# MALARIA VECTOR POPULATIONS ASSOCIATED WITH THE AGRICULTURAL DEVELOPMENT AT MAMFENE, NORTHERN KWAZULU-NATAL, SOUTH AFRICA

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This thesis is dedicated to my family who waited patiently

during my study period in South Africa

# ABSTRACT

The irrigation farming methods on the Makhathini Flats are thought to be responsible for recent increase in malaria cases in the Mamfene area of northern KwaZulu-Natal despite ongoing malaria control activities. Their coincidence with the period of intensive farming is an interesting one. This study was therefore carried out to determine the relationship between larval habitats and adult mosquito population in malaria transmission using Geographic Information System (GIS).

Four types of breeding sites were utilised by malaria vectors in Mamfene, viz; types I, II, III and IV. Habitat type I was tap pools, type II was irrigation spillage in agricultural land, type III was spillage outside but adjacent to the agricultural land and type IV was depression pools located both in the Balamhlanga swamp and inland. The cumulative larval density in all habitats peaked in March 1995 (20/man-hr), with irrigation spillage (type III) recording the highest density index (33/man-hr) as compared to tap pools (type I) which recorded 32.8/man-hr while other waterbodies recorded 13.4/man-hr (type II) and 0.5/man-hr (type IV) respectively.

Subsamples of both larvae and adults of *An. gambiae* group were identified by the Polymerase Chain Reaction (PCR) technique. Of the larvae identified, 94.1% and 5.9% (n=289) were *An. arabiensis* and *An. quadriannulatus* respectively while in the adult component, the composition was 98.7% and 1.3% (n=303) for *An. arabiensis* and *An. quadriannulatus* respectively. This confirmed *An. arabiensis* as the dominant malaria vector in Mamfene area while the exophilic behaviour of *An. quadriannulatus* was portrayed. Of the *An. gambiae* group dissected for parity, 51.5% were found parous. This is an indication that the population was old and was able to maintain transmission locally despite ongoing vector control measures.

The Global Positioning System (GPS) was used to position larval and adult mosquito sampling sites. The spatial distribution of adult mosquitoes from the breeding sites were plotted using GIS soft ware (MapInfo) and the distance between the breeding sites and study houses were measured using a utility distance tool. With the aid of GIS, the adult mosquito density in houses could be used as an indicator to locate the breeding sites in the vicinity. The importance of these findings in terms of application in cost-effective malaria control cannot be over-emphasized.

# PREFACE

This study represents original work done by the author and has not otherwise been submitted in any form for any degree or diploma to any University. Where the author used the work of others it is duly acknowledged in the text.

The experimental work described in this dissertation was carried out in the Department of Zoology and Entomology, University of Natal, Pietermaritzburg and in the laboratories of the National Malaria Research Programme of the South African Medical Research Council, Durban from May 1994 to December 1995 under the supervision of Professor Chris C Appleton (University of Natal), Dr Brian L Sharp and Dr Dave le Sueur (Medical Research Council).

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# CHAPTER ONE

# INTRODUCTION AND LITERATURE REVIEW

In this chapter, the application of GIS is reviewed in terms of its use in health. An attempt has been made to highlight those aspects which make GIS a suitable tool in disease epidemiology in various parts of the World with special reference to North America, Canada, Europe and elsewhere where its application has permeated almost all spheres of life. In disease epidemiology, the community structure, their pattern of life, migratory habits and interactions with the environments combine to form a strong database comprising of both geo-referenced and attribute information. When analysed, this often leads to the elucidation of disease determinants and their point source.

The application of GIS in health in South Africa and in particular its application in the control of malaria in northern KwaZulu-Natal is reviewed. Lastly the problem of malaria and its control in South Africa is highlighted and related to the aggravated breeding of *An. gambiae* group in relation to irrigation development.

## 1.1. The use of Geographical Information Systems (GIS) in Health

The current approach to providing cost-effective health services to the community depends on the ready access to information on current and future health needs. This initiative has generated the need to handle large amounts of spatially referenced information.

This includes the addresses and mobility pattern of people attending a particular health facility, the types of diseases infecting them, their point locations, the spatial distribution in a locality and the factors that are responsible for their presence. Causative agents may include industrial plant pollution as in the case of cancer, landscape topography and vegetational changes which may provide suitable habitat for disease vectors. The addresses of general medical practitioners should also be included (Verhasselt, 1987; Lovett, 1992; Helmut, 1994).

These type of databases are large and require relational components with complex geographically referenced information. GIS has the means of integrating such spatial information and attribute data and can provide output in an easily understandable form. This is because it has the capacity to show the spatial distribution of health needs in a community and can generate presentation formats that may be used to address them (Verhasselt, 1987; Lovett, 1992; Helmut, 1994; Hine 1994).

The potential use of GIS in health is promising as most environment related problems contain a geographical component, but awareness of this is still confined to spatial scientists. Many epidemiologists and environmental health specialists are hardly aware of the possibilities of GIS as a tool that can be used to integrate relevant data from different sources for analysis and presentation (van der Veen, 1992). In spatial epidemiology, disease information may be presented in two forms, firstly where the available disease data are aggregated to a set of spatial units, and secondly that the point location of each individual case is recorded and may be presented as a dot on

a map. These types of data are important in locating high risk areas in an epidemic condition and may be used in redirecting control operations (van der Veen, 1992).

GIS technology has not been confined to health only. It has also found wide applications in many aspects of life that affect man-kind, for example in agriculture, forestry, meteorology and water management. It is being successfully used to manage an exceptionally large and diverse set of data for predicting the onset of food security emergencies for every country on earth, i.e. an ongoing study at the University of Arizona in collaboration with the Global Information and Early Warning System (GIEWS) and the Remote Sensing Centre of the Food and Agriculture Organization (FAO) (Marsh *et al.*, 1994).

**1.2. Mobility and Disease Epidemiology**

Attempts to associate the geographical determinants of disease distributions and the population mobility in Africa are contained in the work of Prothero (1968). This study was done when the author was a research fellow of the West African Institute of Social and Economics Research. He studied the relationships between people and land in Sokoto province, northern Nigeria, and showed that disease transmission among the migrating population rendered the success of the malaria control programme unattainable.

The seasonal migration of the pastoral Fulani community in search of pasture and water for their cattle had inherent health risks. Implications and effects of disease transmission existed for migrant labour both on the home areas of the

migrants and the places they visit to seek work. He observed that a combination of these risk factors greatly increased the possibilities for disease transmission. For example, there was a minor outbreak of small pox in Sokoto province in May 1955 which coincided with the return of migrant labourers some of whom were thought to have been responsible for the epidemic (Prothero, 1962; 1968).

The Garki project which was formulated to study the epidemiology and control of malaria in northern Nigeria was unable to completely realize its main objectives. The control of malaria in the Garki district was not fully achieved although all possible epidemiological parameters and intervention methods were investigated (Molineaux and Gramicia, 1980). Although the human behavioural aspects in the disease transmission were ignored, this study provided the most detailed evidence of the relationship between population mobility and malaria transmission (Prothero, 1987). For example, the study community was highly mobile but vector-human contact was maintained among the migrants especially the Fulani community, because of their pastoralist culture (Molineaux and Gramicia, 1980).

To achieve malaria control in an area and to maintain it, it is necessary to prevent new sources of infection from being introduced. These sources would be infected people from elsewhere on whom mosquitoes might feed, become infected and re-establish the transmission of the disease. In this situation the population movement profile is an important component in understanding the causes and pattern of disease distribution (Molineaux and Gramicia, 1980; Prothero 1962; 1983; 1987).

In the Horn of Africa, there are problems in malaria eradication associated with the movements of the nomadic pastoralists and the international frontiers. For the Somali people who are camel and cattle herding nomads, these boundaries have little significance. The pattern of their lives is determined by the need to find pasture and water for their stock and to this end they cross and re-cross frontiers with the seasons of the year. These movements are unpredictable but are at their maximum during the malaria season (Prothero, 1968).

Another example of mobility that probably affects disease transmission in the frontier region is that of the Masai in Tanzania and the adjacent parts of East Africa. The pastoral Masai who live in the common border area between Tanzania and Kenya, cross the frontiers with each season of the year in search of pasture and water. This makes it impossible for any disease control programme to succeed without the collaboration of the two countries (Prothero, 1962).

Similar frontier problems have been experienced in other parts of Africa where the need to find jobs in countries with stronger economies have been noted among migrant workers. For example, malaria control programmes in South Africa have witnessed mixed fortunes due to the pressing economic needs and political instability in countries that share common borders with South Africa and where control programmes are either ineffective or lacking (Ngxongo, 1993).

Migrant workers from the neighbouring countries, for example Mozambique, Swaziland and Zimbabwe, cross and re-cross the border daily in search of

work in South Africa. This exacerbates the malaria problem in spite of the elaborate control programmes that exist in the relevant provinces of South Africa, for instance; northern KwaZulu-Natal, Mpumalanga and the Northern Province. It has been suggested that for a campaign to eradicate the disease in South Africa, ties needs to be formed with neighbouring countries (Ngxongo, 1993). Not only do these countries have a high prevalence of malaria but there also exists the problem of drug resistance (Isaacson *et al.*, 1984; Sharp and Freese, 1988).

Prothero's work on human mobility and malaria transmission brought about an important shift towards an emphasis on human behaviour as a factor in disease transmission (Stock, 1986 ). This is also partly because habitat conditions for disease agents are frequently created by the human agencies. For example, the ecotones such as the fringes of agricultural fields are rich in vector insect life and in host animals (Meade, 1977).

Mosquito vectors of malaria, filarial worms, yellow fever etc are often associated with agricultural developments which also require farmers to spend many hours exposed to infection in the fields, a component which increases human-vector contact (Meade, 1977; Service, 1982; 1991). In highlighting the study by Pavlovsky on landscape epidemiology (in the 1960s), Meade (1977) associated infectious zoonotic diseases such as scrub typhus, encephalitis, leishmaniasis, bubonic plague, etc, with landscape, vegetation, soil type, animal life and other elements of the natural landscape.

### 1.3. Disease Epidemiology and GIS Application

Katzenellenbogen *et al.* (1991) described epidemiology as the study of the distribution and determinants of health related conditions and events in the population. These authors also noted the limitations of the value of a single indicator and suggested a standard set of indicators which would enable environmental health specialists to compare key health outcomes between areas and over time. This component requires a relational database approach which would also allow routine monitoring of the health status of a population (le Sueur *et al.*, 1994). Spatial research experts are also aware that environmentally related problems in health and quality of life in relation to the environment is not completely new. For example, the cholera and tuberculosis epidemics in western Europe were direct consequences of the poor hygienic circumstances in respect of water supply and the indoor environment (van der Veen, 1992; Glass *et al.*, 1993).

Most of the information related to environment and health has an important spatial component which can be used to explain the spatiality and patterns of health outcomes and environmental phenomena. For example, the study of Lyme disease along the eastern coast of the United States in the early 1980s supported the association between disease and the environment and demonstrated how GIS could be applied to show up risk factors that contribute to disease distribution:

"The study of how environmental factors influence infectious diseases has long involved examining how infections are distributed throughout the population. Despite the obvious spatial component, geographical information systems (GISs) have played a minor role in such studies to date" (Glass *et al.*, 1993).

The Lyme disease project was designed to evaluate the GIS as a practical tool for epidemiologists to study risk factors associated with vector-borne diseases. Point data on disease incidence were collected and the number of cases and geo-referenced data were combined to determine whether environmental factors could be identified and associated with an increased risk of Lyme disease. This information was used to determine how much of the county and which areas of it were at high risk of disease (Dister *et al.*, 1993; Glass *et al.*, 1993).

In Israel Kitron *et al.* (1994) suggested the application of GIS for malaria surveillance. The distance between the breeding points of the vector mosquitoes and the centres where infected immigrants lived were calculated and maps associating epidemiological and entomological data were generated. The risk of malaria transmission was assessed in conjunction with known vectorial capacity and the flight range of each species of *Anopheles*. Localized malaria outbreaks could easily be associated with a likely breeding site, a specific *Anopheles* spp vector and a probable human source. This GIS-based surveillance approach promises to be a cost effective and efficient contributor to controlling malaria outbreaks. The authors recommended that this approach could be tried in holo-endemic malaria environments such as those found in tropical Africa. In The Gambia, GPS and GIS were applied to assist in locating factors associated with malaria transmission. However, the results indicated no correlation between malaria prevalence levels and the soil types and the proximity of the villages to the River Gambia even when they were highly correlated with vector mosquito abundance. Instead the length of transmission period was a more important determinant of malaria transmission (Connors *et*

*al.*, 1994).

Another study associating disease distribution and environmental risk is contained in the review by Twigg (1990). By comparing the observed number of childhood leukaemia cases within different sized buffers around the Sellafield power installation, at Gateshead in northern region of England, with the expected value derived from a simulated random distribution of the cases, a significant cluster of the disease was seen to be concentrated near the power installation. The pattern of disease distribution decreased with the increasing distance from the point source (Gatrell and Lovett, 1990; Twigg, 1990). Guthe *et al.* (1992) also used this computer based technology in New Jersey to assess where there may be greater environmental risks associated with exposure to lead.

### 1.4. Maps and the Spatial Distribution of Diseases

The concept of mapping disease distribution is not new in medical science. Probably one of the early users of maps in health was the renowned Dr. John Snow (1813-1858), a London anaesthetist and Queen Victoria's obstetrician, who used maps to trace the geographical distribution of cholera deaths in the Soho area in London. He demonstrated the striking association between cholera cases and contaminated water supplies and noted their geographical similarities. Primary transmission was interrupted by chaining down the handle of the pump which was identified on the map as the source of a cholera outbreak. He traced secondary transmission to the ingestion of the cholera bacteria in infected food and the handling of the bodies of people who died of the disease (Scholten and de Lepper, 1991).

Mapping the spatial distribution of a disease is a valuable means of epidemiological monitoring. The capability of a GIS facility increases the range of issues that can be addressed within a short time (Lovett, 1992). A map constitutes an excellent method of communication as most people enjoy looking at maps. They display a lot of information quickly and clearly, and allow the reader to see patterns unnoticed when written on paper.

Guthe (1993) in his review paper observed that in a GIS, attribute data are linked to physical features and are easily updated. These different data attributes are stored in separate files and can be recalled on demand. Many decision makers are practically oriented and their decisions will easily be influenced when they see patterns on a map. For example, Zwarenstein *et al.* (1991) in analysing the equitable distribution of hospitals within the catchment areas of KwaZulu-Natal used point data of hospital locations and the potential user population. The authors were able to show the inequitable distribution in patient/bed occupancy in what were previously classified as " black areas ". They observed that hospitals are a vast accumulated capital resource that needs to be utilised with maximum efficiency.

" Spatial efficiency for example, the appropriateness of the citing of a resource in relation to potential users is one of the most important aspects of efficiency because it reduces average travel distance per admission and tends to equalise utilisation. Optimum efficiency results from putting basic services (in this study, general hospital care) within equal reach for everyone, improving the referral network and concentrating sophisticated services (super specialist care) in cities" (Zwarenstein *et al.*, 1991).

## 1.5. Remote Sensing Technology

The Landsat mapping technique uses remote sensing methods to image cloud cover and land topography, for example landscape, soil types, vegetation etc. The remote sensed data has the advantage of covering a large area on a single scene, the ability which reduces unit cost and makes it less labour intensive. But the problems associated with satellite images as a mapping source are those of the imagery, the availability of stereoscopic cover and the detrimental effect of cloud cover upon the frequency with which optimal space-borne sensors can collect (Fox, 1991).

Remote sensing technology was used to map vegetation changes as they affected the populations of rodents. It was applied in tracking the rodent reservoir host of *Punmala* virus in Sweden and *Hyalomma* spp ticks in Africa, the host of Crimean Congo Haemorrhagic Fever virus (Decarlo, 1992). Different data files for example, vegetation, landsat, soils, veterbrate and rainfall data were stored at USAMRIID GIS database in Maryland USA from where many researchers had access to information.

This technique was applied in mapping seasonal and annual vegetation changes and the ways they affected the breeding of *Aedes mcintoshi,* the vector of Rift Valley Fever virus which breeds in dambos. This information was used in locating the breeding habitats of this mosquito species (Decarlo, 1992):
"... the dambos are characterised by tall sedge grass, papyrus and other grasses that usually contrast sharply with surrounding grasslands, making them easy to differentiate with the spectral capabilities of landsat. Dambos also never contain trees because the soils are too wet " ( Decarlo, 1992).

In research highlighted by Decarlo (1992), Major Kenneth Linthicum and co-workers used landsat maps to trace causative agents of disease. These workers used landsat in combination with GPS-GIS technology to associate rainfall patterns and vegetational changes with the breeding of the mosquito vector of Rift Valley Fever (RVF). The project, which was carried out in 1982 at the US Army Medical Research Unit in Kenya, discovered that the RVF virus used *Aedes mcintoshi* as a host. This mosquito species lays its eggs in flood-prone low lying grassland. A system to predict where the *Aedes mcintoshi* and RVF virus were likely to appear was developed by the US Army Medical Research Institute of Infectious Diseases (USAMRIID) in 1982.

Lyme disease in the coast of United States is another study where landsat was used to associate risk factors in the landscape topology with disease transmission ( Glass *et al.*, 1992 ). In this study geographic and public health data were combined to determine the environmental factors associated with an increased risk of Lyme disease, for instance, the risks was higher on loamy soils within certain watersheds in low density residential neighbourhoods. These areas were also home to deer, the reservoir host of the causative bacteria. Conversely, the risks of Lyme disease was significantly lower in highly developed areas and decreased with increasing distance from the forest.

### 1.6. GIS Application in health in South Africa

The application of GIS in health is still a relatively new technology in South Africa and the information regarding its application is limited. This is reflected in its meagre application to disease epidemiology in South Africa. The

available information is contained in a review by Dauskardt (1992). The critical issue outlined in this work was the provision of health care system during the apartheid era.

One example is the study by Zwarenstein *et al.* (1991) in KwaZulu-Natal. This revealed the inadequacies in hospital bed resources allocated to the black community in the province. Among other works in health with a GIS component in South Africa is that of Abdool Karim *et al.* (1992) which investigated the prevalence of HIV in northern KwaZulu-Natal.

The GIS applications in malaria research are contained in the studies by le Sueur *et al.* (1994), Ngxongo (1993) and Stuttaford (1994). All these studies have examined the spatial pattern of the geographic distribution of malaria in northern KwaZulu-Natal. Ngxongo (1993) used GIS to identify high risk malaria areas and showed that 80% of the malaria cases were from the Ingwavuma and Ubombo districts of the KwaZulu-Natal province. GIS was also used in this study to determine the incidence and prevalence of malaria as well as locate the residential areas of malaria infected individuals in northern KwaZulu-Natal (Ngxongo, 1993). A study by Stuttaford (1994) examined the spatial pattern of human settlement and how this related to malaria transmission in Mamfene. This study also linked malaria cases with the paddy scheme and the spillage points in the study area.

### 1.7. The problem of malaria in South Africa

Malaria and mosquito vectors in South Africa occur in the lowveld regions of Mpumalanga (eastern Transvaal), the Northern and North-West provinces,

northern KwaZulu-Natal as well as the north-eastern parts of Namibia (formally South West Africa) including the Caprivi strip. Figure 1.1 shows where malaria occurs in South Africa. Some cases have also been reported from settlements along the Orange River in the district of Gordonia, where in 1909 there was an epidemic of malaria following a period of unusually heavy rainfall. Malaria also occurred in Namaqualand, the Kalahari Desert and the adjoining districts (Pratt - Johnson, 1918; Fripp, 1983).

An epidemic of malaria in Durban in 1905 focused attention on the malaria problem in Natal (Hill and Haydon, 1905). But this probably was not the first time malaria had been experienced in the province. The malaria Medical Officer of the district between Tongaat River (lat. 29° 35'S) and Tugela River (lat. 29° 14'S), testified (Hill and Haydon, 1905), that he had treated malaria in the area for the last thirty years he had been in the district.

Not much was heard about malaria after this until the epidemics of the early 1930's. Dr Park-Ross, a government district surgeon stationed at Nqutu KwaZulu-Natal, had however, carried on with malaria surveillance between 1910 and 1921. He recommended a chemotherapeutic approach to the control of the disease in the designated malaria endemic foci. These included some parts of KwaZulu-Natal (Natal), Mpumalanga (eastern Transvaal), Northern and North-West Province (northern Transvaal). During this time "species" sanitation was introduced for the control of mosquitoes and quinine was used to control the spread of malaria (Park-Ross, 1929).

