The Abundance and Biting Behaviour of *Anopheles merus* (Dönitz) in Gokwe South District, Zimbabwe

By

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DEDICATION

I dedicate this work to my wife Enerth, mum and my younger brother Daniel. Thanks for the support and encouragement during the two-year period I was away from home.

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ABSTRACT

Malaria is a major public health problem in most sub-Saharan countries. *Anopheles merus* (Dönitz), a saltwater breeding member of *An. gambiae* complex, is involved in low rate malaria transmission in this region. In this study, female anopheline mosquitoes were collected at Masakadza in Gokwe South District to determine the biting cycle of *An. gambiae* complex species and the relative abundance of malaria vectors in this area for effective vector sampling and control. Three adult mosquito collection techniques were employed: window trap, knockdown and human-baited tent trap. Peak biting activity by *An. gambiae* complex species and *An. merus* during the three months of sampling showed seasonal variations in the biting cycle of these species at Masakadza. The peak of biting activity for *An. gambiae* complex species occurred at 2200 hours in November 2006, 0300 hours in January 2007, and 2200 hours in March whereas the peak of biting activity for *An. merus* occurred at 2200 hours in November 2006, midnight in January and March 2007. *Anopheles merus* was found to be the predominant species accounting for 70% of total collections based on morphological identification. The study also revealed that the resting behaviour of *An. gambiae* complex species in this area is mainly exophilic with mean hut densities of 0.2 mosquitoes per hut. It is suggested that more insecticide-treated bed nets be used in this area for effective control of malaria vectors and further studies be conducted to determine the role of *An. merus* in malaria transmission.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

1.0 INTRODUCTION

1.1 Background

Of all insects known to man, the mosquito is unquestionably the one which causes most illness, economic loss and discomfort (Snow, 1974). Several mosquitoes belonging to the genera *Anopheles*, *Culex* and *Aedes* are vectors for pathogens of various diseases such as malaria, filariasis, yellow fever, dengue, Japanese encephalitis (JE) and haemorrhagic fever (Ganguly, 2003). Even when mosquitoes do not disseminate disease, they may cause great annoyance and make areas otherwise suitable for human and animal occupation quite uninhabitable. There are some 110 genera and subgenera and about 2426 species of mosquitoes known throughout the world (Goma, 1966).

Malaria is one of the most prevalent diseases in the tropics and sub-Saharan Africa. In Afrotropical region, the *Anopheles gambiae* Giles complex and *An. funestus* group are the major malaria vectors. The *An. gambiae* complex comprises six named and one unnamed species (Coetzee, 2004). Before 1962, *An. gambiae* was considered to be a single biologically, variable species (Coetzee *et al*., 2000). However, reports on large variations in the larval habitat and adult female resting behaviour and feeding preferences among this species were revealed. By 1964, five species had been recognized, with Paterson (1964) presenting evidence that the three freshwater-breeding species did not mate in nature. Evidence of a sixth and seventh species came later from Uganda (Davidson and Hunt, 1973) and Ethiopia (Hunt *et al*., 1998), respectively.

The *An. funestus* group consists of nine species with slight morphological differences. Out of the nine, only two have been incriminated as effective malaria vectors (Braginets *et al*., 2003). These two are often considered as vector species that bridge malaria transmission during the dry season (Mbogo *et al*., 2003). However, vectorial capacity of *An. funestus* can often exceed that of *An. gambiae* in some localities (Braginets *et al*., 2003).

Malaria is an acute and often chronic disease which commonly begins with a brief and indefinite illness, shortly followed by a characteristic shaking chill with rapidly rising temperature, usually accompanied by headache and nausea and ending in profuse sweating (Goma, 1966). The disease constitutes one of the leading causes of mortality and morbidity, particularly among infants. Worldwide, mosquitoes transmit diseases to more than seven million people annually and are responsible for the deaths of 1 in every 17 people (Taubes, 1977). Of these deaths, 90 to 95 per cent are estimated to be in Africa (Wilson, 1991). The disease afflicts pregnant women, young children and migratory populations particularly because of their low or non-existent immunity to the disease.

Malaria is caused by protozoans of the genus *Plasmodium*. Members of this genus occur in reptiles, birds and mammals only, with man being a susceptible host to a number of species (Snow, 1974). Four species of the genus produce clinical symptoms and these include *P. vivax, P. malariae, P. ovale* and *P. falciparum. Plasmodium falciparum* is the most virulent of the malarial parasites with febrile attacks every two days which are less well defined than those of the other human malarial diseases (Snow, 1974). Of the four species, *P. faciparum* and *P. vivax* account for more than 95 per cent of the cases of malaria in the world. *Plasmodium malariae* has an even distribution worldwide while *P. ovale* is found practically only in Africa, particularly West Africa (Pampana, 1969).

Several attempts have been made to eradicate malaria. Malaria eradication means the ending of transmission of malaria and the elimination of the reservoir of infective cases, in a campaign limited in time and carried to such a degree of perfection that, when it comes to an end, there is no resumption of transmission (Pampana, 1969). However, these eradication programmes involve heavy expenditure that developing countries cannot afford. As a result, instead of eradication, most developing countries opt for malaria control programmes. Malaria control means the reduction of the disease to a prevalence where it is no longer a major public health problem and carries the implication that the programme will never end, control having to be maintained by continuous active work (Pampana, 1969).

Malaria control involves larval and adult population reduction of vector mosquitoes. One of the frequently used methods in adult mosquito control in developing countries is the use of insecticides. The insecticides mostly used are DDT, gammexane and dieldrin (Goma, 1966). There are two general methods in which insecticides are used: (a) application of insecticidal residues to surfaces on which adult mosquitoes will come to rest, and (b) direct application of insecticide to the mosquito by means of space sprays, mists, aerosols or dusts (Goma, 1966). However, two principal problems are encountered in mosquito control programmes using residual house-spraying and these are: (i) resistance development to insecticides such as DDT, and (ii) the sorption of insecticides by certain wall materials (Goma, 1966). Apart from resistance of malaria vectors to insecticides, malaria control in sub-Saharan Africa is problematic due to other factors such as the diversity of the parasite that infects humans, gross inadequacy of human and financial resources devoted to malaria control and general decline in GNP/GDP that often results in decrease in health and other social services (Pampana, 1969).

1.2 Malaria in Zimbabwe

Reports on malaria in Zimbabwe invariably attribute the transmission of the parasite to *An. arabiensis* (Crees and Mhlanga, 1995; Taylor and Mutambu, 1986; Masendu, 2004). The distribution of malaria in Zimbabwe is not uniform. The epidemiology of the disease is determined by the country's physio-geography with low altitude and associated high temperatures being the main determining factor (Taylor, 1985 - cited by Masendu, 2004). The areas that lie below 1200 m above sea level are prone to malaria in Zimbabwe (Masendu, 2004). Out of an estimated population of 12 million, 50% are at risk of malaria (Masendu, 2004). Fifty- five percent of the people in Zimbabwe reside in malaria endemic areas and the government spends up to U$ 2 million annually through the National Malaria Control Program (NMCP) (Vundule and Mharakurwa, 1996). By introducing blanket spraying of houses using DDT in the early 1980s, the National Malaria Control Program achieved a significant reduction of the populations of strongly endophilic vectors *An. gambiae s.s*. and *An. funestus* (Masendu, 2004).

