EFFECTS OF TEMPERATURE ON MEMBERS OF THE *ANOPHELES GAMBIAE* COMPLEX (DIPTERA: CULICIDAE) IN SOUTH AFRICA - IMPLICATIONS FOR MALARIA TRANSMISSION AND CONTROL

by

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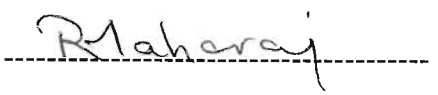
This thesis is dedicated to my late mother.

PREFACE

The experimental work described in this thesis was carried out at the Medical Research Council in Durban from January 1991 to July 1995, under the supervision of Professor C.C. Appleton (University of Natal) and Dr D. le Sueur (Medical Research Council).

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any University. Where use has been made of the work of others it is duly acknowledged in the text.



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ABSTRACT

This study investigated the effects of temperature and relative humidity (both controlled and natural) on the lifecyle and morphology of adults of members of the *Anopheles gambiae* complex in northern KwaZulu-Natal, South Africa.

Laboratory investigations into the effects of simulated temperature and relative humidity regimes concentrated on seasonal differences in longevity, egg hatchability, reproductive potential and adult survivorship of *An. arabiensis*. Differences were found in the life table parameters when these mosquitoes were reared under conditions of seasonal temperature and relative humidity. During the cool season the lifespan and adult survivorship of mosquitoes were greater than those reared during the warm season. In summer, the egg hatchability and reproductive potential were greatest whereas in winter *An. arabiensis* underwent gonotrophic dissociation although these females were found to take blood meals readily.

The influence of seasonal temperature and relative humidity on the body size of *An. arabiensis* was investigated, both in the laboratory and under field conditions. In both environments, these factors were found to significantly influence body size. In winter, there was a 13% increase in wing size compared to summer bred mosquitoes. A comparison of body size of *An. arabiensis, An. merus* and *An. gambiae* reared under laboratory conditions of seasonal temperature and relative humidity showed that the wing size of *An. arabiensis* was greater than that of *An. merus* and *An. gambiae*.

The effect of temperature and relative humidity on morphological criteria used in species separation was also investigated. Seasonal differences in wing spot size were compared for *An. arabiensis*, *An. merus* and *An. gambiae*. From this investigation it was concluded that the pale and dark spots on the wing of *Anopheles* mosquitoes could not be used in species identification due to the large degree of inter-species overlap in the wing spot measurements. The measurement of the pale band at the junction of the 3rd and 4th tarsomere on the hind leg was also investigated for its use in species separation and were found to be useful within the *An. gambiae* complex.

The implications of this study on the transmission and control of malaria are discussed with reference to the late season transmission during March to May that is characteristic of the region.

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CHAPTER 1

GENERAL INTRODUCTION

Malaria is the disease caused by protozoan parasites of the genus *Plasmodium* that are transmitted to humans by certain anopheline mosquitoes. Despite major campaigns to eradicate malaria, the disease remains one of the most serious and widely spread tropical diseases in the world. According to the World Health Organisation, 110 million clinical cases of malaria occur each year, mostly in Sub-Saharan Africa (WHO 1992). The majority of these are infections with *Plasmodium falciparum* which causes the most virulent form of the disease.

1.1. WORLD MALARIA SITUATION

Malaria is a major public health problem in many tropical and subtropical countries, mainly in Africa, Asia and Latin America (Bruce-Chwatt 1987). According to World Health Organisation estimates, 90% of the annual clinical cases of malaria occur in Africa, and 1.5-3 million deaths, one million of them African children less than five years of age. Of the 10% of clinical cases outside Africa, India accounts for 38% of cases, and Brazil accounts for 11% (mainly from Amazonia). Some 70% of cases outside Africa come from just seven countries: in decreasing order of incidence these are India, Brazil, Sri Lanka, Afghanistan, Thailand, Viet Nam, and Colombia (WHO 1992).

1.2. MALARIA IN THE SOUTH AFRICAN CONTEXT

South Africa is at the southern extremity of malaria distribution in Africa. Malaria occurs in limited areas in South Africa (Figure 1.1). Limited focal transmission of malaria may develop in the Northern Cape, along the Orange River, during conditions of favourable temperature and rainfall. The focal malaria areas are the lower altitude areas (below 1000 metres) of Northern Province, Mpumalanga and the north-eastern part of KwaZulu-Natal. Malaria in the former KwaZulu area of the KwaZulu-Natal province is largely endemic and restricted to the two northerly magisterial districts of Ingwavuma and Ubombo. It may however be considered to be endemic to the Ndumu and Makanisdrift areas of the Ingwavuma district (Figure 1.2).