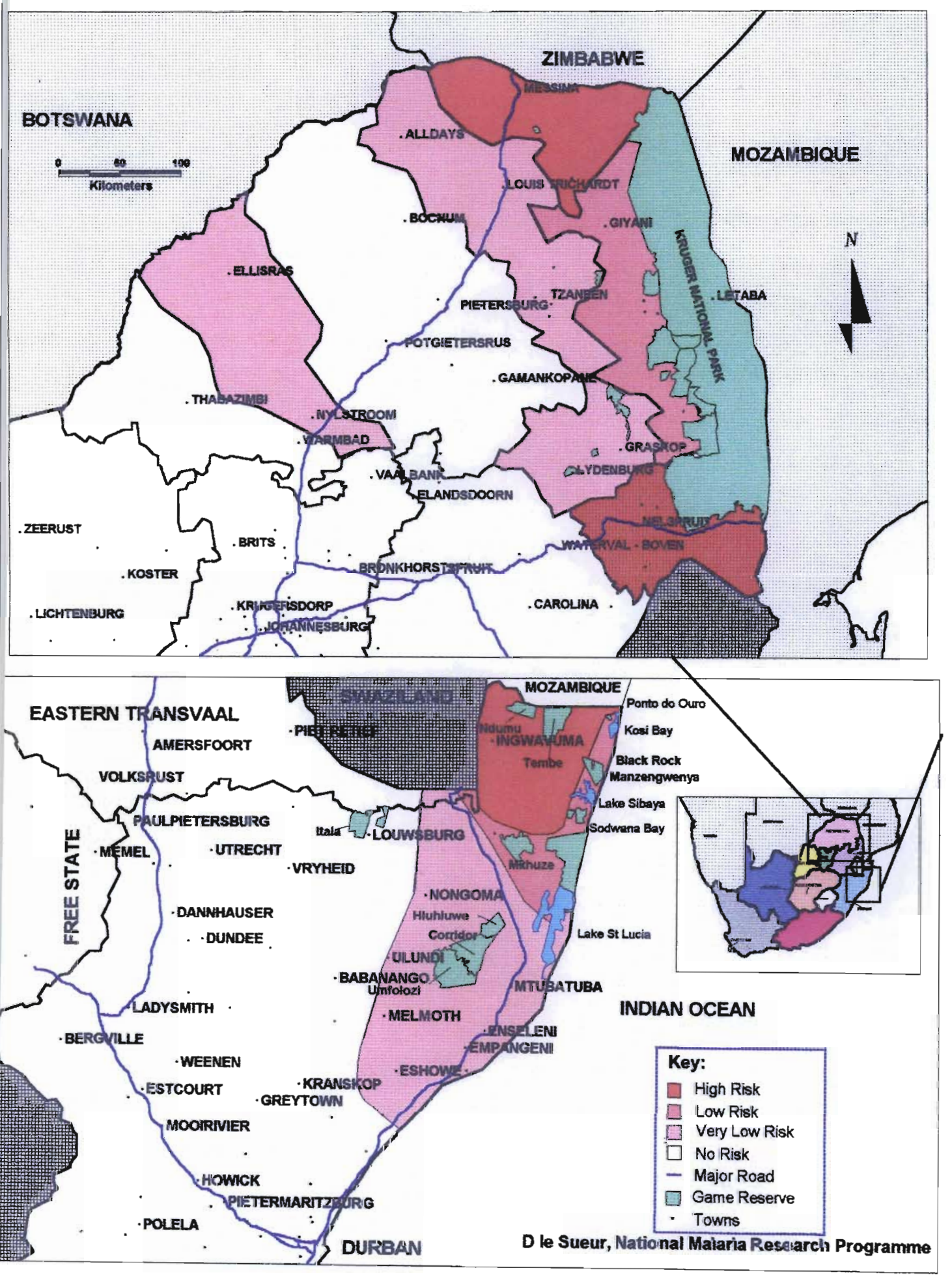



Fig. 1.1. Malaria Distribution in South Africa (le Sueur, 1995, unpublished data)

In 1930 Professor Swellengrebel, a malariologist from Amsterdam University, was commissioned to study and advise on the control of malaria in the provinces where it was endemic. These provinces included KwaZulu-Natal, Mpumalanga, Northern and North-West Provinces. The work of Ingram and De Meillon (1927) on the distribution of the malaria vectors assisted Prof. Swellengrebel in understanding the dynamics of malaria transmission in the endemic foci of the Union of South Africa.

In his report to the government in 1931, later published (Swellengrebel *et al.*, 1931), Prof. Swellengrebel proposed control programmes for the areas affected by the epidemics. He suggested however, the omission from the programmes of some parts of KwaZulu-Natal which were already endemic for malaria. The reason for the exclusion, he argued, was because the communities resident in these localities had acquired some immunity due to repeated attack. He feared the immunity would be lost with the introduction of control programmes in these areas. The three districts included in these areas were Ingwavuma, Ubombo and Hlabisa (le Sueur *et al.*, 1993). These control strategies were later applied in the epidemics of 1932 in KwaZulu-Natal. Both larval and adult mosquito control measures were instituted. Paris green (copper aceto-arsenite) and oil were used for the control of larvae of mosquitoes (De Meillon, 1936).

Pyrethrum was later introduced in 1934 as an intra-domiciliary knock-down insecticide. Spraying was repeated weekly during the main transmission season. Environmental sanitation also formed part of control strategy and together these yielded significant results (De Meillon, 1936; le Sueur *et al.*, 1993). Pyrethrum was replaced by DDT in 1946 both for larviciding and

house-spraying. Anti-larval measures were abandoned in 1956. In 1988 anti-larval measures were re-introduced and Temephos (emulsifiable concentrate) has been used as a larvicide since then to supplement house-spraying by DDT in Mamfene irrigation scheme on the Makathini flats, northern KwaZulu-Natal (Sharp *et al.*, 1990; le Sueur *et al.*, 1993).

## 1.8. The distribution of mosquitoes of the *Anopheles gambiae* group in sub-saharan Africa

The breeding pattern of *An. gambiae* group of species is dependent on suitable breeding habitats. Zahar (1985) in highlighting literature from the early 1950s classified habitats as temporary and permanent water bodies. Temporary water-bodies were more productive than permanent ones as they contained fresh water and were open to the sun which created an abling environment for the breeding requirements for the *An. gambiae* group of species (Blacklock and Evans, 1926; Gillies and De Meillon, 1968; Gillies and Coetzee, 1987; le Sueur and Sharp, 1988).

Six member species of the *An. gambiae* Giles group, namely; *An. gambiae* sensu stricto (Giles), *An. arabiensis* (Patton), *An. merus* (Dönitz), *An. melas* (Theobald), *An. qudriannulatus* (Theobald) and *An. bwambae* (White) occur in Afro-tropical Africa and in the adjacent oceanic islands. While *An. melas* occurs exclusively along the coastline of West Africa, its eastern and southern salt water breeding counterpart, *An. merus*, breeds in the brackish water along the eastern and southern African coast (Muirhead-Thomson, 1951; Gillies and De Meillon, 1968; Mosha and Mutero, 1982; Mosha and Petrarca, 1983). It also occurs further inland in association with salt pans in some localities, for

example in Zimbabwe (Muspratt and Henning, 1983), Swaziland (Paterson *et al.*, 1964), Tanzania (Mnzava and Kilama, 1986) and South Africa (Sharp, 1983; Gillies and Coetzee, 1987; le Sueur and Sharp, 1988; Coetzee *et al.*, 1993).

*An. quadriannulatus* seem to exhibit a discontinuous distribution with confirmed identifications from Zimbabwe, Mozambique, South Africa, Zanzibar and Ethiopia (Sharp *et al.*, 1984; Gillies and Coetzee, 1987; le Sueur and Sharp, 1988; Coetzee *et al.*, 1993). *An. bwambae*, another member of the group, has only been identified from the forest zones of the great Rift Valley between Zaïre and Uganda where geothermal water is found (White, 1973; Gillies and Coetzee, 1987). It is thought to be a vector among the local community of the Bambute tribe (Davidson and Hunt, 1973; Service, 1985).

With the exception of *An. quadriannulatus* which is strictly zoophilic, the three other members of the *An. gambiae* group, namely; *An. melas*, *An merus* and *An. bwambae*, bite man and have been documented as having low malaria vectorial capacities (Coluzzi, 1984; White, 1973; 1985). *An. gambiae* and *An. arabiensis*, the two principal malaria vectors in afro-tropical region, have a much wider distribution than the other members of the group (Petrarca *et al.*, 1987; 1991; Ralisoa-Randrianasolo and Coluzzi, 1987; Coetzee et *al.*, 1993).

### 1.9. *Anopheles gambiae* breeding and human activity

The breeding habits of the *An. gambiae* group of species is changing somewhat as they adapt to new types of water bodies created by human activities, particularly agriculture (FAO, 1984; Gillies and Coetzee, 1987; Service, 1991).

The presence of *An. gambiae* group has been recorded in unusual habitats, for example it has been collected in deep wells especially during the dry season when these are the only water sources. These occurrences have been observed in various localities, such as Aden (Patton, 1905), Mozambique (De Meillon, 1938), Tanganyika (Phipps, 1951) and in the former British Somaliland (Lovett, 1947). De Meillon (1938) attributed this tendency of *An. gambiae* to breed in unusual habitats to absence of suitable breeding sites. He suggested that the possibility of larvae found in wells were the results of overflow from the habitual breeding sites due to overcrowding. Causey *et al.* (1943) also attributed the presence of larvae of *An. gambiae* in unusual breeding sites to overflow.

The primary concern therefore is the ease with which some member species of the group readily colonize the new types of habitats. Many ecological changes have occurred as a result of human settlements and agricultural activities. These include the destruction of forests and the introduction of new methods of farming with the aim of producing more food (Holstein, 1954; FAO ,1984; Service, 1991). One result has been the creation of numerous ephemeral pools where nuisance mosquitoes and those of medical importance can breed.

Studying the breeding habitats for *An. gambiae* group of species Holstein (1954) noted:

"... an essential fact on which entomologists are agreed should be born in mind namely that the pullulation (profuse breeding) of this *Anopheles* spp is closely bound up with human activity..., so that the increase in the incidence of malaria in the region is directly correlated with an intensive development of agricultural

work".

His contention was that important breeding sites for *An. gambiae* group of species are provided by badly planned or poorly constructed agricultural drainage works.

**2.0 Irrigation systems and mosquito breeding**

By 1986 about 270 million ha of land was under irrigation worldwide and between 65-70% of this was in developing countries (Service, 1991). More than 95% of rice grown in developing countries is by irrigation. Service (1991) noted that there have been marked increases in mosquito populations as a result of the rice schemes of both Africa and Asia. A similar occurrence was observed after the introduction of agricultural development at Mamfene, northern KwaZulu-Natal (Sharp *et al.*, 1984; le Sueur and Sharp, 1988).

The introduction of irrigation is often associated with an increase of mosquito population, especially the fresh water breeders and vectors of human malaria namely, *An. gambiae* and *An. arabiensis* (Surtees *et al.*, 1970; Service, 1977; 1989; FAO, 1984; Sharp *et al.*, 1984). Although *An. gambiae* and *An. arabiensis* occur sympatrically over much of their range, the latter is a more successful colonizer in the savanna areas where most of the rice irrigation schemes are located (Service, 1985). Studies done in Kisumu area of Kenya did not detect any significant differences in larval habitats between the two species (Service, 1970). The paucity of catches of adult *An. gambiae* in huts in localities where the two species of the group are sympatric, is perhaps due to its endophilic and anthropophagic behaviour and its resultant susceptibility to house-spraying with residual insecticides.

*An. arabiensis* exhibits a more varied resting and feeding behaviour and this makes it a major health problem which is difficult to control (White, 1974; Service, 1982; Petrarca, *et al.*, 1987; Sharp, 1990).

In Burkina Faso two variants of *An. gambiae* co-existed in the Kou Valley namely, the *mopti* and *savannah* sub-groups. During the biting peaks in the rice irrigation scheme, lower infectivity rates have been recorded than the non-irrigated areas (Roberts *et al.*, 1985). It was established that the *mopti* sub-group which was the dominant of the two in the Kou Valley had a lower infectivity rate than the *savannah* variant (Coluzzi *et al.*, 1985).This situation could have been at least partly responsible for the lower infectivity rates in the irrigation scheme. This phenomenon could also have been a function of high larval density in some breeding sites which produce weak and short lived adults and which are therefore unable to take part in transmission (le Sueur 1995, pers. comm).

In contrast, studies by Coosemans *et al.* (1989) in the Ruzizi valley irrigation scheme in Burundi, observed an increase in malaria transmission coupled with a vectorial capacity 150 times greater than in a nearby cotton-growing area. A study by Service (1977) at Ahero irrigation scheme Kisumu , Kenya, also showed paddy rice pools were more productive in terms of larval mosquito density than naturally occurring pools in the same area.

## 2.1. Malaria vectors breeding in Mamfene, northern KwaZulu-Natal

The malaria vectors, *An. gambiae* group of species, breed in two different types of environments in Mamfene irrigation scheme, namely, (i) irrigation and tap water spillage and (ii) naturally occurring water bodies. The former types of environments occur as a result of irrigation systems while the latter are due to inundation by the seasonal summer rains between January and June every year. The irrigation spillage has also created a permanent wetland, the Balamhlanga swamp. This swamp used to fill only during the rainy season. It is located within section eight of the Mamfene irrigation scheme and together with the spillage from water taps and irrigation canals is a major source of mosquito breeding habitats (le Sueur and Sharp, 1988).

The Mamfene irrigation scheme is situated on the Makathini flats in Maputaland which is close to the southern limits of the distribution of the *An. gambiae* group (Figure 1.2). Only three species of the group occur here namely; *An. quadriannulatus*, *An. arabiensis* and *An. merus* (Sharp *et al.*, 1984; Gillies and Coetzee, 1987; le Sueur and Sharp, 1988; Coetzee, *et al.*, 1993). *An. merus* is found predominantly in association with exposed marine Cretaceous deposits or with saline marsh areas (le Sueur and Sharp, 1988). In Maputaland, these deposits are exposed at several points and as a result are the saline waterbodies in the region (Sharp *et al.*, 1984; le Sueur and Sharp, 1988).

The breeding sites for the two fresh water-breeding members of the group, *An. arabiensis* and *An. quadriannulatus*, are predominantly man-made in Mamfene. This occurrence was also observed by De Meillon (1937) who also noted that the breeding habitats for malaria vectors in northern KwaZulu-Natal

were largely due to soil erosion caused by overstocking by cattle. Keeping cattle is a strong feature of Zulu culture and the sentiments expressed by Dr De Meillon 58 years ago may still be true today.

## 2.2. Motivation and rationale

The Mamfene area of northern KwaZulu/Natal is endemic for malaria. Present control measures include both parasite and vector control. Rigorous control measures have however been in place for the last five decades (le Sueur *et al.*, 1994), and yet malaria is still a public health problem in the area. Among the factors responsible for this quandary include parasite resistance to alternative anti-malarial drugs (Freese *et al.*, 1988; Ngxongo, 1994), hut-leaving behaviour of the vectors (Sharp *et al.*, 1990) and the increased breeding in irrigation spillage in winter of the local vectors due to agricultural developments in Mamfene (Sharp *et al.*, 1984; le Sueur & Sharp, 1988).

For these reasons it has become necessary to re-evaluate current control measures and this includes targeting of the winter breeding sites for larviciding. Data from le Sueur *et al.* (1992) suggest that these habitats are the main radiation points at the onset of summer rains each season. In addition to winter breeding sites, the identification of breeding sites in general would be useful especially in re-directing control strategies based on larviciding. Larviciding is a difficult measure to carry out especially in areas such as Mamfene where breeding sites are not easy to locate but waterbodies are numerous (le Sueur *et al.*, 1994). Vector control is a daunting task when an adult vector population bites indoors and avoids insecticide-sprayed surfaces by leaving to rest outdoors before oviposition.

Attempts were made by Sharp *et al.*, (1984), le Sueur & Sharp (1988) and le Sueur (1991) to study the biology and breeding pattern of malaria vectors in relation to transmission in the agricultural irrigated area of Mamfene. However,

very little was known about the role of different types of breeding sites in the transmission of malaria. This was attributed to the lack of a means by which spatial data could be geo-referenced for analysis (le Sueur *et al.*, 1994).

The development and use of GIS with reference to Mamfene malaria control in recent years has made this feasible (le Sueur *et al.*, 1994; Stuttaford, 1994). The **aim** of this study was to locate and compare the malaria vector breeding sites, to study the spatial distribution of the adult mosquitoes and see how they relate to malaria transmission in Mamfene irrigation scheme using GPS and PC based GIS software.

## 2.2. Broad objectives

1. To estimate the productivity of agricultural spillage and other waterbodies in terms of malaria vector populations in Mamfene.
2. To determine dispersal pattern of adult mosquitoes in the area in relation to breeding sites using GIS.
3. To determine the effects of agricultural developments on vector mosquito populations.

## 2.3. Specific objectives

1. To estimate larval densities in irrigation spillage and other types of waterbodies located using GIS mosquito population data.
2. To identify mosquito vectors collected from different types of waterbodies using two different techniques (taxonomy and Polymerase Chain Reaction [PCR]).
3. To indirectly quantify mosquito emergence from different types of breeding

sites using sentinel surveillance (trapping) points located within 2 km radius buffer zones around the breeding sites.

4. To investigate mosquito distribution spatially using the GIS.

5. To study effects of the biotic and abiotic factors on larval abundance and distribution in the breeding sites.

6. To study the dispersal pattern of adult mosquitoes from breeding habitats over "time" and "space".

# CHAPTER TWO

# MATERIALS AND METHODS

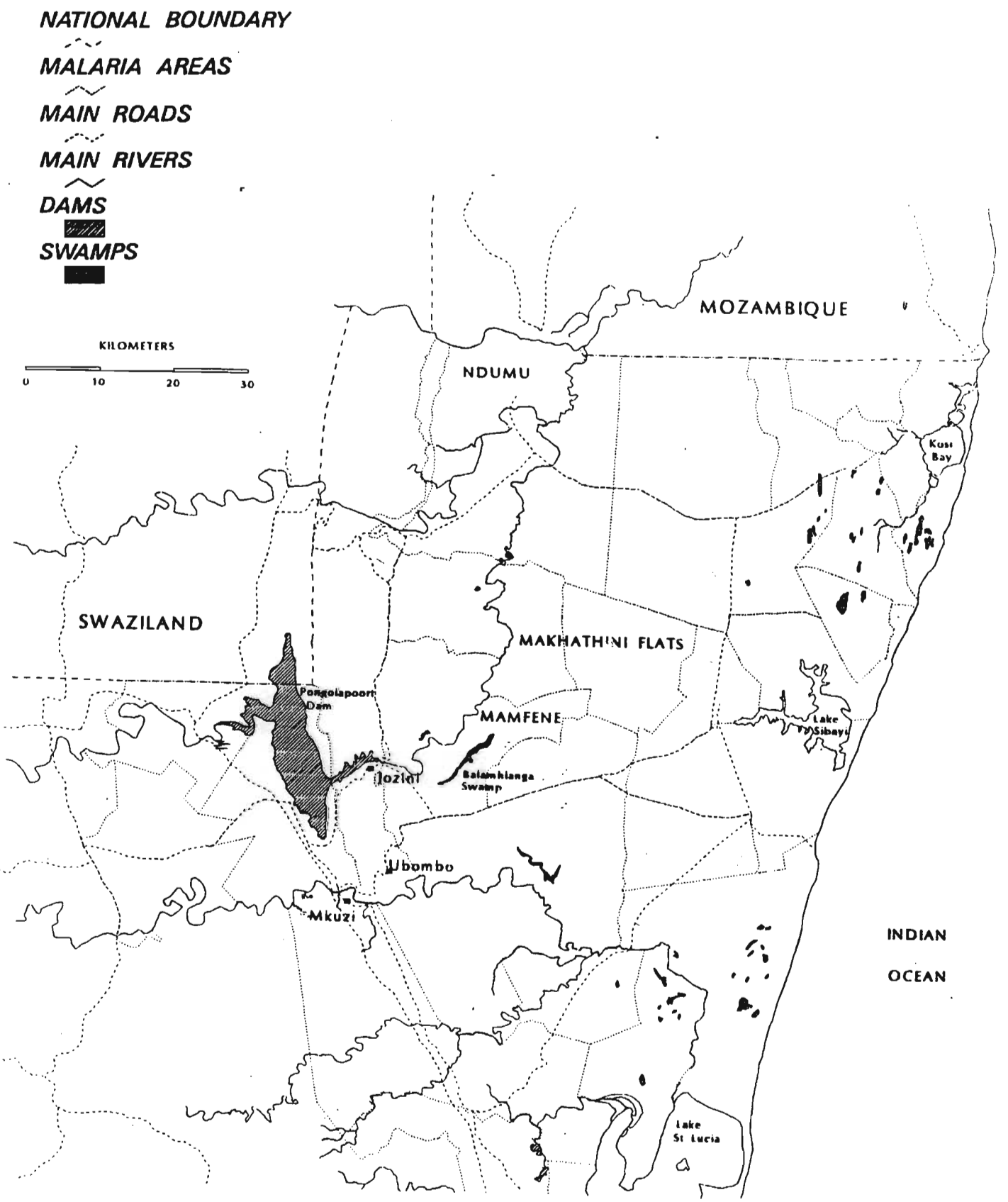
## 2.1 The study area

### 2.1.1 Introduction

Mamfene irrigation scheme on the Makhathini flats is located east of the Lebombo mountains in Ubombo district, northern KwaZulu-Natal (32° E to the Indian Ocean; 26° 50' S to 27° 50' S). The Ubombo and Ingwavuma magisterial districts comprise the area known as Maputaland which is bound by Mozambique in the north, Lake St. Lucia in the south, Swaziland in the west and the Indian Ocean in the east (Figure 2.1). Most of the Lebombo mountain region is characterised by acacia vegetation interspersed by exposed hard core rock and patches of green vegetation suitable for range agriculture. In the low lands to the east, the vegetation changes to that of savannah with low shrub and occasional tall canopy typical of a tropical climate (Furness and Breen, 1980).

The Makhathini flats lies on the alluvial floodplain of the Pongolo river which flows through the Jozini dam in the Lebombo mountains. A rapid change of the river's gradient from 7.5 in 3000 (metres) to a slope of 1 in 3000 after the dam results in a decrease in flow rate. The river flows northwards over the plain until it joins the Usuthu river on the Mozambican border, and on to Maputo bay on the Indian Ocean. As a result, the Pongolo River leaves in its wake numerous seasonal pans (about 90) within a 13 000 ha floodplain (le Sueur 1991; Stuttaford, 1994). These pans play an important role in the breeding pattern of *An. gambiae* group of mosquito species in the region.

**Fig. 2.1.** Maputaland and Makhathini Flats where Mamfene is located (This figure was taken from the thesis of Maria C Stuttaford [1994]).




National Malaria Research Programme, MRC. 1994

### 2.1.2 Soil types, agricultural farming methods and the climate

Alluvial black cotton and sandy loam with patches of red soils are found on most parts of the alluvial plain. These soil types are suitable for farming. Before the irrigation system was established in 1972, subsistence farming was probably the main occupation on what used to be ancestral pasture land (De Meillon 1937; Furness and Breen, 1980). Most families practice mixed farming methods. They grow both subsistence and cash crops and keep livestock. Cattle, sheep and goats can be seen in most homes. Rainfall is expected in summer, but the exact months when it comes vary each year. Recent trends have shown that most rain falls between January and June with some early falls expected in October/November (le Sueur *et al.*, 1992).

Literature cited by le Sueur and Sharp (1988) indicates that the Lebombo mountain region has a largely tropical biota as a result of its low lying topography, some 75 m above mean sea level (Furness and Breen, 1980), and the southward flowing warm Mozambique and Aqulhas currents. The region falls within the 18°C Effective Temperature (ET) isoline, a fact which confirms its position as being on the peripheral boundary of a tropical climate (Sharp, 1983).

