Characterization of *Anopheles gambiae* (Giles) Breeding Sites in Gokwe South, Zimbabwe

By

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DEDICATION

DEDICATED TO MY PARENTS,

BROTHERS,

SISTERS

WHOSE LOVE,

CARE, ENCOURAGEMENT

AND SUPPORT

SAW ME THROUGH

AND MY FIANCÉ, OMAR,

WHOSE LOVE I WILL ALWAYS CHERISH.

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ABSTRACT

Anopheles gambiae Giles larval breeding habitats were characterized in Gokwe South district in Zimbabwe to determine the ecological parameters of *Anopheles* breeding sites and identify their associations with larval density. This was also done to determine abundance, distribution and extent of cohabitation of the anophelines and to identify them to species level. Larval abundance in swamps, drainage channels, a dam and a pool of water were determined through sampling conducted using the dipping technique. Sampled larvae were then reared to adults and identified to species level using morphological characters. Habitats were characterized based on vegetation, water quality, habitat permanence, pH, conductivity, turbidity, dissolved oxygen, temperature, salinity, total dissolved solids, calcium, nitrates and chlorides. Anophelines were found breeding in swamps primarily populated with *Typha* species. The water in the breeding sites was dirty and organically polluted with decaying plant and animal waste. Data analysis using variable selection by stepwise regression followed by multiple regressions of larval densities with significant variables showed that the abundance of mosquito larvae was positively correlated with salinity. Larval density was not affected by pH, conductivity, turbidity, total dissolved solids, dissolved oxygen, temperature, calcium, nitrates and chlorides. Three members of the *An. gambiae* complex were identified: *An. arabiensis, An. merus* and *An. quadriannulatus.* While *An. arabiensis* was the most abundant and widely distributed species, *An. merus* was predominant at Masakadza where breeding sites had high levels of salinity. On the other hand, *An. quadriannulatus* occurred in low densities but in association with *An. arabiensis*.

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1. INTRODUCTION AND LITERATURE REVIEW

1.1 THE MALARIA BURDEN

Malaria is by far the most important insect-transmitted disease in the world. It is an infection characterized by fever, chills, shivering, malaise, headache and sweats. The disease is endemic in 107 countries and territories (WHO, 2005). More than 40% of the people in the world are at risk of malaria but its impact is most evident on the African continent where the disease continues to be the leading cause of death and morbidity. The World Health Organization (WHO) estimates that malaria parasites infect 200-300 million people and kill more than 1 million people each year, primarily those under the age of five (WHO, 2005; Ruiz *et al*., 2006). Based on reported malaria data, estimates of populations at risk and incidence rates, it is estimated that there were 350-500 million malaria cases in 2004 (WHO, 2004).

Malaria transmission in Africa is governed by the existence of the mosquitoes of the *Anopheles gambiae* Giles (Diptera: Culicidae) complex which are highly efficient vectors of malaria (Coetzee, 2004). Approximately 70% of the total sub-Saharan population lives in areas infested with malaria vectors (Keating *et al*., 2003). Thus, it is not surprising that 90% of all clinical cases and nearly 90% of deaths caused by malaria are reported from this region (Jamieson and Toovey, 2006). In eastern and southern Africa, the proportion of deaths caused by malaria is estimated to have increased from 18% in the 1980s to 37% in the 1990s (Killeen *et al*., 2004), with most of these occurring in children under five years (Tanser and Le Sueur, 2002; UNICEF, 2002; WHO, 2005; Cano *et al*., 2006). The overwhelming bulk of the world's malaria burden rests upon the population of sub-Saharan Africa because of the unique coincidence of expanding human populations, weak health systems, the world's most effective vector mosquito species and environmental conditions ideal for transmission (Killeen *et al*., 2004).

Most of the people in Africa live in areas where malaria transmission is relatively intense and continuous, so that with time some degree of immunity to malaria develops (WHO, 2000). In such areas, young children and pregnant women are at greatest risk of malaria infection and death due to their lower levels of immunity. A smaller proportion of people in Africa live in areas of seasonal and less predictable transmission due to lower temperatures or rainfall in highland or desert fringe areas. Thus, populations in these areas generally have lower levels of immunity and all age groups are vulnerable to highly seasonal transmission and epidemics (WHO, 1996).

Anaemia, low birth weight and cerebral malaria are complications of malaria that may compromise the health and development of children in Africa. Severe anaemia among children increases susceptibility to other diseases and hampers growth and intellectual development (WHO, 1996). Pregnant women die as a direct result of severe malaria or as an indirect result of malaria-related severe anaemia. In addition, infection of pregnant women may result in spontaneous abortion, neonatal death and low birth weight (WHO, 2004). Complications in pregnancy such as miscarriage and stillbirth as well as death of the newborn are greatly enhanced by the infection of the mother and contribute to high maternal death rates in tropical malarial areas (Jamieson and Toovey, 2006).

Malaria is both a social and economic burden to the country, the family and the individual. It stands in the way of social progress and better standards of life. The disease, which threatens the livelihood of millions of Africans, has been incriminated in the continual under-development of the continent as a whole. Malaria slows economic growth in African countries by 1.3% of the population annually (Killeen *et al*., 2004). These economic effects are especially noticeable in rural areas where malaria strikes at the time of the year when there is greatest need for labour in agriculture. In general, however, malaria mortality rates are higher among poor families who are less able to afford protection measures and to seek health care when sick (WHO, 1996).

1.2 MALARIA IN ZIMBABWE

Malaria is one of the major health challenges in Zimbabwe just like other parts of sub-Saharan Africa. The disease has remained a major cause of mortality and morbidity despite decades of sustained national control programmes. The country experiences seasonal and potentially epidemic malaria transmission characterized by a population which lacks immunity to the disease (Mharakurwa *et al*., 2004). Forty-five to fifty percent of the 12.5 million people of Zimbabwe are at risk of malaria (Mabaso *et al*., 2006). The population with epidemic risk is 27% while 29% is at endemic risk (WHOSIS, 2002). Estimates from weekly epidemiological reports are that in 1996, nearly 750,000 clinical cases and 1,800 deaths were recorded from January to November in Zimbabwe (Manokore *et al*., 2000).

Season, altitude and associated temperature and rainfall changes are the most important factors governing malaria endemicity in Zimbabwe (Figure 1). The disease is highly seasonal and winter constitutes a low risk period. The seasonal peak of transmission occurs from

February to May each year (Mharakurwa *et al*., 2004). There is very low malaria transmission from July to October (Taylor and Mutambu, 1986).

Malaria in Zimbabwe is highly influenced by altitude. It is endemic mainly within the low and mid altitude zones (below 900 m and between 900-1200 m, respectively) and rarely at altitudes above 1200 m (Mabaso *et al*., 2006). Malaria transmission occurs in three-fifths of the country but the central band from Harare to Gwanda is free from the disease (WHOSIS, 2002). In the low-lying areas of the Zambezi Valley and the south-eastern lowveld, malaria transmission occurs throughout the year (Crees and Mhlanga, 1985).

1.3 MALARIA VECTORS

Malaria is transmitted by the infectious bite of the female *Anopheles* mosquito which needs proteins from a blood meal to produce a batch of eggs. Highly adapted to sucking blood efficiently, the mosquito has a proboscis or long, sucking organ, consisting of six hair-like stylets or probes, two of which are used for piercing the skin, two for sawing the wound open and the third pair sucks out the blood (Dorozynski, 1977). The disease is caused by protozoan parasites of the genus *Plasmodium*. These include *P. ovale*, *P. malariae*, *P. vivax* and *P. falciparum* (Jamieson and Toovey, 2006). The most prevalent and by far the most deadly is *P. falciparum*, accounting for almost all malaria deaths worldwide (Onyabe and Conn, 2001). *Plasmodium falciparum* can kill within hours of noticeable symptoms which include high fever, severe headache, drowsiness, delirium and confusion. Although *P. vivax* infections are severe and debilitating, they are usually self-limiting in healthy individuals. *Plasmodium malariae* and *P. ovale* infections cause little morbidity and almost no mortality (Collins and Paskewitz, 1995). In Zimbabwe, the main malaria parasite is *P. falciparum* Mpofu (1985).