The earliest recorded malaria epidemic in KwaZulu-Natal was in 1905 in Durban (Hill & Haydon 1905). In 1929 a severe epidemic occurred in the coastal districts of northern KwaZulu-Natal. The 1932 season has proved to be the worst experienced in the documented history of the province - the Department of Health estimated that the total number of deaths was 10 000 (Nethercott 1974). This figure was in striking contrast to those produced by the magistrates for the various districts, and which gave a total of 22 132 (le Sueur *et al.*1993). There was enormous controversy over the actual number of deaths and the different aspects of the argument were discussed by le Sueur (1991) and le Sueur *et al.* (1993).

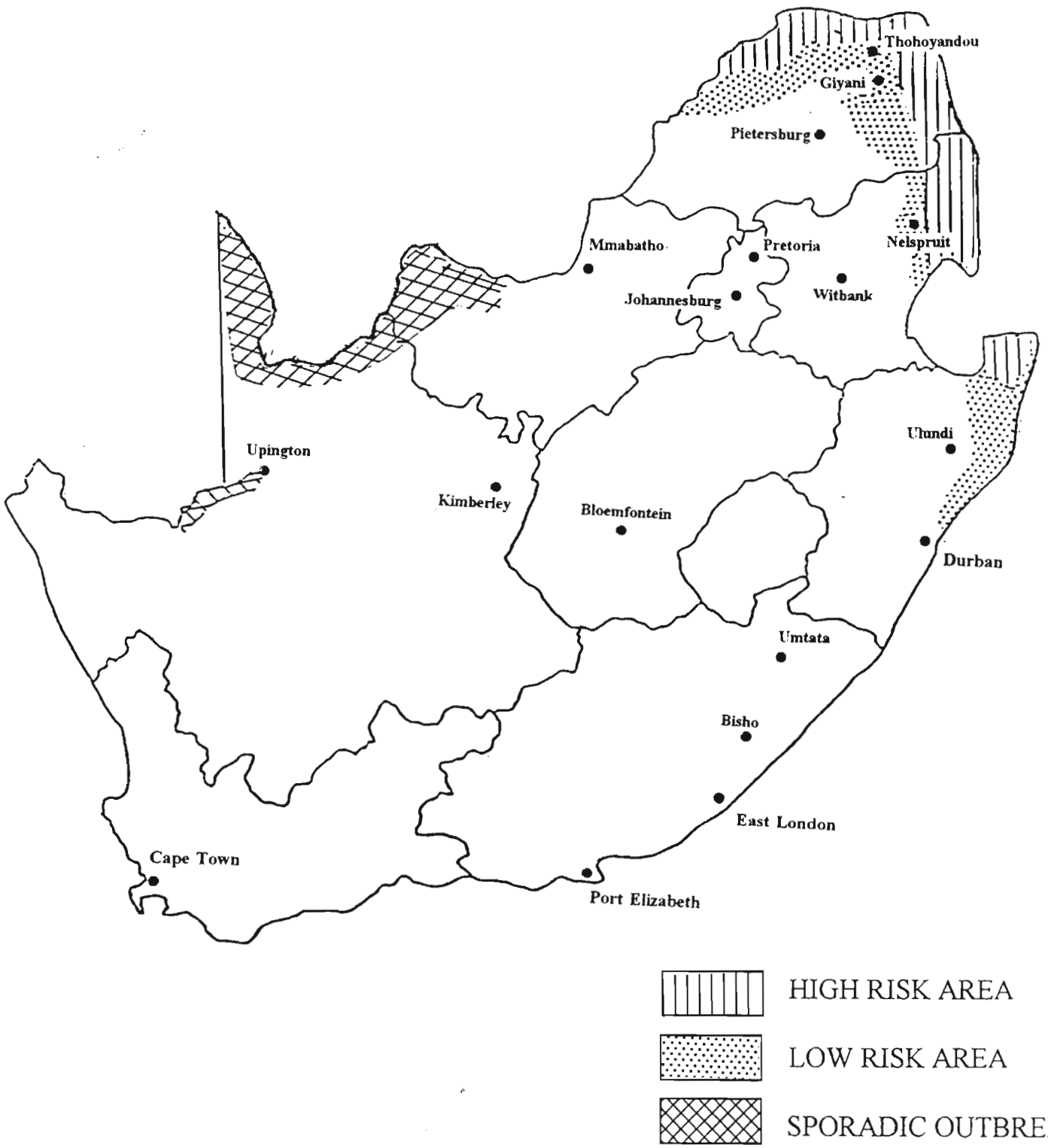

Figure 1.1. The distribution of malaria in South Africa.

