such as swamps and marshes. *Anopheles funestus* feeds both indoors and outdoors and prefers to rest indoors after feeding.

Summary

Malaria has been, and continues to be, one of the world's most important diseases. Despite extensive attempts at eradication and control, the malaria situation has worsened over the past two decades and parasites are re-emerging in areas where they had been successfully or nearly eradicated, such as the Republic of Korea, Iraq, and Turkey (WHO 2000). Because malaria affects many of the world's poorest nations, control of the disease has been difficult due to a lack of a solid health infrastructure. Sub-Saharan Africa is most affected by disease and is home to *An. gambiae,* one of the world's most efficient malaria vectors. It is considered a highly efficient vector because it prefers to feed on humans and will take multiple blood meals (Foster and Walker 2002, Service and Townson 2002).

African malaria vector control and research programs often focus on sampling adult populations of *An. gambiae* s.l. (Giles), *An. funestus* (Giles), and other important vectors. A major constraint in many developing countries where malaria is rampant is the lack of funds available to such programs. Commonly used methods for sampling adult mosquitoes include hand catches with oral or mechanical aspirators, Pyrethrum Spray Catch, and CDC light traps. These methods can become costly and labor-intensive, especially for community-based malaria control programs. The goal of this study was to develop a technique for sampling adult malaria mosquitoes for use in research and community control programs. To accomplish this, the following five objectives were delineated:

1. Test the efficacy of simple resting boxes for sampling *An. gambiae* s.s. mosquitoes resting indoors in semi-field conditions (screen-walled greenhouse). The design of the semi-field test structure is described by Mathenge et al. (2002).

2. Identify the preferred natural indoor resting sites for common malaria vectors, *An. gambiae* s.l. and *An. funestus.*

3. Assess the representative accuracy of the sampling of mosquito specimens through hand catches by an experienced collector using an oral aspirator.

4. Compare the accuracy of sampling common malaria vectors using a technique utilizing indoor resting boxes and bednets to 15 minutes of hand catches by an experienced collector using an oral aspirator.

5. Test the efficacy of resting baskets for sampling *An. gambiae* mosquitoes indoors in semi-field conditions (screen-walled greenhouse).

CHAPTER 3
MATERIALS AND METHODS

Site Descriptions

All study sites were located on the shores of Lake Victoria in the Suba District, within Nyanza Province, Western Kenya (altitude 1100-1300m)(Fig. 3.1). All semi-field trials were conducted at the International Centre for Insect Physiology and Ecology's (ICIPE) Biological Station in the town of Mbita Point. Fields trials were conducted in the nearby village of Lwanda Nyamasare. The two sites are within approximately 9 km of each other. The area has two rainy seasons from March to June and October to December with an average annual rainfall of 700-1800mm. Average temperatures range from 16° to 34° Celsius. Malaria is considered holoendemic in the region. Suba District is home to around 156,000 people with the majority of these people belonging to the patrilineal Luo ethnic group. Luos make a living primarily through fishing and subsistence agriculture. Description of the area is given in Mathenge et al. (2002), Geissler et al. (2000), Okech et al. (2003), and Knols et al. (2002).

Survey of Indoor Resting Habitats of Mosquitoes

From March 27th to April 18th 2004 at varying times of the day (8:25 to 16:45 hr.), thirty-two dwellings were visited for inspection by a local expert mosquito collector with eight years experience in collecting resting mosquitoes with a plastic tube oral aspirator. This method for sampling adult mosquitoes resting indoors, often referred to as hand collection or hand catches, is a widely used method and provides a suitable technique to quantify the resting site of each collected mosquito (Service 1993, WHO 1992).

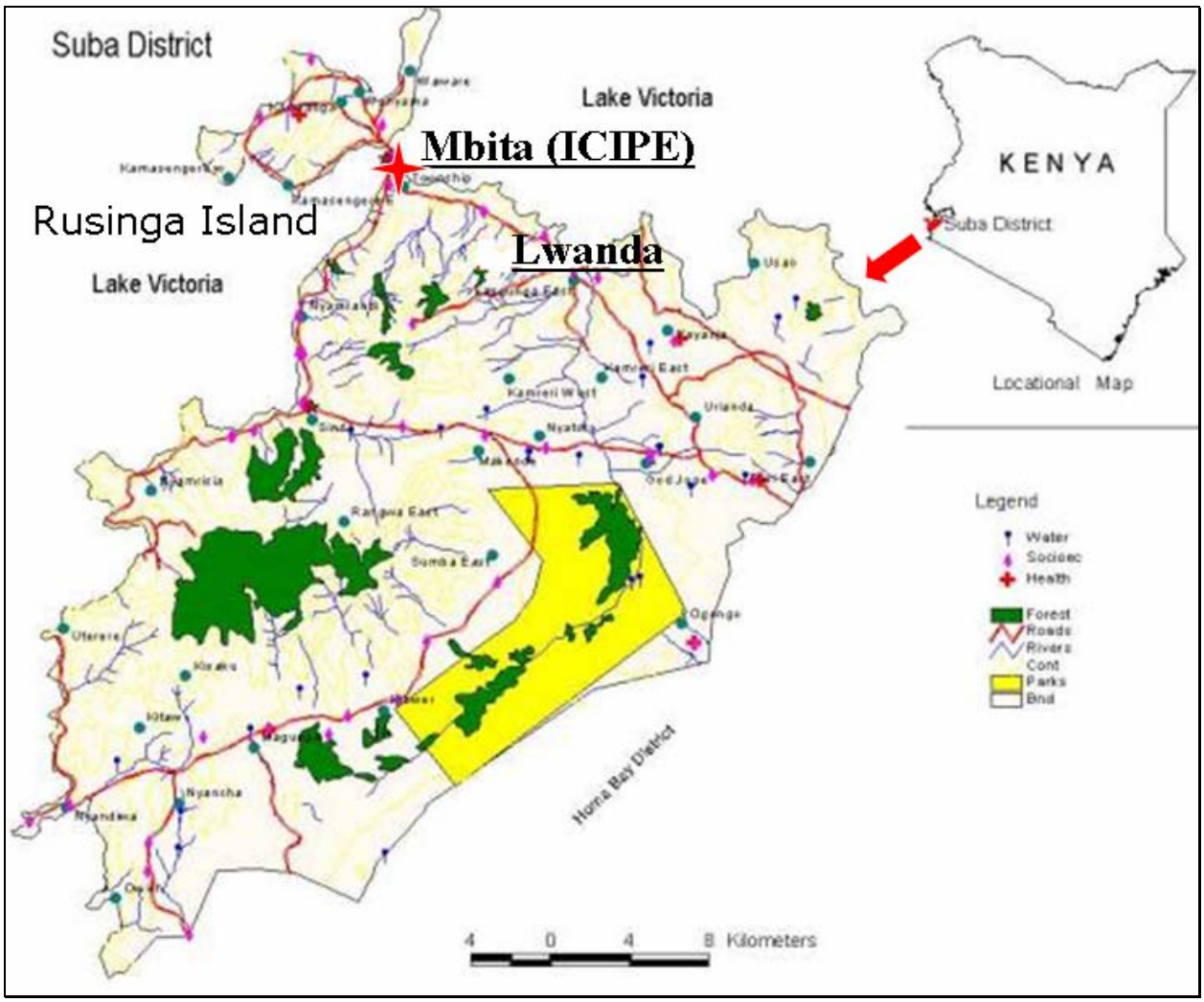


Figure 3-1. Map of Kenya showing the study sites.