### 2.1.3 *An. gambiae* group distribution in the Lebombo mountain region

The Lebombo mountain region in northern KwaZulu-Natal lies on the southernmost limit of *An. gambiae* group of species distribution. Only three species of the group occur here, namely *An. quadriannulatus*, *An. arabiensis* and *An. merus* (Sharp *et al.*, 1984; Gillies and Coetzee, 1987; le Sueur and Sharp, 1988; Coetzee *et al.*, 1993). While two members of the group, *An.*

*quadriannulatus* and *An. arabiensis*, are fresh water breeders, *An. merus* is found predominantly in association with exposed marine Cretaceous deposits or with saline marsh areas (le Sueur, 1991). In Maputaland these deposits are exposed at several points the result of which is the saline nature of many water bodies in the region (Sharp, 1984; le Sueur and Sharp, 1988).

## 2.2 Study design

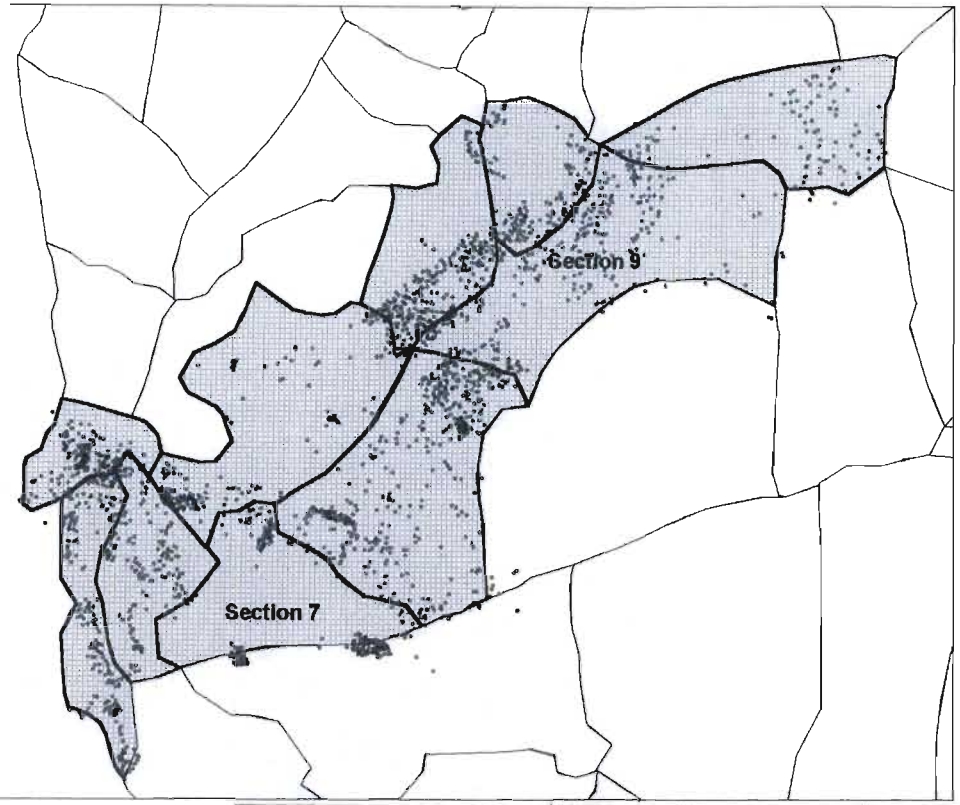
### 2.2.1 Baseline data on malaria vector larvae

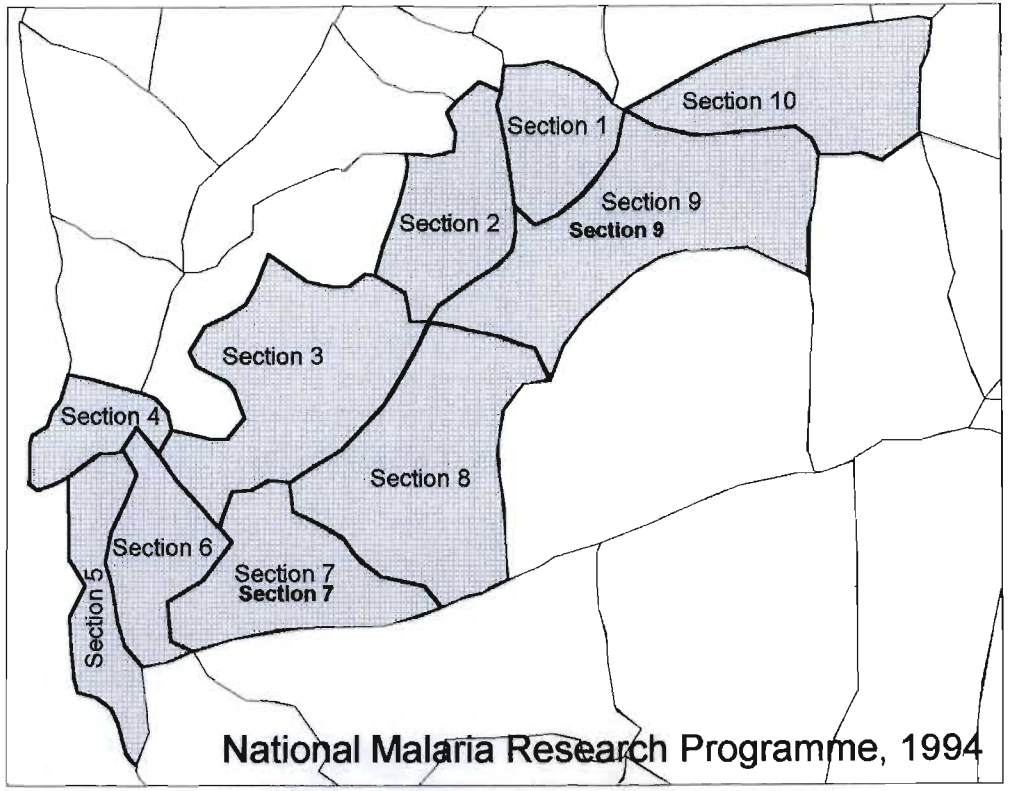
Baseline data (Appendix 1a-c) were collected between September and November 1994 to assess the productivity of winter breeding sites which constitute the main radiation points at the onset of summer rains (le Sueur *et al.*, 1992). The main project was carried out in summer, from January to May 1995, the time during which rainfall was expected in the Lebombo mountain region.

### 2.2.2 Larval sampling and data processing

#### 2.2.2.1 Selection of breeding sites

For malaria control purposes, the Mamfene area comprises 10 sections out of which section no. 8 was selected for this study. This section had the highest number of mosquito vectors during the 1993 malaria season as compared to other sections within Mamfene for the same period (Fig. 2.2). It includes the irrigation spillage from the adjacent irrigation scheme and the Balamhlanga swamp. This swamp used to fill only during the rainy season but now contains water all year round, though sometimes intermittently, due to irrigation spillage. As a result, breeding habitats occur in the area in both winter and summer and the malaria vectors are able to breed in them all year round.




| Owner | Area | Section | Houseno | Facility | Longitude | Latitude | Maltotal |
|---|---|---|---|---|---|---|---|
| MOURICE NXUMALO | MAMFENE | 2 | 256 | | 32.1908 | -27.4033 | 0 |
| DLIWAYO GUMEDE | MAMFENE | 2 | 257 | | 32.192 | -27.4039 | 1 |
| GEORGE MAKHANYA | MAMFENE | 2 | 258 | | 32.1928 | -27.4034 | 0 |
| JOSEPH GWALA | MAMFENE | 2 | 259 | | 32.1916 | -27.4033 | 0 |
| VICTOR MAHLOBO | MAMFENE | 2 | 260 | | 32.1934 | -27.4029 | 0 |
| ELLIAS NKOSI | MAMFENE | 2 | 261 | | 32.1924 | -27.4018 | 0 |
| CALALINI NTSHANGASE | MAMFENE | 2 | 262 | | 32.194 | -27.4028 | 0 |
| MSESHI MABAASO | MAMFENE | 2 | 263 | | 32.1944 | -27.4037 | 0 |
| THEMBISA STORE | MAMFENE | 2 | 264 | SHOP | 32.1951 | -27.4034 | 0 |
| MABASO'S COMPOUND | MAMFENE | 2 | 265 | COMPOUND | 32.1948 | -27.4028 | 0 |
| FANO MKHABELA | MAMFENE | 2 | 266 | | 32.1945 | -27.4024 | 0 |
| BETHUEL BUTHELEZI | MAMFENE | 2 | 267 | | 32.1957 | -27.4018 | 0 |
| ANTHONY GUMBI | MAMFENE | 2 | 269 | | 32.1963 | -27.4007 | 1 |
| CLOPHAS SITHOLE | MAMFENE | 2 | 270 | | 32.1972 | -27.4017 | 0 |

Fig. 2.2. Map of Mamfene malaria control area, showing division into sections and distribution of homesteads. Section 8 was selected for the study. A sample of the household geo-linked database is shown.

Larval sampling sites were selected using a Geographic Information System (GIS-MapInfo). Potential breeding sites in Mamfene section eight were identified on ortho-photo maps (1:10 000). These sites were located on the ground in the study area by means of a hand-held path finder Global Positioning System (GPS, Ensign model, Trimble Co. Ltd) (Plate 2.1). The digital coordinates of the breeding sites were databased in dbaseIV, imported into MapInfo (vector based) and overlaid on physical features digitised from ortho-photos.

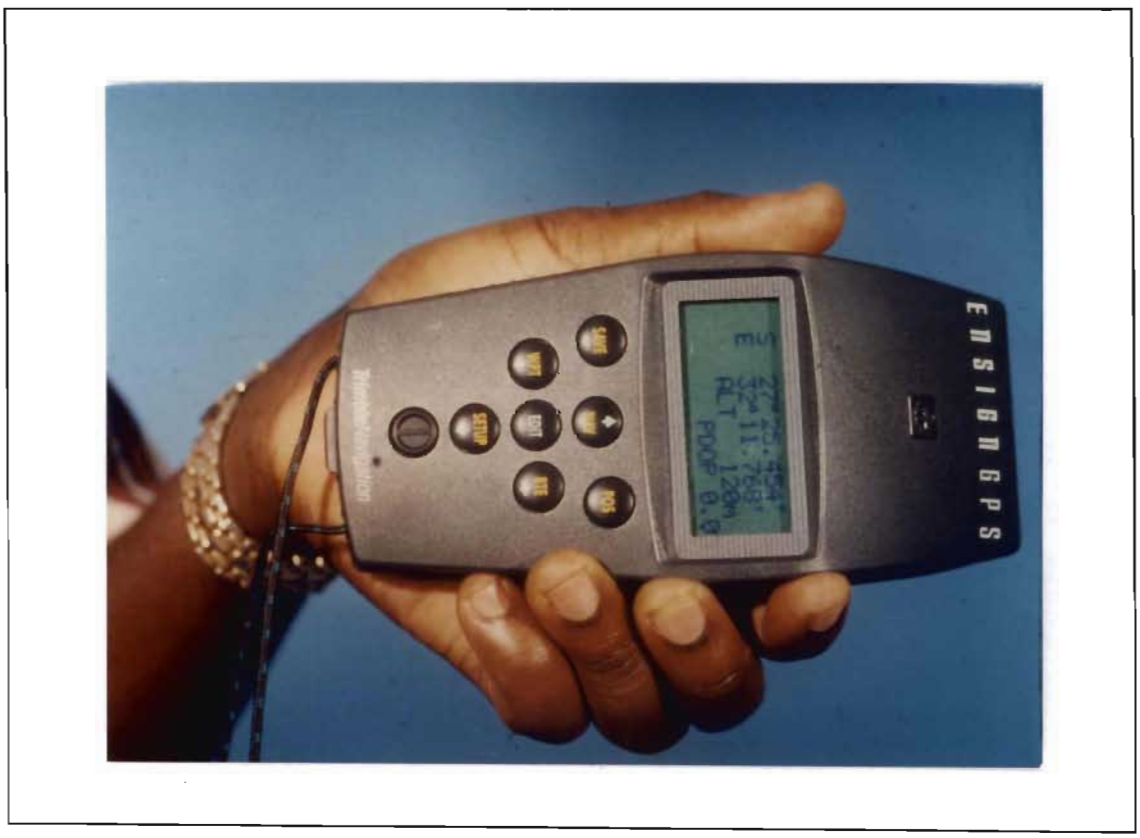



**Plate 2.1.** Global Positioning System (GPS) unit used in locating and taking digital coordinates of the study sites.

The larval sampling sites were selected around homes for which high numbers of adult mosquitoes were recorded in 1993 (Sharp *et al.*, 1993, unpublished). Figure 2.3 shows the mosquito population and distribution in Mamfene during the 1993 malaria transmission season. Two sampling sites each were selected in eight different localities in Mamfene section 8. These habitats included: tap pools, irrigation spillage within and outside but adjacent to the agricultural land, ground depression pools in the Balamhlanga swamp and elsewhere inland and road side pools as a result of rainfall.

### 2.2.2.2 Types of breeding sites

**The breeding sites were categorised into four main types**:

Type I was tap-water pools created by defective taps and inadequate provision for drainage of water spilled by the community during use. These taps are communal and cattle also frequent them, mainly in winter and during summer dry periods when they are the only source of water. Resultant foot and hoof prints were utilised by the malaria vectors for breeding. Vegetation cover was emergent with occasional algae blooms.

Type II breeding sites were drainage systems into which excess water drained from the agricultural land. Pools created by cattle and tyre tracks were numerous and were also utilised by malaria vectors for breeding both in winter and in early summer. Vector mosquitoes were found in association with emergent vegetation at these sites.

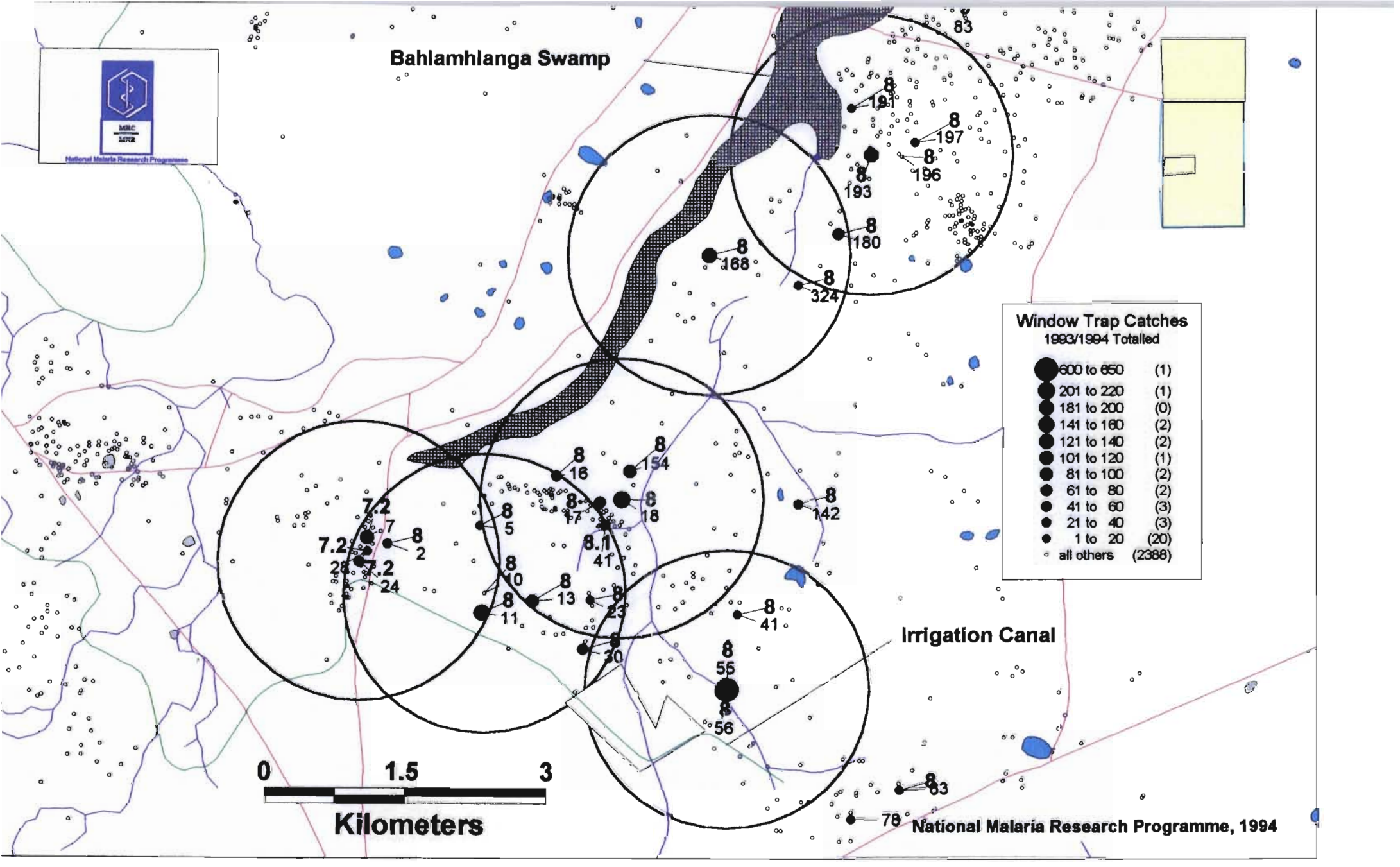



**Fig. 2.3. Mosquito Sentinel Surveillance Points for Hazard Points Created by the irrigation scheme.**

**(Composite Data for 1993 and 1994 Window Trap Catches)**

Type III was irrigation spillage outside but adjacent to agricultural land. These habitats were the first contact for mosquitoes radiating from winter breeding sites. These sites were considered the initial radiation points from which malaria vectors spread with the onset of summer rains. Larvae were found in association with emergent vegetation and algae blooms at several points.

Type IV was depressions in the Balamhlanga swamp and elsewhere inland and roadside pools which occurred as a result of seasonal rains or water spillage. This type of habitats was scarce because rainfall was irregular.

### 2.2.3 Breeding site characteristics

The breeding site characteristics determined were the size of the waterbody and the biotic and abiotic factors that were thought likely to influence larval densities and distribution. The months when breeding commenced and ceased were also recorded for each site.

#### 2.2.3.1 Size of the breeding site and sampling time

The measurement of the water surface to be sampled was done for each site by means of a graduated one-metre rod and expressed in square metres ($m^2$). This was tabulated and compared to the number of larvae collected. The time taken to sample larvae at each site was recorded and used to calculate larval density collected per man-hour.

#### 2.2.3.2 Biotic and abiotic factors

Predators collected during larval collection were identified to genus only. These were counted and recorded according to the site where the collection

was done.

Abiotic factors determined included temperatures, substratum type and water quality. Temperatures were taken by means of an alcohol-in-glass thermometer inserted for five minutes in the pool at the beginning of each larval collection. The type of vegetation of each breeding site was recorded as: none, creeping weed, emergent, emergent with algae bloom or dense. Substratum type was also recorded, e.g. the presence of rotting vegetation, mud or sand. These records were entered at the start of every larval collection in each habitat throughout the study period.

### 2.2.4 Mosquito larval collections

Larval densities were determined in the irrigation spillage pools, tyre tracks, tap water pools and in other water bodies (for example, depressions) within Mamfene section 8. Collections were carried out in each of the selected breeding sites according to the method described by Service (1976). A soup ladle (12.0 cm diameter) with its inner portion painted white for easy visibility of larvae was used. An average of 20 ladles full of water was put into a five-litre plastic container (Plate 2.2). Larvae collected were immediately taken to Medical Research Council (MRC) field laboratory in Jozini where the initial processing was done.

### 2.2.5 Larval processing in the laboratory

Larval instars were separated and recorded according to the collection site. A sub-sample of these larvae were reared in the laboratory at Jozini to adult stage and identified using Gillies and De Meillon (1968) and Gillies and Coetzee

(1987). Mosquitoes identified as belonging to the *An. gambiae* group were preserved in 75 µℓ vials containing iso-propanol for later identification into species by means of the Polymerase Chain Reaction (PCR) method (Paskewitz and Collins 1990, Bredenkamp and Sharp 1993) at the MRC laboratory in Durban.

**Plate 2.2.** Sampling larvae at a breeding habitat. Some of the equipment utilised in this study are shown.

Attempts were made to have equal numbers of larvae from each site processed for both rearing and PCR identification respectively. This was not possible as some breeding sites had too few larvae and the risk existed of losing them while trying to rear them in captivity. Where larvae were less than 10, all were

preserved in iso-propanol for PCR identification for species of the *An. gambiae* group. This option had the disadvantage of not allowing for the identification of other anophelines but a sub-sample of larvae reared to adulthood in captivity afforded the investigator the opportunity to identify the other *Anopheles* spp breeding in the same sites as the *An. gambiae* group. Where culicine larvae were found to breed in sympatry with anophelines, the stages and total numbers were recorded and the larvae discarded.

## 2.3 Adult mosquito collections and processing

### 2.3.1 Selection of houses for adult mosquito collections

Productivity in the breeding habitats was indirectly determined by the numbers of adult mosquitoes collected in the sentinel homes and other homes in the immediate vicinity of the breeding sites. The study houses were selected within 2 km buffers of the larval collection sites. Sentinel houses utilised by Sharp *et al.* (1993, unpublished) during the 1993 transmission season which were located within two kilometre buffers were used. These houses were chosen from ortho-photo maps (1:10 000) and were located aid of by GPS in the field.

The digital coordinates of the houses selected were databased in dbaseIV, imported into MapInfo (vector based) and overlaid on physical features digitised from ortho-photos (Figure 2.4). Where additional houses were required, GPS was used to position them so that the spatial distribution of the mosquito population could be assessed relative to breeding sites. A cluster of 3 houses were selected within 2 km buffer zone around each type of breeding sites. A total of 24 houses were used in this study.