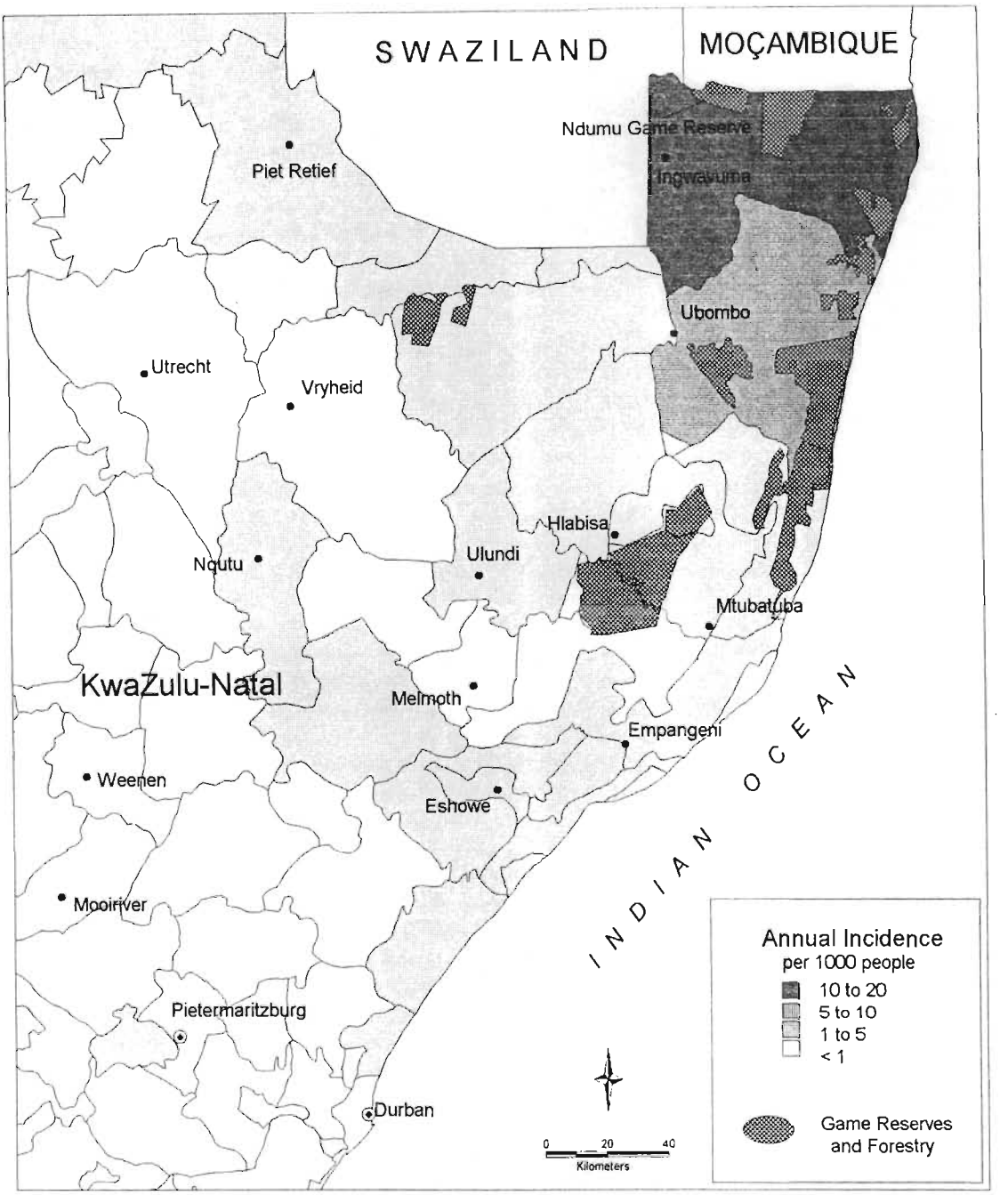

Figure 1.2. The malaria areas in KwaZulu-Natal. Average annual malaria case incidence, 1987 to 1993 (indigenous and imported malaria). Source: National Malaria Research Programme, Medical Research Council, Durban.