The technician thoroughly searched each dwelling (Fig. 3.2) collecting mosquitoes and aspirating them into paper cups covered with netting. Searching involved not only walking around the dwelling and collecting visible mosquitoes, but also looking under and behind furniture, behind curtains, around and under pots and pans, etc. Cups containing live collected specimens were placed in a sealable container with cotton balls soaked with ethyl acetate to prepare the specimens for processing. Nine dwellings were revisited five to ten days after the first collection. Two separate rooms, each of similar size to a single dwelling, were checked in the same large house. The technician was given

as much time needed to collect all mosquitoes seen. The time spent collecting at each house was noted.

Dwellings were made of wood and mud with a thatch or a corrugated iron sheet roofs or made completely out of wood and corrugated iron sheets. All dwellings were built without ceilings with the underside of the roof exposed. Dwellings were all rectangular except for one circular house. The lengths of two adjacent walls inside all rectangular dwellings were measured to find the total area inside. For the circular dwelling, the diameter was measured to find the area. The height from the floor to the highest point of all dwellings was also measured from the inside.

 A)  B)

Figure 3-2. Dwellings in Lwanda. A) Single family structure B) Multiple family structure.

The height where each mosquito was collected was recorded as high, medium, or low. A mark of "high" required the technician to lift his arm above his shoulder (roughly 161 to 240 cm from the ground). "Medium" was designated as any collection made from the technician's shoulders to his waist (approximately 81 to 160 cm from the ground). Mosquitoes caught below the waist of the technician, causing him to bend over or kneel, were designated as "low" (0 to about 80 cm from the ground). The object (wall, jug, pots, hanging shirt, etc.) the mosquito was collected from was also recorded. The objects

on which the mosquitoes were collected were later divided into 5 different categories (Fig. 3.3):

1. wall/floor/door
2. plastic (plastic items such as bottles and basins)
3. cloth (items such as clothes and bed-nets)
4. furniture (more permanent items less likely to be moved around such as a bed, a cabinet, chairs, etc.)
5. temporary (found on items likely to be disturbed or moved such as lanterns or bicycles).

Finally, the number of all mosquitoes that were collected in an area deemed "hidden" or protected was noted (Fig. 3.3). These categories are similar to the studies completed by Pal et al. (1960) and Wattal and Kalra (1960).

Figure 3-3. Objects in dwellings on which mosquitoes can be found resting.

Representative Accuracy of Hand Collection

Twenty-one dwellings of similar size and make were visited by the expert collector and the investigator from April 1st to April 16th 2004. The walls were constructed of either wood and mud or wood and corrugated iron sheets. The roofs were made from either corrugated iron sheets or thatch. All dwellings were without ceilings. Fourteen were in multiple family units and seven were single family units. Mosquitoes were collected by hand using a plastic tube oral aspirator by the collector until an entire search of the house was completed (no more mosquitoes found). The time needed to hand collect all mosquitoes was noted. The time of day the collection took place ranged from

8:25 to 16:30 hr. Immediately after finishing a search of the dwelling, a Pyrethrum Spray Catch (PSC) was performed. A commercial-grade pyrethroid insecticide (Doom Fast Kill™, manufactured by Mortein, Australia) in 300g/494 mls containers were substituted for pyrethrum because it could be easily purchased and was safer (higher dilution of chemicals than the industrial grade) to use for the investigator and families living in the dwellings. The active ingredients of the insecticide were d-phenothrin (Pyrethroid) 1.0 g/kg and imiprothrin (Pyrethroid) 0.4 g/kg. For each dwelling the combined number of mosquitoes collected from the PSC and hand collection was found giving a total complete catch for each dwelling. From this combined number, the percentage collected by hand was found.

Development of Cloth Indoor Resting Box in Semi-Field Conditions

Semi-field trials were conducted from April 3rd to June 15th, 2004 at the ICIPE, Mbita Point Biological Station at Mbita Point, western Kenya (00° 25'S, 34° 13'E). A 30cm × 30cm × 30cm cloth resting box, similar to the design described by Crans (1989) was tested for its ability to sample indoor resting mosquitoes. The box was made of a plain 2 cm thick galvanized wire frame with blue cotton cloth. Blue cloth was sewn to cover the outside of the box with black cotton cloth sewn to cover the inside. The attractiveness of dark colors to mosquitoes is discussed in Bidlingmayer (1994). A flap made of mosquito netting (mesh size 196) with a sleeve was sewn to the top of the box. This was closed to facilitate capture when the number of mosquitoes in the box was relatively high.

The box was hung approximately 50 cm from the ground in the opposite right corner from the door of an experimental hut made of plywood (3.2m x 2.7m x 1.7m) inside a modified screen-walled 11.4m x 7.1 m x 4.2m greenhouse (Cambridge Glass

House Co. Ltd., U.K.) (Fig. 3.4). Both hut and greenhouse are described by Mathenge (2002). The box was hung from the far right corner because it was the opposite corner from the door, a large source of light in the hut. Each night one of three different densities of female *An. gambiae* s.s. mosquitoes from Mbita strain colony at ICIPE (low = 50, medium = 100, and high = 200, as in Mathenge et al. 2002) was released into the greenhouse at approximately 21:00 hr. For each density, mosquitoes were allowed to feed on 10% sugar solution for at least 24 hours after emergence. Half of the females were not allowed a bloodmeal and half were allowed to feed on blood for ten minutes daily for three nights prior to release. This adult rearing protocol is approved by the Kenya Medical Research Institute and the Kenyan National Ethical Review Committee (2001). The resting box was checked hourly from 7:00 to 12:00 hr and again at 15:00 hr. All mosquitoes caught in the box were counted and removed each time. The number of mosquitoes collected each hour was noted. After the final collection of the day, two technicians searched and collected the remaining females in the greenhouse and then disposed of them. One similarly designed resting box treated with deltamethrin (the cloth soaked in 2 liters of water with a 25% m/m concentration) was also tested in preliminary tests but was discarded when almost 15% fewer mosquitoes were caught. The deltamethrin was chosen for testing because it could be purchased nearby and in preliminary trials some of the released mosquitoes were found dead in the box making collection of mosquitoes easier.

Comparison of Accuracy of Resting box/Resting Net to Hand Collection

A comparison of accuracy of the collection methods was performed in Lwanda Nyamasare village from April 13th to June 30th 2004.


A.

B.

C)

Figure 3-4. Components of the experimental tests A) Resting box hung in experimental hut. B) Experimental hut in modified greenhouse. C) Modified greenhouse.