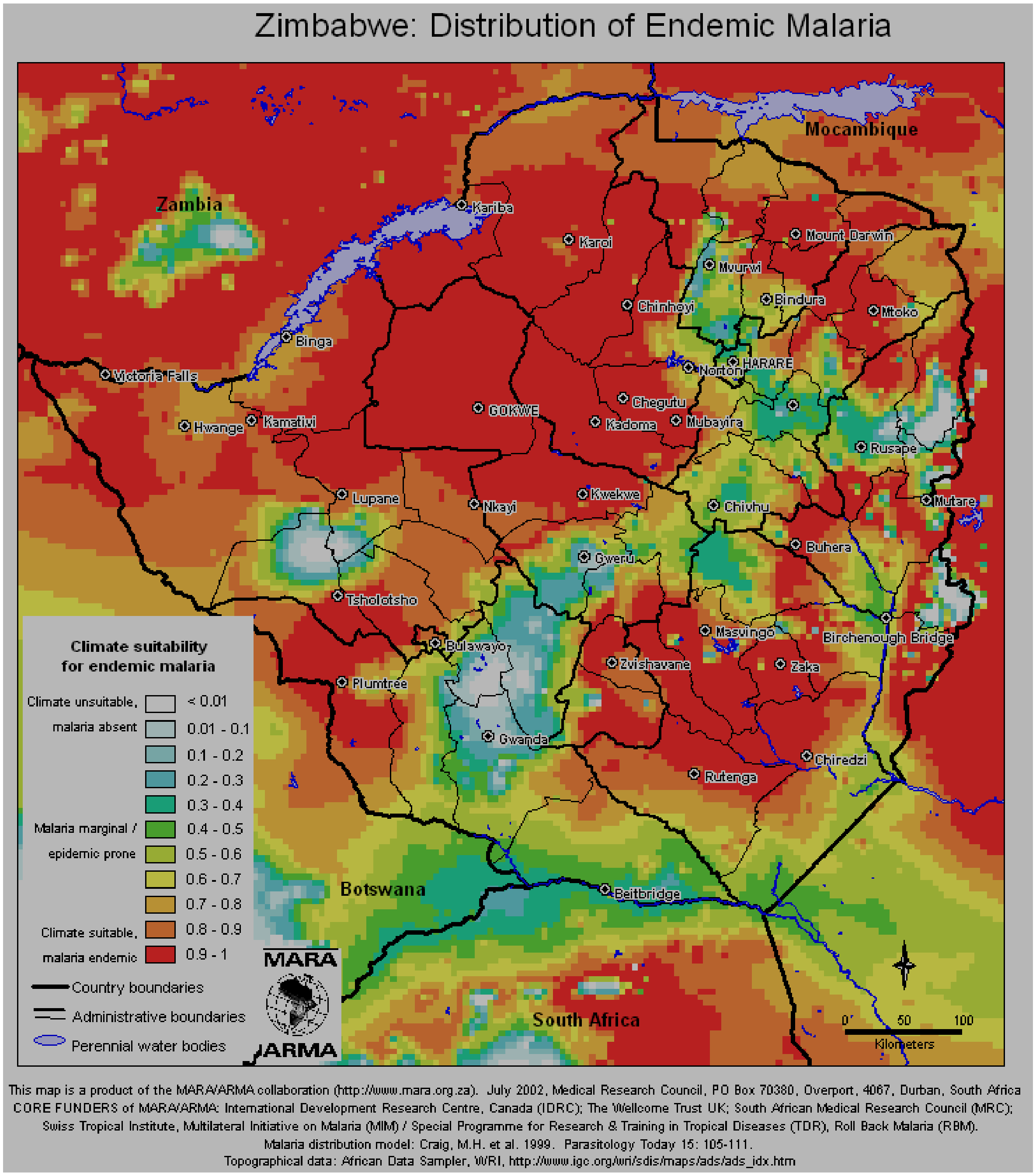

Figure 1. The distribution of endemic malaria in Zimbabwe according to Mapping Malaria Risk in Africa, MARA, 2001

Members of the *An. gambiae* complex are the most important vectors of malaria in sub-Saharan Africa (Onyabe and Conn, 2001). The complex contains what may be considered to be the most efficient malaria vector mosquito species in the world (Coetzee, 2004). The *An. gambiae* complex consists of seven morphologically indistinguishable sibling species which include *An. arabiensis* Patton, *An. gambiae* Giles *sensu stricto*, *An*. *bwambae* White, *An. melas* Theobald, *An. merus* Donitz and *An*. *quadriannulatus* Theobald species A and species B (Coetzee, 2004). These species vary in distribution and composition from one geographical location to another, this diversity being greatly influenced by the prevailing climatic conditions. They also vary in their ability to transmit malaria.

Of the seven sibling species of the complex, *An. gambiae s.s.* and *An*. *arabiensis* are the most widely distributed and efficient vectors of malaria in sub-Saharan Africa (Coetzee, 2004; Edillo *et al*., 2004). These two species are broadly sympatric but there are areas where only one or the other may be found (Edillo *et al*., 2004). *Anopheles arabiensis* is largely zoophagic (feeding on animal blood) but endophilic, that is, it rests inside houses during the time required for blood digestion and maturation of the ovaries. On the other hand, *An. gambiae s.s.* is highly endophagic (frequently entering houses to feed) and anthropophagic (feeding on human blood) (Mutuku *et al*., 2006). *Anopheles arabiensis* will feed on humans and rest indoors as well as feed on cattle and rest outdoors making this a more difficult mosquito to control by conventional means (Coetzee *et al*., 2000). On the other hand, *An. gambiae s.s*. is predominantly endophilic. *Anopheles arabiensis* is the most widely distributed member of the complex found throughout the Afro-tropical region except in the Equatorial forest belt. In addition, it shows a greater tolerance to dry environments and is a major vector in Sahelian savannas (Morlais *et al*., 2005). However, *An. gambiae s.s.* is more commonly distributed in West and East Africa and has a patchy distribution in southern Africa (Coetzee *et al*., 2000; Masendu *et al*., 2005). *Anopheles arabiensis* also tends to be more frequent where it is hot and dry (Edillo *et al*., 2004). Because of its greater anthropophily, *An. gambiae s.s.* is considered the more important vector and is the predominant vector when malaria transmission is at its highest during the rainy season (Schneider *et al*., 2000).

Anopheles quadriannulatus species A and B are recognized as allopatric members of the *An. gambiae* complex and occur in southern Africa and Ethiopia, respectively (Fettene *et al.*, 2002). However, they are mainly cattle feeders and do not play any role in malaria transmission (Coetzee *et al*., 2000; Fettene *et al*., 2002). Because of its markedly zoophilic behaviour, *An. quadriannulatus* has not been implicated as a vector of malaria. Two other members of the complex, *Anopheles melas* in West Africa and *An. merus* in East Africa are found in coastal mangrove forests and salt marshes where they avoid the potential competition from the freshwater members of the complex (Coetzee *et al*., 2000). Locally, *An. merus* and *An. melas* can be important vectors of malaria. *Anopheles bwambae* is a minor vector of malaria and is restricted to the mineral springs in western Uganda where it is associated with water high in mineral content (Berzosa *et al*., 2002).

In Zimbabwe, the main vectors of malaria are members of the *An. gambiae* complex (Mpofu, 1985). The wide distribution of *An. arabiensis,* often found in association with the non-vector *An. quadriannulatus,* has confirmed its status as the principal vector in Zimbabwe (Masendu *et al*., 2005). At Masakadza village in Gokwe South, the salt water breeder *An. merus* has been implicated in malaria transmission.

1.4 MALARIA VECTOR LARVAL HABITATS

Understanding where mosquitoes breed and why they prefer certain water bodies over others is vital for designing mosquito control strategies. Knowing the ecology and behaviour of a vector is essential to determine its role in disease transmission and the type of control measures that may be appropriate for it (Shililu *et al*., 2003). Habitats that support breeding by the vectors of human malaria are extremely diverse. Mosquito larvae are found in a great variety of different habitats, ranging from large expanses of water such as swamps and rice fields to small collections of water as found in snail shells and fallen leaves (Service, 1976). *Anopheles gambiae* normally breeds in shallow, temporary, bare-edged pools or puddles fully exposed to sunlight (Muirhead-Thomson, 1951). According to Minakawa *et al.* (1999), there are three reasons why the larvae choose to breed in these habitats. Firstly, the females prefer to select small open habitats for oviposition. Secondly, larval predation is less prevalent in temporary habitats than it is in large permanent habitats. Thirdly, the larvae exploit the increased resources of warmer, open habitats that tend to produce more algae (the main food source) than do shaded habitats. Warmer temperatures encountered in small and open habitats (during daytime hours) shorten larval-pupal development time while also reducing mortality associated with desiccation.