1.3 Malaria Vectors

Mosquitoes of the *An. gambiae* complex have been recognized as including some of the most efficient vectors of malaria in the Afrotropical Region (White, 1974). This complex consists of *An. gambiae sensu stricto* Giles, *An. arabiensis* Patton, *An. merus* Dönitz*, An*. *melas* Theobald, *An*. *bwambae* White*, An*. *quadriannulatus* Theobald species A and B (Hargreaves *et al*., 2003). *Anopheles gambiae s.s*. and *An. arabiensis* are the main malaria vectors in tropical Africa.

The *An. funestus* group consists of nine sibling species, of which *An. funestus* and *An. rivulorum* are the only ones that have been incriminated as malaria vectors (Braginets *et al.*, 2003). In Tanzania, *An. rivulorum* has been implicated as a vector (Wilkes *et al*., 1996; Masendu, 2004). However, *An. gambiae* s.s., *An*. *arabiensis* and *An*. *funestus* are the primary vectors of malaria in sub-Saharan Africa (Gimning *et al*., 2001).

Previous studies indicate that *An. arabiensis* is the main vector in the Southern Africa region including Zimbabwe (Mahon *et al*., 1976; Mpofu, 1985; Masendu, 2004) but few studies have been carried out to quantify its transmission capacity in areas where other species of the *An. gambiae* complex are predominant. Previous studies by Masendu (2004) showed that *An. merus* is predominant at Masakadza, Gokwe District (Midlands province), accounting for 89.6 and 72.3 per cent of human landing collections indoors and outdoors, respectively. The corresponding proportions for *An. arabiensis* were 10.4 indoors and 25.5 per cent outdoors.

1.3.1 *Anopheles gambiae sensu stricto*

Distribution records of *An.gambiae s.s*. indicate that the species is more common in West and East Africa while its distribution in Southern Africa is patchy (Masendu, 2004). It is often localized in areas with closed canopies. *Anopheles gambiae* breeds in a great variety of shallow open sunlit pools. Such pools come into being in a variety of ways and may range from borrow-pits, drains, brick-pits, ruts, car-tracks, hoof prints, round ponds and water holes (Gilles and De Meillon, 1968). Although *An. gambiae* thrives under rather cool conditions, it is tolerant of relatively high temperatures. In general terms, seasonal changes in *An. gambiae* populations tend to follow the seasonal pattern of rainfall. Thus, in savanna zones with a single rainy season per year, numbers start to rise explosively soon after the first main falls,

reaching a peak in the middle of the rains and declining steadily thereafter as the levels of water become stabilized and vegetation and predators become established (Gilles and De Meillon, 1968).

Anopheles gambiae s.s. is regarded as the most efficient malaria vector in sub-Saharan Africa, particularly in West Africa (Della Torre *et al*., 2002). Before the introduction of blanket insecticide spraying in Zimbabwe, the species was found in several localities (Mahon *et al*., 1976; Masendu, 2004). Female *An*. *gambiae s.s.* are highly anthropophilic, feeding preferentially on humans (White, 1974; Coluzzi *et al*., 1979), although in West Africa they are less discriminating and will feed readily on other animals like horses and cattle (Diatta *et al*., 1998; Bøgh *et al*., 2001). The endophilic and anthropophagic behaviours of *An. gambiae s.s* create an intimate association between human reservoirs and the insect vectors of malaria. It has also been shown that *An. gambiae s.s.* is much more catholic in its feeding habits. Savanna populations, especially East and South-Eastern Africa tend to show higher levels of zoophily (Gilles and De Meillon, 1968).

1.3.2 *Anopheles arabiensis*

Anopheles arabiensis has a wide distribution in Africa, ranging from Madagascar in the east to Senegal in the west (Coetzee *et al*., 2000). The range and relative abundance of *An. arabiensis* tend to be influenced by climatic factors, especially total annual precipitation (Lindsay *et al*., 1998). The species preferably breeds in fresh temporary sunlit or rain water pools.

Anopheles arabiensis is more tolerant of higher temperatures and is able to survive in drier conditions. This explains why it is found biting in dry seasons (Petrarca *et al*., 2000). *Anopheles arabiensis* has a number of strategies which allow it to persist in arid conditions. Adult females tend to lay their eggs on damp surfaces, rather than water, with hatching being delayed in a proportion of eggs (Lindsay *et al*., 1998), and females aestivate during periods of prolonged dryness (Omer and Cloudsley-Thomson, 1970).

Anopheles arabiensis has a more opportunistic feeding behaviour although it can be entirely zoophilic, as recent studies from Madagascar have shown (Duchemin *et al*., 2001). The species also tends to be more exophagic and exophilic. The variable behaviour of *An. arabiensis* females, being anthropophilic and zoophilic as well as endophilic and exophilic, makes them incompletely vulnerable to house-spraying (White, 1974). Seasonal abundance of *An*. *arabiensis* with peaks following the onset of rains makes it largely responsible for malaria transmission in Southern Africa (Hargreaves *et al*., 2003). *Anopheles arabiensis* is regarded as the main malaria vector in Zimbabwe (Crees and Mhlanga, 1995; Mpofu, 1985). This species has also been described as the main malaria vector in Namibia, Botswana, Southern Mozambique and South Africa (Masendu, 2004).

1.3.3 *Anopheles quadriannulatus*

The two zoophilic members of the *An*. *gambiae* complex, *An. quadriannulatus* species A and B, have a limited distribution associated perhaps with more subtropical climates than the other members of the complex (Hunt *et al*., 1998). Species A represents *An*. *quadriannulatus* s.s., widespread in Southern Africa (Hunt *et al*., 1998), whereas *An*. *quadriannulatus* species B occurs in Ethiopia (Fettene *et al*., 2002). In general, *An*. *quadriannulatus* is a cattle-feeding,

outdoor-resting member of the *An. gambiae* complex and is not known to transmit malarial parasites (Coetzee, 1989). Both *An. quadriannulatus* species A and B are freshwater species and are adapted to lower developmental temperatures.

1.3.4 *Anopheles merus*

Anopheles merus is a saltwater breeding member of the *An. gambiae* complex which has been shown to be involved in low rate malaria transmission (Sharp, 1983). Its distribution is limited to the East coast of Africa, as well as the adjacent inland areas and coastal islands (Sharp, 1983). Larvae of *An. merus* are found in coastal Avicennia mangrove swamps, saltpans, brackish ponds (Gilles and De Meillon, 1968) or mineral springs (Coetzee and Le Sueur, 1988). In the absence of domestic animals, *An. merus* bites man readily both indoors and outdoors.

Gilles and De Meillon (1968) observed that in the presence of other hosts *An. merus* is more attracted to cattle. Although both Mahon *et al.* (1976) and Masendu (1996) documented the occurrence of *An. merus* in several localities in Zimbabwe, its biting and resting behaviour has not been investigated locally.

1.3.5 *Anopheles melas*

Anopheles melas is the West Coast salt-water member of the *An. gambiae* complex. It occurs in patches of salt grass in tidal swamps and in pools, ponds or lagoons flooded by spring tides, and also within the mangrove belt in Avicennia orchards (Gilles and De Meillon, 1968). In East Africa, *An. melas* is only found in the coastal areas, with the exception of Mozambique,

where it is also found far inland along the Zambezi and Save river systems (Coetzee *et al*., 2000). Even in the presence of other domestic animals, *An*. *melas* strongly attacks man and appears to feed as readily indoors as outdoors (Gilles and De Meillon, 1968). *Anopheles melas* is an important vector of malaria at many points along the West African coast. It is also capable of transmitting brancoftian filariasis (Gilles and De Meillon, 1968).