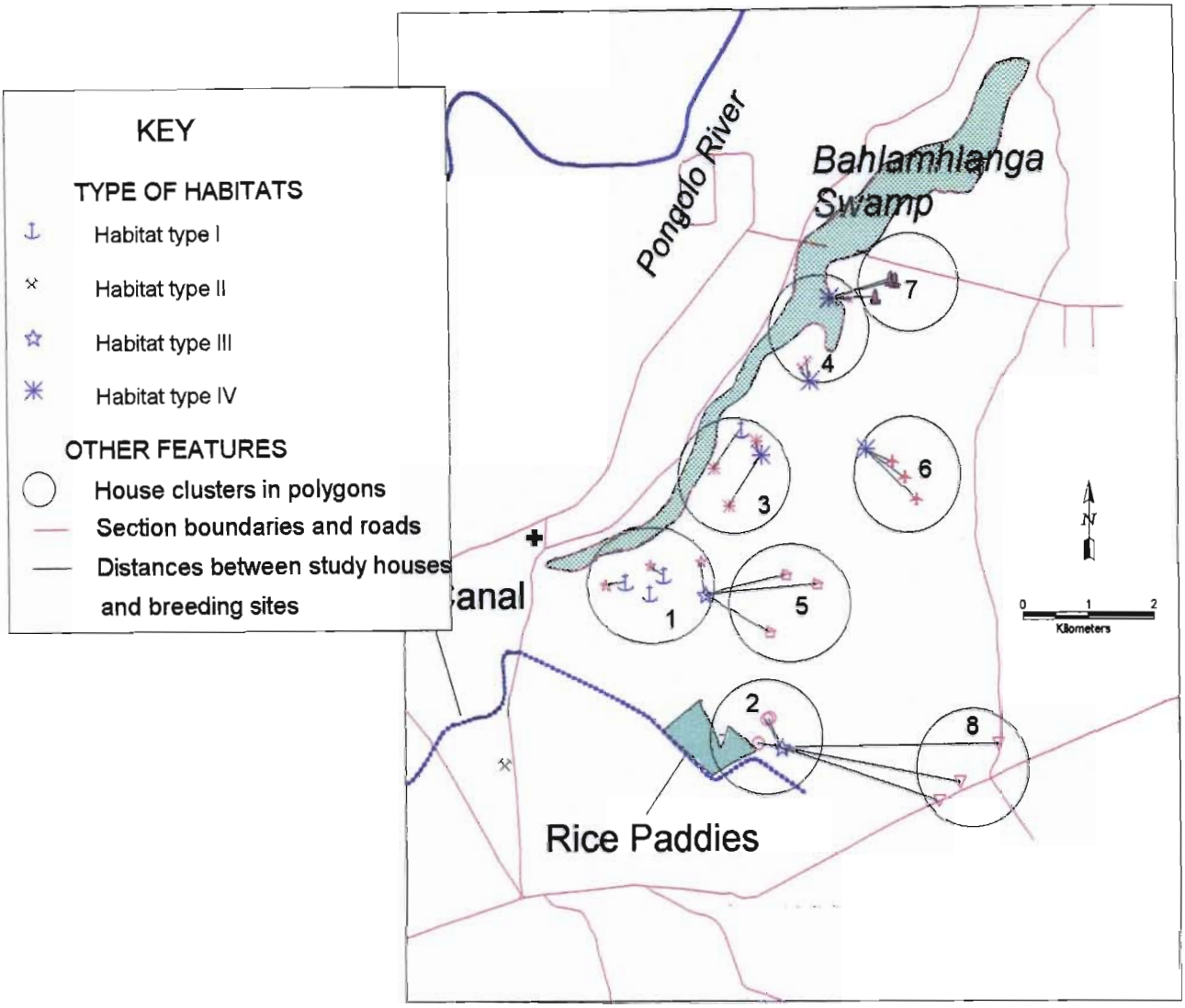



Fig. 2.4: Mamfene section 8 showing distance links between breeding sites and study houses (in polygons). A sample of geo-linked database between households and the breeding sites is shown.

| Area | Sectio | House9 | Long | Lat | distance |
|---|---|---|---|---|---|
| MAMFENE | 8 | 8 | 32.1635000 | -27.4520278 | 0.30 |
| MAMFENE | 8 | 16 | 32.1704444 | -27.4493333 | 0.24 |
| MAMFENE | 8 | 55 | 32.1888333 | -27.4700278 | 0.48 |
| MAMFENE | 8 | 56 | 32.1883889 | -27.4702500 | 0.48 |
| MAMFENE | 8 | 56B | 32.1870278 | -27.4734722 | 0.37 |
| MAMFENE | 8 | 92 | 32.2182778 | -27.4788056 | 2.75 |
| MAMFENE | 8 | 99 | 32.2242222 | -27.4737222 | 3.30 |
| MAMFENE | 8 | 122 | 32.2118333 | -27.4406667 | 1.06 |
| MAMFENE | 8 | 127A | 32.2100278 | -27.4377222 | 0.70 |
| MAMFENE | 8 | 128A | 32.2081389 | -27.4356389 | 0.43 |
| MAMFENE | 8 | 142 | 32.1963611 | -27.4520556 | 1.71 |
| MAMFENE | 8 | 143 | 32.1916111 | -27.4507500 | 1.28 |
| MAMFENE | 8 | 149 | 32.1890000 | -27.4584722 | 1.14 |
| MAMFENE | 8 | 154 | 32.1783333 | -27.4488889 | 0.50 |
| MAMFENE | 8 | 157 | 32.1828056 | -27.4414722 | 0.90 |
| MAMFENE | 8 | 161 | 32.1805000 | -27.4364167 | 0.72 |
| MAMFENE | 8 | 168B | 32.1871667 | -27.4328333 | 0.22 |
| MAMFENE | 8 | 175B | 32.1942222 | -27.4229722 | 0.24 |
| MAMFENE | 8 | 177 | 32.1952500 | -27.4219722 | 0.32 |
| MAMFENE | 8 | 191 | 32.2019444 | -27.4140000 | 0.32 |
| MAMFENE | 8 | 245 | 32.2089444 | -27.4114444 | 1.04 |
| MAMFENE | 8 | 248 | 32.2082222 | -27.4112222 | 0.98 |
| MAMFENE | 8 | 262 | 32.2058611 | -27.4136111 | 0.70 |

### 2.3.2 Adult mosquito collections in the field

The adult mosquito collections were carried out using exit window traps of the Muirhead - Thomson (1947) design modified by Sharp (1990). Plate 2.3 shows an exit trap fitted to a window. The traps were emptied each morning between 6.00 am and 8.00 am each day. This timing was necessary to avoid mortalities due to heat and unnecessary gonotrophic changes that would occur with the increasing temperature after 8.00 am. Mosquitoes collected were taken back to the field laboratory in Jozini where taxonomic identifications were done using the keys of Gillies and De Meillon (1968) and Gillies and Coetzee (1987).

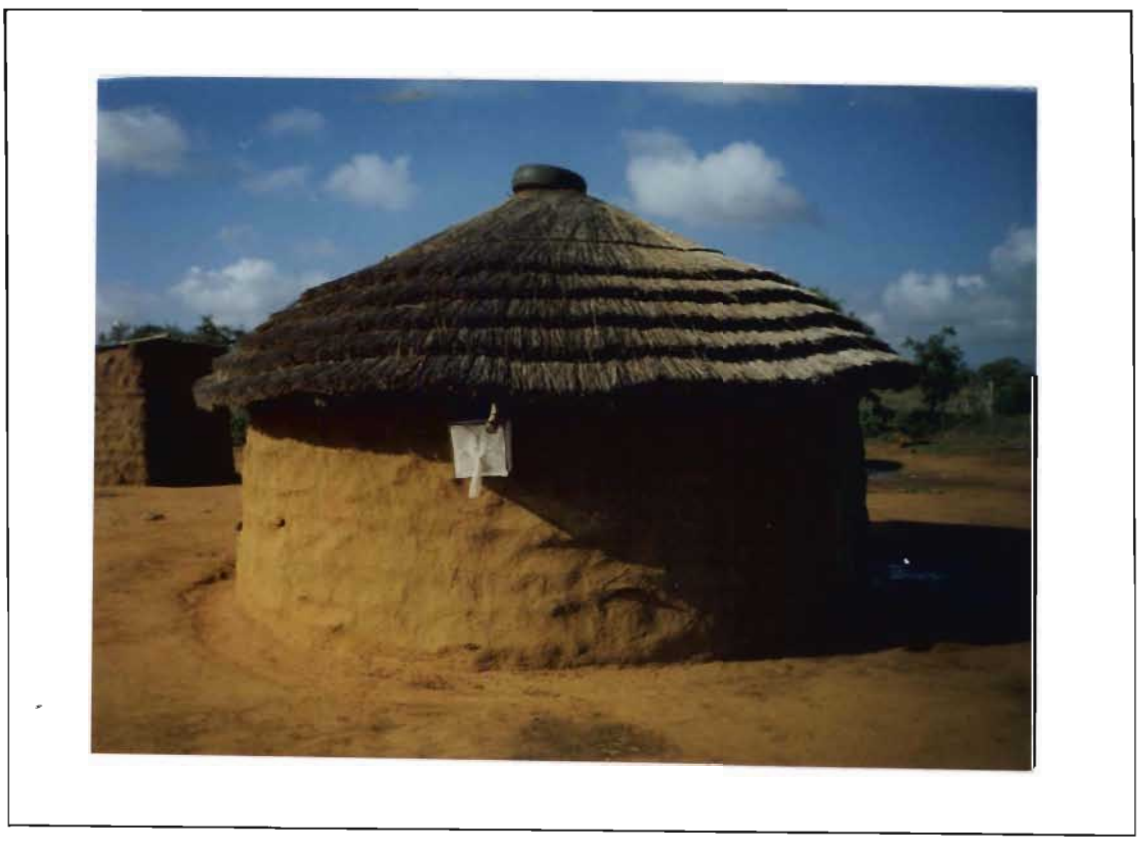


**Plate 2.3.** An exit trap fitted to a window in one of the study houses used in this study.

### 2.3.3 Processing of adult mosquitoes in the laboratory

Adult anophelines and culicines were separated in the laboratory. Anopheline adults caught were separated into males and females and abdominal conditions of the females scored as unfed, blood-fed, half-gravid and gravid. The total number of anopheline mosquitoes caught in each hut was recorded. Culicine adults collected in study houses were graded according to the abdominal status. The numbers of male and female culicines caught in each hut were recorded after which they were discarded.

### 2.3.4 Parity dissections

Parity dissections were carried out on sub-samples of unfed, blood fed and half gravid females of the *An. gambiae* group. These preparations were air dried and read under high-dry field magnification [x40] and scored for parity status at a later date. The remaining portions and the rest of the mosquitoes caught were preserved in iso-propanol for further identification to species level by the PCR method in Durban.

### 2.3.5 Species identification by the Polymerase Chain Reaction (PCR) technique

The polymerase chain reaction (PCR) is based on the enzymatic amplification of DNA fragments. This technique has been described by several workers, for example by Collins *et al.* (1987), Gale and Crampton (1987), Gale and Crampton (1988), White *et al.* (1989), Paskewitz and Collins (1990). More recently in South Africa, Bredenkamp and Sharp (1993) introduced slight modification because of different reaction components supplied by Promega

(Promega corporation, Madison, USA). The change introduced was the optimisation of temperatures at the annealing stage to 51°C instead of 30°C.

A portion of adult mosquito or a whole larva (first or second instar) was put in 300 µℓ or 20 µℓ of water in a pre-labelled vial respectively. Third and fourth stage larvae and pupae were processed as adult mosquitoes. The preparations were boiled for three minutes after which they were removed and each specimen was macerated with an Eppendorf tip in the vial.

One microlitre of the DNA sample was added to 24 µℓ of a master mix of reagents comprising of the following: 2.5 µℓ of a solution containing 50.0 nmol of each dATP, dCTP, dGTP and dTTP; 0.625 µℓ (20 ng $\mu\ell^{-1}$) of each of the primers; 0.125 µℓ (0.625 units) Taq DNA polymerase; 3.0 µℓ $MgCl_2$ (25 mM) and 13.2 µℓ distilled water. Reaction vials were placed in the thermal cycler (ESU Electronics, Cape Town [plate: 2.4]); and after initial denaturation at a temperature of 94°C for 1 min, the preparations were taken through a cycle of 94°C for 30 s, 51°C for 30 s and 72°C for 30 s, repeated 30 times. Amplified product (25 µℓ) was electrophoresed in 2% agarose gel (Molecular Biology Grade, Promega), dissolved in Tris-acetate (40 mm $l^{-1}$) buffer containing EDTA (2 mmol $l^{-1}$) and visualized over a Ultra-Violet transilluminator (Plate 2.5). Fragment size was estimated by comparison with size markers (pGEM, Promega).

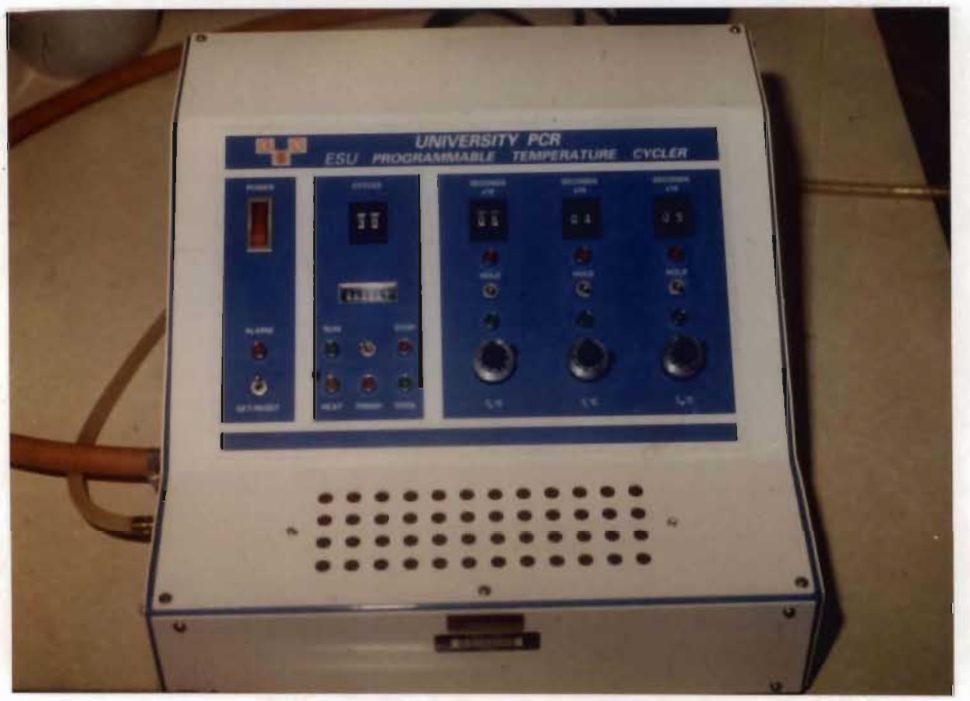



**Plate 2.4**. Locally manufactured (ESU Electronics Capetown) thermal cycler unit used for the identification of the *An. gambiae* group of species.

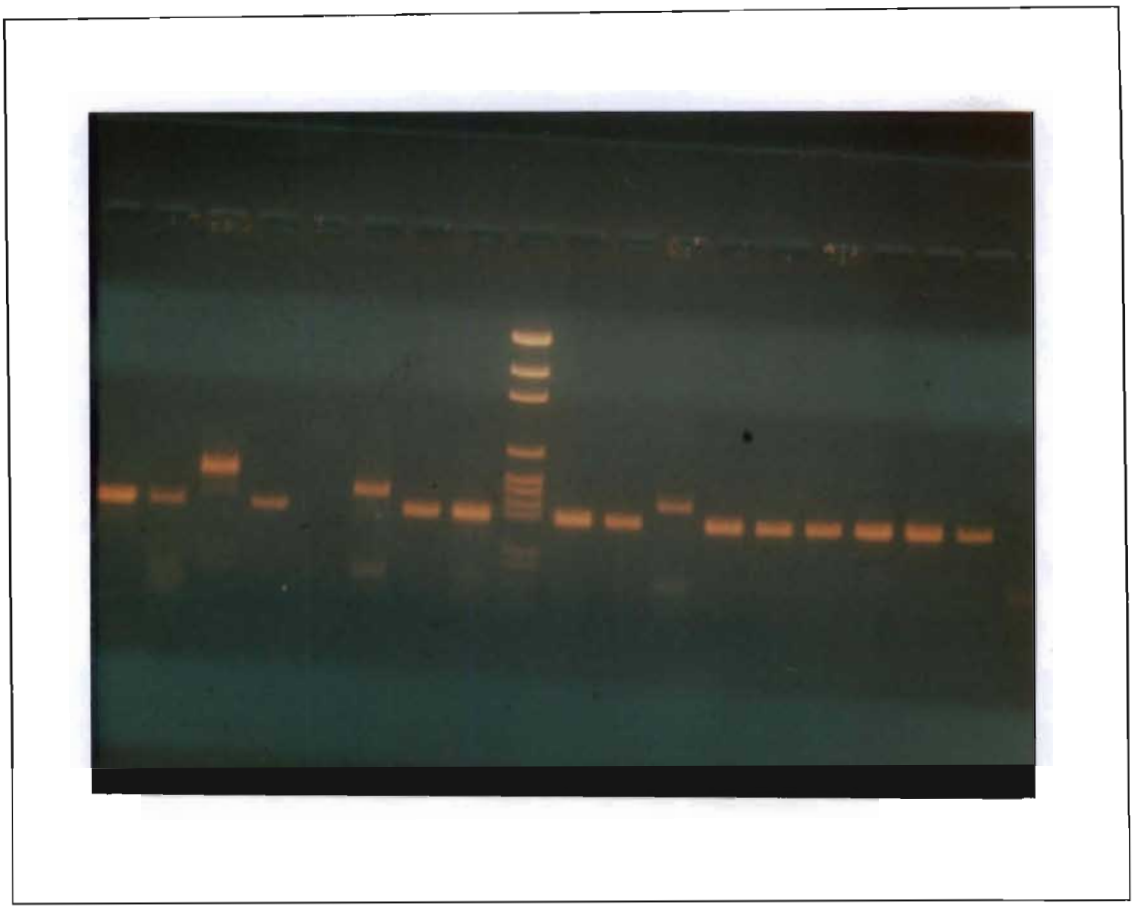


**Plate 2.5.** Electrophoresis of amplified DNA from field and known insectary specimens. Marker and negative control were identified lanes 10 and 20 respectively. *An. gambiae* ss were identified at lanes 7 and 13, *An. merus*are at lanes 1 and 4 while *An. arabiensis* were identified at lanes 2, 3, 5, 8, 9, 11, 12, 14-19. Lane 6 was a negative result.

### 2.3.6 Malaria cases for the study period (January - May 1995)

The malaria data reported in this study were taken from the KwaZulu/Natal Health department (courtesy of Mr. S M Ngxongo, Chief health inspector and the malaria coordinator KwaZulu/Natal).

# CHAPTER THREE

# RESULTS

# PART ONE

## 3.1 Mosquito larvae in four types of habitat

### 3.1.1 Mosquito breeding in each type of habitat

Mosquito larvae were collected in each of four types of habitat as described in chapter 2 section 2.2.2. Figure 3.1.1 shows the breeding pattern in each type of habitat where larval collections were done. Type II habitats were the only productive sites in January and February 1995 but breeding occurred in all habitats from March to April after which larvae were only recorded in habitat types III and IV in May. This was a reflection of the rainfall pattern in Mamfene during the study period. Figure 3.1.2 shows the monthly rainfall and larval abundance from January to May 1995.

Spearman correlation coefficient test was used to assess the association between monthly larval densities and the climatic variables, for example rainfall (previous month), mean maximum temperature and relative humidity. There was moderate correlations between previous month rainfall and monthly larval density ($r=0.46382$, $p=0.3542$). The correlations between monthly larval densities and temperature was weak ($r=-0.25714$, $p=0.6228$) while strong association between monthly larval densities and relative humidity was detected ($r=0.71429$, $p=0.1108$).

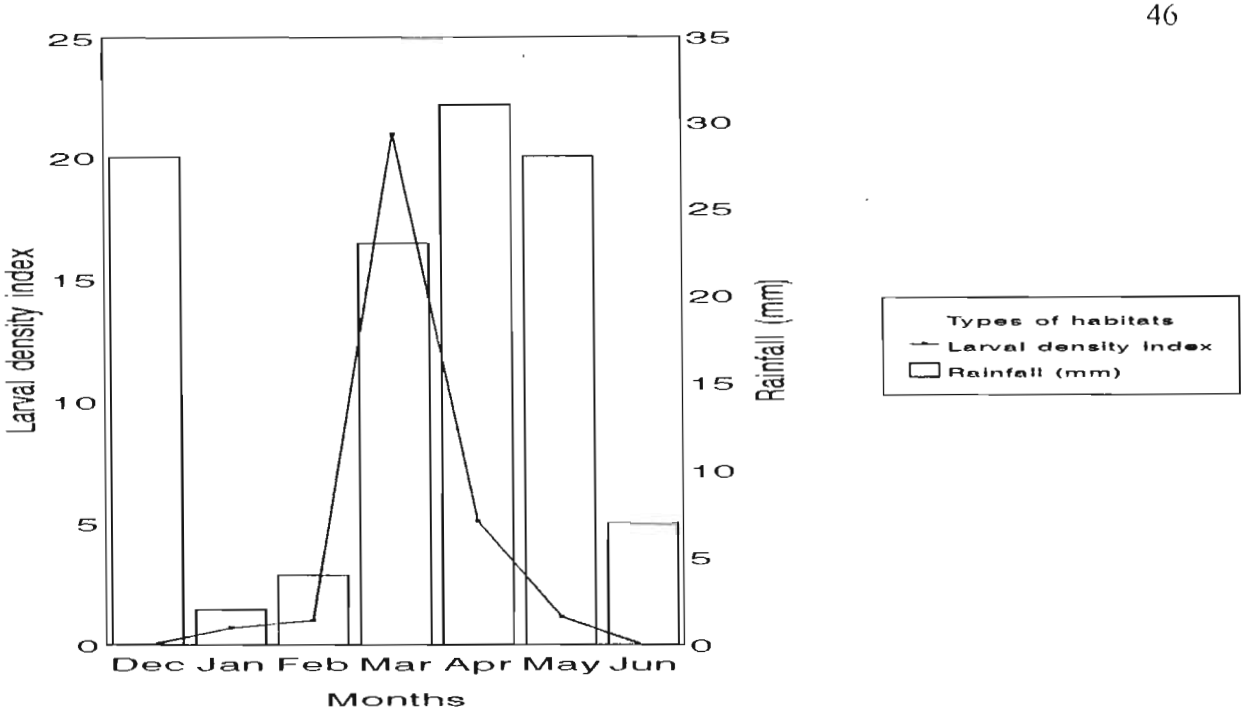



Fig.3.1.2: Larval density indices and rainfall (Jan-May 1995)

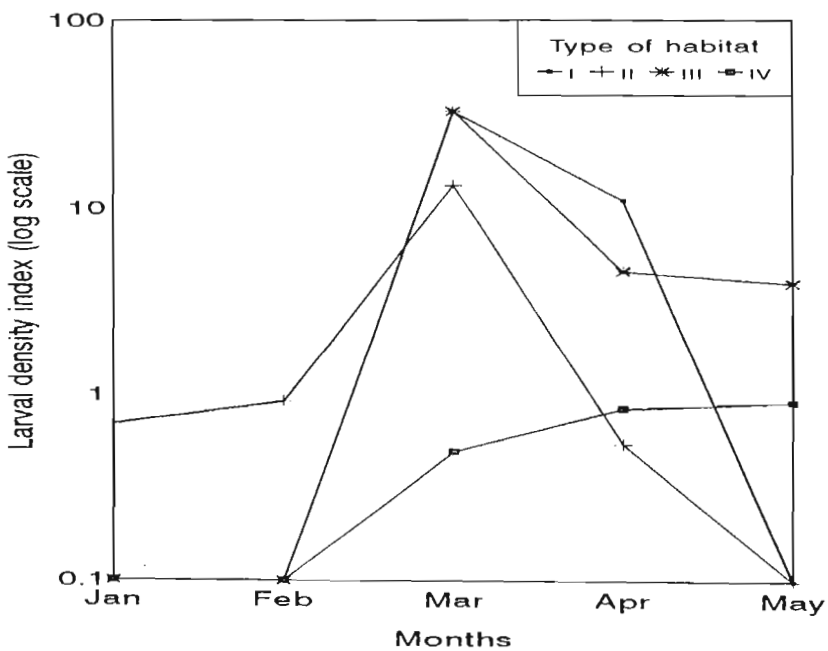



Fig.3.1.1: Larval density indices in habitats (Jan-May 1995)

### 3.1.2 Factors affecting larval densities in the breeding sites

The larval density indices in each habitat were the products of the following parameters:

i) **values per man-hour**: the number of larvae collected divided by time taken (in minutes) searching and collecting them,

ii) **values per dip**: the number of larvae collected divided by the number of dips used,

iii) **values per unit area ($m^2$)**: the number of larvae collected divided by the surface area of water where collections were done. Table 3.1.1 shows these indices for each type of habitat.