Water temperature in the breeding habitats has a great influence on the development of larvae. Optimal breeding temperatures for mosquitoes are between 25^{o}C and 30^{o}C with humidity levels of over 60% (Jamieson and Toovey, 2006). They can still breed in temperatures as low as 20^{o}C but cooler conditions will severely hamper the hatching of larvae. The minimum temperature for mosquito development is between 8-10^{o}C.

1.5 CURRENT MALARIA CONTROL STRATEGIES

1.5.1 Anti-malarial Drugs

Globally, the malaria situation is worsening with large scale epidemics and increasing mortality. Current strategies for malaria control in sub-Saharan Africa involve treating infected individuals with anti-malarial drugs to clear the parasites and reducing human-mosquito contact rates through vector control efforts. However, the incidence of malaria has tripled across Africa due to the emergence of parasites resistant to standard treatments such as chloroquine, sulfadoxine/pyrimethamine (Fansidar) and amodiaquine (MCMEP, 2004). In Zimbabwe, a combination of chloroquine and Fansidar is used to treat uncomplicated malaria. Fansidar is used for prevention of malaria in pregnancy while quinine is the second-line drug and is also recommended for severe malaria (WHOSIS, 2002).

Resistance of *P. falciparum* to chloroquine in Africa was first documented in Kenya in 1979 (Fogh *et al*., 1979). Anti-malarial drug resistance is defined as the ability of a parasite strain to survive and multiply despite the administration and absorption of the drug given in doses equal or higher than those usually recommended (WHO, 2004). One of the major contributing factors to resistance build-up in Zimbabwe is that most cases of malaria are diagnosed on the basis of clinical symptoms and treatment is presumptive, rather than based on laboratory confirmation. A study carried out in north eastern Zimbabwe showed that of the 104,000 malaria cases diagnosed, less than 30% of those made by trained nursing staff operating at rural clinics were slide-positive (Shiff, 2002). Thus, the widespread resistance of *P. falciparum* to chloroquine around the world has meant that this cheap drug has lost its place in the fight against the parasite. Furthermore, anti-malarial drugs have little impact on the intensity of transmission at the community level because most drugs do not reduce the production of *Plasmodium* gametocytes, the parasite stage responsible for initiation of infection in mosquitoes (Mushinzimana *et al*., 2006). Individuals who receive treatment can soon become infected. An ideal anti-malarial drug will not only kill the parasite but also the gametocytes, thus blocking the transmission of the infection as well as treating the infection.

Combination therapy with drugs which have different modes of action is now the preferred approach to malaria treatment to inhibit the emergence and spread of parasites resistant to one component of the combination. Artemisinin Combination Therapies (ACTs) are effective anti-malarial drugs which produce a very fast response in patients and are active against multi drug-resistant *P. falciparum* malaria. They are also well tolerated by patients and have the potential to reduce malaria transmission by decreasing gametocyte carriage (Cohuet *et al*., 2003). However, Medicines for Malaria Vectors' (2004) annual report indicated that these drugs were still not ideal because they contain processed plant extracts and their production is lengthy and relatively costly. Anti-malarial combination therapies are ten times more expensive than the drugs currently used and as such are not affordable to the majority of the poor.

1.5.2 Vector Control

Vector control remains the most effective measure to prevent malaria transmission. There are basically two kinds of mosquito vector control. These comprise control strategies directed towards the adults, such as the use of Insecticide-Treated Nets (ITNs) and Indoor Residual Spraying (IRS), and larval control which is targeted towards the aquatic stages. Currently in Zimbabwe, the mainstays in malaria prevention are ITNs and IRS.

The use of ITNs has so far produced good results in Zimbabwe. The ITNs provide a barrier against the attacks of indoor, night-biting mosquitoes, thus preventing disease transmission. These nets protect their occupants by diverting host-seeking vectors to look for a blood meal elsewhere and by killing those that attempt to feed (Killeen *et al*., 2002). However, in much of the world, malaria remains uncontrolled since household use of ITNs is currently low, averaging only 1% as the nets are still prohibitively expensive or unavailable in some areas (UNICEF, 2002). There are several challenges that come with the use of ITNs. Firstly, a key element in the ITNs programme is the need for nets to be retreated regularly with insecticide, which is costly. Without impregnation, the nets will lose their effectiveness as vector control agents (Shiff, 2002). Secondly, mosquitoes have developed resistance to pyrethroids used to impregnate the nets. Thirdly, the provision of an improved supply of affordable ITNs requires huge investments. Fourthly, community use of ITNs in most epidemic-prone areas is limited and the distribution of the nets may not be practical given the urgency of implementing epidemic control measures. Finally, ITNs may not reduce the overall population of malaria vectors. In Zimbabwe, the distribution of ITNs has been greatly hampered by the poor state of roads in the rural areas. This has led to the rural population having limited access to the nets. Also, the high cost if ITNs has led to less people having access to them.

Control strategies for African malaria mosquitoes largely involve methods that kill or prevent female mosquitoes from having a blood meal and one such method is indoor residual spraying (IRS) (Mutuku *et al*., 2006). Indoor Residual Spraying is the process of spraying the inside of walls and ceilings of houses and buildings with insecticides. The intention of house spraying is that mosquitoes will rest on the insecticide deposit before or after biting and remain long enough to pick up a lethal dose. The use of IRS is a powerful method for vector control and is especially effective for the prevention and control of malaria. However, the key constraint to implementing IRS programmes is that they are costly due to the need for long-term human and financial resources for regular spraying campaigns (Shiff, 2002). Furthermore, IRS is relatively demanding in terms of planning, skills required and coverage levels that are needed for a successful operation. Indoor Residual Spraying methods are only effective when at least 80% of all structures in a neighbourhood have been sprayed and an insecticide that is effective against the specific mosquito vector is correctly applied (WHO, 2005). Reaching areas without roads, particularly in the rainy season, may also be exceedingly difficult. Other major constraints are mosquito resistance to insecticides and the resistance of people to having their homes sprayed because of cultural and religious beliefs (Manokore *et al*., 2002). This has therefore led to the relatively low efficiency of the spray programmes.

Preliminary consultations with the health workers in Gokwe South revealed that the World Health Organization in conjunction with the Ministry of Health and Child Welfare has reintroduced the organochlorine insecticide, dichlorodiphenyltrichloroethane (DDT), for IRS in the area. In low dosages, DDT can be applied to the interior walls of homes. Following an indoor blood meal, female *Anopheles* mosquitoes often rest on nearby walls and absorb the DDT through their feet and die. The treatment kills *Anopheles* females after a single blood meal. By feeding only once, they are thus never able to transmit malaria sporozoites.

The role of DDT in combating mosquitoes has been a subject of controversy. While some argue that DDT damages biodiversity, others maintain that it is the most effective weapon in combating mosquitoes and hence malaria (Chevillon *et al.*, 1999). DDT was banned many years ago in many parts of the world including Zimbabwe, for use in public health and agriculture on the grounds of environmental pollution and potential toxicity to humans. DDT does not break down into harmless forms (it persists in the environment long after use) but remains harmful for many years and with ingestion can be passed along the food chain (Jamieson and Toovey, 2006). Inhabitants of sprayed houses will continue to inhale it from the air inside the houses. Among the harmful effects of DDT are neuro-developmental delays in early childhood, reproductive disorders, reduced birth weights and premature deliveries of babies (Calson and O'Brian, 1988).

The development of resistance to DDT among malaria vectors also necessitated the change of tactics because higher doses were required to control the mosquitoes resulting in unacceptably high levels of DDT in humans and the environment. DDT resistance has since been detected in *An. arabiensis* mosquitoes collected from Gokwe South (Masendu *et al*., 2005). Despite this, WHO still recommends the use of DDT for disease vector control provided it is used only for indoor spraying and not for agricultural purposes (WHO, 2000).