Preliminary trials suggested that a double size blue bednet (Supanet ™) hung from the highest point inside a dwelling and then spread out and tied to the top part of the eaves could facilitate in capturing resting mosquitoes out of reach from the technician performing hand catches. To aid in collection this resting net could be lowered within reach of the technician when needed. All dwellings were made with mud walls; two had thatched roofs, the other had a roof composed of corrugated iron sheets. Three dwellings of similar size and make were chosen to compare the efficacy of three methods of indoor resting collection in a Latin Square design. The three collection methods compared were (Fig. 3.5):

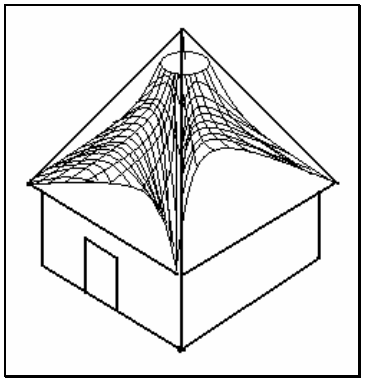
1. A cloth resting box (described in previous semi-field trials) and a resting net

2. A plain cardboard box of similar size to the cloth resting box (42 cm by 21.5 cm by 25 cm) and resting net

3. Aspiration for 15 minutes using a plastic tube aspirator.

The time (15 min) chosen for hand collection was based on the results from the previous survey and recommended in WHO (1992). When trials involved hand collection, the resting net was tied in a knot and placed out of the way for the homeowner. Both the cardboard box and bednets (for resting nets) are items that can easily be found in Lwanda. The blue cloth resting box was made from materials found in Mbita (12 km away). Immediately after mosquito collections at each dwelling, a PSC was performed (as described previously). Following the completion of spray catches at each house the collection methods were rotated. Dwellings were tested every 3 days to allow ample time for the insecticide sprayed during spray catches to dissipate. The number of days was chosen based on preliminary data from the Latin square. Trials showed that numbers of mosquitoes caught by PSC increased from the previous spray catch after two days. The sex and gonotrophic status of all captured mosquitoes was noted. The categories for gonotrophic status (bloodfed, half gravid, gravid, and unfed) was taken from Service and Townson (2002). The mosquitoes collected were identified as either *An. gambiae* s.l., *An. funestus*, Culicine, or unidentified Anopheline. The Latin square was run for a total of 27 experimental days in 3 complete rotations. The time taken to complete sampling of a dwelling using the resting box (cloth or cardboard) with a resting net was also noted.


A)

B)

C)

D)

Figure 3-5. Treatments used in the Latin Square. A) Cloth resting box hanging in dwelling B) Cardboard box hanging in dwelling C)Diagram of a resting net spread out in a dwelling D) Expert collecting mosquitoes by aspiration.

Development of an Alternative to Cloth Box in Semi-Field Conditions

Field Trials

To evaluate the possibility of using a wicker basket (30 cm tall with an opening of 28 cm in diameter) with an attractive black cotton cloth lining inside (Bidlingmayer 1994) as method to sample indoor resting mosquitoes, a preliminary field trial was run from April 23rd to June 30th 2004 in the village of Lwanda Nyamasare (Fig. 3.6). The 'resting basket' was hung approximately one meter from the floor in a corner of a bedroom in a single family dwelling. The opening was faced toward the middle of the room. The dwelling had walls made of mud and stick with an iron corrugated sheet roof.

Two to five people slept there nightly. Collections from the basket were made once at approximately 10:00 hr at least every three days. The number of mosquitoes collected was noted.

Semi-Field

From May 15 to July 13, 2004 trials were run in semi-field conditions using a resting basket of equal size and make to the one tested in the field. The basket was hung approximately 50 cm from the ground placed in the far right corner opposite the door of an experimental hut made of plywood (3.2 m x 2.7 m x 1.7 m) (Fig. 3.6) inside a modified screen-walled 11.4 m x 7.1 m x 4.2 m greenhouse (Cambridge Glass House Co. Ltd., U.K.). Both hut and greenhouse are described in Mathenge et al., (2002). Each night one of three different densities of female *An. gambiae* s.s. mosquitoes (low = 50, medium = 100; and high = 200, as in Mathenge et al. 2002) from the ICIPE Mbita strain colony was released into the greenhouse at approximately 21:00 hr. The method for testing the recapture rate of the resting basket was the same as the previously described trials using the cloth resting box. For both low (50 females) and high (200 females) densities, ten trials were run while thirty-two trials were run for the medium (100 densities).


A)

B)

Figure 3-6. Alternative to the cloth resting box. A) Cloth resting box and resting basket B) Resting basket hanging in dwelling

CHAPTER 4
RESULTS AND DISCUSSION

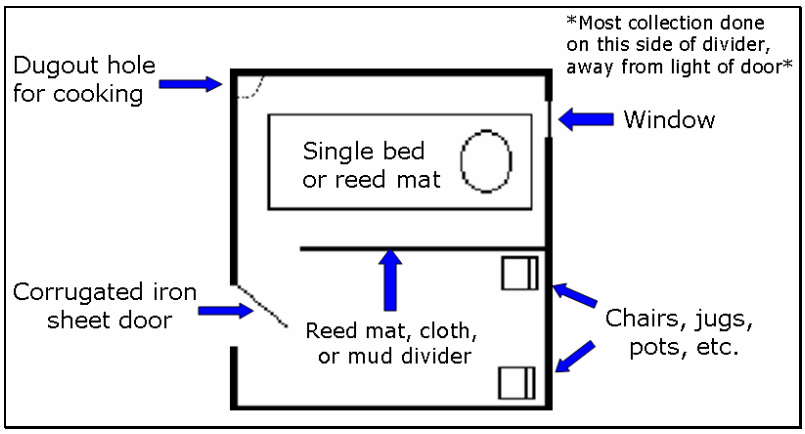
Results

Survey of Indoor Resting Habitats of Mosquitoes

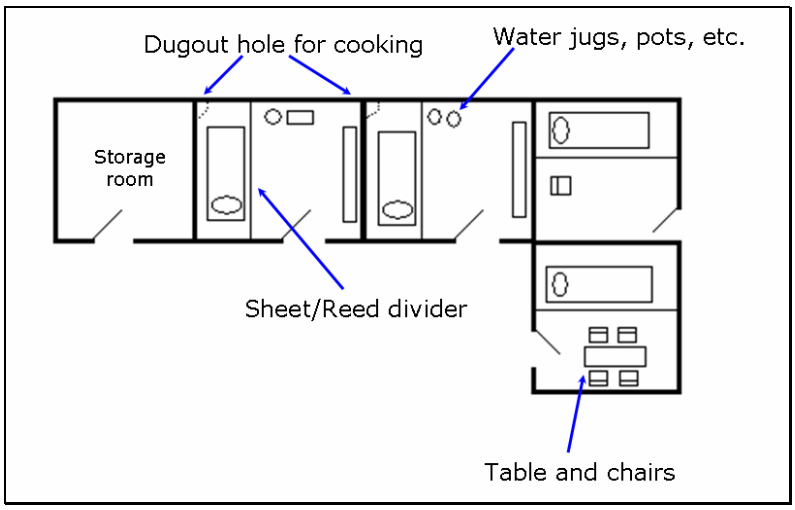
Dwellings typically held one family. Some dwellings were part of a single large structure which consisted of at least three or more dwellings (rooms with doors to the outside) connected under the same roof (Fig. 4.1).