#### 3.1.2.1 Vegetation cover

Vegetation cover on water surface was found to affect the breeding preference of the anopheline mosquitoes. The vegetation of water surface was not quantified, however, the presence or absence of the vegetation on the water surface were recorded. The changes that occurred, i.e. change in height of vegetation were recorded. The results showed that larval densities were high in waterbodies with emergent vegetation than those with dense. Dense vegetation cover on water surface showed an inverse relationship to larval abundance in each habitat (Table 3.1.2). Anopheline larvae were frequently recorded in habitats with emergent or no vegetation.

| Type of habitat | Larvae and predator density | | | | | |
|---|---|---|---|---|---|---|
| | Density/unit area ($m^2$) | | Density/dip | | Density/man-hr | |
| | larvae | predator | larvae | predator | larvae | predator |
| I | 898 | 28 | 4.28 | 0.13 | 10.95 | 0.34 |
| II | 38.3 | 10.83 | 1.02 | 0.29 | 1.74 | 0.49 |
| III | 111.9 | 6.04 | 5.67 | 0.31 | 20.45 | 1.1 |
| IV | 4.9 | 4.68 | 0.57 | 0.55 | 0.83 | 0.8 |

**Table 3.1.1:** Larvae and predator densities in four types of habitats expressed in three different parameters.

Larval density (man-hr) were high when habitats had emergent vegetation as opposed to dense growth. For example, habitat type three which was the most productive, recorded man-hour density indices of 33.0 and 4.0 in March and May respectively. The vegetation cover in this habitat was emergent and interspersed with algae blooms at some points in March but the number of larvae was low in May when the vegetation was dense . Table 3.1.1 shows larval density indices and the type of vegetation in each habitat among other parameters.

### 3.1.2.2 Predators

The effects of the presence of predators were investigated and the results presented in Table 3.1.2 and Figures 3.1.3a-d. Three out of four habitats studied showed reduced larval density indices as predator densities increased. The most commonly encountered predators were *Granarius* spp (Notostraca) and the *Anisops* spp (Notonectidae) which comprised 34% and 25% of the total number (n=156) collected respectively. Other groups accounted for 40.2% and comprised of other insects, for example; water beetles and larvae of mayflies (Ephemeroptera), damselflies (Zygoptera) and dragonflies (Anisoptera). These predators were frequently encountered in habitats with long standing water (Figures 3.1.3a-c) as opposed to pools which were transient and dried out after a short while (Figure 3.1.3d).

| Months | Larval density, biotic and abiotic factors | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Larval density (man-hr) | | | | Mean (=) temperatures (°C) | | | | Predator density (man-hr) | | | | Type of vegetation | | | |
| | Type of habitat | | | | Type of habitat | | | | Type of habitat | | | | Type of habitat | | | |
| | I | II | III | IV | I | II | III | IV | I | II | III | IV | I | II | III | IV |
| Jan | - | 0.7 | - | - | - | 27 | - | - | - | 0.3 | - | - | - | N | - | - |
| Feb | - | 0.98 | - | - | - | 28 | - | - | - | 0.33 | - | - | - | N | - | - |
| Mar | 32.8 | 13.4 | 33 | 0.5 | 26 | 33 | 31 | 31 | 0.2 | 1.4 | 0.5 | 1 | E | E | E/A | E |
| Apr | 11.8 | 0.55 | 4.63 | 0.85 | 27 | 28 | 31.5 | 32 | 0.27 | 0.47 | 2.13 | 1.1 | E | TG | E/W | E |
| May | 0 | 0 | 4 | 0.92 | 27 | 28 | 26 | 26.5 | 0.6 | 0.5 | 1.3 | 0.4 | TG | TG | TG | TG |
| Totals= | 11 | 1.85 | 20.2 | 0.7 | 26.7 | 28.8 | 29.5 | 29.8 | 0.34 | 0.49 | 1.1 | 0.8 | | | | |

**Table 3.1.2:** Bioti and abiotic factors affecting larval density in each habitat between January and May 1995.

Key
Types of vegetation
N----none
E----emergent
E/A--emergent/blue green algae (algae bloom)
E/W--emergent/weed
TG--tall/grass

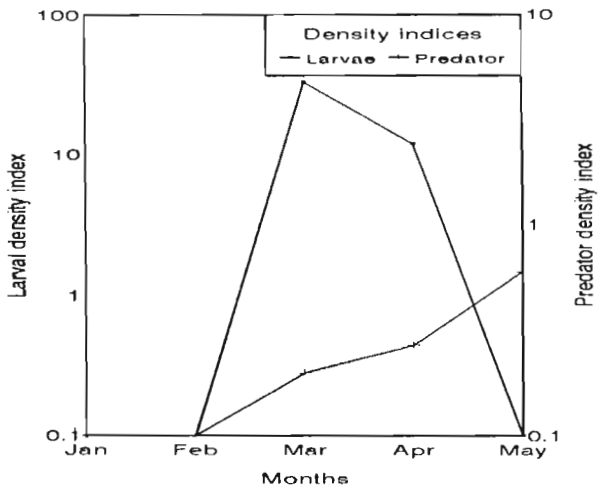



Fig 3.1.3a Habitat type I larvae and predator density indices.

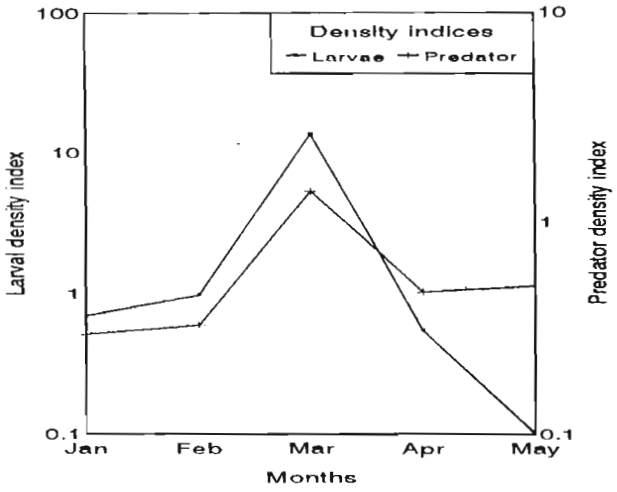



Fig 3.1.3b Habitat type II larvae and predator density indices.

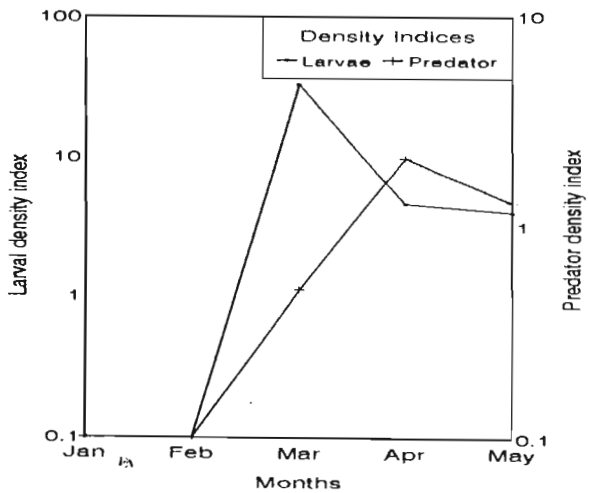



Fig 3.1.3c Habitat type III larvae and predator density indices.

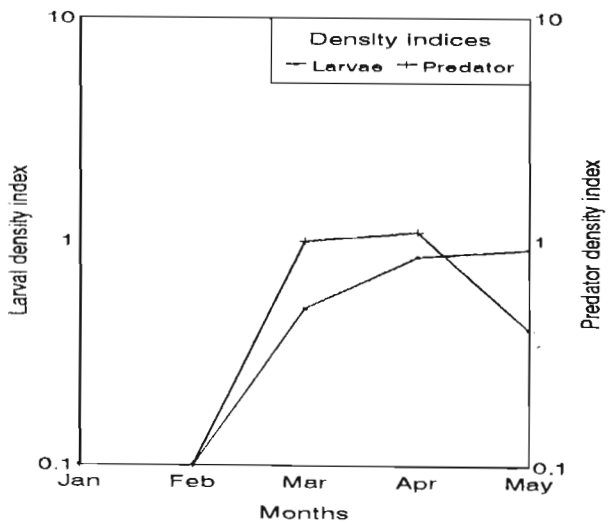



Fig 3.1.3d Habitat type IV larvae and predator density indices.

### 3.1.2.3 Temperature regimes and larval abundance

The effects of temperature variations on the larval density in the breeding habitats were investigated (Figure 3.1.4 and Table 3.1.1). The mean temperatures were calculated for each habitat from recordings taken each time larvae were collected. Habitat type III recorded the highest index (33.0/man-hr) in March at a mean temperature of 31°C.

There was a significant reduction of larvae in April with a density index of 4.63/man-hr when the mean temperatures increased to 31.5°C. Overall, larval density was high (20.9/man-hr) in March when the mean average temperatures were also high (30.3°C) as compared to 1.2/man-hr recorded in May when the mean temperature was low (26.9°C). Figure 3.1.4 shows mean average temperature plotted against larval density indices for each month.

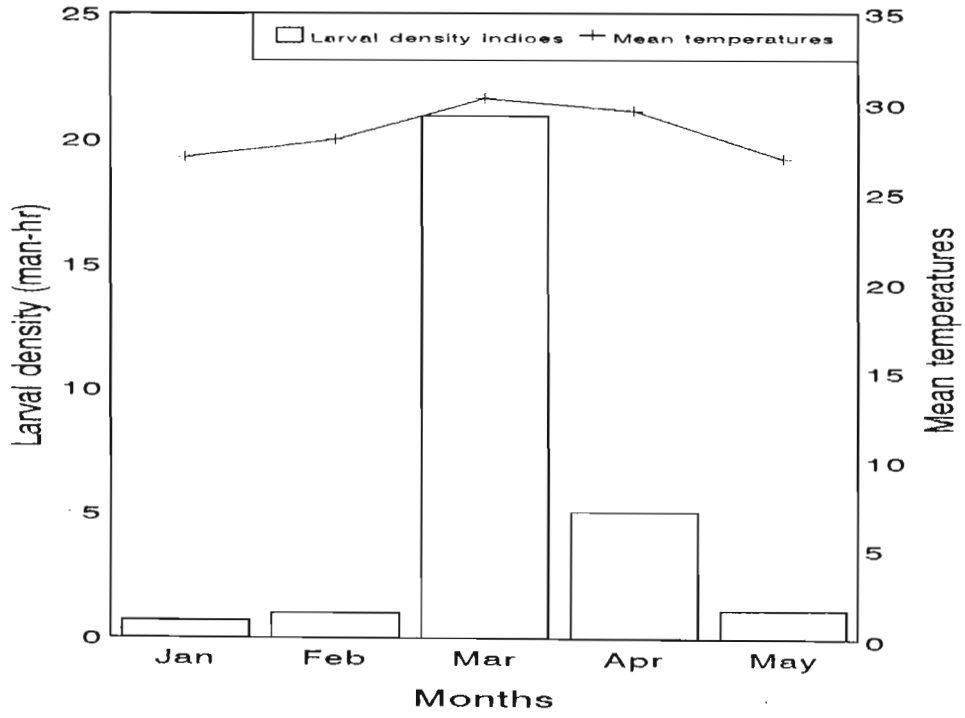



Fig. 3.1.4 Larval density and mean temperatures (Jan-May 1995)

### 3.1.2.4 Other abiotic factors affecting larval abundance

Other factors which possibly affected larval abundance were observed. Although these were not quantified for statistical analysis, the edaphic components of the substrata of the breeding habitats seemed to influence larval density and distribution. For example, anopheline larvae were commonly found in association with habitats which had muddy substrata. Conversely, culicine larvae were frequently collected from habitats where water was foul and rotting vegetation common.

### 3.1.3 The geographic distribution and density of larvae in each habitat type

Larval abundance in each type of habitat was determined (in man-hr) for the entire period of study. These values were used to develop a thematic map of larval density for each habitat type. Density ranges were represented by varied dot shading for each type of habitat. Figure 3.1.5 shows Mamfene section eight overlain with different dot shading representative of ranging densities for each type of habitat.

### 3.1.4 Larval survival in each habitat type

The total numbers of larvae in all instars collected from each type of habitat were corrected for instar duration as recommended by Service (1971) and le Sueur (1991). The instar durations used were those recommended by le Sueur (1991) since this project was undertaken in similar geographic area.

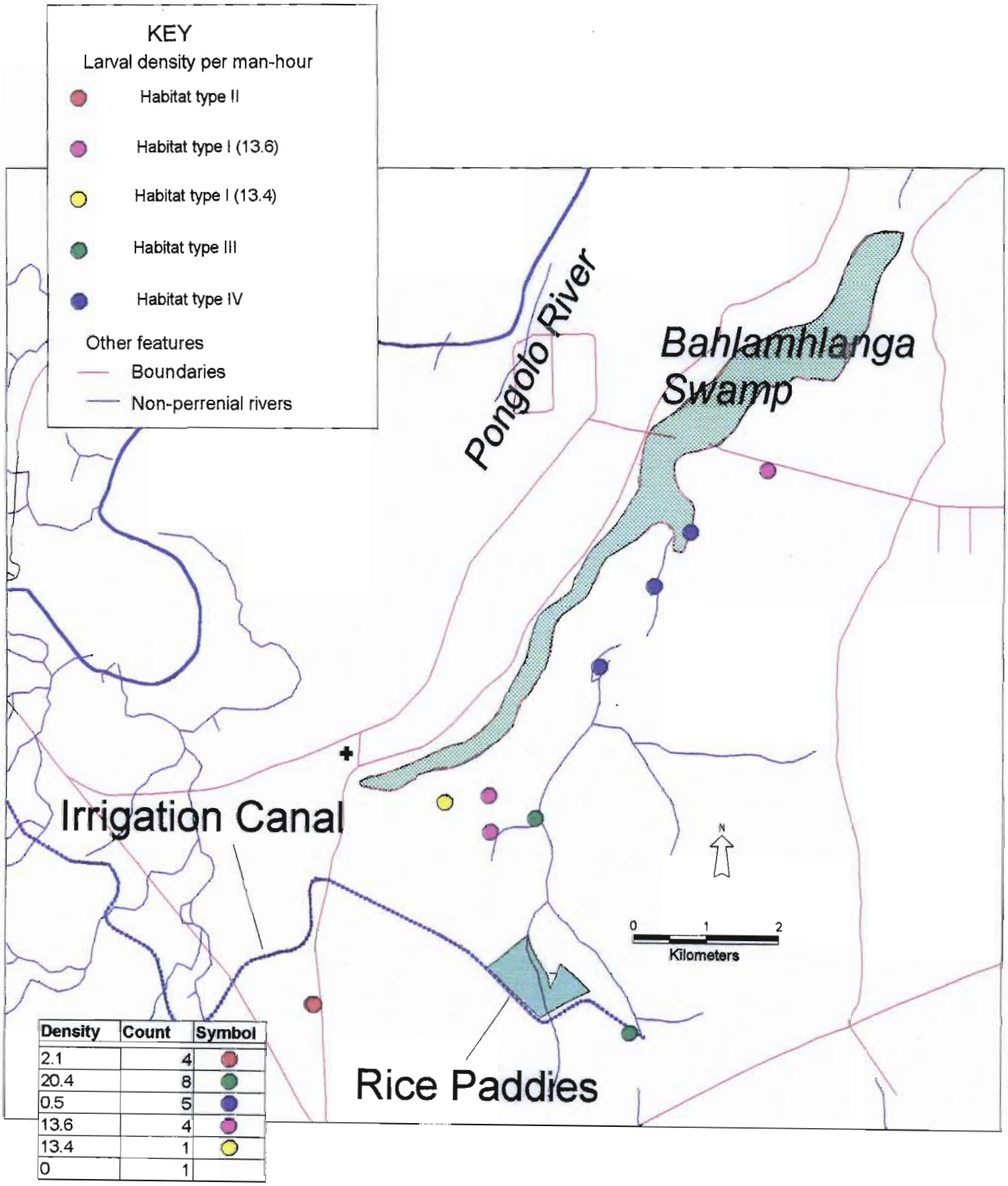



Fig. 3.1.5: Larval density and distribution in each type of habitat between January and May 1995

Figures 3.1.6a-d are survivorship curves for habitat types I, II, III and IV respectively. The assumption made while plotting these curves was that the population was stable and that the larval input into each type of habitat was constant when the collections were made. All curves plotted show irregular trends, especially due to increased numbers of 2nd and 3rd instars.

The survivorship curves for type III habitats in the months of March, April and May are presented in figures 3.1.7a-c. There were more 2nd instar larvae in March and May while April showed an increase in 3rd instars. These asynchroneous larval stages will be discussed in terms of the rainfall pattern, temperature variations and effects of predator in each habitat type.

### 3.1.5 Identification of larvae by the Polymerase Chain Reaction (PCR) technique

The PCR technique as described in Chapter 2 section 2.2.3.5 was used to identify a subsample of larvae collected in each type of habitat the results of which are presented in Table 3.1.3. Of the total number of larvae processed (n=331), 94.1% were identified as *An. arabiensis* while *An. quadriannulatus* constituted 5.9%. *An. merus* was not identified among the specimens processed. Habitat type II produced the most (n=6) *An.quadriannulatus.*

Habitat types I and III produced only 6.02% and 2.8% of the *An. quadriannulatus* identified respectively. No *An. quadriannulatus* were identified in type IV habitats. Negative results accounted for 12.7% of the total number of larvae processed for PCR identification but the proportion of negative results varied for each type of habitat as follows: 19.3%, 10%, 10.6% and 9.1% for habitat types I, II, III and IV respectively.