Judging from the thorough investigation conducted by Park Ross (le Sueur 1991), it is blatantly apparent that the official figure of 10 000 deaths is indeed incorrect.

Anti-larval measures using oil and Paris Green were introduced in 1932 and continued to be the main means of control until 1946 (le Sueur *et al.* 1993). In 1934, intradomiciliary spraying using pyrethrum was introduced; spraying was repeated weekly during the main transmission season - from October to May (De Meillon 1936). In 1946 the use of pyrethrum was discontinued and replaced by DDT for house spraying and larviciding (Sharp *et al.* 1988). After 1970 the malaria control programme became more structured and more efficient. Surveillance methods for malaria parasite detection were enhanced through the introduction of active surveillance, laboratory diagnosis was improved through the training of staff and equipment and facilities were upgraded. More than 85% of the notified cases of malaria in South Africa are attributed to infection by *Plasmodium falciparum* (Medicines Information Centre 1994).

1.2.1. **Seasonal Malaria Transmission**

As has been determined by Sharp *et al.* (1988), peak transmission occurs in autumn, whereas one would expect it to occur in mid-summer when mosquito population numbers are at their maximum. Transmission efficiency by the vector is a combination of population size and longevity (le Sueur 1991). le Sueur (1991) hypothesised that although population turnover may peak in mid-summer, longevity may be reduced due to the

production of smaller, weaker individuals at high temperatures. Bates (1941) recorded a decrease in the *An. maculipennis* adult population in Albania, during summer and suggested that this might be related to temperature and saturation deficit. This reduced longevity in summer may result in the peak in the adult population occurring in late summer. It could thus be theorised that the late season transmission of malaria is a result of an optimal balance between population size and longevity, at cooler temperatures.

1.3. MALARIA CONTROL

The control of malaria is two pronged, through the control of the mosquito vector and through the elimination of the blood stage of the parasite within infected human hosts.

1.3.1. **Vector Control**

The efficient control of vector species of mosquitoes is central to the containment of malaria. Vector control aims to reduce transmission to the lowest possible level or even to interrupt it (Ravaonjanahary 1992). Vector control can be achieved through two processes:

I. adult mosquito control through residual spraying

ii. larval control through larviciding

1.3. 1.1. **Adult Mosquito Control**

Spraying of residual insecticides under eaves and on the inside walls of houses has proved to be one of the most effective methods of controlling malaria vectors that feed indoors. The mosquitoes rest on the treated surface either before or after feeding and pick up a lethal dose of insecticide. DDT remains the insecticide of choice due to its long residual effect, low toxicity, relatively low cost (Table 1. 1) and its effectiveness against the target vector (Capel-Williams 1991; Mellanby 1992). Table 1. 2 gives the approximate number of structures annually sprayed with insecticides in South Africa.

Table 1.1. The cost of insecticides used for adult mosquito control*.

INSECTICIDE (Wettable powders)	CONCENTRATION (g/active ingredient [a.i.])	APPLICATION RATE (a.i./m²)	COST/KG (Rands)	COST/m² (Cents)
DDT	750	2	20.07	5.35
Bendiocarb	800	0.4	394.40	19.70
Cyfluthrin	100	0.02	577.20	11.50
Deltamethrin	50	0.02	178.12	7.12
Lambda-cyhalothrin	100	0.031	324.00	10.04
Fenitrothion	400	1	42.52	10.60

*Department of Health, Pretoria

Table 1.2. Number of structures sprayed with DDT during the 1994/95 season.

DISTRICT	DDT SPRAYED STRUCTURES	DDT USED (kg)
Mpumalanga	208 587	21 793
Northern Province	841 260	94 506
KwaZulu-Natal	216 235	27 309
Northern Cape	1 180	18
TOTAL	1 267 262	143 624

*Source: Provincial Departments of Health.

The structures are sprayed before the main transmission season, usually starting in September for high risk areas in KwaZulu-Natal. DDT is applied to mud-plastered wall surfaces once annually. However, some respraying may be necessary where re-plastering, re-thatching or new construction has taken place. Pyrethroids or carbamates are applied on cement-plastered and painted surfaces.