1.5.3 Malaria Vaccines

Vaccine development against malaria is ongoing. The search is underway for transmission-blocking vaccines against *P. falciparum and P. vivax.* These vaccines are designed to prevent mosquitoes that feed on vaccinated individuals from becoming infected, thus reducing transmission and providing indirect protection to the entire population (Greenwood *et al*., 2005). The search is also underway for vaccines that protect against the erythrocytic stages as well as those that prevent infection by acting against the pre-erythrocytic stages. However, these vaccines will not be available for several years and their chances of success have been seriously questioned. Nevertheless, even if highly effective vaccines were available, problems of poverty, lack of public health infrastructure and community mobilization may contribute to incomplete coverage of the populations at risk (Collins and Paskewitz, 1995).

1.6 LARVAL CONTROL

Larval control is a control technique which is targeted towards aquatic stages of mosquitoes. It can be achieved by environmental management and the use of larvicides or larvivorous fish. Larval control should always be part of an integrated vector management strategy. Larviciding and environmental management have a major advantage in that they control mosquitoes before they disperse and transmit disease (Killeen *et al.,* 2002).

The insect vectors of malaria are linked to aquatic ecosystems. The life cycle of each individual anopheline mosquito vector proceeds through four stages: three immature stages which occur in a water body (egg, larva and pupa) and then the mature stage, a flying adult (Depinay *et al*., 2004). Mosquito eggs, larvae and pupae are confined within relatively small aquatic habitats and cannot easily escape control measures (Killeen *et al*., 2002). On the other hand, adults are highly mobile flying insects that can readily detect and avoid many intervention measures such as excito-repellent insecticides, including bed-net impregnation treatments or indoor residual sprays. Therefore, promising strategies for malaria control should include insecticide-treated nets and prompt access to treatment as well as measures targeted against the larval stage of the mosquitoes (Sattler *et al*., 2005).

Malaria interventions such as breeding site reduction and insecticide use have been considered the most effective and practical ones for reducing malaria transmission (Depinay *et al*., 2004). Larval control strategies against the vectors of malaria in sub-Saharan Africa could be highly effective, complementary to adult control intervention, and should be prioritized for further development, evaluation and implementation as an integral part of rolling back malaria (Killeen *et al*., 2002). The effectiveness of malaria control programmes is crucially dependent upon not only the extent of coverage but also the ability to target the most intense foci of transmission. Thus, adulticide-based control may be limited because of constantly shifting distributions of biting vectors and their ability to avoid interventions.

Larval control is not an entirely new strategy for managing malaria. Historically, many successful campaigns of mosquito eradication relied heavily on management of larval habitats. Successful programmes to control malaria transmission by *An. gambiae* were initiated in the 1930s and 1940s and their effects sustained for two decades or more in Brazil, Egypt and Zambia (Utzinger *et al*., 2002). The features common to these successes included integrated control and emphasis on environmental management. Environmental management of mosquito breeding sites is a promising approach with which to control malaria, but it has seen little application in Africa.

1.7 MAPPING OF BREEDING SITES AND MOSQUITO ECOLOGY

Malaria control methods are affected by several factors such as endemicity, vector species and behaviour, seasonality and disease patterns (Sattler *et al*., 2005). Since these factors are not distributed equally in space, accurate and timely information is required before malaria control can be planned and resources allocated properly. With regard to transmission reduction, attention must be paid to the areas of greatest vector abundance and precise risk maps of *Anopheles* species breeding sites are of major importance.

Mapping of malaria risk on the basis of breeding sites plays an important role in malaria control programmes. In addition, identification of ecologic risk factors that lead to high case density could allow intervention programmes to be targeted at areas high in risk factors, reducing the need for costly and extensive evaluations (Ernst *et al*., 2006). Vector ecological studies have to be undertaken to locate risk areas for malaria and formulate an appropriate strategy for vector control. Factors that play a role in transmission can be studied using computerized mapping with Global Positioning Systems (GPS) and Geographical Information Systems (GIS). GIS technologies are software systems that give researchers the ability to manipulate, visualize and analyze multiple geospatial data forms simultaneously. The application of GIS to the study of vector-transmitted diseases considerably improves the management of the information obtained from field surveys and facilitates the study of the distribution patterns of the vector species (Cano *et al*., 2006).

1.8 SPECIES IDENTIFICATION OF *AN. GAMBIAE* COMPLEX

The identification of species of the *An. gambiae* complex is vital to the efficient management of malaria vector control programmes in Africa. The occurrence of cattle-feeding species in a given area also makes species identification essential so that control measures can target the actual vectors and not look-alike non-vectors (Coetzee *et al*., 2000).

Morphological identification of *Anopheles* species is quick and can be carried out in the field with the minimum of equipment using the method developed by Coetzee (1989). This technique separates the *Anopheles* on the basis of characters such as the palpus ratio which is the length of the fourth and fifth segments divided by the third, the size of the pale band at the joint of the hind tarsomeres 3 and 4 as well as the number of the coeloconic sensilla on antennal flagellomeres (Coetzee, 1989). The hind leg pale band at the junction of the tarsomeres is a good character for grouping *gambiae*/*arabiensis* and *quadriannulatus*/*merus*. The pale bands on hind tarsi 3 and 4 in *An. arabiensis* and *An. gambiae s.s.* are generally narrower than those of the sibling species *An. merus* and *An. quadriannulatus* where the pale band tends to overlap the joints of the adjacent segments. The palpus ratio is useful and effective in separating *An. merus* from the freshwater breeders *An. gambiae s.s.*, *An. arabiensis* and *An. Quadriannulatus* (Sharp *et al.,* 1989).

1.9 RESEARCH PROBLEM AND JUSTIFICATION

Malaria transmission is strongly associated with specific mosquito breeding sites and normally can only be transmitted within certain distances from them (Carter *et al*., 1990). A precise knowledge of the location of breeding sites is important for a cost-effective malaria control programme. Accurate information and knowledge on ecological features of mosquito breeding sites is a potential key element for implementing efficient and effective larval control which is an important tool for reducing malaria endemicity. Malaria vector ecological studies have to be undertaken in order to locate risk areas for malaria and to formulate an appropriate strategy for vector control. Information on the spatial distribution of anopheline mosquito habitats would allow vector control efforts to target the most productive larval habitats resulting in reduction of operational costs (Mushinzimana *et al*., 2006).

The purpose of this research was to locate and characterize *An*. *gambiae* larval habitats in Gokwe South, and determine any associations between the presence of anopheline larvae and ecological parameters such as pH, temperature, turbidity, conductivity, total dissolved solids, dissolved oxygen, salinity, calcium, nitrates and chlorides. Understanding the factors that regulate the size of the mosquito populations is considered fundamental to the ability to predict transmission rates and for vector control. For example, certain levels of physicochemical attributes such as pH, turbidity or salinity can facilitate the reproduction and survival of the vectors (Bond *et al*., 2004). *Anopheles* species use and exploit a variety of habitats that vary considerably in physicochemical properties, surface areas, vegetation and productivity, thus the need to study breeding sites (Weidong and Novak, 2005). In addition, simple methods for ranking larval habitats according to larval presence and abundance provide a powerful basis for consistent monitoring and targeting of specific aquatic habitats for larval control strategies on a temporal scale (Shililu *et al*., 2003).

The study was aimed at identifying vectors from each site to species level so that malaria control measures could target the actual vectors. With species identification comes the associated knowledge of the biology and behaviour of that species which in turn dictates appropriate control measures (Chen *et al*., 2006). The distribution of *An*. *gambiae* and the extent to which cohabitation exists among the species was determined.

Research Questions

- What is the association between larval densities and ecological parameters such as pH, turbidity, conductivity, total dissolved solids, salinity, temperature, dissolved oxygen, calcium, chlorides and nitrates in the breeding habitats?
- What is the abundance of *An*. *gambiae* in the breeding sites?
- What is the distribution of the *An. gambiae* species complex in Gokwe South?
- To what extent do the members of the *An. gambiae* complex cohabit?

1.10 OBJECTIVES

General Objective

The purpose of this study was to characterize *An. gambiae* breeding sites in Gokwe South and to determine the association between breeding site characteristics and larval abundance.