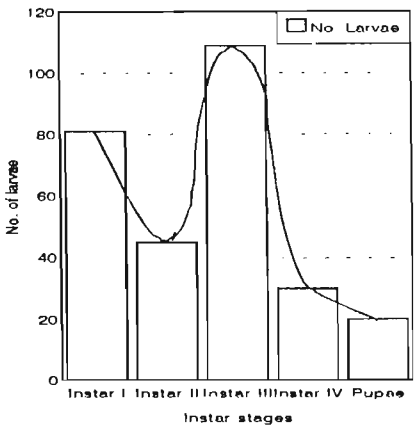



Fig.3.1.6a: Survivorship curve for habitat type one

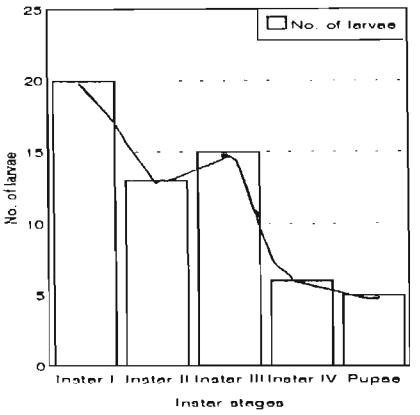



Fig.3 1.6b: Survivorship curve for habitat type two

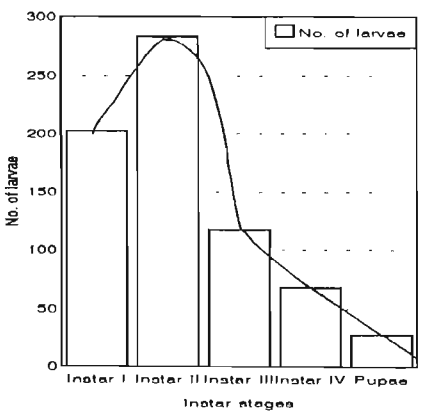



Fig. 3.1.6c: Survivorship curve for habitat type three

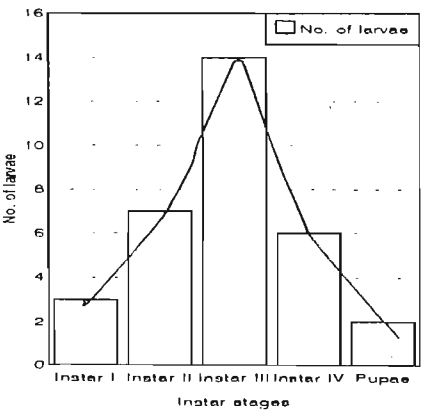



Fig. 3.1.6d: Survivorship curve for habitat type four

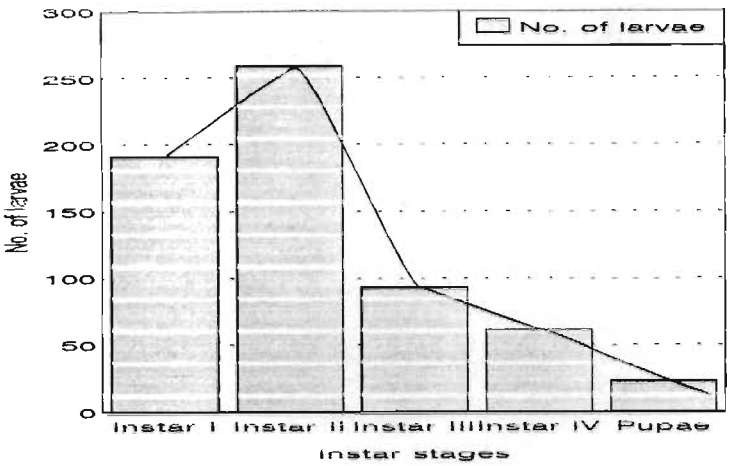



Fig.3.1.7a: Survivorship curve for habitat type three in March 1995

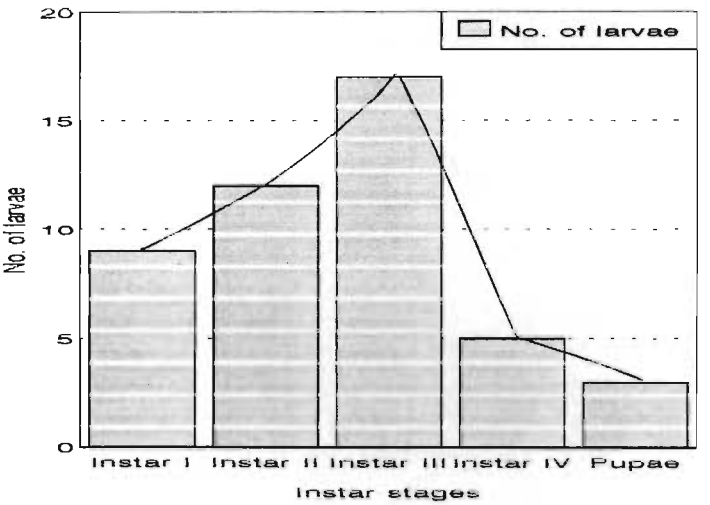



Fig. 3.1.7b: Survivorship curve for habitat type three in April 1995

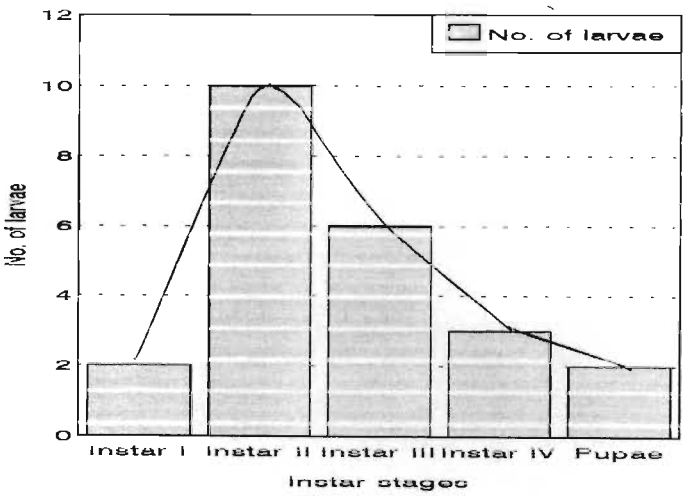



Fig.3.1.7c: Survivorship curve for habitat type three in May 1995

| Type | Number of larvae | Number processed for PCR | PCR results: | | |
|---|---|---|---|---|---|
| | | | *An. arabiensis* | *An. quadriannulatus* | Neggative. Result |
| I | 452 | 83 | 62 | 5 | 16 |
| II | 98 | 20 | 12 | 6 | 2 |
| III | 1171 | 217 | 188 | 6 | 23 |
| IV | 50 | 11 | 10 | 0 | 1 |
| Totals= | 1771 | 331 | 272 (94.1%) | 17 (5.9%) | 42 |

**Table 3.1.3:** PCR results of larvae collected from four different types of breeding sites.

PART TWO

## 3.2 Adult mosquito collections

### 3.2.1 Adult mosquito density per house

Relative population density per month was calculated based on window trap catches for each house for the study period (January to May 1995) and are presented in Tables 3.2.1a-c. The cumulative density index was low (0.22) for all the houses in the first quarter of 1995. There was, however, an increase in the mosquito population to an index of 2.05 per house in April with a peak of 4.15 in May 1995. This change in adult mosquito density in April and May may have been the result of the increased rainfall in Mamfene area (Figure 3.2.1). There was moderate correlation between rainfall in the previous months and the mosquito population each month when tested for correlation by Spearman correlation coefficient test ($r=0.46382$, $p=0.3542$).

The malaria case rate from January to July was plotted against mosquito population density and rainfall in Mamfene area. Two peaks in transmission occurred in the area in January and May 1995 (Figure 3.2.1). These were a reflection of increases in mosquito numbers which occurred as a result of increased rainfall in December 1994 and March/April 1995 respectively.

There were variations in mosquito density between and within houses. For example, house no.3 recorded a high density index of 22.7 in April 1995 whereas no.10 recorded an index of 1.1 after nil returns in February and March. The mosquito population in these two houses changed dramatically in May when out of 697 mosquitoes collected, house no.10 accounted for 462

(66.3%) as compared to only 5 (0.6%) in no.3. These two houses are approximately 3.5 km apart. House no.3 is located within the 2 km buffer zones of a type III breeding habitats, while no.10 is within the range of a type IV breeding habitats. The mosquito density index of 154 and 1.3 in these two houses in May 1995 represented a 140-fold increase for no.10 and a reduction of more than 17-fold for no.3 respectively. The density indices for house nos.3 and 10 in April and May respectively are presented in Figure 3.2.2.

| Months | House numbers | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 10(1) | 194(2) | 190(3) | 69(4) | 70(5) | 73(6) | 201(7) | 204(8) |
| Jan: | 0.2 | 0.2 | 0.6 | 1.4 | 0 | 0 | 0 | 0 |
| Feb: | 0.3 | 0 | 0.67 | 0 | 0 | 0 | 0 | 0 |
| Mar: | 0.25 | 0.25 | 0.75 | 0 | 0 | 0 | 0 | 0 |
| Apr: | 1 | 1.7 | 22.7 | 9.3 | 1.7 | 2.4 | 0.57 | 0.29 |
| May: | 1.3 | 1.67 | 1.3 | 3.67 | 0.67 | 6 | 0.3 | 0 |
| Totals | 0.64 | 0.86 | 7.7 | 5.5 | 1.4 | 3.5 | 0.23 | 0.09 |

**Table3.2.1 (a):** Monthly mosquito house density indices from January to May 1995. House codes are in parentheses.

| Months | House numbers | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 214 (9) | 222 (10) | 223 (11) | 330 (12) | 180 (13) | 181 (14) | 186 (15) | 156 (16) |
| Jan | 0 | 3.6 | 0 | 0.6 | 0 | 0 | 0 | 0 |
| Feb | 0.3 | 0 | 0 | 0 | 0 | 0 | 0.3 | 0 |
| Mar | 0 | 0 | 0 | 0 | 0 | 0.3 | 3 | 0 |
| Apr | 3.7 | 1.1 | 0 | 0 | 0.4 | 1.7 | 2.1 | 0 |
| May | 26.7 | 154 | 9.7 | 17.3 | 0 | 1 | 0 | 0 |
| Totals | 4.86 | 22.2 | 1.3 | 2.5 | 0.14 | 0.73 | 1.3 | 0 |

**Table3.2.1b:** Monthly mosquito house density indices from January to May 1995. House codes are in parentheses

| Months | House numbers | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 163 (17) | 165 (18) | 310 (19) | 313 (20) | 319 (21) | 120 (22) | 122 (23) | 130 (24) |
| Jan | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Feb | 0 | 0 | 0 | 0 | 0 | 0.3 | 0 | 0 |
| Mar | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Apr | 0 | 0.1 | 0 | 0 | 0.1 | 0 | 0.1 | 0 |
| May | 0 | 2 | 0 | 0 | 6.7 | 0 | 0 | 0 |
| Totals | 0 | 0.32 | 0 | 0 | 0.95 | 0.05 | 0.05 | 0 |

**Table 3.2.1c:** Monthly mosquito house density indices from January to May 1995. House codes are in parentheses.

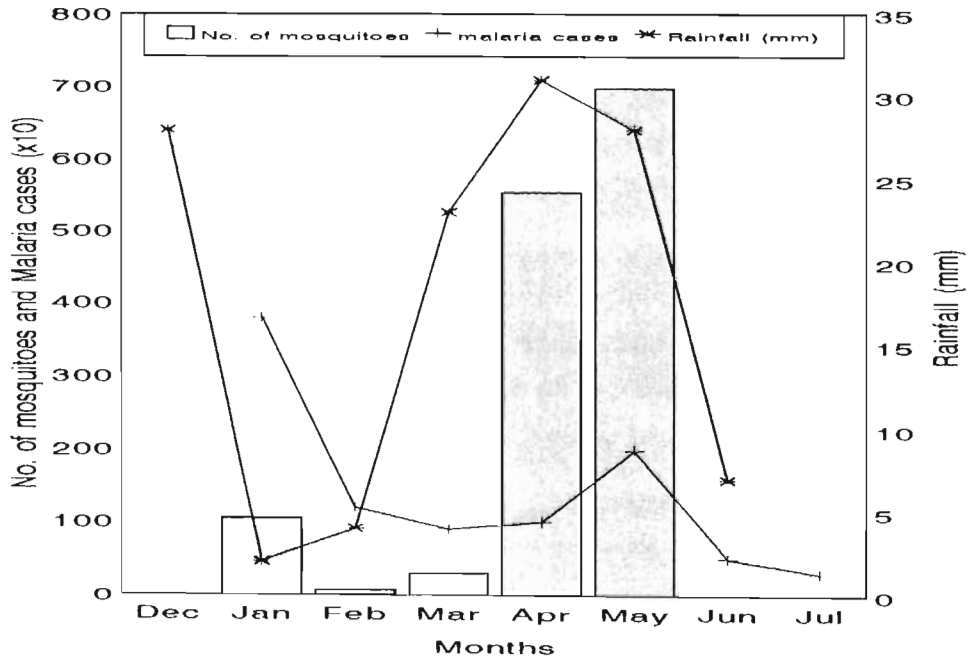



Fig.3.2.1: Mosquito numbers, malaria cases and rainfall in Mamfene.

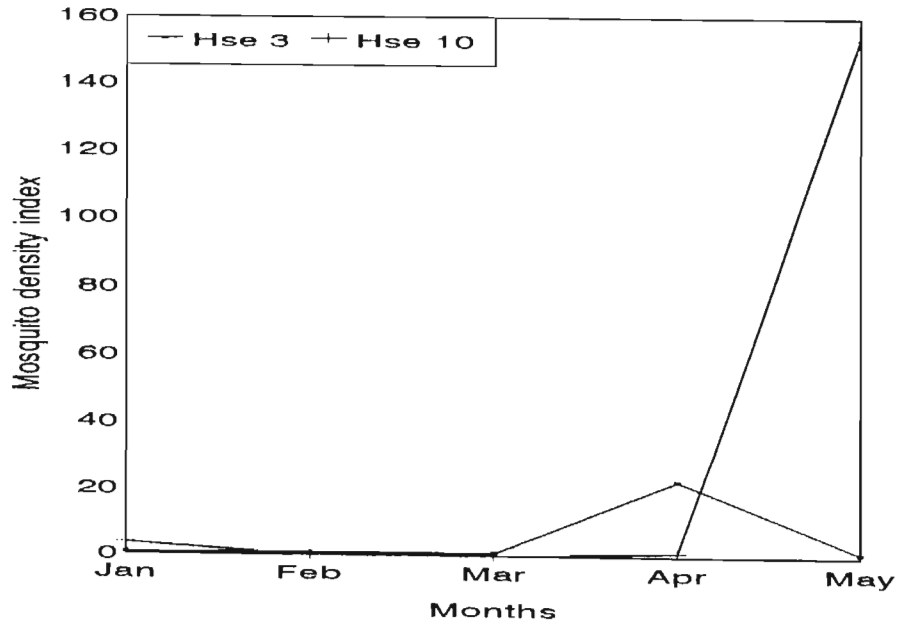



Fig. 3.2.2: Mosquito density index in houses 3 and 10 (Jan-May 1995)

### 3.2.2 Adult mosquito density in house clusters

The distribution of mosquitoes and mean average density index per clusters of houses (see Figure 2.2.4) from January to May 1995 are presented in Figure 3.2.3. The density index was a product of the total number of mosquitoes collected per cluster divided by the number of traps in each cluster for the period of study. There were 3 houses in each cluster located around each set of breeding habitats where larval sampling was done.

Figure 3.2.4 shows a topographic map of Mamfene section eight overlain with thematic shading of mosquito mean average densities per cluster. The intensity of the shading in each cluster of houses was dependent on the mean average density index. The range of shading was from light to dark, those which had highest indices shaded more intensely than those with less. These constrasting views were also a reflection of the productivity of each type of breeding habitat. The density indices were high in house clusters within the buffer zones of the most productive types of habitats.

The mosquito collections for first quarter of 1995 constituted only 5.2% of the total collections (1099) for all house clusters. Cluster 4 recorded the highest number (63.6%) of total collections in January (33) with a mean average cluster density index of 1.4. This was followed by a marked increase in mosquito population with density indices of 3.7 and 8.7 (Figure 3.2.5) for clusters 2 and 4 in April and May 1995 respectively.

Of the total number (1099) of mosquitoes collected from January to May, cluster 4 recorded 52.0% as compared to 18.6% returns in cluster 1. House

clusters 2 and 3 recorded 12% and 10.4% of the total number of mosquitoes collected respectively. The relatively low number of mosquitoes collected in cluster 2 was probably because there were no collections in this cluster in February and March 1995. It was included in the study after a large number of larvae was collected in irrigation spillage habitats (type III) adjacent to cluster 2 houses. House clusters 5, 6, 7 and 8 together accounted for only 7% of the total collection.

Differences also existed within clusters in terms of mosquito density per house. This is illustrated in nos.10 and 11 which had indices of 154 and 9.7 respectively in May 1995 representing 66.3% and 4.2% of the total number (697) of mosquitoes collected that month. These two houses are close together (0.1km apart) and both were located on the periphery of the water where an irrigation spillage stream joins the Balamhlanga swamp. The site where larval collections were done was classified as type 4.

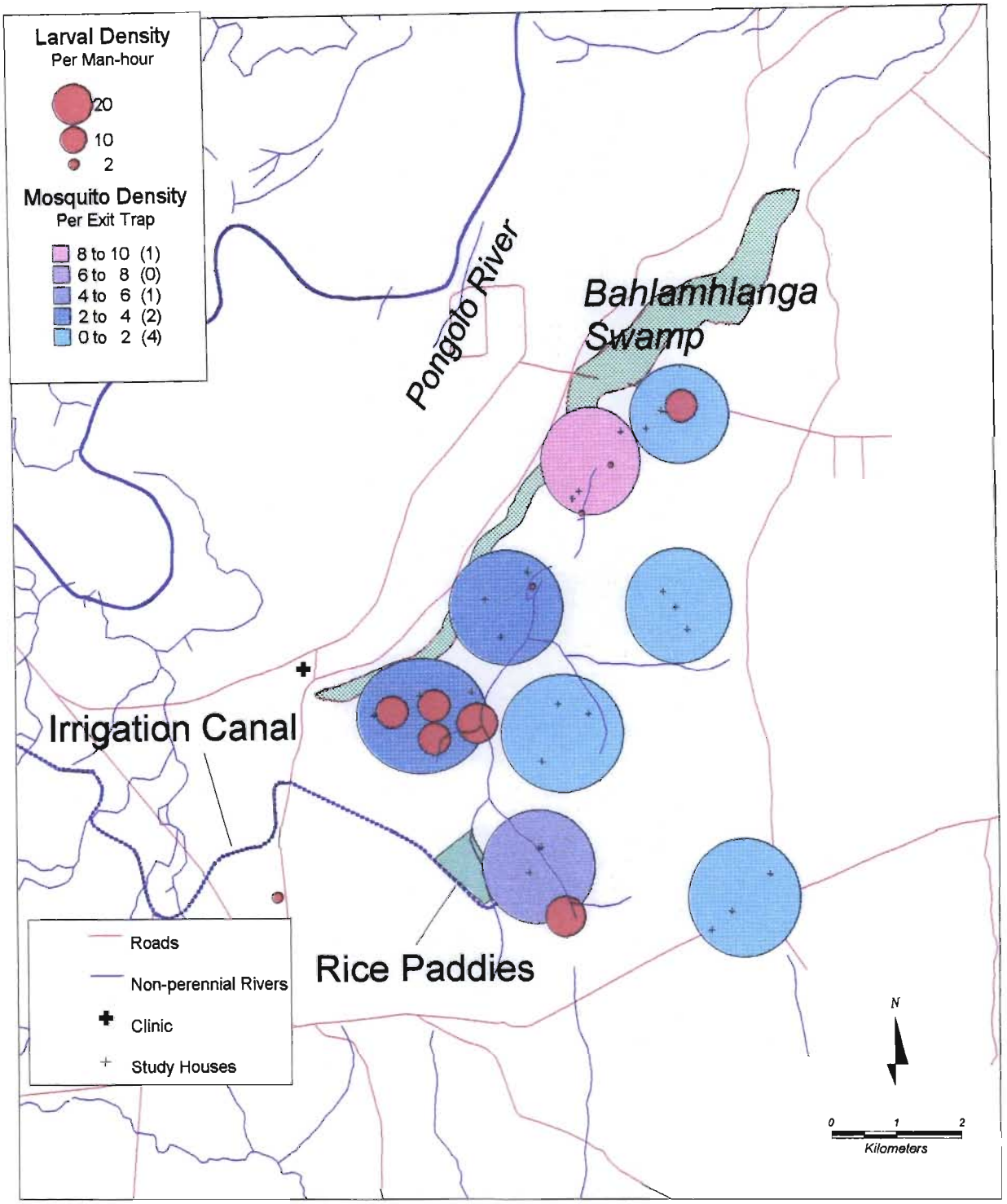



Fig. 3.2.4: Mamfene section 8 overlain with thematic shading of density ranges of larvae and adults.

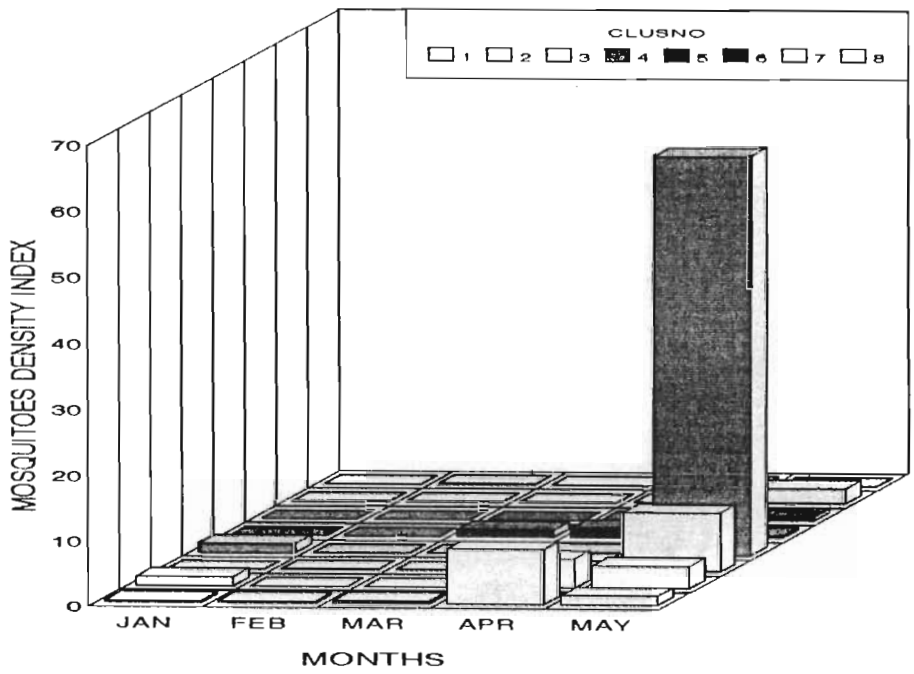



Fig.3.2.3: Mosquito density index in house clusters (Jan-May 1995)

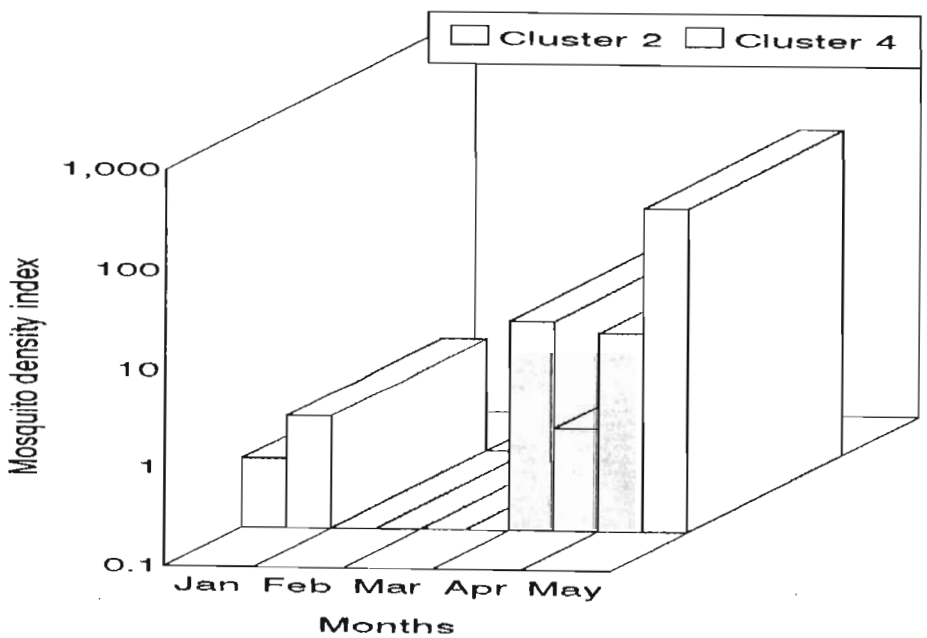



Fig.3.2.5: Mosquito density index in cluster two and four (Jan-May 1995)

### 3.2.3 Abdominal status

Subsamples of the mosquitoes whose abdominal status were determined were collected from sentinel houses used by the National Malaria Research Programme team during the 1993 season. As a result, the total number of mosquitoes whose abdominal conditions were determined were 1348 instead of 1099. A large component of mosquitoes collected during the study period (January and May 1995) were unfed females, i.e. 57.2% of the total collection of 1348 as shown in Table 3.2.2. A total of 45 male mosquitoes were collected but were not considered in the calculations because they do not feed on blood and hence their abdominal status are irrelevant in this study.