However, in some areas where DDT is sprayed, bed bugs present a problem because the DDT irritates the bed bugs and increases their activity (Rafatjah 1971; Newberry *et al.* 1984). Fenitrothion is also applied in these dwellings to control the bed bugs (Newberry 1991).

The use of DDT in early control programmes successfully eliminated *Anopheles funestus* from South Africa (De Meillon 1986). However, controlling *Anopheles arabiensis* effectively is difficult because it feeds indoors and rests outdoors (Sharp *et al.* 1990; Sharp & le Sueur 1991).

1.3.1.2. **Larviciding**

The breeding sites of the main malaria vectors are numerous, widely distributed, difficult to locate, often temporary and exposed to sunlight (le Sueur & Sharp 1988). They include rice fields, cattle hoof-prints and borrow-pits. Due to the temporary nature of some breeding sites, the application of either chemical or bacterial (*Bacillus thuringiensis*) larvicides (Rishikesh *et al.* 1983) are not used except in particular situations where breeding sites are well localised. For larval control surface water is treated using temephos at 200 ml 50% emulsifiable concentrate per hectare of water (Department of National Health and Population Development 1988). Environmental control such as drainage or biological control using fish (Alio *et al.* 1985; Roberts & Sampson 1987) is not used in South Africa.

1.3.2. **Parasite Control**

The main efforts of malaria prophylaxis are aimed at *P. falciparum*. Infection with *P. vivax, P. ovale* and *P. malariae* may cause severe morbidity but rarely death (Breckenridge 1989). No antimalarial drug can guarantee

protection against malaria. Nevertheless, the only way to suppress the blood stage parasites is through the use of drugs.

Prophylaxis has little influence on the control of malaria transmission but is important for individual protection. For prophylactic purposes, a combination of chloroquine and proguanil is recommended during the months of October to May (press release by the Department of Health, 28 November 1994). The therapeutic treatment of malaria consists of sulfadoxine/pyrimethamine compound (Fansidar) or halfan (Halofantrine) (Department of Health 1994).

1.4. SHORTCOMINGS OF MALARIA CONTROL

The control of malaria is becoming more difficult because the malaria parasites are becoming increasingly resistant to antimalarial drugs (Sharp & Freese 1990). Furthermore, the behavioural patterns of mosquito vectors are such that they are able to avoid the residual insecticides (Sharp & le Sueur 1991). There is increasing migration of infected people between malarious regions and non-malarious areas (Ngxongo 1994). Lack of appropriate research and modifications of the environment have also contributed to the declining effectiveness of malaria control programmes throughout Africa.

1.4.1. **Drug Resistance**

Chloroquine resistant parasites were first reported in East Africa in 1979 (Fogh *et al.* 1979) and have since spread to other countries. In 1985 chloroquine resistance was confirmed in KwaZulu-Natal (Freese *et al.* 1988). In the Mpumalanga malarious areas, 1.2% resistance to

chloroquine has been established (Hansford 1989). In KwaZulu-Natal resistance has increased from 0% in 1983 to 20% in 1987 (Hansford 1989). In Mpumalanga, Deacon *et al.* (1994) found seven out of a total of twelve isolates to be resistant or partially resistant to chloroquine. The level of resistance is considered to be influenced by the dosage and duration of use of the drug. Continued use of chloroquine in areas where resistance occurs may lead to a higher degree of stable resistance (Sharp & Freese 1990).

1.4.2. **Population Movement**

Since the late 1950s considerable agricultural and industrial development has taken place in malarious areas. This has attracted local and foreign workers some of whom are asymptomatic and carry parasites with them. Infected mosquitoes are also transported in their vehicles. The highest number of cases reported in KwaZulu-Natal is from Ingwavuma. This district is bordered by Mozambique in the north and ongoing cross-border population migration is a major contributing factor to the deterioration of malaria control in this region (Ngxongo 1993). Civil war in Mozambique, strong family ties between people living on either side of the border, job seekers from Mozambique and the availability of health and commercial services in Ingwavuma are considered to be responsible for this population circulation (Ngxongo 1994). The adverse effects of population migration on malaria transmission, epidemics and the spread of the drug resistant strains has been well documented by several authors (Prothero 1961; Gascon *et al.* 1984; Macques 1986).