1.3.6 *Anopheles funestus*

Anopheles funestus generally exhibits patchy distribution patterns because its larvae require long-standing aquatic habitats, such as swamps. Since these habitats are limited to lower valleys, members of the group are generally discontinuous in their distribution (Braginets *et al*., 2003). The species is widespread and locally abundant over almost the whole of Africa wherever there is sufficient permanent water and residual insecticides have not been intensively used (Gilles and De Meillon, 1968).

Anopheles funestus is one of the most anthropophilic mosquitoes known, attacking man in many areas even in the presence of abundant alternative hosts such as sheep and cattle (Gilles and De Meillon, 1968). However, because it is strongly endophilic, *An. funestus* is most vulnerable to attack with residual insecticide sprays (Gilles and De Meillon, 1968).

1.4 Vector Species Identification

Species identification of members of the *An. gambiae* complex is vital to the efficient management of malaria vector control programs in Africa (Sharp *et al*., 1989). The identification brings the associated knowledge of the biology of that species which, in turn,

dictates appropriate control measures (Coetzee, 2004). Thus the development of reliable species identification tools and an understanding of population structure play an important role in development of vector control strategies (Collins *et al*., 2000). One of the common identification methods used today is chromosomal identification. In this technique, banding sequences on the giant polytene chromosomes show specific differences between the species (Hunt, 1972 cited by Coetzee, 1989). Another commonly used identification method is electrophoresis. In this technique, a mixture of proteins from a mosquito is separated by differential mobility in a magnetic field. This technique can be used for the analysis of many different kinds of proteins and is especially useful for studying enzymes. The results obtained are more or less qualitative since bands are seen to be present or absent (Rarr, 1974). The biochemical key for identification by electrophoresis is adequate when studying large populations.

When the identity of individuals is required, the electrophoresis method needs to be correlated with chromosomal identification (Coetzee, 1989). The other modern and effective technique currently used is the Polymerase Chain Reaction (PCR) technique. The technique has a 99.68 per cent probability of correct identification (Masendu, 2004). The PCR technique amplifies the number of copies of a specific region of DNA to produce enough DNA to be adequately tested (Brown, 2001).

In general, chromosomal, electrophoresis and PCR identification techniques require a high level of expertise and sophisticated laboratory equipment. Even though not very accurate, the least complex method for species identification is the use of morphological characters. This technique is quick and can be carried out in the field with minimum of equipment (Coetzee, 1989). In a comprehensive morphological study of the *An. gambiae* complex, Coetzee (1989)

revealed some characters that can be used to separate salt-water breeders from the fresh water breeders. In the current study, morphological characters were employed to identify *An. gambiae* complex species that co-exist at Masakadza.

2.0 RESEARCH PROBLEM AND JUSTIFICATION

Anopheles merus is involved in malaria transmission (Gilles and De Meillon, 1968; Sharp, 1983), which is a major public health problem in most Sub-Saharan countries including Zimbabwe. Complexes containing morphologically cryptic species that vary in their behaviour and vectorial capacity present a real problem in malaria control programmes. *An. gambiae* complex consist of the most effective malaria vectors (White, 1974). Correct identification of malaria vectors in specific areas is vital when carrying out studies on indoor resting and host-seeking behaviour (Coetzee *et al*., 2000). This study was aimed at establishing *An. gambiae* complex species that co-exist at Masakadza based on their morphological characters to determine their resting and biting pattern. Previous studies by Masendu (2004) showed that *An. merus* is predominant at Masakadza, Gokwe District (Midland province). Knowledge of the resting and biting behaviour of this species in this area can limit wastage of scarce resources on controlling species that do not transmit malaria parasite. Basing on the resting behaviour the most effective control strategy will be recommended for this area. The biting pattern and host-seeking behaviour of the species would reveal the most effective sampling time and techniques and thus save time and resources.

Masakadza is prone to malaria and falls within the annual house-spraying program for malaria vector. In order to assess the impact of the control measures currently being implemented in this area, it is necessary that the relative abundance of *An. merus* be determined.

3.0 OBJECTIVES

3.1 General Objective

The aim of this study was to investigate the abundance and biting behaviour of *An. merus* at Masakadza in Gokwe South district and recommend appropriate vector control strategies.

3.2 Specific Objectives

- To determine the abundance of malaria vectors occurring at Masakadza.
- To determine *An. gambiae* complex and *An. merus* biting cycles.
- To study the resting and house-leaving behaviour of *An. gambiae* complex.

4.0 MATERIALS AND METHODS

4.1 Study site

The study was conducted at Masakadza (28^{o} 36′ E 17^{o} 49′ S) in Gokwe South district (Midlands Province) (Figure 1). Adult mosquito collections were conducted in November, 2006 and in January and March, 2007. The study area falls within the annual house-spraying program for Malaria Vector Control. The study area falls under Natural ecological Region IV, which is characterized by low rainfall (450-650 mm per annum), with only extensive farming being the most appropriate (Masendu, 2004).

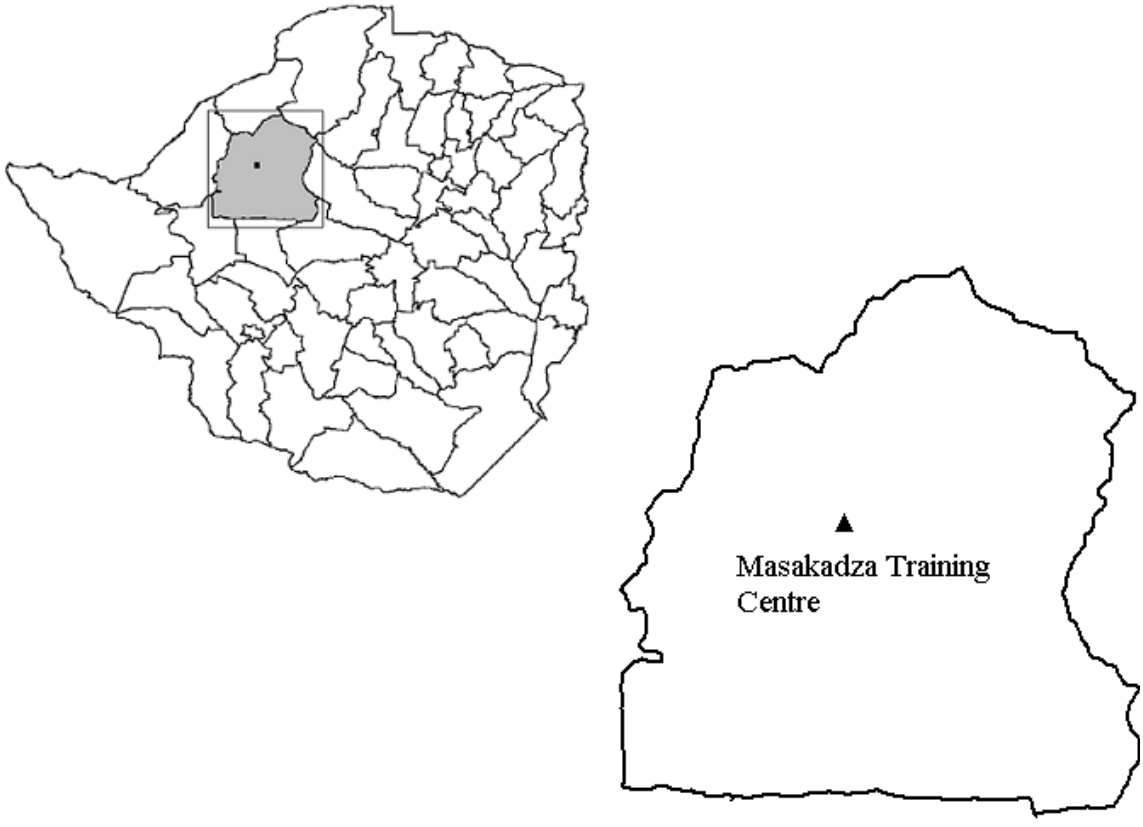

Plate 1. Detailed map of Gokwe District (insert) showing the relative location of the study site.