Specific Objectives

1. To determine ecological parameters of *Anopheles* breeding sites and identify their associations with larval density.
2. To determine abundance of anophelines in the breeding sites.
3. To identify *Anopheles* mosquitoes to species level.
4. To determine the distribution of the *Anopheles* species and the extent of their cohabitation.

2. MATERIALS AND METHODS

2.1 STUDY SITES

Gokwe South is a savannah region located in the Zambezi valley in the Midlands province of Zimbabwe at an altitude ranging from 737 to 1179 m above sea level. The study was conducted at five localities within the district: Rugora ($17^{o}45'$ S, $28^{o}34'$ E), Masakadza ($17^{o}49'$ S, $28^{o}36'$ E), Kamhororo ($17^{o}51'$ S, $28^{o}39'$ E), Gwave ($17^{o}55'$ S, $28^{o}41'$ E) and Sesame ($18^{o}10'$ S, $28^{o}49'$ E) (Figure 2).

The climate in the area is typically sub-tropical. Three seasons characterize the area and these are the cold and dry from June to August, hot and dry from September to November and the warm and wet season from December to March. Annual precipitation averages 400-1200 mm with the highest falls occurring in January, February or March.

The district has poorly drained soils which allow water stagnation thus resulting in the formation of large swamps. The area is generally dry with no source of ground water since the rivers in the area are seasonal. In order to allow access to the nearby Sengwa coal field, a road network was constructed in the area. This led to the drilling of artesian wells as a source of water for the road construction workers. The leakages from these artesian wells have led to the formation of extensive swampy areas. Combined with the hot climate, these factors have resulted in creating excellent breeding opportunities for *An. gambiae* mosquitoes thus favouring malaria transmission.

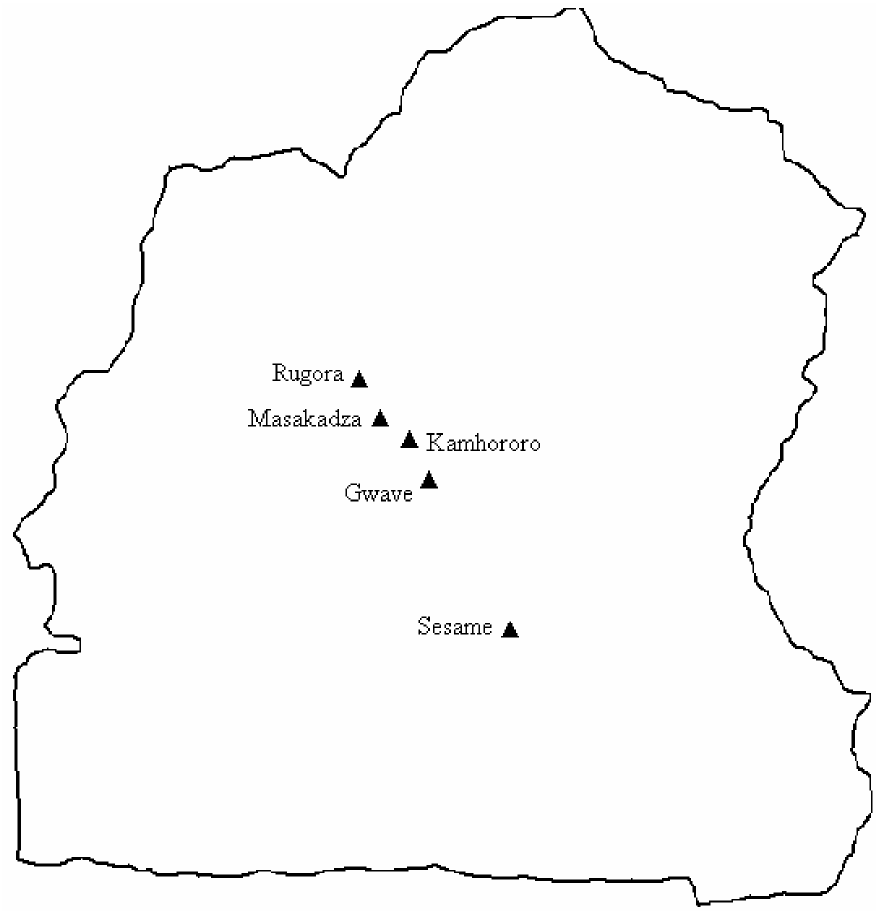

Figure 2: Map of Gokwe South showing the relative locations of the different study sites.

Larval sampling was conducted at six sites. Three of the sites were in large swamps and they included Masakadza, Kamhororo and Gwave. The artesian wells and the resultant swamps are shown in Plates 1, 2, 3, 4, 5 and 6. Rugora (1) was a pool of water formed as a result of brick moulding while Rugora (2) was a drainage trench for an artesian well (Plate 7). At Sesame, the site was a dam which is used for agricultural and domestic purposes.

The rural population of Gokwe South comprises mainly subsistence farmers who cultivate maize and cotton and keep livestock which include cattle, goats and donkeys. Malaria is highly endemic in the district and occurs all year round due to the prevailing high temperatures although the incidence usually increases shortly after the onset of rains (Mpofu, 1985). The principal malaria vectors in the area belong to the *An. gambiae* complex (Masendu *et al*., 2005).

The study was conducted during the period 20th November 2006 to 1st March 2007 at which time breeding habitats are easier to detect due to the rains. In total, three trips were made to the study sites.

2.3 LARVAL COLLECTIONS

Anopheles larvae were sampled using the standard dipping method (Service, 1976). The larvae were sampled along the margins of the swamps. Standard dippers (350 ml) were dipped over the surface of water or at the edge of the aquatic vegetation to collect the larvae.

Plate 1: The artesian well at Masakadza.

Plate 2: The resultant swamp at Masakadza formed by leakages from the artesian well

Plate 3: The artesian well at Kamhororo

Plate 4: Resultant swamp at Kamhororo with *Typha* species

Plate 5: The artesian well at Gwave

Plate 6: The resultant swamp at Gwave.

Plate 7: The artesian well and the drainage trench at Rugora (2)

In very shallow water, the dipper was pressed firmly down into the bottom mud and debris to allow water to flow into it. The presence of larvae at high or low densities was determined by the number collected per dip (Sattler *et al*., 2005). If larvae were seen without dipping or nearly every dip contained *Anopheles* larvae, the breeding site was said to have a high density. Sites where only one or two dips out of 10 had larvae were described as having a low density. Sites where no larvae could be found in 10 dips were recorded as empty. Sampling consisted of about 20-40 dips depending on the size of the breeding site. The samples were emptied into a flat white pan in order to count the larvae caught. The larval density index was calculated by dividing the total number of larvae by the sampling effort (i.e. the total number of dips). Sampled larvae were taken to the insectary at the University of Zimbabwe and reared to adults for species identification. The larvae were fed once daily on a mixture of dog biscuits and baker's yeast ground to a powder form. On adult emergence, females were preserved in eppendorf tubes with silica gel for species identification using morphological characters outlined by Coetzee (1989).

2.4 CHARACTERIZATION OF *ANOPHELES* BREEDING SITES.

Ecological parameters of the breeding sites of *An. gambiae* which included pH, temperature of the water body, turbidity, salinity, conductivity, total dissolved solids, dissolved oxygen, calcium, nitrates and chlorides were determined on site at the time of larval sampling using a Horiba Water Quality Monitor multi-probe, model w-2030. The breeding sites were also characterized according to the water condition, exposure to sunlight, habitat permanence and type of vegetation. A hand-held GPS unit was used to determine the coordinates and elevation at each of the breeding sites.

2.5 SPECIES IDENTIFICATION

A total of 100 *An. gambiae* complex mosquitoes were identified to species level using morphological characters outlined by Coetzee (1989). Measurements were taken of the pale band at the junction of hind tarsomeres 3 and 4 using a compound microscope (× 100 magnifications) fitted with an eyepiece micrometer. To determine the palpus ratio, the third, fourth and fifth segments of the palps were measured. The key below was used to identify the members of the *An. gambiae* complex according to Coetzee (1989).