All style of dwellings in the study area remained quite constant among single and multiple family structures. Dwellings consisted of a rectangular room divided by a hanging cloth sheet, reed mat, or mud wall. The sleeping area was always on the opposite side of the divider from the door. Structures containing a single family tended to be larger in area and had a higher roof than dwellings in multiple family structures (Table 4.1).

In the dwellings mosquitoes were collected off a variety of different substrates with around a quarter of those found in areas hidden from plain view (Table 4.2). The category "*Hidden (protected)*" was noted in addition to the substrate. For example, a mosquito collected from a *plastic item* was found in an area on the item that was "hidden" from plain view of the collector. A General Linear Model procedure using a Wilcoxon rank sum test and a Kruskal-Wallis test was performed to analyze the percent of mosquitoes caught at low, mid, or high heights in the dwellings (SAS 2001). There was no significant difference between the heights the mosquitoes were found (Table 4.3).



A.



B.

Figure 4-1. Layout of dwellings. A) Single Family B) Multiple Family

Table 4-1. Mean area and height of dwellings in study area

Structure	Mean area (± Std)	Mean Height (± Std)	Number
Single Family	14.4 ± 4.2 m^2	3.4 ± 0.4 m^2	13
Multiple Family	9.5 ± 2.5 m^2	2.7 ± 0.4 m^2	14

Representative Accuracy of Hand Collection

Out of the 531 mosquitoes caught in 31 dwellings by both hand collection and the PSC performed afterwards, hand collection caught 32.2% of the total of the two methods.

Development of Cloth Indoor Resting Box in Semi-Field Conditions

Trials using a treated box were stopped once it became apparent that the percent of the mosquitoes released that were captured was less than the untreated. The mean percentages for each of the densities tested on the untreated box were similar (Table 4.4).

The total mean percent of mosquitoes recaptured from all trials of the untreated box (Table 4.4) was similar to the percent caught by hand collection in 31 dwellings (32.2%).

Table 4-2. Percent of total actual number of mosquitoes (437) collected from each location.

Wall/Floor/Door	34%
Furniture (more permanent)	30%
Cloth/Bednet	24%
Temporary items	8%
Plastic items	4%
Hidden (protected)	26%*

*The category "Hidden" was noted separately.

Table 4-3. Total actual number of mosquitoes collected at each height. Note: Numbers followed by the same letter are not significantly different ($P \leq 0.05$), df = 2, Chi-square = 0.97.

Height dwelling where collected (in cm)	Number of mosquitoes caught
0 to 80 "Low"	176[a]
81 to 160 "Medium"	111[a]
161 to 240 "High"	119[a]

Table 4-4. The mean percent of mosquitoes recaptured in semi-field conditions using a resting box either treated or untreated with deltamethrin. (Mean ± Standard Error)

Type of box	Density-50 females	Density-100 females	Density-200 females	Total percentage
Untreated	39.4% ± 10.6 N=10	38.3% ± 8.9 N=10	30.7% ± 8.5 N=10	36.1% ± 9.9 N=30
Treated*	15.3% ± 3.0 N=3	25.3% ± 10.9 N=3	27.3% ± 6.2 N=3	22.6% ± 9.6 N=9

Note: (N=number of trials performed). *Trials with the treated box were stopped early once the it was shown that the it caught fewer mosquitoes than the treated.

Comparison of Representative Accuracy of Resting Box/Resting Net to Hand Collection

The total number of mosquitoes collected during the Latin Square experiment using each method (hand collection, cloth resting box and resting net, and cardboard box and resting net) was calculated (Table 4.5). For each method, the total number of mosquitoes caught by a method was added to the numbers caught by Pyrethrum Spray Catch (PSC) performed after the same method giving an actual overall total (Table 4.5). The percent

of the overall total number of mosquitoes collected using each method was found by dividing the total actual number mosquitoes caught a method by the overall total number of mosquitoes caught for each method (Table 4.5)

Table 4-5. Comparison of actual number of mosquitoes collected by the three methods tested. N=27

	Hand Collection	Blue resting box and resting net	Cardboard box and resting net
Overall total mosquitoes caught (PSC + method)	1166	1020	1060
Total mosquitoes caught using method	166	282	256
Percent of overall number of mosquitoes collected using method	14.2%	27.6%	24.1%

Note: (N=number of trials performed).

The cloth resting box/resting net caught 1.7 times the number of mosquitoes and almost twice the percentage of the total mosquitoes as hand of collection (Table 4.5). The cardboard box/resting net caught 1.5 times the number of mosquitoes and almost 10% more of the percentage of the total mosquitoes as hand collection (Table 4.5). The percentages captured by each of the methods on each test day were transformed to achieve linear model assumptions by:

$$\arcsin\sqrt{x}$$

where x is the percentage of mosquitoes captured by a method on each test day. The results were then analyzed using the Tukey Multiple Comparison Procedure (SAS 2001). The catches from the cloth resting box/resting net method were significantly different than hand collection (significance was set at $P \leq 0.05$) (Table 4.6). There was no significant difference between the cloth resting box/resting net and cardboard box/resting

net methods nor between the cardboard box/resting net and hand collection. The time taken to complete sampling using a resting box/resting net method was slightly shorter than the 15 minute time suggested by WHO (1992) for hand collection (Table 4.7).

The results of the identification to species, sex, and gonotrophic status showed An. gambiae s. l. to be the most collected of the four types of mosquitoes (An. gambiae s.l., An. funestus, Culicine, and an unidentified anopheline) identified from the Latin Square catches (Tables 4.8-4.11). The highest numbers of An. gambiae s.l. mosquitoes found in the dwellings were bloodfed (882 mosquitoes) and unfed females (884 mosquitoes) (Table 4.8). This trend was also seen with An. funestus (Table 4.9). For both Culicine and unidentified Anopheline mosquitoes, males were the most collected in the dwellings (Tables 4.10 and 4.11)

Development of an Alternative to Cloth Box in the Semi-Field

The resting basket caught a mean of 4 mosquitoes daily in one dwelling in the field (Table 4.12). A General Linear Model procedure was performed on the percentages of mosquitoes recaptured using a Type III sum of squares test for the total percent of mosquitoes recaptured (SAS 2001). There was no significant difference ($P \leq 0.05$) in the total percent recaptured between the two methods (Table 4.13). There was no interaction between the density of mosquitoes and the methods used but a difference was found among the densities of mosquitoes. For further analysis, a Tukey-Kramer adjustment for multiple comparisons was performed on the pooled (resting box and resting basket) percents of mosquitoes recaptured by each density (SAS 2001). Results showed a significant difference between low and high densities (Table 4.14

Table 4-6. Comparison of the accuracy (percent of the overall number of mosquitoes found in each dwelling) over the 27 test dates for the three methods tested. Methods followed by the same letter are not significantly different (P≤0.05). Note: F= 3.37, df = 2, 50.