At Masakadza there is an artesian well (Plate 1), which was drilled during the construction of the main road linking Gokwe, town and the Sengwa coal mine. The artesian well discharges salty water which is unsuitable for gardening or fish farming. This water accumulates around wells forming perennial swamps (Plate 2) and thus creating a breeding ground for mosquitoes. The salinity of the water increases down stream of the swampy area from the source (Masendu, 2004).

4.2 Materials

During the study, the following materials were used: human-tent trap, torches and batteries, sucking tubes, plastic containers, cotton wool, eppendorf tubes, silica gel, forceps, window trap, calico cloth sheets, insecticide aerosols (Baygon and Doom Super), eyepiece micrometer and a microscope, specimens, cover slips, glass slides, nail vanish, recording sheets and pen, storage boxes for mounted specimens and a scissors.

4.3 Research Methods

The study was aimed at determining adult biting and resting behaviour of *An. merus*. Three collecting techniques were employed, namely: window trap, knockdown and human-baited tent.

Plate 1: The artesian well at Masakadza

Plate 2: Perennial swamps at Masakadza.

4.3.1 Knockdown technique

Daytime resting mosquitoes were collected using the pyrethrum knockdown method (spray sheet collection as described by Rishikesh (1966). A total of 20 houses were sprayed during the field trips: five in November 2006, five in January 2007 and 10 in March, 2007. The knockdown method involved the laying of calico cloth on the floor and on surfaces of immovable household furniture. The houses were sprayed using the following aerosol insecticides: DOOM SUPER (0.138% d-tetramethrin, 0.092% d-phenothrin, 0.040% prallethrin and 99.730% inert ingredients) or BAYGON (1.0% propoxum, 0.1% imiprothrin and 98.9% propellant/solvent).

After 10 minutes, the sheets of calico cloth were removed and inspected outdoors for knocked down mosquitoes (Plate 3). This technique was conducted in the morning hours since it has been observed that there are about 30% more endophilic mosquitoes in the morning than in the afternoon (Masendu, 2004). The female anopheline mosquitoes collected were classified by abdominal condition (WHO, 2002). Knockdown space spraying with pyrethrum is now used as a standard, quick and easy method of catching mosquitoes resting in huts and animal shelters (Service, 1976). The collections from knockdown technique were used to determine the mean indoor resting densities of *An*. *gambiae complex* species.

4.3.2 Human-baited tent trap collections

Adult mosquitoes were collected from resting surface of the tent trap (Mpofu and Masendu, 1986) with mouth-operated glass aspirators (sucking tubes) with the help of battery-operated torches (Service, 1976). All night landing catches were carried out from 1800 to 0500 hours

(Rishikesh, 1966). The only exception was the first field trip where catches were carried out from 1900 hours to 2200 hours. Hourly mosquito catches were counted and stored in separate containers. Human baits (students, a technician and the supervisor) collected the mosquitoes from 1800 to 0500 hours from the tent trap (Plate 4). In the morning, all female mosquito catches were then preserved in eppendorf tubes containing silica gel. The mosquitoes and silica gel were separated by a layer of cotton wool in the eppendorf tubes. These human landing collections were used to determine the biting cycles of *An*. *gambiae* complex species and *An*. *merus*.

Plate 3: Calico cloth sheet with knocked down mosquitoes at Masakadza.

Plate 4: Human-baited tent trap at Masakadza.

From human-baited tent trap collections data, mean hourly catch rate (Sharp, 1983) were calculated. In turn, these catch rates were used to determine the percentage bite per hour of mosquitoes prior to and after midnight. The following formulae were used as described by Sharp (1983).

The mean hourly catch rate:

$$\%\ \text{per hour prior to midnight} = \frac{\%\ \text{of total catch biting prior to midnight}}{\text{number of hours catching before midnight}}$$

$$\%\ \text{per hour after midnight} = \frac{\%\ \text{total catch biting after midnight}}{\text{number of hours catching after midnight}}$$

4.3.3 Window trap technique

The most widely used window trap is probably that developed by Muirhead- Thomson (1948). This consists of a cage made from 1 ft^3 framework of wire covered with mosquito netting. One side is inverted to form an entrance funnel narrowing to about ¼ in diameter opening. The funnel is supported by a string tied from its narrow end to the four corners of the trap (Service, 1976). The window traps (Plate 5) were installed in 12 houses at Masakadza Training Centre and nearby villages within a 1 km radius from the breeding site. The window traps were inspected daily and mosquitoes collected between 0700 and 0800 hours. Mosquitoes collected were morphologically identified as anophelines and culicines then counted. The female anopheline mosquitoes were classified by abdominal condition. The following classes were used: unfed, freshly fed, half gravid or gravid (WHO, 2002). The collection of blood-engorged adults is particularly useful as they can be used to study natural host preference. The objective of the exercise was to determine the house leaving behaviour and indoor resting densities of anopheline mosquitoes found at Masakadza.

Mosquitoes caught from huts are usually expressed in terms of mean hut densities (Service, 1976). The window trap and knockdown collection were used to calculate the mean hut densities as described by WHO (2002) as shown below:

$$\textbf{Mean hut density} = \frac{\text{Total number of females of particular species}}{\text{Total number of huts inspected}}$$

Plate 5: An exit window trap at Masakadza.

The hut densities are commonly used to measure changes in the seasonal and annual abundance of mosquitoes, to compare house resting densities in different villages or area and to assess the impact of control measures on endophilic species (Service, 1976).

4.4 Morphological identifications

A randomly chosen representative sample from the female anopheline mosquito collection was identified using morphological characters as described by Coetzee (1989). For each adult insect, the characters examined were the palpus ratio (Coluzzi, 1964) - length segments (IV +

V/III), and the length of the pale band at the joint of hind tarsomeres 3 and 4. Measurements of morphological characters were taken using an eyepiece micrometer (at ×40 magnification). Below is the modified key of Coetzee (1989) which was then used to identify mosquitoes to species level as outlined below:

Identification key

1. Pale band at the joint of hind tarsomeres 3 and 4, 0.1 mm or more…….….2

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

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

- This ratio 0.84 or lower……..….*quadriannulatus*

*3. The sum of coeloconic sensilla on flagellomeres 5+6+9 of both antennae 13 or more……...…...*arabiensis*

- This sum 12 or less……...…….*gambiae*

* Since *An. gambiae* is absent in Gokwe south district (Masendu, 1996; 2004) couplet 3 was substituted by *An. arabiensis* which is present in this area.