1.4.3. **Ecological Changes**

Agriculture practices, such as rice cultivation, have provided more favourable breeding conditions for the mosquito vectors of malaria. In KwaZulu-Natal the Makhathini Irrigation Scheme was the direct cause of the increase in the incidence of malaria in Ubombo district in 1987 (Ngxongo 1994). Sharp *et al.* (1992) found that excess water spilled from this irrigation scheme into the Balamhlanga pan created and maintained mosquito breeding sites throughout the year. Irrigation schemes such as this offer a suitable environment for *Anopheles* (Rwamakuba 1991; Service 1991) and extend the availability of vector breeding sites (Bradley 1991), resulting in an increased incidence of malaria and other vector-borne diseases (Thitai 1991).

1.4.4. **Behaviour of the Vector**

The diverse behavioural attributes of *An. arabiensis* throughout its geographical distribution suggests that this species should be investigated at each relevant locality in order to assess the efficacy of regional control measures (Sharp & le Sueur 1991; Sharp *et al.* 1993). Sharp *et al.* (1993) found that the human biting component of *An. arabiensis* exits the huts after feeding. This has serious implications regarding the efficacy of control measures using residual insecticides since the vector does not come into contact with the sprayed surface.

1.4.5. **Controversy over the use of DDT**

The use of DDT in the malaria control programme in South Africa has

created considerable controversy since it was found that DDT can accumulate in biological systems because of its stable, lipophilic properties (Bouwman *et al.* 1994). Bouwman *et al.* (1990a,b) found that the mean intake of DDT from breast milk by infants in KwaZulu-Natal exceeded the acceptable daily intake of 0.02 mg/Kg. However Bouwman *et al.* (1991) concluded that the levels of DDT in the blood did not present a health risk to the people in KwaZulu-Natal.

Although DDT has been the insecticide of choice for the malaria control programme, alternative insecticides are being tested to find a suitable alternative to DDT (minutes of the inaugural meeting of the Malaria Advisory Group's subcommittee for Vector Control, April 1995). Laboratory trials conducted by the National Malaria Research Programme of the South African Medical Research Council have shown that Deltamethrin wettable powder has the potential to replace DDT (le Sueur *et al.* 1995) but tests have to be conducted to test its effectiveness under field conditions.

1.5. THE PRESENT STUDY

1.5.1. **Motivation for Study**

In South Africa, *Anopheles arabiensis* transmits *Plasmodium falciparum*. An investigation of the population dynamics and overwintering by le Sueur (1991) has shown that water temperature influences adult morphology and some biological characteristics of the *An. arabiensis* and *An. merus* larval cycles. This study is aimed at continuing this work and will examine the effects of temperature and relative humidity (both controlled and natural) on

adult *An. arabiensis*, *An. merus* and *An. gambiae* so as to obtain an overall view of the roles of these abiotic variables on the mosquitoes' entire life-cycles and, as a consequence malaria transmission.

le Sueur (1991) found that temperature influences larval instar duration and consequently mosquito population numbers. This author found that in winter larval densities in breeding pools were low and the pre-imaginal growth rate was reduced with the resulting adults being large (body size was measured in terms of wing length). In summer, larval densities increased, larval instar duration decreased and the adults produced were smaller than those produced in winter. A study by le Sueur and Sharp (1991) confirmed that there were significant differences in the body size of *An. merus* produced in winter and summer. The present study was initiated to determine the effect of seasonal and monthly temperature on *An. arabiensis*, the principal vector of malaria in South Africa.

The literature (Haramis 1983, Nasci 1986, Kitthawee *et al.* 1990) indicates that body size may influence vector competence. Since it was established that temperature influences body size (le Sueur 1991), it was hypothesised that differences in the body size of the vector may explain the late season peak in malaria transmission in South Africa. There was thus a need to determine the effect of temperature on body size and subsequently the influence of body size on longevity and malaria transmission. To transmit malaria, a mosquito needs to survive the necessary incubation period of the parasite to become infectious. Therefore, mosquitoes capable of longer

survival after they become infectious have the potential for greater malaria transmission.

The temperature of its aquatic and aerial environments not only affects the mosquito directly, but also determines the part it plays in malaria transmission by its influence on the development of *Plasmodium* sporozoites in the body of the female anopheline. There is a direct relationship between the environmental temperature and the speed with which sporogony proceeds in the mosquito (Detinova 1962). This implies that the development of the parasite within the mosquito will differ from one season to the next and malaria transmission will occur at different rates across seasons. Therefore, the influence of temperature on the size, longevity and fitness of all stages of the mosquito is of interest from the point of view of designing control strategies tailored to particular sets of ecological conditions.