There were variations in abdominal conditions between months with a high proportion of unfeds recorded in May. Chi-square test was used to compare the abdominal status of female mosquitoes collected in January, April and May (1995). These variations were found to be significant ($x^2$=198.198, $p>0.001$). Out of 697 mosquitoes collected in May, 72.6% were unfed females. The proportion of unfed female *An. gambiae* group collected in April was 43.2% (225/521). The unfed female components in the other months, especially February and March were negligible because of the low numbers collected.

| Months | Totals | | %age proportion of female abdominal status | | | |
|---|---|---|---|---|---|---|
| | males | females | unfed | bloodfed | h/gravid | gravid |
| Jan: | 4 | 101 | 37.6 | 17.8 | 14.9 | 29.7 |
| Feb: | 1 | 6 | 0 | 50 | 50 | 0 |
| Mar: | 7 | 23 | 8.7 | 17.4 | 30.4 | 43.5 |
| Apr: | 32 | 521 | 43.2 | 28.2 | 18.8 | 9.8 |
| May: | 1 | 697 | 72.6 | 7.2 | 13.6 | 6.6 |
| Totals | 45 | 1348 | 57.2 | 16.5 | 16.2 | 10.1 |

**Table 3.2.2:** The percentage abdominal status of female *An. gambiae* collected from January to May 1995.

### 3.2.4 Parity dissection results

The results of parity dissections on subsamples of the pre-gravid female *An. gambiae* group of species done between January and May 1995 are shown in Table 3.2.3. From a total of 163 dissections, 63.2% were parous and 36.8% nulliparous. There were no significant difference in parity rates between the months ($x^2$=0.781, p>0.667). However, the rates for February and March were based on very low numbers of mosquitoes and therefore inflated, and were disregarded for the purpose of this report.

| Months | Parity dissections: | | Totals |
|---|---|---|---|
| | Nullipar | Parous | |
| January | 10 | 14 | 24 (58.3) |
| February | 0 | 3 | 3 |
| March | 3 | 3 | 6 |
| April | 40 | 42 | 82 (51.2) |
| May | 26 | 22 | 48 (45.8) |
| Totals | 79 | 84 | 163 (51.5) |

**Table 3.2.3:** Parity dissections of mosquitoes collected between January to May 1995. Percentage parity (Jan, April and May ) in parentheses.

### 3.2.5 *An. gambiae* group identifications by the polymerase chain reaction (PCR) technique

A subsample of total adult mosquito collections was processed for species identification by the PCR method. The results are presented in Table 3.2.4. A total of 340 *An. gambiae* group were processed out of which 303 were positively identified as either *An. arabiensis* (98.7%) or *An. quadriannulatus* (1.3%). A small proportion (10.9%) of the total number processed did not yield any results and were considered negative. These negative results could have been due partly to human error both during preservation and processing. *An. merus* was not identified among the specimens processed.

| House Cluster No | PCR Results: | | | Totals |
|---|---|---|---|---|
| | *An. arabiensis* | *An. quadriannulatus* | Negative Results | |
| 1 | 76 | 2 | 7 | 85 |
| 2 | 33 | 2 | 4 | 39 |
| 3 | 29 | 0 | 0 | 29 |
| 4 | 141 | 0 | 25 | 166 |
| 5 | 11 | 0 | 1 | 12 |
| 6 | 2 | 0 | 0 | 2 |
| 7 | 5 | 0 | 0 | 5 |
| 8 | 2 | 0 | 0 | 2 |
| Totals | 299 (98.7%) | 4 (1.3%) | 37 | 340 |

**Table 3.2.4**: *An. gambiae* group of species identification by PCR technique.

### 3.2.6 Malaria cases from January to July 1995

Figure 3.2.6 shows malaria cases in relation to rainfall pattern for the Mamfene region while the geographic distribution of cases in Mamfene area is presented in Figure 3.2.7. These data were obtained from the KwaZulu-Natal Health Department, Republic of South Africa (courtesy of Mr. S M Ngxongo, Chief Public Health Officer and Malaria Coordinator).

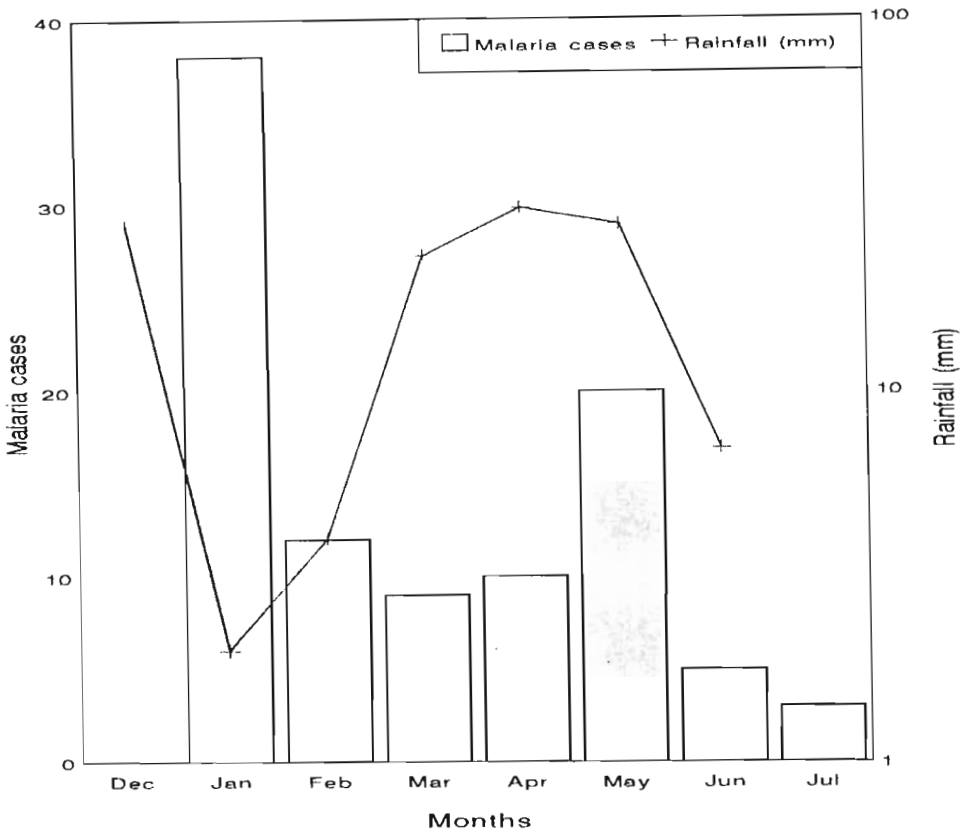



Fig.3.2.6: Malaria cases and rainfall in Mamfene (1994/5).

There was a mean average of more than 17 cases per month between January and May 1995 with 38 and 20 cases reported in January and May 1995 respectively. June and July had a monthly mean less than 5 cases each. The distribution of malaria cases seem to be concentrated around the winter breeding sites (type II) with a few located within the buffer zone of the irrigation spillage breeding habitats (type III). This observed pattern is probably a reflection of the vector population build up from the winter breeding habitats. Two malaria peaks occurred as a result of cumulative effects of rainfall between October/December 1994 and March to May 1995.

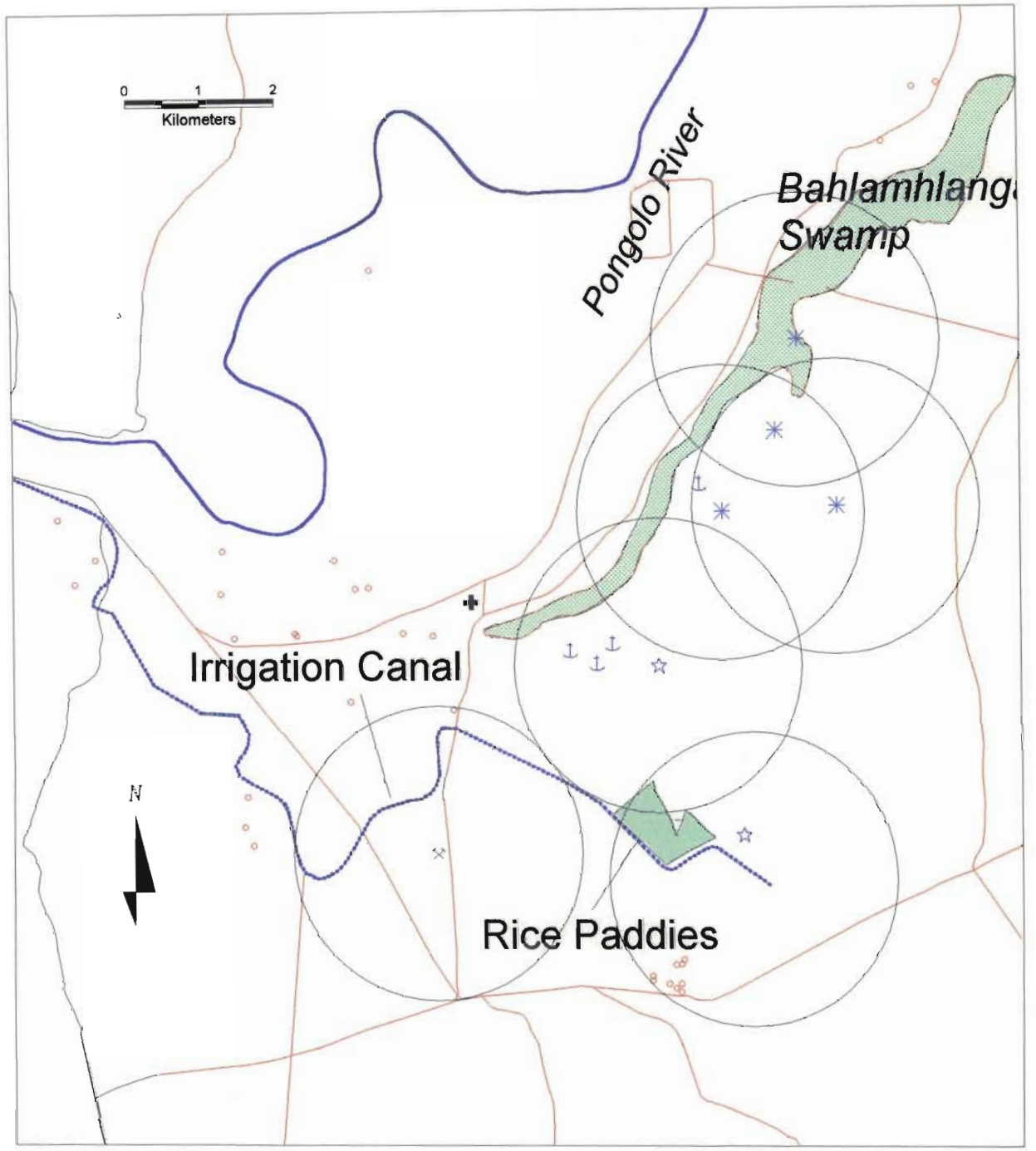
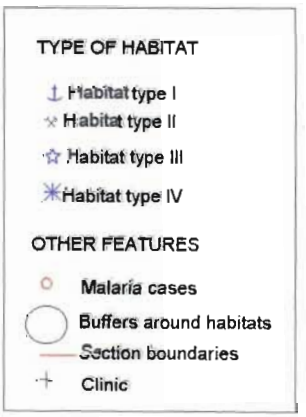



Fig. 3.2.7: The geographic distribution of malaria cases in Mamfene area between January and June 1995.



### 3.2.7 Mean rainfall, temperature and humidity schedules

#### 3.2.7.1 Rainfall pattern

Rainfall data for the period of this study showed that a moderate amount of rainfall was experienced in December 1994. January and February 1995 were dry with low mean average of 3 mm. March, April and May recorded an average of 27.3 mm per month. With the onset of winter, rainfall dropped to a low of 7 mm in June (see Figure 3.2.1).

#### 3.2.7.2 Air temperatures and relative humidity

January and February 1995 were dry as indicated by the rainfall pattern in Figures 3.2.1 and 3.2.6 and mean maximum air temperatures were 33.2°C for both months. The mean average air temperature for January and February was 22.8°C. These two months also recorded a lower mean average relative humidity of 67.2% (Figure 3.2.8).

The relative humidity of 77% recorded for April and May was the highest during the study period with a mean air temperature of 26.8°C. Figure 3.2.8 shows relative humidity (RH), mean air temperature and mosquito numbers during the study period. The association between mean relative humidity and maximum temperature and the mosquito monthly density was tested for associations by Spearman correlation coefficient test. There was weak correlation between mean maximum temperature and mosquito density (r=-0.05406, p=0.9084) while moderate correlation existed between relative humidity and mosquito density (r=0.48651, p=0.2682). There were correlations between these climatic variables and mosquito monthly density.

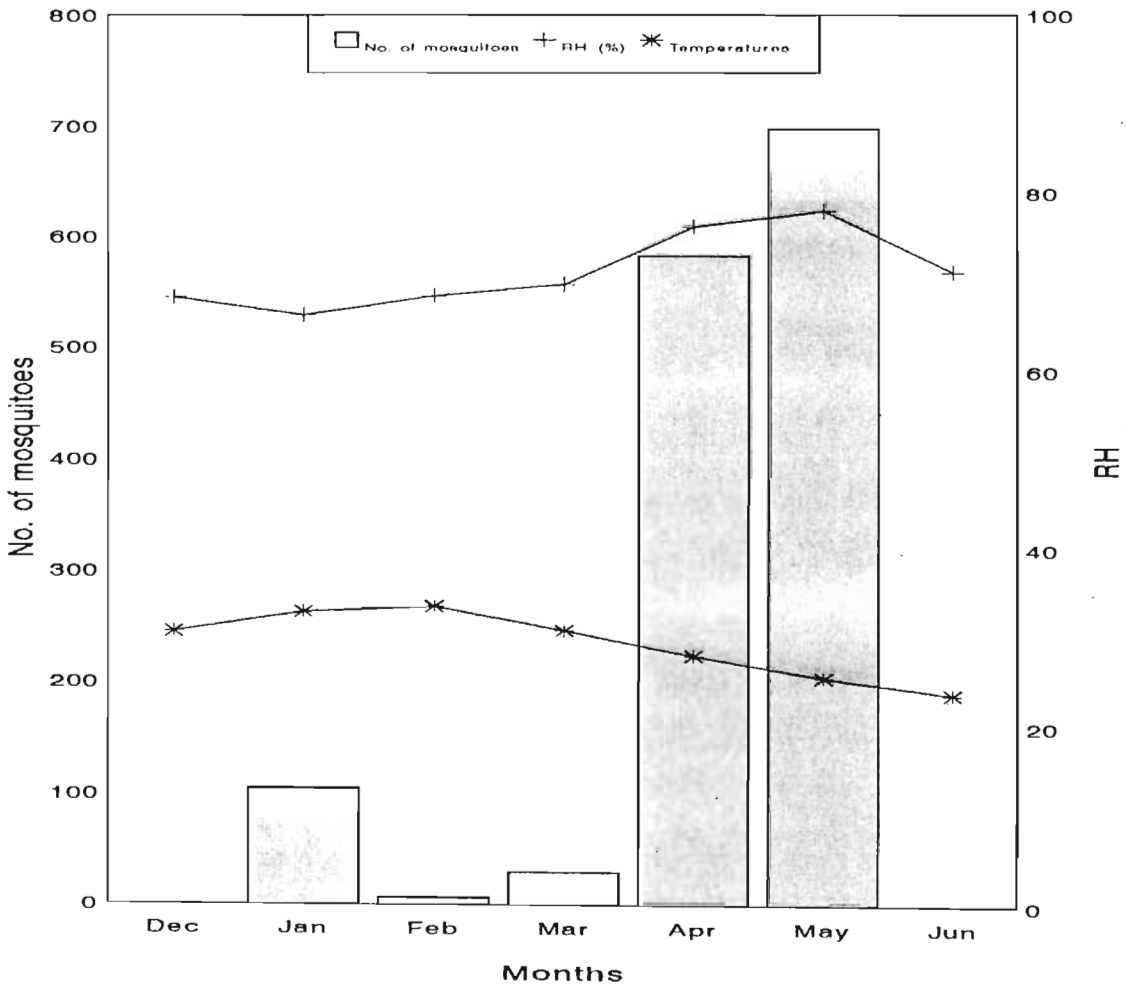



Fig.3.2.8: Total catch, temperatures & RH (Dec-Jun 1994/5)

# CHAPTER FOUR

# DISCUSSION

## 4.1. Anopheline vector mosquito breeding in Mamfene: characteristics and factors affecting larval abundance

The larval density data obtained in this study showed that the irrigation spillage habitats were more productive in terms of larval numbers per man-hour when compared to other breeding sites in summer (Figure 3.1.1). Rainfall was irregular and as a result the mosquito vector radiation was limited. The seasonality of mosquito breeding is depicted by the rainfall pattern experienced in southern Africa in the past decade (le Sueur, 1991; le Sueur *et al.*, 1995). Recent published work on the bionomics of the *An. gambiae* group in this region indicate a seasonal peak between March and May each year (le Sueur and Sharp, 1988; le Sueur *et al.*, 1992). Unpublished data by Dr le Sueur (Appendix III) indicate a seasonal peak in May for most of the southern Africa states except in Zimbabwe where an average rainfall data showed most rain was experienced in March between 1972-81. The results obtained in the present study concur with the above findings.

The cumulative larval density peaked in March (20.9/man-hour) with breeding habitats types I and III recording high density indices of 32.8 and 33.0 /man-hour in same month respectively. These habitats were considered to be among the first contacts for mosquitoes radiating from the winter breeding sites during the summer rains. Breeding ceased in May in habitats types I and II, but type III recorded low larval densities while type IV habitats recorded a higher density per man-hour (0.92), an improvement from an index of 0.5/man-hour

recorded in March (Figure 3.1.1 and Table 3.1.1). Type IV breeding sites were rain dependent and it is possible breeding ceased in them when rains stopped. However, due to irregular and scarce rainfall, these type of habitats were less productive. They were mainly ground depression pools as a result of storm water from the non-perennial rivers. From the results obtained, it seemed possible that with adequate and regular rainfall sizeable mosquito breeding would have occurred inland in type IV habitats. The associations between climatic variables and monthly larval densities were tested by Spearman correlation coefficient which showed moderate correlations between monthly larval densities and the previous month rainfall ($r=0.46382$, $p=0.3542$) but the correlations between larval density and mean maximum temperature was weak ($r=-0.25714$, $p=0.6228$) while the association between larval density and relative humidity was strong ($r=0.71429$, $p=0.1108$). The weak correlations between mean temperatures and monthly larval density could have been due to minimum temperature variations experienced during the study period (Fig. 3.1.4). Larval density and distribution in the breeding sites are a direct consequent of rainfall. Increased rains often result in numerous breeding sites which are a pre-requisite for malaria vector breeding. The malaria vector breeding occurred mostly in the irrigation spillage (type III habitats). This occurrence was also reported by le Sueur *et al.* (1995) and Sharp *et al.* (1992) who observed that breeding was concentrated in irrigation spillage in winter and that these sites provide breeding habitats in summer when the rains are not sufficient and mosquito vector radiation is limited to available waterbodies.

Increased vegetation cover and high predator densities in three types of habitats (I, II and III) had negative correlations with mosquito larval density

(Figures 3.1.3a-d). The vegetation cover in habitat types I, II and III increased in May but type IV habitats which were temporary water bodies supported emergent vegetation and consequently fewer predators. These results are consistent with those reported elsewhere, for instance in Tanzania by Njunwa (1993) who showed that excessive shade had a negative correlation with larval density, while in Swaziland Kelly and Cory (1984) attributed low numbers of larvae in habitats to increased predator densities. In Mamfene, South Africa, le Sueur and Sharp (1988) observed that the increasing size of rice plant was negatively correlated with larval density and distribution in the paddy pools.

The vertebrates and invertebrates encountered in the breeding habitats were similar to species implicated as predators by Service (1977) at Ahero irrigation scheme, Kenya. Service (1977) examined the gut contents of some of these predators for the presence of *An gambiae* group antibodies by precipitin testing and incriminated several of them, for instance different species of Ephemeroptera, Zygoptera, Anisoptera, Notostraca and *Anisops* spp. were found to be predators of anopheline immatures. Service (1977) also observed that larval mosquito numbers were higher compared to those of predators in temporary waterbodies which concurs with results obtained in this study. In this study it was observed that the predator numbers increased in permanent waterbodies as compared to temporary ones. This could have been due to mosquito eggs hatching faster as compared to those of other aquatic animal species which required a period of desiccation before hatching and therefore were unable to colonise these habitats simultaneously. From these findings it can be concluded that increased predator densities were inimical to larval densities in three of the four habitats studied (Figures 3.1.3a-d).