4.5 Data Analysis

The proportions of mosquito bites prior and after midnight from Human-baited tent trap collection were subjected to Two Sample Student *t*-test to determine their differences. The abundance of *An. gambiae* complex species co-existing at Masakadza based on morphological identification were evaluated by Chi Square (χ^2) (Fettene *et al*., 2004). MINITAB statistical package was used for the analyses and differences were considered significant at $P < 0.05$.

6.0 RESULTS

The biting cycle of Anopheles gambiae complex species

A total of 539 female anopheline mosquitoes were collected using the human-baited tent trap (Table 1) and all the female mosquitoes caught were unfed. The results of hourly collections of female anopheline mosquitoes during the three months of study are summarised in Figures 2 to 4.

Table 1: Summary of female anopheline mosquitoes collected during each sampling month.

Month	Collection method			
	Human-baited tent trap	Window trap technique	Knockdown technique	Total
November 2006	35	0	0	35
January 2007	144	6	0	150
March 2007	360	107	3	470
Total	539	113	3	655

In November 2006, the host-seeking activity of *An. gambiae* complex species increased with time from 0600 hours to 2200 hours (Figure 2). In January, there were no significance differences between mosquito bites prior to and after midnight ($P > 0.05$, Appendix 1.1). However, the peak of host-seeking activity of this species occurred after midnight (Figure 3). There was a significant difference between mosquitoes caught prior to and after midnight in March ($P = 0.020$, Appendix 1.2). The peak of host-seeking activity by *An. gambiae* species

occurred at 2200 hours (Figure 4) and a larger proportion of mosquitoes were caught before midnight with 10.2 % mean bites per hour (Table 2).

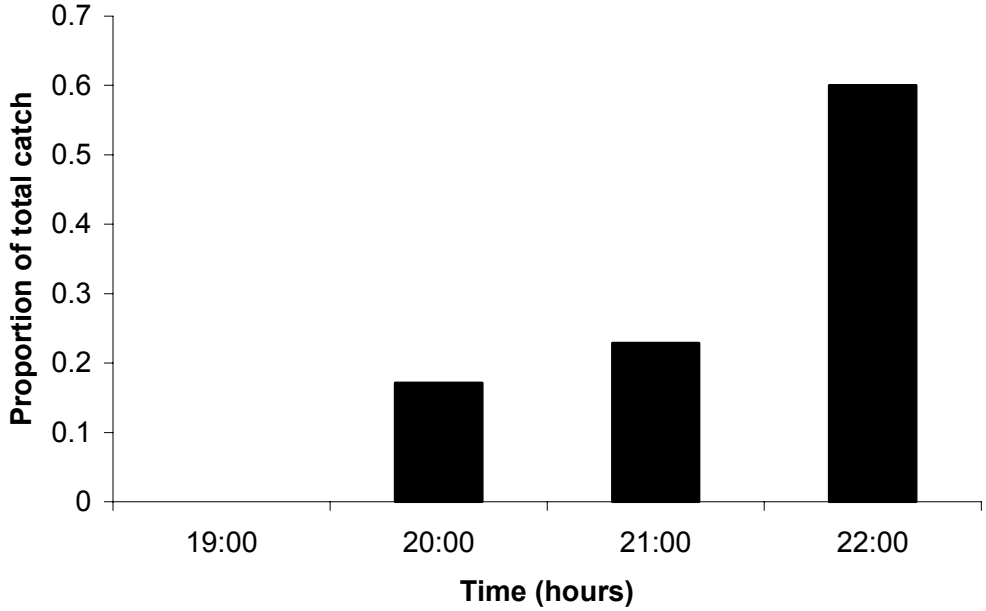

Figure 2*:* *Anopheles gambiae* complex biting behaviour prior to midnight at Masakadza in November 2006.

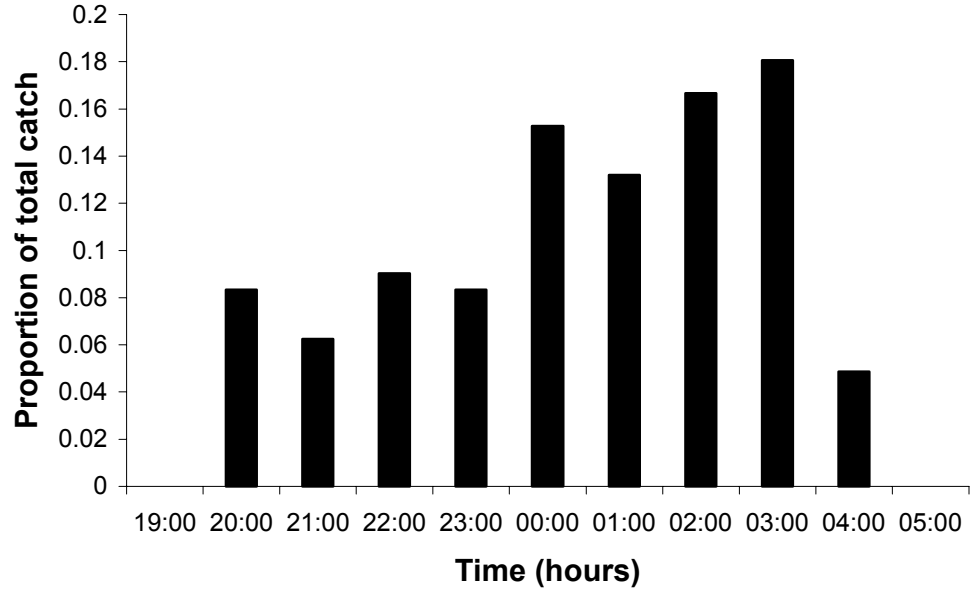

Figure 3: *Anopheles gambiae* complex biting cycle at Masakadza in January 2007.