1.5.2. **Objectives**

The main objectives of this study are therefore to ascertain whether or not (I) adult size is influenced by temperature, (ii) longevity is dependent on adult body size, indirectly through temperature, (iii) adult fitness is affected by breeding-pool temperatures, (iv) to test the hypothesis that the late season peak in malaria transmission which is so characteristic of the north-eastern KwaZulu-Natal endemic area, can be explained in terms of the vector's bionomics. Ultimately it is hoped that this information will contribute

to the designing of a specific larvicide/adulticide mosquito-control component of an integrated control program aimed at limiting the transmission of malaria. This could possibly be achieved by tailoring chemical applications to periods of the mosquito's population cycle that can be identified as particularly vulnerable. These could include periods of optimal temperature and/or relative humidity which could be conducive to increased adult longevity and sporozoite development. Such a control measure would cause maximum mortality of both mosquito and parasite coupled with minimum use of pesticide and expense.

1.5.3. **Choice of mosquito species used**

The *An. gambiae* complex consists of six species: *An. gambiae s.s.* Giles, *An. arabiensis* Patton, *An. quadriannulatus* Theobald, *An. bwambae* White, *An. merus* Dönitz and *An. melas* Theobald. *Anopheles gambiae s.s., An. arabiensis* and *An. quadriannulatus* are freshwater species, *An. bwambae* breeds in mineralised water, whilst *An. merus* and *An. melas* breeds in saltwater (Muirhead-Thompson 1951; Gillies & Coetzee 1987). *Anopheles merus* is essentially a coastal species (Mosha & Subra 1982) but thriving populations can be found at considerable distances from the coast usually in salt pans (Cross & Theron 1983).

Anopheles gambiae s.l. is the most important vector of malaria in Africa. Three members of the *An. gambiae* complex commonly occur in the endemic malaria area of KwaZulu-Natal, namely *An. arabiensis, An. merus* and *An. quadriannulatus*. A single record exists for *An. gambiae s.s.* in

South Africa (Miles 1978). Of the three commonly occurring members of the *An. gambiae* complex only *An. arabiensis* and *An. merus* have been implicated in the transmission of malaria whereas *An. quadriannulatus* is of no medical interest because of its zoophilic tendencies (White *et al.* 1980). Although *An. gambiae s.s.* does not occur in KwaZulu-Natal (Miles 1978), it was used in this study because it is an efficient vector of malaria in the rest of sub-Saharan Africa and many studies on the *An. gambiae* complex focus on *An. gambiae s.s.*

1.6. THE STUDY AREA

KwaZulu-Natal, situated entirely within the sub-tropics (27°-31°S; 29°-32°E), is bordered by the Indian Ocean in the east, Mozambique and Swaziland in the north and by other provinces of the Republic of South Africa in the south and west. The province of KwaZulu-Natal, covers an area of 90 327 km^2. The endemic malarious region of the province encompasses the districts of Ingwavuma, Ubombo and Hlabisa. These districts are in the north-eastern part of the province, a coastal plain, bordered by the sea in the east, Mozambique in the north and Lake St Lucia in the south. Due to its low lying topography as well as the southward-flowing warm Mozambique and Agulhas currents, this region has a tropical biota (Bruton 1980). Marine deposits laid down during the Cretaceous period are responsible for the saline nature of certain water bodies within the area.

This area experiences seasonal malaria transmission and Freese *et al.* (1988) have found that the malaria case rate in this region has increased over the years, due in part to chloroquine resistance in *Plasmodium falciparum*.

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CHAPTER 2

OVERVIEW OF MALARIA AND MALARIA CONTROL IN SOUTH AFRICA

2.1. INTRODUCTION

Anopheles arabiensis, a member of the *An. gambiae* Giles complex, is the major vector of malaria (mainly *Plasmodium falciparum*) in southern Africa (White 1974); its congener *An. merus* may be a minor one (Gillies & Coetzee 1987). Work done by le Sueur and Sharp (1991, 1992) has shown that temperature influences various morphological and biological characteristics of the *An. arabiensis* and *An. merus* larvae and has contributed greatly to a quantitative understanding of the bionomics of these species in South Africa.

2.2. MEDICAL IMPORTANCE OF MOSQUITOES

Mosquitoes create a public health hazard by transmitting causative agents of various diseases such as malaria, filariasis, yellow fever, dengue and many other viral diseases (Begum *et al.* 1986). *Anopheles* mosquitoes have long been known as the primary vectors of Plasmodia throughout the tropical and temperate world. According to Briegel (1990), mosquitoes in the genus *Anopheles* have been neglected in laboratory studies, despite their tremendous importance as vectors of malaria.