Identification Key

1. Pale band at the joint of hind tarsomeres3 and 4, 0.1 mm or more……………..2

   This pale band 0.09 mm or less…………………………………………….…...3

2. Palpus ratio of 0.85 mm or higher……………………………………………*merus*

   This ratio 0.84 mm or lower…………………………………………*quadriannulatus*

3. *Anopheles gambiae*/ *Anopheles arabiensis*

When the pale band was 0.09 mm or less the specimen was identified as *An. arabiensis* since previous studies by Masendu *et al*. (2005) indicated that there are no *An. gambiae s.s*. species in Gokwe South. The relative abundance of each species was expressed as the corresponding percentage of the total number of *Anopheles* identified.

2.6 DATA ANALYSIS

Data were analyzed using variable selection by stepwise regression followed by multiple regressions of significant variables with *Anopheles* larval densities. The statistical package SPSS version 11.0 (for windows) was used for the analysis. Prior to analysis, turbidity data were first normalized by $\log_{10}$ (n+1) transformation. Variable selection by stepwise regressions was employed to determine the effect of pH, conductivity, salinity, total dissolved solids, dissolved oxygen, turbidity, calcium, nitrates and chlorides on *Anopheles* larval density. Variables were then removed if their adjusted partial correlation coefficients were not significant ($P > 0.05$). Multiple regression analysis was used to determine the importance of significant ecological variables for explaining the different mosquito larval densities.

The underlying assumptions of the regression model were that the residuals were distributed normally and that the relationship between larval density and the significant variables was linear. The assumption of normality of the regression model was checked by plotting a scatter plot and a normal probability plot of regression standardized residuals (Appendix 3). Pearson’s coefficients of regression were calculated in order to determine the correlations between the larval density and the ecological variables.

3. RESULTS

3.1 Larval Habitat Diversity and Abundance

Of the six larval breeding habitats that were sampled, anopheline larvae were found at the swamps at Masakadza, Kamhororo and Gwave, the pool at Rugora (1) and the drainage trench at Rugora (2). No evidence of larvae was seen at the dam at Sesame. The highest abundance of larvae was recorded at Masakadza followed by Gwave while the lowest was at Rugora (1) (Table 1). The *Anopheles* larvae were found in habitats that were very muddy and organically polluted by rotting vegetation and animal waste (Plates 8 and 9). The larvae were also found in animal footprints (Plates 9 and 10). Rugora (1), but was later invaded by macrophytes (*Lemna* sp.) (Plates 11) and became unproductive for anopheline larvae.

3.2 *Anopheles* Breeding Site Characteristics

Physicochemical parameters

Anopheles larvae were collected from water bodies which had pH values all in the basic range (Table 2). The breeding sites at Masakadza and Kamhororo were extremely turbid while at Rugora (1) and Rugora (2) the water bodies were slightly turbid. As a result, the water from these sites had to be diluted several times in order to get the turbidity readings. In contrast, the water was clear at Sesame. Water temperatures in all the breeding sites were generally high (above 30^{o}C).

Table 1: Mosquito larval density indices from *Anopheles* breeding habitats in Gokwe South

Breeding Site	Larval Density Index			Density of Larvae
	Minimum	Mean	Maximum	
Rugora (1)	0.00	0.089	0.267	Low
Rugora (2)	0.00	1.95	2.65	High
Masakadza	17.00	22.50	27.50	High
Kamhororo	1.00	3.67	7.50	High
Gwave	3.10	5.36	7.50	High
Sesame	0.00	0.00	0.00	Empty

Plate 8: The breeding site at Rugora (2) polluted with decaying matter.

Plate 9: The breeding site at Masakadza with animal footprints and water polluted with animal and plant waste.

Plate 10: The site at Gwave with animal footprints

Plate 11: *Lemna* species at Rugora (1).

Table 2: Ecological parameters (mean±SD) recorded at different *Anopheles* breeding sites in Gokwe South

Ecological Parameters	Rugora (1)	Rugora (2)	Masakadza	Kamhororo	Gwave	Sesame
pH	7.45±0.56	8.91±0.06	9.27±0.33	9.33±0.24	9.34±0.39	7.61±1.14
Temperature (^{o} C)	29.81±3.70	32.82±2.15	35.49±1.34	34.84±3.57	33.75±3.70	30.55±3.90
Conductivity (S/m)	0.11±0.05	0.74±0.09	1.65±0.71	0.50±0.22	0.21±0.03	24.85±7.42
Salinity (%)	0.07±0.06	0.40±0.00	1.00±0.46	0.30±0.10	0.10±0.00	0.00±0.00
Turbidity (NTU)	2.84±0.00	2.29±0.30	3.25±0.78	2.73±0.83	2.81±0.19	0.00±0.00
Dissolved O_2 (g/l)	0.77±1.15	3.80±0.71	2.10±1.42	3.00±1.51	3.00±1.31	3.00±3.11
TDS (g/l)	0.65±0.32	4.65±0.64	10.43±4.36	3.40±1.37	1.07±0.25	0.15±0.03
Calcium	22.90±3.90	0.39±0.02	0.73±0.33	42.62±39.40	16.56±13.60	1.09±0.56
Nitrates	34.80±16.00	67.80±27.60	67.15±22.41	66.70±26.50	56.9±20.8	38.35±35.1
Chlorides	0.57±0.40	3.43±1.45	0.43±0.18	0.70±0.29	1.03±0.07	87.70±0.00

The abundance of *Anopheles* larvae was positively correlated with the levels of salinity (r = 0.915, $P < 0.05$). Salinity varied significantly among the larval habitats ($F = 20.679$, d.f = 5, $P < 0.05$). The regression model for the relationship between the larval density and the concentrations of total dissolved solids and salinity was represented by the equation, LD = 20.917Sali – 0.791. Salinity accounted for 79.7% of the total variation in the *Anopheles* larval density among breeding sites.

The normal probability plot of regression standardized residuals indicated points lying along a straight line thereby satisfying the assumption of normality of data in the regression model. The scatter plot of larval density versus salinity showed the points clustered about zero and no noticeable systematic pattern thereby indicating that the data was normal (Figure 3). Stepwise regression analysis revealed that larval density was not significantly associated with pH, temperature, conductivity, dissolved oxygen, turbidity, total dissolved solids, calcium, chlorides and nitrates ($P > 0.05$) (Table 3). Pearson's coefficients of regression for the ecological variables are shown in Table 4.

Habitat size and permanence

The breeding habitats of anophelines were extremely extensive. The largest breeding habitat was the swamp at Kamhororo which was the size of four football pitches followed by the swamp at Masakadza which was equivalent to two football pitches. The smallest breeding habitat was the drainage trench at Rugora (2) covering a total area of 75 m^2.

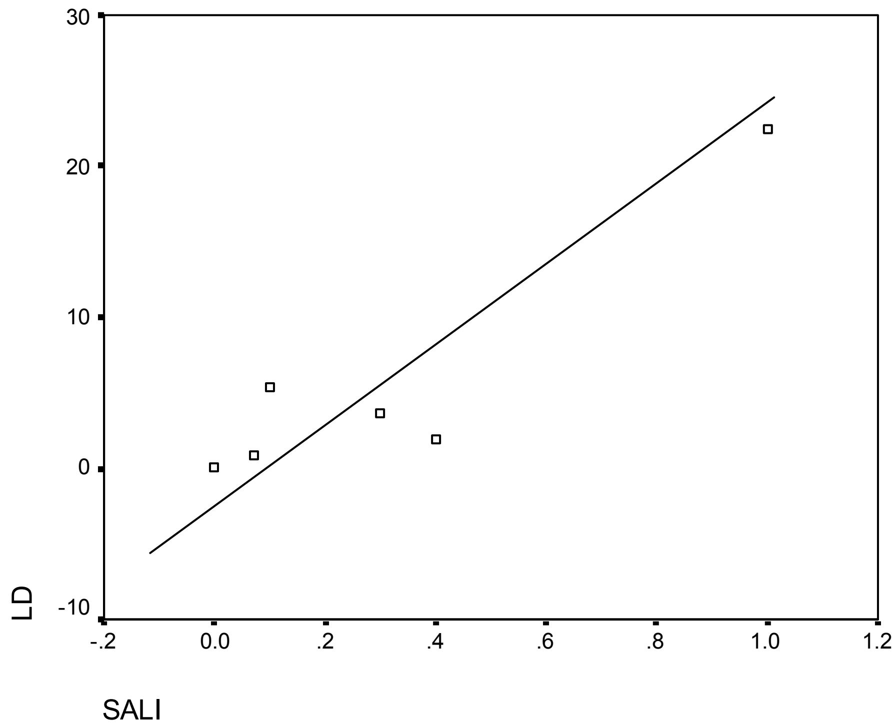

Figure 3: The relationship between larval density (LD) and salinity (SALI)