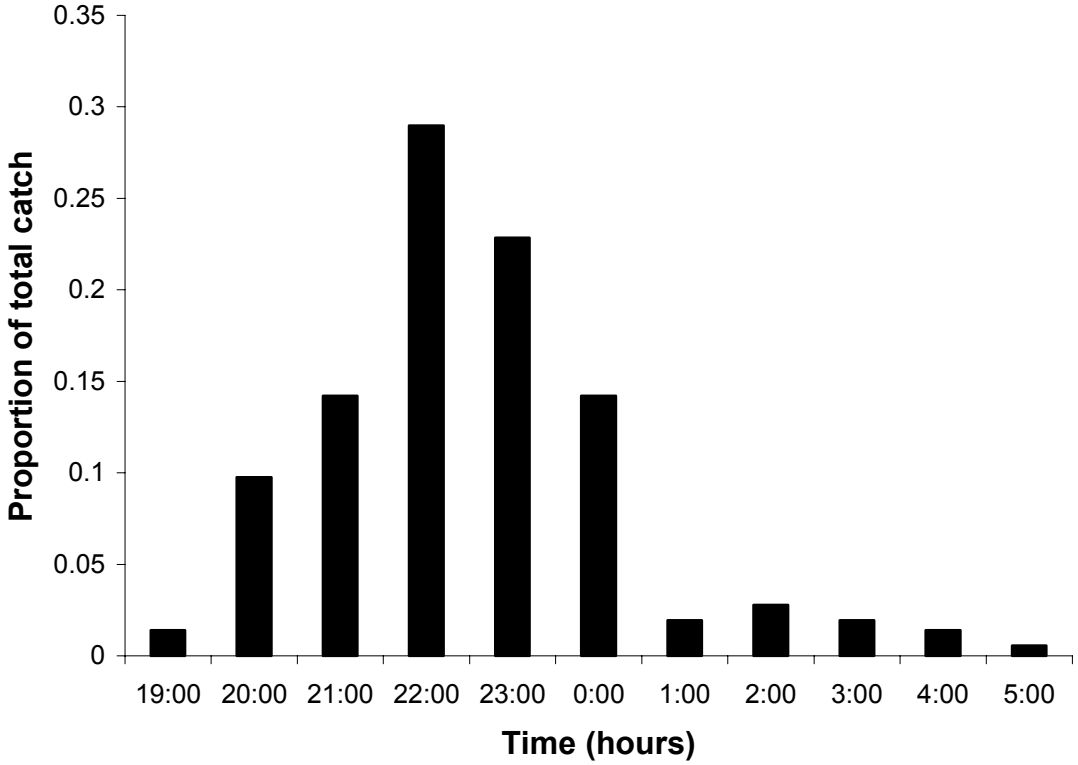

Figure 4: *Anopheles gambiae* complex biting cycle at Masakadza in March 2007.

Table 2: Percentage catch per hour of *An. gambiae* complex species by month prior to and after midnight‡.

Month	prior to midnight	after midnight
January	7.9	10.6
March	10.2	1.2

‡ Results for November are not presented because mosquito sampling was conducted up to 2200 hours due to unavailability of some essential materials.

Anopheles merus biting cycle at Masakadza

Figures 5 to 7 indicate the hourly proportions of *An. merus* collected within the months November 2006, and in January and March 2007. Representative samples of 27, 75 and 98 were identified from November, January and March collections, respectively.

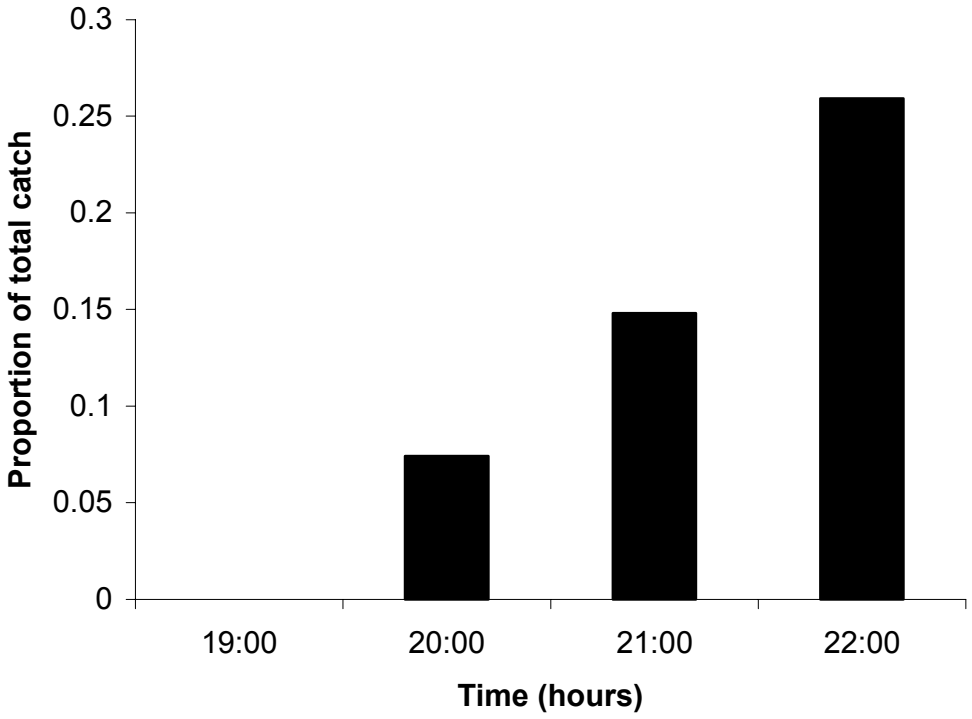

Figure 5: *Anopheles merus* biting cycle at Masakadza in November 2006.

In November, the host-seeking activity of *An. merus* increased with time from 0600 hours to 2200 hours (Figure 5). There were no significant differences in the proportions of *An. merus* caught prior to and after midnight in January ($P = 0.57$, Appendix 2.1). However, the peak of biting occurred at midnight (Figure 6). There were significant differences between *An. merus* caught prior to and after midnight in March ($P = 0.0046$, Appendix 2.2). During this month,

the peak of host-seeking activity by *An. merus* occurred at midnight but a larger proportion of mosquitoes were caught before midnight (Figure7) with 9.4 % mean bites per hour (Table 3).

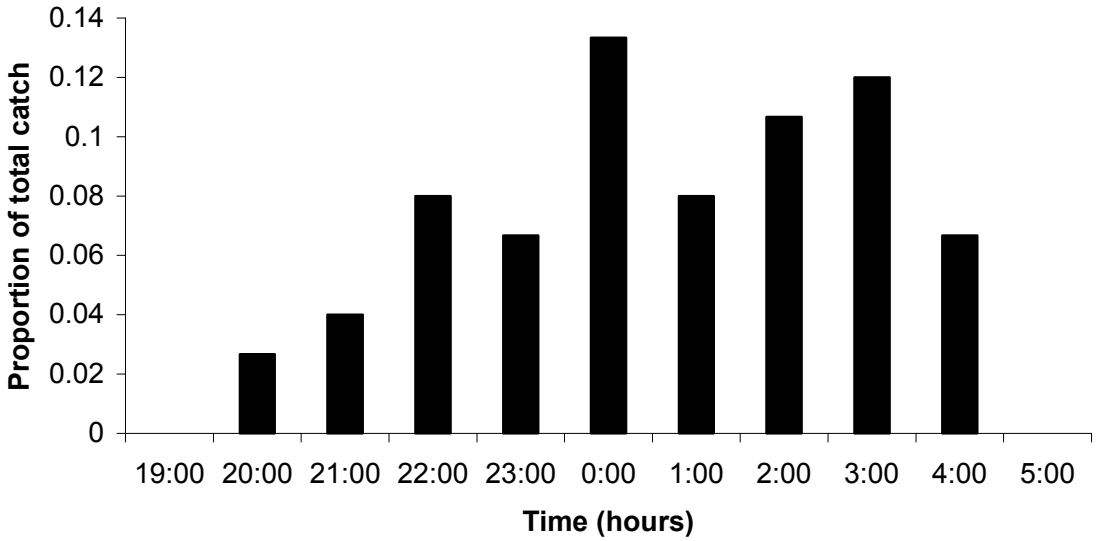

Figure 6: *Anopheles merus* biting cycle at Masakadza in January 2007.