Date	Hand Collection[a]	Cloth Box/ Resting net[b]	Cardboard Box/Resting net[ab]
13-Apr	6.7%	56.0%	2.2%
16-Apr	9.6%	12.0%	85.7%
19-Apr	48.1%	34.6%	18.9%
22-Apr	14.7%	14.2%	9.3%
25-Apr	14.5%	25.0%	20.5%
28-Apr	28.5%	28.7%	11.9%
1-May	9.0%	63.2%	5.2%
4-May	0.0%	25.0%	14.2%
7-May	8.3%	0.0%	31.6%
10-May	4.6%	35.7%	0.0%
13-May	0.0%	0.0%	23.6%
16-May	37.0%	10.7%	21.5%
19-May	13.3%	38.4%	3.2%
22-May	17.2%	17.3%	42.3%
25-May	34.7%	20%	45.6%
28-May	3.8%	20%	15.0%
31-May	14.8%	23.2%	48.0%
3-Jun	28.8%	21.4%	20.8%
6-Jun	14.4%	66.6%	18.7%
9-Jun	5.1%	19.4%	30.7%
12-Jun	11.1%	0.0%	17.0%
15-Jun	33.3%	60.7%	12.5%
18-Jun	4.1%	32.5%	37.5%
21-Jun	33.3%	10%	37.5%
24-Jun	22.8%	10.5%	9.0%
27-Jun	6.6%	29.7%	46.1%
30-Jun	30%	14.2%	16.6%
Mean percentage for each method ± SE	15.9 ± 12.9%[a]	27.1 ± 18.5%[b]	23.3 ± 18.6%[ab]

Note: F= 3.37, df = 2, 50

Table 4-7. Comparison of time taken to complete sampling using a resting box (cloth or cardboard) and resting net to hand collection.

Number of trials using a resting box and resting net	Mean time taken to complete sampling using resting box and resting net ± Standard Error	Time suggested by WHO (1992) to complete a search with hand collection
31	8.0 ± 3.9 min	15 min

Table 4-8. Actual number of An. gambiae mosquitoes caught by method, including sex and gonotrophic status, during the 27 test days of the Latin Square.

Method	Bloodfed	Gravid	Half Gravid	Unfed	Male	Total
Cloth box	18	4	8	7	9	46
Cardboard box	6	4	4	3	0	17
Hand Collection	60	21	10	30	12	133
Resting Net	163	53	26	103	35	380
PSC	635	265	138	741	243	2022
Total	882	347	186	884	299	2598

Note: For the resting net method, the numbers from both nets used in the Latin Square were calculated together.

Table 4-9. Actual number of *An. funestus* mosquitoes caught by method, including sex and gonotrophic status, during the 27 test days of the Latin Square

Method	Bloodfed	Gravid	Half Gravid	Unfed	Male	Total
Cloth box	1	0	3	0	0	4
Cardboard box	0	0	2	0	0	2
Hand Collection	4	1	2	1	1	9
Resting Net	7	2	5	2	2	18
PSC	26	16	8	29	14	93
Total	38	19	20	32	17	126

Note: For the resting net method, the numbers from both nets used in the Latin Square were calculated together.

Table 4-10. Actual number of Culicine mosquitoes caught by method, including sex and gonotrophic status, during the 27 test days of the Latin Square.

Method	Bloodfed	Gravid	Half Gravid	Unfed	Male	Total
Cloth box	0	1	0	0	0	1
Cardboard box	0	0	0	0	0	0
Hand Collection	0	0	0	0	1	1
Resting Net	0	2	2	2	3	9
PSC	8	27	4	17	24	80
Total	8	30	6	19	28	91

Note: For the resting net method, the numbers from both nets used in the Latin Square were calculated together.

Table 4-11. Actual number of unidentified anopheline mosquitoes caught by method, including sex and gonotrophic status, during the 27 test days of the Latin Square.

Method	Bloodfed	Male	Unfed	Total
Cloth box	0	0	0	0
Cardboard box	0	0	1	1
Hand Collection	1	0	0	1
Resting Net	0	0	1	1
PSC	4	15	5	24
Total	5	15	7	27

Note: For the resting net method, the numbers from both nets used in the Latin Square were calculated together.

Table 4-12. Mean number of mosquitoes caught daily by a resting basket placed in a house in the village of Lwanda.

Mean number of mosquitoes caught	Number of days tested
3.9 ± 3.3	33

Table 4-13. Comparison of the resting basket to the cloth resting box of the total percentage of mosquitoes recaptured in semi-field conditions.

Type of trap	Total mean percent of mosquitoes recaptured (± SE)
Resting Basket	33.8% ± 8.9[a] N=52
Resting Box	36.1% ± 9.9[a] N=30

Note: N=Number of trials run for each method, percentages followed by the same letter are not significantly different ($P \leq 0.05$), $F = 0.51$, df = 1, 76)

Table 4-14. Mean percent of mosquitoes recaptured by each density used for both the resting box and resting basket.

Density	Mean Percent of Mosquitoes Recaptured
High	31.3%[a]
Low	39.1%[b]
Medium	35.6%[ab]

Note: N=Number of trials run for each method, percentages followed by the same letter are not significantly different ($P \leq 0.05$), $F = 41.47$, df = 1, 76)

Discussion

Indoor Resting Places of Mosquitoes

The most important malaria vector in Africa, *An. gambiae*, will readily rest in the dwellings of rural villages (Service and Townson 2002). A common method for sampling populations of these resting mosquitoes is a search of a dwelling by a trained technician using an oral aspirator (Service 1993, WHO 1992). A properly trained technician performing the search is essential as the mosquitoes are difficult to find and catch. This idea is reinforced by the results of this study. Since it is also generally thought that both male and female mosquitoes are attracted to darker areas indoors, one would expect to find mosquitoes resting at low heights around the dwelling since they would be further away from a main light source, the eaves. Although a larger percent of mosquitoes were found at heights from 0 to 80 cm, the number of catches was not significantly different from those higher than 80 cm. About a quarter of the mosquitoes (26%) were also found in an area not in plain view of the collector. The results of this study would suggest that it would be difficult to accurately pinpoint an area in a dwelling to focus the aspiration as mosquitoes. The mosquitoes were found on a broad variety of substrates at various heights with many resting in areas that would require the collector to search under, above, or around household items. Many times during the study the collector needed to move furniture, pots, clothes, and other household items to be able to

reach the mosquitoes. This emphasizes the need for adequate training for anyone attempting to sample resting mosquitoes by hand collection using an oral or mechanical aspirator.

Comparison of Sampling Techniques

The local expert technician had eight years experience with hand catches, likely more than most people attempting that method. This would suggest a high quality of collection in comparison to other hand collectors. Since the expert was given as much time needed to complete a search of each dwelling during the initial part of the study, it was assumed that the majority of the mosquitoes collected in the PSC, performed after the search, were resting above the reach and out of the sight of the collector. Since the expert caught only approximately a third (32%) of the total number found, it was decided that any sampling method developed should account for mosquitoes resting on the exposed underside of roofs (made of thatch or corrugated iron sheets) as this would be a likely area to find them (Lindsay et al. 1995, Schofield and White 1983).