The irregular larvae numbers in the habitats (Figures 3.1.6a-d), and especially so with instar stages II and III could have been partly that none of the breeding habitats was oviposition rate constant probably due to inconsistent production of adult mosquitoes. This prevented larval survivorship to be accurately estimated by this technique. However, le Sueur (1991) demonstrated the occurrence of seasonal contraction and expansion of the malaria vector population during winter and summer respectively. He showed that the vector mosquito population is localized in winter and that larval cycle increases from approximately 8 days in summer to 44 days in winter and as a result, the adult population is reduced and that malaria vectors are concentrated as larvae in localized winter habitats. This study was conducted in summer and extreme temperature variations were not encountered, although fluctuations did occur between and within habitats in different months during the study period.

## 4.2. Adult mosquito distribution pattern

The distribution of adult mosquitoes and the mean average density in house clusters from January to May 1995 in Mamfene showed a population concentration around irrigation spillage breeding sites (Figure 3.2.4). The mosquito density in each house cluster was higher in houses within the buffer zones of habitats which had high larval densities. For instance, it was observed that the adult mosquito numbers were high in houses around type III habitats which were irrigation spillage, an occurrence also reported by le Sueur *et al.* (1995) and Sharp *et al.* (1992). The trend of mosquito distribution reported here depicted that of rainfall pattern in the region (Appendix III) although there were no marked increases in population due to irregular rainfall.

The adult mosquito population was generally low during the current study but high density was noted in house clusters located within the 2 km buffer zones of the most productive larval habitats (Figure 3.2.4). The distance from the point source of adult mosquito emergence had a negative correlation with mosquito density in house clusters that were located outside the buffer zones. For example, house cluster eight which was furthest from any productive habitats recorded only two adult mosquitoes during the entire study period. Due to rainfall irregularity in Mamfene between January and May 1995 there was limited mosquito vector radiation from the spillage habitats. No breeding occurred inland in temporary waterbodies or at least they did not last long enough to sustain successive larval generations. This could also partly account for very few adult mosquitoes collected in clusters nos. 6 and 7 which were inland.

The striking difference in mosquito density between house nos.10 and 11 in May (Table 3.2.1b) could be attributed to one or several factors. The dispersion and distribution of adult *An. gambiae* group are affected by several variables for example, Zahar (1985) in his review noted that the intensity and height of vegetation, strength and the direction of wind influenced the adult mosquito dispersion pattern in some localities in west Africa. The difference in house density of adult mosquito population between nos. 10 and 11 separated by a distance of only 0.2 km could also have been partly the result of high vegetation between house no. 11 and the point from which adult emergence occurred. Dense and high vegetation act as physical barriers to mosquito flight. Mamfene has an irregular terrain and supports dense and high vegetation interspersed with clearings in which most homesteads are located. However, there was no high vegetation between house no. 10 and the adult mosquitoes emergence source except for the cotton plantation and a low hedge which possibly facilitated the mosquito dispersion into this house. Zahar (1985) reporting on some work done in west Africa also noted that low vegetation sometimes facilitated the dispersal of *An. gambiae* group which fly in short hops close to the ground. Low bush provides temporary resting sites enabling them to cover long distances that they could not otherwise traverse in open land.

The characteristics of individual houses could also have been responsible for the differences in mosquito population in the two houses. For example, the number of people sleeping in each house and their ages, the size and position of eave gaps and the availability of cattle, goats, sheep etc. were also considered as possible confounders that may have acted as bait to attract

mosquitoes to individual houses. But these factors would have been relevant if the difference in mosquito density existed between houses in the same compound.

### 4.3. *An. gambiae* group of species identification

The species composition of a subsample (n=331) of larvae identified by the PCR technique show that 94% were *An. arabiensis* while *An. quadriannulatus* comprised of 6%. The negative samples which accounted for 13% of the total number processed, could have been other species of *Anopheles* not belonging to *An. gambiae* group. The adult mosquitoes caught in exit traps were predominantly *An. arabiensis* (98.7%) with *An. quadriannulatus* accounting for only 1.3% of the total number positively identified by the PCR method.

The results of adult composition of the *An. gambiae* group are consistent with those reported by Sharp and le Sueur (1990) who also reported *An. arabiensis* to be the dominant species accounting for more than 96% of a total of 692 specimens processed for identification by iso-enzyme electrophoresis and a small number (99) confirmed by the chromosomal identification technique. More *An. quadriannulatus* were identified in the larval stages (5.9%) than from the exit trap adult collections which comprised only 1.3%. This has confirmed the exophilic behavioural characteristics of this species (Gillies and de Meillon, 1968; Sharp *et al.*, 1990). These results also showed that PCR technique compared favourably with more established ones, for example iso-enzyme electrophoresis and the polytene chromosomal identification techniques. But PCR has the advantage of being easy to perform and does not require a lot of expertice to identify the DNA amplified bands of different

species of the *An. gambiae* group on the electrophoresed agarose gel. However, the initial cost of setting up PCR technique is prohibitive but the need for the identification of specific malaria vector species in an endemic environment is overwhelming, especially so when vector control programmes are envisaged.

**4.4. Malaria vector behaviour, transmission and control**

The parity results obtained in this study showed that 51.5% female *An. gambiae* group caught in the months of January, April and May were parous. The variation between the months January, April and May was tested for significance by chi-square test but this was insignificant ($x^2$=0.781, p=0.677). The parity rate reported here are lower than those reported by Hoc and Wilkes (in press) who recorded a parity rate of 55.2% in a rural village in Muheza, Tanzania. However, there was no mention of any control activity by these authors and the higher parity rates reported could have been due to lack of intervention to malaria transmission in this village. le Sueur *et al.* (1992) reported a higher proportion of nulliparous components in Mamfene during the transmission season between February and March 1991 and 1992. But this difference could have been as a result of a more consistent rainfall between January and March 1991/2 which resulted in substantial recruitment of the newly emerged adult mosquitoes and hence a shift towards the nulliparous components.

The aim of malaria control measures is to alter the population structure by reducing the average life span of individual mosquitoes and as a result that of the entire mosquito population. For a vector mosquito to be infective, it must

be able to live long enough to support the development of malaria parasites. Each blood meal taken exposes adult female mosquito to infection or infecting its hosts with malaria parasites. The infectivity rate of mosquito vectors is a product of a positive number dissected multiplied by a hundred. But dissection for sporozoite rates is a laborious and time consuming procedure that would only be appropriate in an area with high transmission. This technique would not be appropriate in Mamfene where the transmission is low. However, some advanced laboratories have adopted a relatively reliable immunological technique based on the detection of circum-sporozoite protein (CS), the enzyme-linked immunosorbent assay (ELISA) technique which is expensive (Beier *et al.*, 1987). Therefore, in the absence of sporozoite rates, the parity status which partially determines the longevity of vector mosquitoes becomes an important indicator in malaria epidemiology (Detinova, 1962). This has implications for the malaria transmission because any parous female vector *An. gambiae* seeking a blood meal is likely to be infective and may transmit malaria. The parity reported in this study showed that there were more parous females than the nulliparous components, an indication that there could be active malaria transmission.

The exit trap collections of adult mosquitoes showed a substantial number leave houses either as unfed, bloodfed or half-gravid with only a small proportion exiting as gravids. However, there were variations in abdominal status of exit trap collections between months during the study period, but this difference was tested by chi-square statistical analysis and was found to be insignificant ($p<0.001$). The unfed females accounted for over 57% of the total collections. But the proportion of the unfed components could have been

inflated as a result of a large number of unfeds caught in a single house (no. 10) which accounted for 72.6% of total collection in May 1995. It is possible the unfeds caught in exit traps in house no. 10 may have entered the house for shelter due to close proximity to the breeding sites rather than for blood meal purposes alone. However, these result differ from those reported by Sharp *et al.* (1990) who caught more than 50% bloodfeds in exit traps in control and replastered huts and 32.6% in DDT sprayed huts as compared to only 16.5% reported in this study for all houses irrespective of the spray status. There was an increase in the proportion of gravids caught in exit traps (10.1%) as compared to 0.3% (DDT sprayed huts) and an average of 4% (for replastered and control huts) reported by Sharp *et al.* (1990). These results showed that some degree of indoor resting occurs after a blood meal in all houses irrespective of the spray status possibly in alternative resting surfaces provided by microhabitats, for instance clothes and linen hung on walls and rails, cooking pots etc. Sharp (1995, pers. comm.) observed an association between the amount of alternative resting sites and numbers of mosquitoes caught resting indoors in Mamfene.

The behaviour of a vector mosquito is a key factor in determining the outcome of a malaria control strategy based on house-spraying with residual insecticides. *An. arabiensis*, which is the main malaria vector in Mamfene, exhibits behavioural polymorphism and has varied resting and feeding behaviours (Service, 1985; Sharp *et al.*, 1990). Although the mosquito numbers reported in this study were relatively low and the study period too short to warrant any conclusive observations to be made, nonetheless these results may be indicators of changing circumstances and therefore require

further research to confirm. The most important findings are those of the gravid components which suggest there could be more indoor resting than has been reported to date in the Mamfene area. However, this study was carried out in one village only as compared to that of Sharp *et al.* (1990) which comprised the whole of Mamfene area and the results obtained in the current study needs to be interpreted cautiously until proven by further research.

The highest number of malaria cases occurred within a 2 km radius along the irrigation canal, Balamhlanga swamp and some sporadic cases along the Pongolo River (Figure 3.2.7). These results are consistent with observations by le Sueur *et al.* (1995) that spillage from cracks along the canal and the water courses that were blocked during the construction of the canal are major breeding sites both in summer and in winter. It also possible breeding occurred in seepage from crucks as a result of inadequate maintenance of the main irrigation canal. There were extremely few malaria cases recorded within the study site except for a few located within the buffer zone of type III breeding habitats. But it will be noted that type III habitats were the first contact breeding habitats for mosquitoes from the winter sites and mosquito breeding could therefore have occurred in them throughout most of the year. The malaria transmission was localized in those areas which had continuous adult mosquito production both in winter and summer. This was for the obvious reason that malaria transmission was continuous among the local community and because of the year-round availability of the vector mosquitoes. A similar occurrence was observed in The Gambia, west Africa, where no correlation between malaria prevalence levels and the soil types and the proximity of the villages to the River Gambia could be found even when

they were highly correlated with vector abundance. Instead the length of the transmission season was a more important determinant of malaria transmission (Connors *et al.*, 1995).

### 4.5. GIS in monitoring mosquito breeding and dispersal

The distances between the breeding habitats and the houses where mosquito collections were done (Fig. 2.5) and the number of mosquitoes caught in each house were tested for correlations the result of which showed significant correlations (Exponential curve, log (mosquitoes)=Distance and $R^2$=0.567, where distance is the dependable variable). The mosquito densities were high in houses around the breeding habitats and were less so in those further away from productive sites. The relative density of mosquitoes in house clusters also showed this trend. For example, house clusters nos.1, 2 and 4 recorded higher numbers of mosquitoes than cluster nos. 3, 5 and 6 which were further away from productive habitats. However, confounding factors such as the number of houses around the breeding sites, the placement of the door *viz a viz* the position of the breeding sites, the strength and direction of wind and the height of vegetation between the breeding sites and study houses affected mosquito house densities. Based on results obtained in the current study, I concur with the suggestion of Kitron *et al.* (1994) that it is possible to locate and target breeding sites from where adults emerge once the distribution pattern and the flight range of the *Anopheles* spp. involved are known. Other authors, for example Twigg (1990) and Connors *et al.* (1995), have documented that the intensity of disease distribution decreases with the increasing distance from the point source. The results obtained in this study indicate a similar occurrence, for instance a high number of mosquito population was located around the

breeding habitats. The malaria cases also depicted a similar pattern in the study locality.

The attribute data (mosquito density) were linked to house clusters and thematic maps of these were developed and overlain on the map of Mamfene section 8. When mapped, this gave a clear picture of mosquito distribution. The mapping concept is a powerful tool for displaying the spatial distribution of geo-linked information and is a valuable means of epidemiological monitoring which reveals information unnoticed when written on paper (Lovett, 1992). Guthe (1993) also in a review, noted that decision makers are practically oriented and are easily influenced when patterns are presented on a map. The results obtained in the current study concur with views expressed by these authors.

# CHAPTER FIVE

# CONCLUSIONS AND RECOMMENDATIONS

## 5.1 Conclusions

### 5.1.1 Malaria vector breeding

5.1.1.1 The most important breeding sites were the irrigation spillage which almost independent of rains, sustained the malaria vectors breeding in Mamfene. Other breeding sites, for example tap and depression pools were secondary breeding sites.

5.1.1.2 Balamhlanga swamp provide important isolated breeding pockets especially during periods of irregular rainfall as opposed to when there is regular rains and waterbodies become numerous and profuse breeding of malaria vectors occurs. It is possible to target these isolated breeding habitats for larviciding both in winter and early summer.

5.1.1.3 These results confirm *An. arabiensis* as the main malaria vector species in Mamfene and was the dominant species identified in all types of breeding sites studied. *An. quadriannulatus* were infrequently identified even in the larval stages. Sharp *et al.* (1984) reported *An. quadriannulatus* as the dominant of the two, but there has been systematic reduction of this species since then as indicated by results obtained by Sharp *et al.* (1990) and le Sueur (1991)

### 5.1.2 Adult mosquito population

5.1.2.1 The increase in the gravid component of adult mosquito population caught in exit traps could be an indication that there is increased indoor resting probably on unsprayed surfaces, for instance on cloths and linen hung on walls, cooking pots and under the beds.

5.1.2.2 The parity dissections showed a proportionately large number of adult mosquitoes caught in exit traps are old and are able to transmit malaria in spite of control programmes in Mamfene.

5.1.2.3 GIS plotted spatial distribution pattern of malaria vectors show a high concentration in house clusters adjacent to the breeding sites than those located further away. Important observation made here was that high density of mosquito population in house clusters was correlated to the proximity of the mosquitoes emergence source. These findings are consistent with those of Connors *et al.* (1995) who observed a strong correlation between high density of mosquito population in huts adjacent to mosquito emergence source.

## 5.2 Recommendations

### 5.2.1 Control measures

#### 5.2.1.1 Larviciding

Winter breeding habitats which act as the main radiation points during summer could possibly be singled out for larviciding before onset of summer rains. This point had also been elucidated by le Sueur *et al.* (1992). It is cost-effective and important to locate and carry out control measures in these habitats in winter before summer vector radiation occurs each season because in summer, the breeding sites are numerous and larviciding would be ineffective.

#### 5.2.1.2 Environmental management

5.2.1.2.1 Water spillage from the canal should be reduced by returning it to the natural watercourses which have uninterrupted flow and terminate in the Pongolo river. This would require the construction of suitable canals which are concrete lined. In the long term this will prove more cost-effective than larviciding strategies which may have long term adverse environmental implications.

5.2.1.2.2 Regular maintenance of the canal, for instance, deweeding and repair along the edges. This will allow un-interrupted water flow and the avoidance of spillage from crucks which in turn ensures no mosquito breeding occurs at these points. The GIS plotted malaria cases (Fig. 3.2.7) show a distinct pattern along the irrigation canal which reflect the state of lack of repair in the last few years.

5.2.1.2.3 Flash points which result in sudden adults mosquito emergence needs to be got rid of, for instance, there was sudden increase of adult population at certain houses in the vicinity of a breeding site which did not have high number of larvae previously, for example house no.10, house cluster 4. This was clearly the result of storm water carrying larvae down-stream. The larvae, probably in their penultimate stages were dumped at this site from where the adults emerged. This point at which water stagnate should be dug out to allow free flow of water down-stream.

5.2.1.2.4 Tap pools were among the most important breeding sites, at least in the early stages of vector mosquito radiation. The maintenance and drainage systems of these important water sources were not adequate and consequently the resultant stagnant water pools were utilised by the malaria vectors for breeding. The maintenance of these taps needs to be on regular basis and sewage systems provided to mop up excess water which spill from the taps during use. Each tap needs to be reconstructed with concrete slub, secured and provided with a gate to keep off cattle. A french drain needs to be built from the tap leading to a small reservoir tank which would be used as water point for cattle especially in section 8A, a settlement scheme where many taps are located. Each tap could be entrusted to an elder who would liaise with field officers from the Department of Agriculture for their maintenance.

**5.2.2 Research activities**

Data collection of malaria vectors should be sustained as a result of which better understanding of malaria transmission dynamics will be elucidated. This will also assist in assessing the gains made in the malaria control programme.

## 5.3 Limitations of this study

The limitations encountered when carrying out this study were two folds, for instance, those that were inherent in the study design and those which were imposed on the study by unfavourable climatic conditions.

### 5.3.1 Limitations due to study design

5.3.1.1 This study did not investigate the indoor resting component of *An. gambiae* group to be able to assess the effectiveness of the residual insecticide and as such it was not possible to draw conclusion on the status of insecticide against the malaria vectors.

5.3.1.2 The study period was too short to allow for seasonal variations to be fully assessed with respect to climatic variables that could possibly have affected the mosquito population.

5.3.1.3 Culicine mosquitoes were not exhaustively studied. When the current study was carried out, numerous culicine larvae and adults were encountered. Their possible role in arboviral transmission needs to be assessed.

### 5.3.2 Limitations due to weather conditions

Rainfall was the most important factor that affected mosquito population in Mamfene. The rains were irregular and seasonal inundation was erratic. The vector radiation from the winter breeding sites was limited due to non-availability of waterbodies inland. The data collection was inconsistent especially in February and March when the mosquito population was too low and could not be used for data analysis.

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| | | | *Anophelines:* Instar stages | | | | | Culicines |
|---|---|---|---|---|---|---|---|---|
| Breeding sites | No. of dips | Time taken | 1st | 2nd | 3rd | 4th | Pupa | |
| Irrigation spillage | 85 | 125 | 66 | 45 | 34 | 2 | 0 | 34 |
| Balamhlanga swamp | 95 | 80 | 6 | 5 | 3 | 1 | 0 | 58 |
| Totals= | | | 72 | 50 | 37 | 3 | 0 | 92 |

**Appendix 1a:** Table showing *Anopheline* and *Culicine* larvae collected from different breeding sites in Mamfene irrigation scheme between September and November 1994.

| | | *An. gambiae*: PCR results | | |
|---|---|---|---|---|
| Breeding sites | No. processed | *An. arabiensis* | *An. quadriannulatus* | Totals |
| Irrigation spillage | 41 | 38 | 3 | 41 |
| Balamhlanga swamp | 10 | 4 | 1 | 5 |
| Totals = | 51 | 42 | 4 | 46 |

**Appendix 1b:** Table showing PCR results for the identification of *An. gambiae* group of species.

| | *An. gambiae* group | | *An. rufipes* | | *An. ziemmani* | |
|---|---|---|---|---|---|---|
| Breeding sites | females | males | females | males | females | males |
| Irrigation spillage | 27 | 16 | 13 | 25 | 1 | 1 |
| Totals | 43 | | 38 | | 2 | |

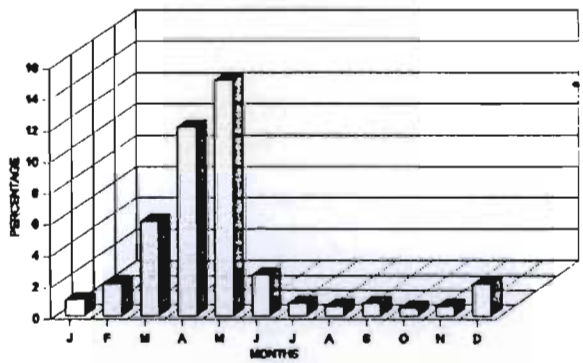
**Appendix 1c:** Table showing *Anopheline* species profile collected between September and November 1994.

| Habitats | | Predators | | | | Total number of larvae | Total number of dips | Total time (mins) | Mean Temp. (°C) |
|---|---|---|---|---|---|---|---|---|---|
| Type | Area ($m^2$) | Triops | Notonectidae spp | Other Hemipteran spp | Total predators | | | | |
| I | 0.5 | 14 | 0 | 0 | 14 | 449 | 105 | 41 | 26.7 |
| II | 2.4 | 0 | 2 | 0 | 26 | 92 | 90 | 53 | 28.8 |
| III | 10.6 | 15 | 22 | 27 | 64 | 1186 | 209 | 58 | 29.5 |
| IV | 11.1 | 17 | 9 | 26 | 52 | 54 | 95 | 65 | 29.8 |
| Totals= | 24.6 | 46 | 33 | 53 | 156 | 1781 | 499 | 217 | 28.7 |

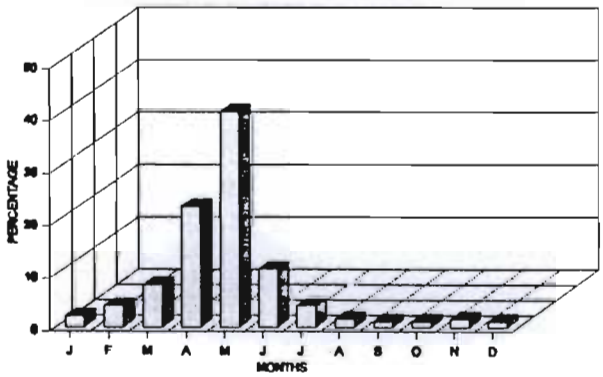
**Appendix 2:** Total number of larvae and predators and other characteristics of each type of habitat.

**Appendix 3:** Mean annual rainfall in southern African countries (le Sueur, 1995, unpublished).

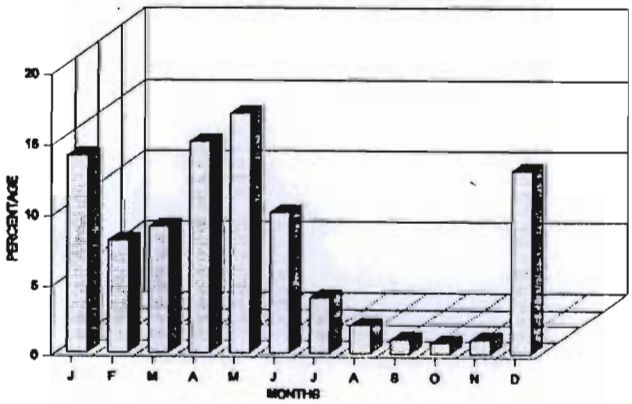
## NAMIBIA 1990




## BOTSWANA 1988




## KWAZULU/NATAL (SOUTH AFRICA) 1985-1988




## ZIMBABWE 1972-1981