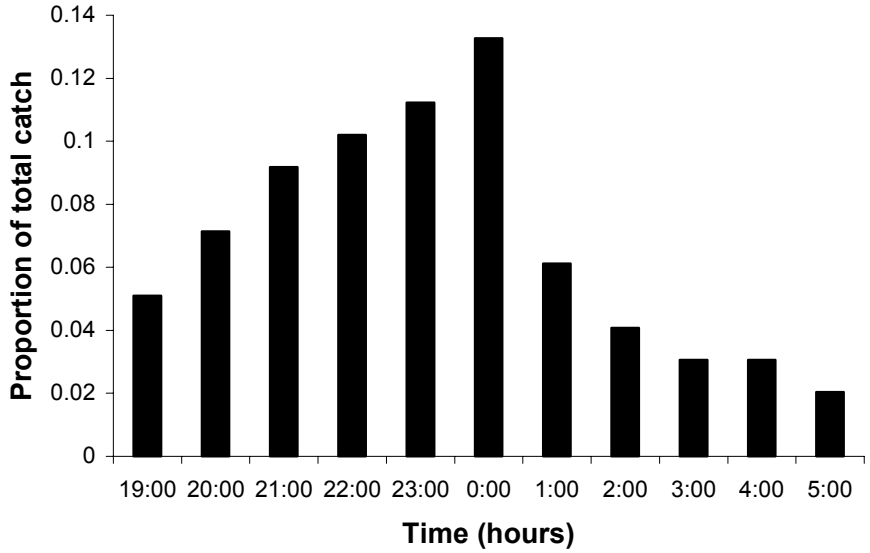

Figure 7: *Anopheles merus* biting cycle at Masakadza in March 2007

Table 3: Percentage catch per hour of *An. merus* mosquitoes by month‡.

Month	% per hour prior to midnight	% per hour after midnight
January	5.8	7.5
March	9.4	3.7

‡ Results for November are not presented because mosquito sampling was conducted up to 2200 hours; because some materials were not yet bought at this time.

Table 4: Abundance of malaria vectors at Masakadza – trap technique

Month	N‡	*An. gambiae* complex species[a]	
		An. merus	*An. arabiensis*
November	27	13 (48.1)	14 (51.9)
January	75	54 (72.0)	21 (28.0)
March	98	73 (74.5)	25 (25.5)
Total	200	140 (70.0)	60 (30.0)

‡, Total number of female anopheline mosquitoes identified.
[a] Percentage values in parentheses.

The abundance of malaria vectors at Masakadza

Due to the large number of anopheline mosquitoes collected during this study, only a sub-sample (n = 200) of specimens was used for species identification using morphological characters. Table 4 gives a summary of species identified.

The proportions of *An. merus* and *An. arabiensis* collected by Human-bait tent trap based on morphological identification were significantly different from each other (χ^2 = 7.223, *P* =

0.027; Appendix 3). The greatest number of *An. merus* was collected in March (74.5%) while that of *An. arabiensis* was in January. Thus, *An. merus* was the abundant species at Masakadza, accounting for 70% of the total number of mosquitoes collected.

The resting and house-leaving behaviour of An. gambiae complex species at Masakadza

Only three mosquitoes were collected by the indoor resting sampling technique (Table 5). This indicated that very few mosquitoes rest indoors in this area with mean hut density of 0.2 mosquitoes per hut. These indoor resting mosquitoes were only collected in March. A greater proportion of mosquitoes in this area leave houses in the morning and rest outdoors as evidenced by female mosquitoes collected by the window trap technique, which is used to assess house-leaving behaviour of mosquitoes. In total, 113 female mosquitoes were collected during the three-month period of the study.

Table 5: Indoor resting collection of *An. gambiae* complex species by month.

Month	Mosquitoes caught [b]	Mosquitoes caught [a]	Huts inspected	Mean hut density[a]
November	0	0	5	0
January	6	0	5	0
March	107	3	10	0.3
Total	113	3	20	0.2

[a] Mosquitoes collected by knockdown technique.

[b] Mosquitoes collected by Human-baited tent trap.

7.0 DISCUSSION

Climate is the major factor governing the distribution and relative abundance of insects (Surthest and Maywald, 1995 - cited by Craig *et al*., 1999). Some members of the *An. gambiae* complex occur sympatrically over a large area of their distribution. This study revealed that *An. merus* and *An. arabiensis* occur sympatrically at Masakadza. This finding agrees with what Masendu (2004) observed in the same area. *Anopheles merus* was the predominant species at Masakadza accounting for 70% of indoor collections while *An. arabiensis* made up the remainder. Studies conducted by Masendu (2004) also revealed that *An. merus* is predominant at Masakadza, accounting for 89.6 and 72.3% of human landing collections indoors and outdoors, respectively, with 10.4% and 25.5% being the corresponding respective proportions for *An. arabiensis*.

The ecological conditions, such as salinity of the breeding sites and relative availability of different host species, which support malaria vectors, primarily determine the intensity of the disease (Gallup and Sachs, 2000). Masakadza is one of the areas with high malaria prevalence and falls under the Ministry of Health and Child Welfare's yearly spray programme introduced in the early 1980's (Masendu, 2004). This study revealed the biting cycle of *An. gambiae* complex species. The biting cycle interpreted in the broad sense covers host-seeking as well as the act of feeding (Gilles and De Meillon, 1968).

It was shown that biting of *An. gambiae* complex species commenced at 1900 hours and ceased at 0500 hours with biting peaks at 2200 hours in November and March, and 0300 hours in January. In Uganda, the forest-dwelling *An. bwambe* had a peak of biting activity in the last two hours before sunrise; in Kenya the biting activity of *An. gambiae* s.l gradually

increased throughout the night with a peak being attained three hours before dawn (Braack *et al*., 1994). The basic cycle of *An. gambiae* complex is dynamic and varies in response to changes in environmental factors such as wind and rainfall (Sharp, 1983). The differences in the mean biting rates for *An. gambiae* complex species in January and March exhibit seasonal variations in biting cycle of these species at Masakadza. Data collected by other workers show that 3.9-7% per hour bite of *An. gambiae* complex species occur before midnight and 9.75-12.5% per hour bite after midnight (Sharp, 1983). This, in general, partly agrees with the findings of this study at Masakadza where 5.8-7.9% per hour bite occurred before midnight and 7.5-10.6% after midnight in January. However, in March the inverse was observed where more bites per hour occurred before midnight. The studies conducted show that members of *An. gambiae* complex species bite throughout the night, although peak-biting activity may vary according to species.

An endogenous rhythm in the biting cycle of *An. merus* was observed during the study. Biting activity commenced at 1900 hours and steadily increased to a peak from midnight to 0300 hours in January 2007, and midnight in March 2007 and decreased thereafter and ceased at 0500 hours. In Kenya, the peak of biting activity of *An. merus* occurred between midnight and 0100 hours whereas in Natal Province, South Africa the biting peak of this species was between 2300 and 0200 hours (Braack *et al*., 1994).

Sharp (1983) reported that the biting cycle of *An. merus* can be markedly disrupted by changes in environmental factors during the night, e.g. rain and wind. Wind has a direct effect on mosquito flight (Snow, 1980, Gilles and Wilkes, 1981 - cited by Sharp and Quike, 1984). During the study, *An. merus* activity decreased after midnight in March as a result of increase in rainfall intensity. Masakadza received more rain in March (with wet-hot nights) compared

to January when the weather conditions were dry and hot. Sharp (1983) also observed that there was a decrease in numbers of mosquitoes collected when rain increased from light to strong showers and biting activity completely ceased when the heaviest rain of the night fell.