Resting boxes have been a well established method for sampling mosquitoes (Service 1993). Few, however, have been used indoors. The results from the semi-field trials suggest that the cloth resting box could collect close to the same percentage of mosquitoes (36.1%) as the expert hand collector in the field (32.2%). However, it is likely that a cloth resting box placed near a similar location in a dwelling could not accurately sample mosquitoes that prefer to rest in the underside of the roof. This assumption is based on the idea that since the expert technician was not able to sample mosquitoes resting so high, a stationary trap would also have the same difficulty. For this reason, a resting net was tested with the cloth resting box. The results of the Latin Square trial suggest that the cloth resting box/resting net method could catch a significantly

higher percentage of the total population resting in a dwelling than that of an expert performing hand catches using an oral aspirator.

To increase the practicality of the method the resting basket was tested in the semi-field. Because there was no significant difference in recapture rates between the resting basket and the cloth resting box, the resting basket could be an acceptable alternative to the cloth resting box. The cloth resting box (or resting basket) and resting net method has a number of advantages over hand collection:

1. It can more accurately sample the population resting in a dwelling (a higher percentage of mosquitoes will be collected).
2. The materials needed for construction are inexpensive and can be easily found in many rural areas as opposed to the time and money to needed to train and/or hire a technician to perform hand catches.
3. It requires less training, not only because mosquitoes are easier to see in the black cloth of the box and in the bright blue of the net, but also because the investigator only needs to search a set area (the trap and the net) rather than the whole house.
4. The time needed to complete a search is shorter than 15 minutes, which is commonly assigned for hand catches (WHO 1992).

The use of resting boxes and nets also has advantages over the PSC technique. Although a greater percentage of mosquitoes can be caught with spray catches, the spray technique is too expensive and impractical for use on a regular basis for local control programs. Pyrethrum spray catches also expose the investigators and the members of the tested household to potentially harmful chemicals that may take several days to dissipate (Service 1993). This study showed that cloth resting boxes or resting baskets used with resting nets can be a practical method for local malaria control programs to sample indoor resting mosquitoes.

Summary

Malaria continues to be one of the world's most important human diseases. Although, has been eradicated in some areas, it continues to be a threat to 40% of the world's population. Many of those people affected by the disease live in some of the poorest nations in the world. The lack of resources and infrastructure has produced major obstacles to malaria control in those countries. Because malaria affects many people living in impoverished nations, many of the more expensive and technical methods utilized by developed countries for mosquito and disease control are not feasible.

An initiative to create sustainable malaria control programs at a community level has been implanted by international organizations such as the WHO (2000). As local malaria control programs become more accepted, technology is needed to keep such programs sustainable. Developing new methods and tools for malaria surveillance and control, which are both user-friendly and economically viable, are essential for this to happen. Because the most important vector of malaria in Africa, *An. gambiae* s.l., readily rests indoors after feeding, developing practical ways to sample populations of those mosquitoes would be valuable.

The sampling technique tested in this study, was developed specifically for malaria control programs based at community levels. The materials needed for the technique can be found inexpensively in rural villages where community malaria control programs are run. The technique provides a more user-friendly alternative to both PSC and hand catches, both of which are commonly used in malaria control programs and research (Service 1993, WHO 1992).

Members of control programs sampling with the new technique have only to collect from a defined area (box or basket and net) compared to hand catches where a complete

search of a dwelling is required. Mosquitoes can also be seen easier on the black cloth in the box or basket and on the blue cloth of the net than on the walls, thatch, and other items in the dwellings. Since mosquitoes can be found and collected more easily than hand catches, less training and experience is needed to effectively sample a dwelling.

Although sampling with PSC captures most, if not all, mosquitoes in a dwelling, the cost of the materials needed (insecticide, sheets, protection against chemicals) is often beyond the means of community malaria control programs. The use of such a method could not be sustained for very long due to its expense and the constant need for insecticide. The safe use and storage of the chemicals needed for PSC is also an important issue for the safety of the people doing the sampling and the homeowners. Since no chemicals are needed with the new technique, it can be performed safely by members of control programs with no danger to the homeowners. Because the materials needed for the new technique can be inexpensively purchased once, it can be used indefinitely.

Further research should focus on developing more inexpensive and practical methods of malaria surveillance and control that are accessible to the people facing the greatest disease load. Such research should be broadly based in multiple disciplines due the complexity of the malaria problem. Things like access to healthcare, economic stability, and cultural behavior also play important roles in the transmission of malaria and need to be considered. Even though much of the world's malaria control stems from international organizations, a larger global effort is needed to have a lasting effect on malaria control.

CHAPTER 5
NOTES ON THE DEVELOPMENT OF A PRACTICAL *ANOPHELES* GRAVID TRAP

Introduction

The ability to sample the populations of host-seeking mosquitoes is essential to the evaluation of malaria control programs. The exposure to potentially infectious bites on people sampling host-seeking mosquitoes makes many such sampling methods ethically questionable. As an alternative to sampling host-seeking adults, gravid traps can provide similar data (reproductive capacity of mosquito populations and the dispersal patterns of mosquitoes as they seek larval habitats after biting humans) without the risks of exposure. Such information is of great importance to vector control programs as many programs focus on and have seen success with larvicide application in potential or known larval habitats.

Gravid traps that have been tested and used in research and mosquito control programs, sample only Culicine mosquitoes (Service 1993, Ritchie et al. 2004). Many of these traps focus specifically on collecting gravid *Culex* mosquitoes (Service 1993, Mboera et al. 2000). Although a variety of methods exist to sample the eggs of malaria-carrying Anopheline mosquitoes, no technique has been devised to effectively trap and sample these mosquitoes solely when they are gravid (Service 1993).

Gravid traps, like those used to sample Culicine mosquitoes, would become problematic in many malaria endemic regions because they require a significant degree of skill and maintenance to ensure they are working correctly. The commonly used, CDC Gravid Trap (J. W. Hock Co. Gainesville, FL.), requires a net collection bag, motorized

trap with a fan, pan with water, a battery, and a battery charger. They would also be quite costly to programs, particularly in African countries where resources available for control are usually very limited. This study was undertaken to develop a much more practical gravid trap that effectively samples egg-laying adults. A simple trap would have a number of advantages over present traps:

1. It would not require skilled personnel for sampling mosquitoes
2. It would be far less expensive than current gravid traps
3. Data collected from traps will most likely reflect a more realistic composition and behaviour of the egg-laying mosquito population since no artificial attractants will be used.
4. It could be easily replicated and therefore be more likely to be used in mosquito surveillance programs; since collected adults and eggs would be disposed, it could directly aid malaria control programs.

The objective of this investigation was to develop a practical gravid trap using materials readily available in rural areas where malaria is endemic. Such a trap would be beneficial to malaria control programs at a community level where resources are often scarce.