Table 3: The T and *P*-values of stepwise regression analysis of *Anopheles* larval density and the ecological variables of breeding sites

Ecological parameters	T-values	*P*-values
pH	0.740	0.043*
Temperature	0.260	0.148
Salinity	4.547	0.010*
Turbidity	0.197	0.113
Conductivity	-0.215	0.123
Dissolved oxygen	-0.752	-0.398
TDS	-2.833	-0.853
Calcium	-0.029	-0.017
Nitrates	-0.749	-0.397
Chlorides	0.173	0.099

*indicates statistically significant ecological variables at $P = 0.05$

Table 4: Pearson Correlation Coefficient values determining whether the abundance of larvae was correlated with the ecological variables

Ecological parameters	Pearson's correlation coefficients	Significance
pH	0.526	0.284
Temperature	0.701	0.120
Conductivity	-0.285	0.584
Salinity	0.915	0.010*
Turbidity	0.513	0.298
TDS	0.896	0.016*
Dissolved oxygen	-0.165	0.755
Calcium	-0.280	0.591
Nitrate	0.509	0.303
Chloride	-3.440	0.504

*indicates statistically significant ecological variables at $P = 0.05$

The swamps at Masakadza, Kamhororo and Gwave were perennial or permanent *An. gambiae* breeding habitats and exist all year round. The dam at Sesame was also perennial. The pool at Rugora (1) and the drainage trench at Rugora (2) were semi-permanent breeding habitats which appear just at the onset of the rainy season. Habitat permanence was determined by preliminary sampling that had been conducted.

Exposure to sunlight and water condition

The breeding sites at Rugora (2), Masakadza, Kamhororo, Gwave and Sesame were completely exposed to sunlight (Table 5). However, Rugora (1) was partially shaded due to the surrounding trees. The water in the breeding sites was extremely polluted with organic matter. At Rugora (2), Masakadza, Kamhororo and Gwave the water in the breeding habitats was polluted with organic matter as indicated by extensive algal growth, decaying plant and animal waste and very dirty. At Sesame, on the other hand, the water was relatively clean and uncontaminated. In general, the water in all the breeding sites was stagnant due to the poorly drained soil strata.

Vegetation

The swamps at Masakadza, Kamhororo and Gwave were populated by the reed *Typha* sp. (Plate 4). The pool surface at Rugora (1) was entirely covered by *Lemna* species and the water muddy and dirty. The drainage trench at Rugora (2) had grass growing in it which caused water stagnation due to the reduced water flow. At Sesame, there was no vegetation growing in the breeding site except on the edges of the dam.

Table 5: Physical characteristics of *Anopheles* breeding and non-breeding sites in Gokwe South

Site	Altitude (m)	Depth (cm)	Shade	Habitat permanence	Vegetation cover
Rugora (1)	737	15	Partially shaded	Semi- permanent	Floating, *Lemna* sp
Rugora (2)	738	5	Sunlit	Semi-permanent	Submerged grass
Masakadza	769	7	Sunlit	Permanent	Emergent, *Typha* sp
Kamhororo	772	1	Sunlit	Permanent	Emergent, *Typha* sp.
Gwave	803	10	Sunlit	Permanent	Emergent, *Typha* sp.
Sesame	1179	15	Sunlit	Permanent	Vegetation on edges

Table 6: *Anopheles* species abundance in Gokwe South

LOCALITY	SPECIES ABUNDANCE (%)			SAMPLE SIZE (N)
	An. arabiensis	*An. merus*	*An. quadriannulatus*	
Rugora	86	0	14	100
Masakadza	28	72	0	100
Gwave	100	0	0	100
Kamhororo	90	0	10	100

4. DISCUSSION

Anopheles larval breeding occurred in small habitats such as footprints on the margins of the water bodies. The habitats were often numerous, exposed to sunlight, very turbid and close to human households. This was in line with the findings by Mutuku *et al.* (2006) that the principal vectors of malaria in tropical Africa inhabit small water bodies that are often numerous, scattered, sunlit, temporary and close to human dwellings. Muirhead-Thomson (1951) observed that *An. gambiae* breeding is mainly confined to fairly clean water and larvae are seldom found in those heavily polluted habitats. However, contrary to conventional thinking that the *An. gambiae* complex breeds in rather clean and clear water, larvae sampled in the current study were found in habitats that were very muddy and organically polluted by rotting vegetation and animal waste. A study by Sattler *et al.* (2005) also found *An. gambiae* breeding in a swamp extremely polluted with organic matter.

Turbidity in the breeding habitats was overwhelming as it could not be read by the Horiba multi-probe until after several dilutions. The most turbid habitat was found at Kamhororo. Gimning *et al.* (2001) also found *An. gambiae* larvae in extremely turbid breeding sites. The presence of anopheline larvae in habitats with turbid water is an indication that physical quality of water may not play a significant role in the propagation of larvae. The water in all the breeding sites was alkaline in nature. Members of the *An. gambiae* complex have a tendency to occur in alkaline waters (Sueur and Sharp, 1988). The water temperatures in the breeding habitats were generally high, above 30^{o}C, in all the habitats thus promoting development of the anophelines. Water temperature of breeding sites has a great influence on the development of immature anophelines. Increase in temperature is accompanied by increase in the rate of development (Muirhead-Thomson, 1951). Conversely, low temperatures prolong the development of larvae.

In Gokwe South, the highest densities of anopheline larvae were found in swamps. The highest larval density index was found at Masakadza. The importance of swamps as *Anopheles* habitats and their consequent role in malaria transmission has been noted by various researchers (Minakawa *et al*., 2004; Mutuku *et al*., 2006). Swamps increase the availability of conducive habitats for the malaria vector. The size of the breeding sites in Gokwe South calls for immediate urgent larval control measures in order to stop malaria transmission.

There was an invasion of the macrophyte *Lemna* species at Rugora (1) that formed a mat on the entire surface of the rock pool. The species grow on quiet and sluggishly moving waters of ponds, pools or swamps and may form a mat on the surface of the water and shade out aquatic organisms (Tarver *et al*., 1986). The macrophytes become dominant and significantly reduce the presence of *Anopheles* larvae. Some plants like the *Lemna species* act by covering the entire water surface so thickly they prevent respiration of the larvae (Bates, 1949). These plants colonize calm or slow moving water provided salt concentrations are not excessive. The swamps at Masakadza, Kamhororo and Gwave were populated with *Typha* species. It is a genus of rhizomatous perennial macrophytes that occur in swamps (Asaeda *et al*., 2005). Depending on the *Typha* species, the plants may reach a height of 2½ m above the surface of the water. These emergent aquatic plants can restrict and almost totally block water flow. The reduced flow results in water stagnation suitable for mosquito breeding.

Anopheline larval density was influenced by the levels of salinity. High densities of larvae were found in habitats that had high concentrations of salinity. These sites included Masakadza, Kamhororo and Gwave. Salinity was highly positively correlated with total dissolved solids. In Gokwe, the primary sources of high concentrations of total dissolved solids in water bodies are agricultural runoffs and leaching of soil contamination. Salinity is a measure of all salts dissolved in water whereas total dissolved solids are the combined content of all organic and inorganic substances in water. Therefore, an increase in total dissolved solids causes a rise in salinity concentrations. Temperature, pH, total dissolved solids, conductivity, dissolved oxygen, turbidity, calcium, chlorides and nitrates did not have any influence on the anopheline larval density.

In Gokwe South, *An. arabiensis* was the most extensively distributed member of the *An. gambiae* complex and occurred in a wide range of habitats. Gokwe district is a low rainfall area (400-1200 mm per annum). Under such areas of low rainfall, *An. arabiensis* is likely to predominate (Coetzee *et al*., 2000; Wondji *et al*., 2005). *Anopheles merus* was the most predominant at Masakadza which had recorded the highest salinity level of 1%. *Anopheles merus* is a salt water breeder and its larvae develop in brackish water. *Anopheles quadriannulatus* often found in association with *An. arabiensis* occurred in low densities.