The knowledge of the biting cycle of species is essential in ascertaining whether peak biting is coincident with the outdoor activity of the local human population or whether house entering by a mosquito would be necessary to establish significant contact (Sharp, 1983). In March, where the biting peak occurs prior to midnight, the possibility of outdoor transmission should be very high as this is a period when a larger proportion of the rural population is still active outdoors. However, in January, where the peak of biting activity occurred after midnight — a time when the local human population is asleep in houses — the endophilic behaviour would be a prerequisite for significant contact between the vector species and man. In the latter case, use of bed nets is desirable for effective vector control.

It is further recommended that mosquito sampling at Masakadza, where *An. merus* is the abundant species, be conducted between 2100 hours to 0300 hours, a period encompassing peak activity of host-seeking by this species.

The resting behaviour of the *An. gambiae* complex species at Masakadza, where *An. merus* is predominant, is mainly exophilic. It was observed that a larger proportion of female mosquitoes both unfed and fed leave the houses early in the morning, presumably to rest in the nearby vegetation as evidenced by window trap collections in January and March. The mean hut density of *An. gambiae* complex species at Masakadza was very low as revealed by the knockdown collections. During the three months of study, only March yielded positive results in indoor resting collections. Hut densities are commonly used to measure changes in

the seasonal and annual abundance of mosquitoes and to assess the impact of control measures on endophilic species (Service, 1976).

The results from window trap collections showed variation in house-leaving behaviour and densities of *An. gambiae* complex species. More mosquitoes were collected in March than in January. This difference could be attributed to changes in weather conditions since in January 2007, Masakadza was mostly dry and hot with temperatures of over 27^0C. In March the rainfall intensity increased thereby creating favourable conditions for mosquito breeding since additional temporary swamps were created. Temu *et al*. (1998) also observed that the anopheline density increased with increase in rainfall intensity at Bagamoyo district in Tanzania with peaks during April to May and November. Large variations in numbers of mosquitoes leaving different houses in the same compound at Masakadza were observed. In general, unfed mosquitoes are attracted to huts in numbers related to the number of human occupants (Haddow, 1942 cited by Sharp, 1976) but no general arithmetic relationship has been established between catch size and number of occupants.

Service (1964) also noted that many other variables such as the presence of open doorways, fires and large gaps between the eaves also affect the number of mosquitoes resting in them. Village huts which are near larval habitats may contain more mosquitoes than those further away.

Based on the exophilic behaviour of *An. gambiae* complex species at Masakadza, conventional residual insecticidal treatment of houses would not be ideal for vector control since it is mostly designed to kill indoor resting fraction of malaria vector population for

example the strongly endophilic species like *An. gambiae s.s* (Takken, 2002). The use of insecticide treated bed nets would thus be more effective in this area.

8.0 CONCLUSIONS

Several conclusions can be drawn from the current study. Firstly, *An. merus* and *An. arabiensis* co-exist at Masakadza in Gokwe South district**.** Secondly**,** *An. merus* is the predominant species in this area. The biting pattern of *An. gambiae* complex species and *An. merus* at Masakadza is dynamic and varies in response to changes in environmental factors such as wind and rainfall. The differences in the mean biting rates for *An. gambiae* complex species and *An. merus* during the three months of sampling exhibit seasonal variations in the biting cycle of these species at Masakadza. The peak of biting activity for *An. gambiae* complex species occurred at 2200 hours in November 2006, 0300 hours in January 2007, and 2200 hours in March whereas the peak of biting activity for *An. merus* occurred at 2200 hours in November 2006, midnight in January and March 2007. The resting behaviour of *An. gambiae* complex species (*An. merus* being predominant) at Masakadza is mainly exophilic. Very low indoor resting densities with mean hut density of 0.2 mosquitoes per hut indicate that a greater proportion of mosquitoes in this area are exophilic The results from window trap collections also showed variation in house-leaving behaviour and densities of *An. gambiae* complex species. More mosquitoes were collected in March than in January 2007**.**

9.0 RECOMMENDATIONS

Based on the results obtained from the current study, the following are recommended:

- Insecticide treated bed nets need to be enhanced since the resting behaviour of the *An. gambiae* complex species — *An. merus* — being predominant, is mainly exophilic in this area.
- The infection rates of *An. merus* need to be determined by Sporozoite ELISA in order to establish its role in malaria transmission in this area.
- Host-preference for *An. merus* in this area need to be determined by Blood meal ELISA in order to establish the propotion of these mosquitoes that feed on humans.
- Based on the biting cycles of *An. merus* in this area, it is recommended that vector sampling be conducted between 2100 and 0300 hours to get reliable data. The above period encompasses peak activity of host-seeking by this species.

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11.0 APPENDICES

Appendix 1: Two Sample *t*-test for *An. gambiae* complex mosquitoes collected by Human-baited tent trap.

Appendix 1.1: Two Sample *t*-test and Confidence Interval for *An. gambiae* complex in January, 2007.

Two sample t-test for BM[a] vs AM[b]

TIME	N	Mean	St Dev	S.E. Mean
BM	6	11.33	7.09	2.9
AM	5	15.2	11.3	5.0

[a]BM= Bites before midnight; [b]AM= Bites after midnight

95% C.I. for mu BM - mu AM: (-18.1, 10.3)

t-test mu BM = mu AM (vs not =): t = -0.67 *P*= 0.53 df = 9

Appendix 1.2: Two Sample t-test and Confidence Interval for *An. gambiae* complex in March, 2007.

Two sample t-test for BM[a] vs AM[b]

TIME	N	Mean	St Dev	S.E. Mean
BM	6	54.8	35.1	14
AM	5	6.20	2.95	13

[a]BM= Bites before midnight; [b]AM= Bites after midnight

95% C.I. for mu BM - mu AM: (12, 85.6)

t-test mu BM = mu AM (vs not =): t = 3.38 *P*= 0.020 df = 9

Appendix 2: Two Sample t-test for *An. merus* collected by Human-baited tent trap based on morphological identification.

Appendix 2.1: Two Sample t-test and Confidence Interval for *An. merus* in January, 2007.

Two sample t-test for BM[a] vs AM[b]

TIME	N	Mean	St Dev	S.E. mean
BM	6	4.33	3.50	1.4
AM	5	5.60	3.51	1.6

[a]BM= Bites before midnight; [b]AM= Bites after midnight

95% C.I. for mu BM - mu AM: (-6.2, 3.6)

t-test mu BM = mu AM (vs not =): t = -0.60 *P*= 0.57 df = 9

Appendix 2.2: Two Sample t-test and Confidence Interval for *An. merus* in March, 2007.

Two sample t-test for BM[a] vs AM[b]

TIME	N	Mean	St Dev	S.E. mean
BM	6	9.17	2.86	1.2
AM	5	3.60	1.52	0.68

[a]BM= Bites before midnight; [b]AM= Bites after midnight

95% C.I. for mu BM - mu AM: (2.4, 8.76)

t-test mu BM = mu AM (vs not =): t = 4.13 *P*= 0.0046 df = 9

Appendix 3: Chi-Square test for *An. merus and An. arabiensis* collected by Human-baited tent trap basing on morphological identification.

Month	*An. merus*	*An. arabiensis*	Total
Nov.	13	14	27
	18.90	8.10	
Jan.	54	21	75
	52.50	22.50	
Mar.	73	25	98
	68.60	29.40	
Total	140	60	200

NB: Expected counts are printed below observed counts

ChiSq = 1.842 + 4.298 + 0.043 + 0.100 + 0.282 + 0.659 = 7.223

df = 2, $p = 0.027$