Preliminary Semi-Field Trials in Florida

Semi-field trials were conducted weekly from June 8th to October 1st 2004 at the United States Department of Agriculture's (USDA) Center for Medical, Agricultural, and Veterinary Entomology (CMAVE) located in Gainesville, Florida. Traps were tested singly in screened cages (2m x 2m x 2m). Prior to placement of the traps, 70 gravid *Anopheles albimanus* females were released into each cage and allowed to acclimatize for 3-4 hours. The females were left in each cage with a trap for 24 to 48 hours after which the number of females caught was noted. After collections were made the cage was left open for at least four days to release any females left in the cage.

One design was tested and modified from oviposition basins from CDC Gravid Traps (J. W. Hock Co. Gainesville, FL.), plastic funnels, and plastic 40 ounce peanut butter jars. All designs were spray-painted with a matte finish primarily of black and white (Fig. 5.1). Spray-painted traps were left outside for at least six weeks prior to testing to remove any odors. Some variations of trap designs had modifications such as the addition of black plastic mesh, the presence of cotton soaked in sugar water, and various amounts of hay infusion (Fig. 5.2). All designs tested had approximately 2 liters of well water placed in the basin.

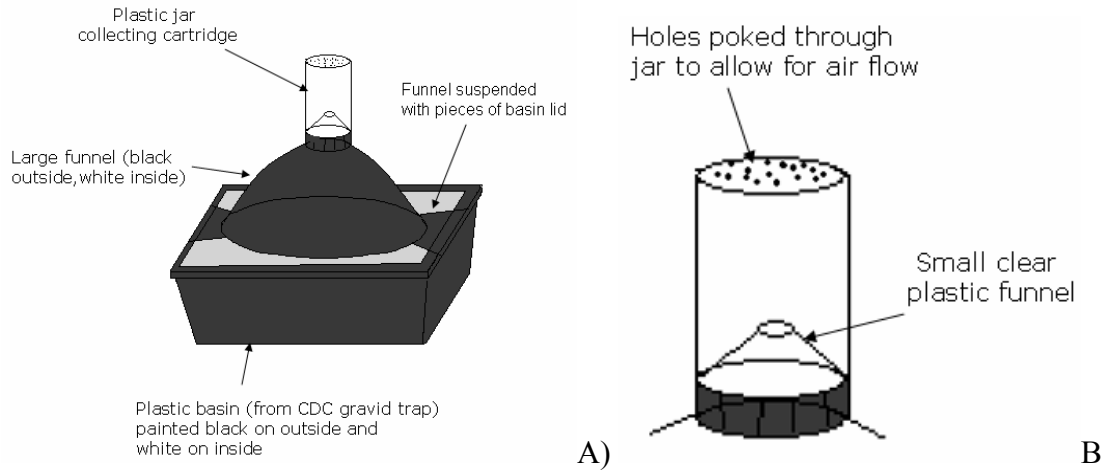


Figure 5-1. Design of oviposition trap. A) Full view. B) Collecting cartridge

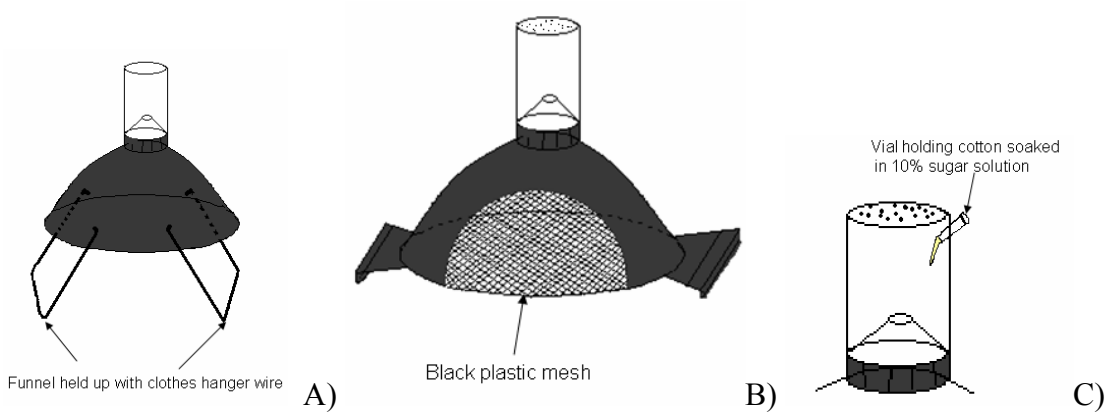


Figure 5-2. Modifications to design. A) with clothes hangers B) with black mesh C) with vial with sugar solution.

Semi-Field Trials in Kenya

From February 17th to March 11th 2004, 1 to 3 color variations of 4 trap designs were tested daily in a screened cage (3m x 3m x 3m) at the ICIPE, Mbita Point Biological Station at Mbita Point, western Kenya. A black plastic bucket (35cm x 30cm) was placed in a hole in the middle of the screened cage. The bucket was placed so that the top was even with the ground level. Some of the soil removed from the hole was placed back into the bucket and water from Lake Victoria (of varying amounts depending on the trap design) was also placed in the bucket. The water was changed daily. Traps were made of 2cm thick plain galvanized wire, white mosquito netting and either black or blue colored cotton cloth. Daily, fifty gravid *An. gambiae* s.s. females were collected from the ICIPE Mbita strain colony and released into the screened cage at 6 pm. The following day from 6:30 to 12:00 hr, traps were checked for any females. From14:00 to 16:00 hr. the remaining females were aspirated out of the screened cage. Following colony protocol all gravid females had been allowed to blood feed on the investigator's arm for ten minutes daily for three consecutive days prior to their release. A colony egg trap was

placed in the colony cage from which the tested gravid females were taken to note the ovipositional success of that batch of females.

The first experimental day, no trap was placed on the bucket and eggs were noticed the following morning. On February 21st the bucket was removed from the screened cage, fresh lake water placed in it and then positioned in a sunlit area for 6 hours to "cook." The bucket was checked for any mosquito eggs that may have been laid by wild mosquitoes and then returned to the screen cage prior to the release of that day's colony mosquitoes. This "cooking" was done for each of the remaining trials. On February 23rd, the soil in the bucket was replaced by mud from a known *Anopheles* larval site in Mbita that was not subject to larval control. On March 5th, dark blue and black cotton cloth was draped on the outside of the screened cage and water was poured on it prior to release of mosquitoes to increase the humidity. This was again continued daily. On March 7th, filter paper used in the colony egg traps was attached to the lower part of one of the traps and positioned half in the water to entice the females into ovipositing. On March 8th, the screened cage was moved into a modified greenhouse (Mathenge et al. 2002). On March 12th a miniature version of the black trap (Fig. 5.3) was placed in a colony cage over a standard egg trap used for maintaining colonies. Only one gravid female was captured during all semi-field trials.