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

In Gokwe South, members of the *An. gambiae* complex were found breeding primarily in small, temporary, sunlit habitats which were extremely turbid and organically polluted with rotting vegetation and animal waste. The extensive swamps in Gokwe South play a major role in malaria transmission since they provide breeding sites for large densities of malaria vectors. Managing these larval habitats throughout the year would have an impact on mosquito abundance and therefore reduce the risk of malaria transmission. The abundance of anopheline larvae was closely associated with the concentrations of salinity. High densities of larvae were found in habitats with high levels of salinity. *Anopheles* larval densities were not influenced by pH, conductivity, total dissolved solids, turbidity, temperature, dissolved oxygen, calcium, nitrates and chlorides. *Anopheles arabiensis* was the most abundant and widespread species while *An. merus* predominated at Masakadza where there was a high concentration of salinity. In contrast, *An. quadriannulatus* was found in low densities in association with *An. arabiensis.*

5.1 RECOMMENDATIONS

1. There is need to develop a model in Gokwe South to determine the relationship between *An. gambiae* complex densities, incidences of malaria and ecological factors. The model can be used for forecasting, prediction and early detection and warning for malaria epidemic management.
2. Environmental factors should be coupled with clinical malaria data, so that it may be possible to identify populations or households or areas that carry the heaviest burden of

malaria or are the most important potential contributors to malaria transmission. Consequently, the impact of malaria control efforts can be maximised by implementing control measures to selected areas.

3. The swamps in Gokwe South play a major role in malaria transmission, therefore, the size of these water bodies calls for immediate urgent larval control measures in order to stop malaria transmission. Larval control should be implemented by source reduction which may involve ditching to enhance drainage, thus, eliminating breeding sites and interrupting mosquito life cycles.
4. Larvicide operations should be implemented in the extensive swamps by applying natural or formulated agents to control mosquito larvae and pupae.
5. There is need for mechanical removal of the *Typha* species in the swamps at Kamhororo, Gwave and Masakadza as the grass reduces the flow rate of water creating a suitable environment for *Anopheles* larvae. This can reduce the larval densities remarkably as the amount of standing water is reduced.
6. Biological control of mosquito larvae by use of environmentally friendly and powerful microbicides such as *Bacillus thuringiensis* and *B. sphaericus* should be implemented in order to reduce malaria transmission.

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APPENDICES

Appendix 1: An SPSS output of stepwise regression analysis between *Anopheles* larval density and the independent variables pH, temperature, TDS, salinity, turbidity, dissolved oxygen calcium, chloride, nitrate and conductivity.

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-2.001	1.829		-1.094	.316
	SALI	20.522	4.149	.896	4.946	.003

a. Dependent Variable: LD

Excluded Variables[b]

Model		Beta In	t	Sig.	Partial Correlation	Collinearity Statistics
						Tolerance
1	TEMP	-.071[a]	-.270	.798	-.120	.554
	TURB	-.095[a]	-.437	.680	-.192	.809
	PH	-.078[a]	-.331	.754	-.146	.697
	DO	-.119[a]	-.625	.560	-.269	.999
	TDS	-5.234[a]	-2.152	.084	-.693	3.458E-03
	CAL	-.018[a]	-.084	.936	-.037	.866
	CHL	.119[a]	.568	.595	.246	.837
	NITR	-.252[a]	-.999	.364	-.408	.516
	COND	.126[a]	.619	.563	.267	.878

a. Predictors in the Model: (Constant), SALI

b. Dependent Variable: LD

Appendix 2: An SPSS output of the regression analysis of *Anopheles* larval density versus salinity.

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	SALI[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: LD

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.915[a]	.838	.797	3.79892

a. Predictors: (Constant), SALI

b. Dependent Variable: LD

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	298.433	1	298.433	20.679	.010[a]
	Residual	57.727	4	14.432		
	Total	356.160	5			

a. Predictors: (Constant), SALI

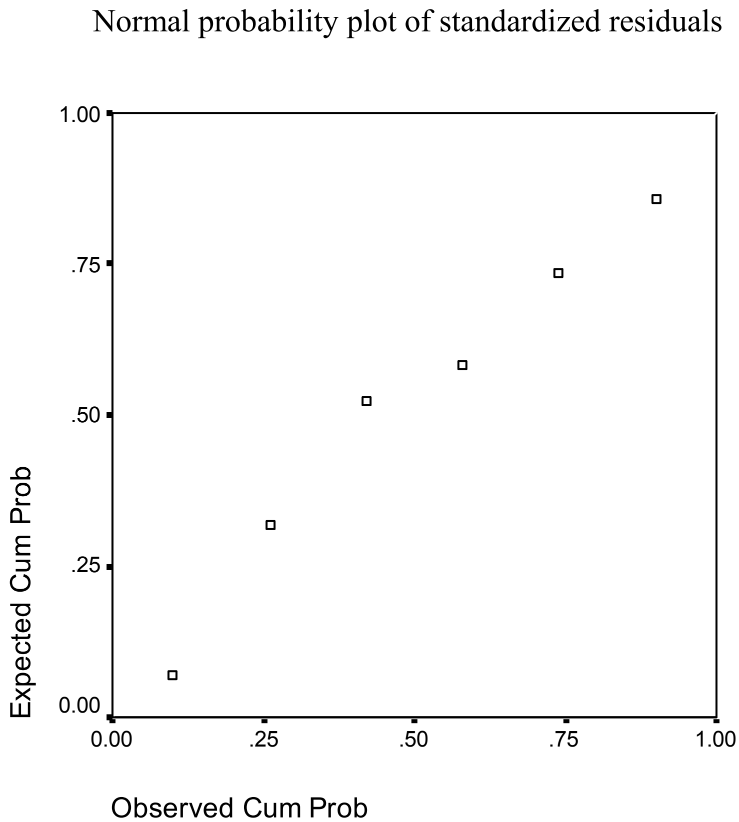
b. Dependent Variable: LD

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-.791	2.112		-.374	.727
	SALI	20.917	4.600	.915	4.547	.010

a. Dependent Variable: LD

Appendix 3: An SPSS output of a normal probability plot of regression standardized residuals with larval density as the dependable variable.



Appendix 4: An SPSS output of Pearson's Correlation Coefficients of *Anopheles* larval density and ecological parameters.

	LD	**TEMP**	**TURB**	**PH**	**TDS**	**DO**	**CHLO**	**CALC**	**COND**	**NITR**	**SALI**
LD	1	.701 .120	.513 .298	.526 .284	.896 .016	-.165 .755	-.344 .504	-.280 .591	-.285 .584	.509 .303	.915 .010
TEMP	.701 .120	1	.555 .253	.952 .003	.729 .100	.369 .471	-.501 .312	.140 .791	-.464 .354	.905 .013	.721 .106
TURB	.513 .298	.555 .253	1	.575 .233	.505 .307	-.327 .527	-.972 .001	.359 .485	-.957 .003	.505 .307	.518 .292
PH	.526 .284	.952 .003	.575 .233	1	.573 .235	.498 .314	-.576 .232	.163 .757	-.556 .252	.925 .008	.559 .249
TDS	.896 .016	.729 .100	.505 .307	.573 .235	1	.039 .942	-.412 .417	-.302 .560	-.360 .483	.710 .114	.999 .000
DO	-.165 .755	.369 .471	-.327 .527	.498 .314	.039 .942	1	.202 .701	-.210 .689	.188 .721	.569 .239	-.005 .993
CHL	-.344 .504	-.501 .312	-.972 .001	-.576 .232	-.412 .417	.202 .701	1	-.388 .447	.998 .000	-.540 .268	-.415 .413
CAL	-.280 .591	.140 .791	.359 .485	.163 .757	-.302 .560	-.210 .689	-.388 .447	1	-.402 .429	.030 .954	-.299 .565
COND	-.285 .584	-.464 .354	-.957 .003	-.556 .252	-.360 .483	.188 .721	.998 .000	-.402 .429	1	-.520 .291	-.362 .481
NITR	.509 .303	.905 .013	.505 .307	.925 .008	.710 .114	.569 .239	-.540 .268	.030 .954	-.520 .291	1	.683 .135
SALI	.915 .010	.721 .106	.518 .292	.559 .249	.999 .000	-.005 .993	-.415 .413	-.299 .565	-.362 .481	.683 .135	1

N = 6