Field Trials in Kenya

From February 19th to March 17th 2004 field trials of the traps designs were conducted. Field sites were chosen in the Kamasengre district of Rusinga Island, about 12 km away by road from Mbita. The trap bucket was placed in a hole dug in the ground approximately 4m from a single family dwelling. Soil from the hole and fresh lake water were added to the bucket. Four days later mud from a nearby larval site was placed

in the bucket instead of soil from the hole. The design placed on or in the bucket was checked daily at approximately 7:00 hr. A week after these preliminary trials two more sites were added; one had a bucket placed approximately a meter from a large larval site (about 10m long) and another placed far from any larval sites or homes. This location was considered a control. Mud from the larval site was placed in both of these new buckets as well as lake water. The lake water was changed daily. Trap designs were rotated daily among the three field sites and the semi-field site. On March 17th the field sites were closed.

Results

Florida Trials

Out of 31 traps tested from June 8th to October 1st, 14 traps captured mosquitoes. Out of those 14 traps, an average of 2.36 ± 1.74 mosquitoes was caught. The mean recapture (± SE) rate of the mosquitoes released for the 14 traps was 3.3 ± 2.3%.

Kenya Trials

In the semi-field experiment only one gravid female was captured. This was by the emergence design with black cloth. Only one mosquito (a Culicine) was caught in trials in the field. The emergence design with blue cloth placed near the larval habitat caught the mosquito.

Discussion

The description of larval habitats for *An. gambiae* s.l. has been well studied (Warrell and Gilles, 2002). Larvae are typically found in sunlit temporary pools often associated with humans. They develop in roadside ditches, wheel ruts, hoof prints from wild and domestic animals, concrete holes, and village pots. Larvae can even to survive close to five days on damp soil (Koenraadt et al. 2003).

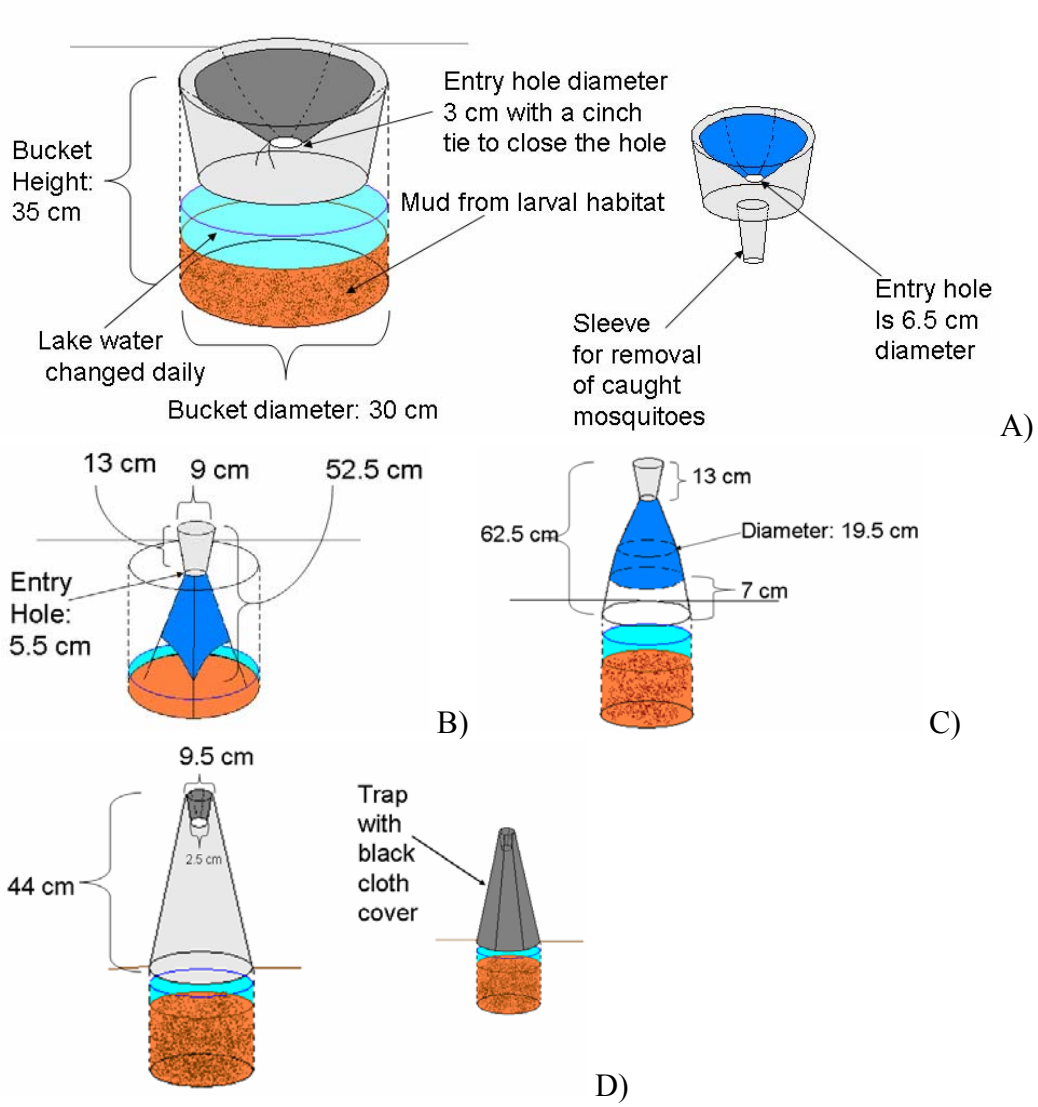


Figure 5-3. Trap designs in Kenya. A) Mbita (two variations) B) Emergence C) Florida D) Cone (two variations).

Most larval sites contain fresh water without much debris. Preliminary trials conducted by other investigators in Mbita Point, Kenya showed that *An. gambiae* will readily oviposit in plastic tubs and buckets used for washing clothes and commonly found around the town and nearby communities (Pers.comm. U. Filinger, University of Durham, England). Although the identification of larval sites can be accomplished without much difficulty, the identification of oviposition attractants has yet to be determined.

One of the obstacles faced by testing a practical gravid trap out in the field is that because An. gambiae will lay eggs in such a wide variety of habitats that are commonly found in great numbers around the area, the trap would need to be at least as attractive to females as the natural habitats. By placing a trap over a bucket or other potential larval habitat the site no longer becomes sunlit and therefore probably less attractive. Further investigations into oviposition chemical and visual attractants would be needed to overcome the deterrent of shade created by the trap.

A problem faced by semi-field experiments is that gravid colony females that have emerged from the same batch of colony pupae may not oviposit at the same time if at all. If fifty gravid females of the same age and exposed to the same amount of blood are released into a semi-field cage only a small number of females if any may actually try to oviposit. More time spent rearing test mosquitoes should be taken to insure that conditions are created to enhance the number gravid females that attempt to oviposit each day.

Although a practical method for sampling gravid *Anopheles* has yet to be developed, such a tool would be of great use to malaria control programs. A practical gravid trap could be a safer alternative to other sampling methods involving chemicals or sampling host-seeking mosquitoes. Identifying oviposition attractants for *Anopheles* mosquitoes could not only aid the development and use of gravid traps but provide insight to the ecology of the mosquito and create more options for mosquito monitoring programs.

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BIOGRAPHICAL SKETCH

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