Anopheles gambiae s.s. and *An. arabiensis* are more important vectors of malaria than is *An. merus* (Coetzee *et al.* 1982). *Anopheles quadriannulatus*, a patchily distributed

species, is not a vector as it feeds on animals other than man. *Anopheles bwambae* is known from one locality on the Ugandan/Zaire border and is both anthrophilic and endophilic and can therefore contribute to malaria transmission (Mahon *et al.* 1976; White 1985). According to Gilles and Coetzee (1987), house-spraying campaigns have failed to eradicate malaria in many parts of Africa and it appears that the presence of *An. arabiensis* as a vector exacerbates the problem. A major factor contributing to this failure is that a significant proportion of populations of both *An. gambiae s.s.* and *An. arabiensis* rest outdoors after feeding and such behaviour reduces contact with the insecticide and therefore its longevity is normal (Gillies & Coetzee 1987). According to Sharp *et al.* (1990) *An. arabiensis* is capable of feeding indoors in DDT sprayed huts, exiting and still surviving. During their investigation on *An. arabiensis* Sharp and le Sueur (1991) found that exit trap catches showed a significantly higher human blood index (HBI) than indoor resting catches. This trend was unaffected by whether or not the hut had been sprayed with DDT (Sharp *et al.* 1990). The high degree of behavioural diversity in *An. arabiensis* throughout its geographical distribution suggests that, as noted in the previous chapter, this species should be investigated at each locality to assess the effectiveness of regional control measures (White 1974; Sharp & le Sueur 1991).

Begum *et al.* (1986) found that the wet and/or dry seasons and/or the availability of different types of breeding habitats influenced the seasonal fluctuations of mosquito population density in Bangladesh. In KwaZulu-Natal, Sharp *et al.* (1988) found the incidence of malaria to be distinctly seasonal, consistently showing a peak during the

months of April, May and June.

2.3. THE EFFECTS OF TEMPERATURE AND HUMIDITY

2.3.1. **Adult Behaviour**

Mosquitoes are very susceptible to desiccation and are thus affected more by the evaporative power of their surroundings than by the wetness of the air. Consequently, saturation deficit is likely to play a more important role in dictating the mosquitoes' behaviour than relative humidity (B.L. Sharp 1992, pers. comm[1].). According to Sharp (1983) temperature and humidity influence the biting cycle of *An. merus* in that biting was recorded within a temperature range of 16 - 25°C and a RH range of 80 - 100%. The rate at which blood digests and the ovaries develop is also influenced by temperature (Day *et al.* 1990). Towards the lower end of the temperature scale in the cool season or in high altitudes, these processes may be so prolonged that breeding comes to a standstill (Bar-Zeev 1958).

2.3.2. **Longevity of Adults**

Temperature and humidity are also thought to play important roles in determining the longevity of adult mosquitoes (Clark & Rockstein 1964; Nayar 1972). McCombs (1980, in Haramis 1983) found that in the laboratory the longevity of adult female *Aedes triseriatus* increased with size. However in this study McCombs (1980, in Haramis 1983) obtained the different size categories through

[1] Dr. B.L. Sharp, Medical Research Council, Durban, South Africa.

manipulating nutritional factors and not as a result of temperature. Nayar (1972) found that the mean lifespan of adult *Aedes taeniorhynchus* maintained at constant temperatures was temperature dependent, i.e. the mean life span of males was 12 days at 32°C and 21.9 days at 22°C while that of females was 22 days at 32°C and 41.2 days at 22°C. This author also concluded that the rate of mortality was greater at high temperatures than at low temperatures, thus indicating a distinct temperature dependence for longevity.

2.3.3. **Body Size**

Temperature is also known to influence the size of various morphological characters of the larvae and is suspected of affecting adult size and "robustness" too (le Sueur & Sharp 1991). These authors found that the mean head capsule width of first to fourth instar *An. merus* larvae collected during summer and winter differed significantly. The mean head capsule width of all instars was greater in winter than in summer. le Sueur and Sharp (1991) also found that the mean wing lengths for *An. merus* females decreased as summer approached. The increase in mean wing length between the months of January (summer) and July (winter) was 19.6%.

2.3.4. **Distribution**

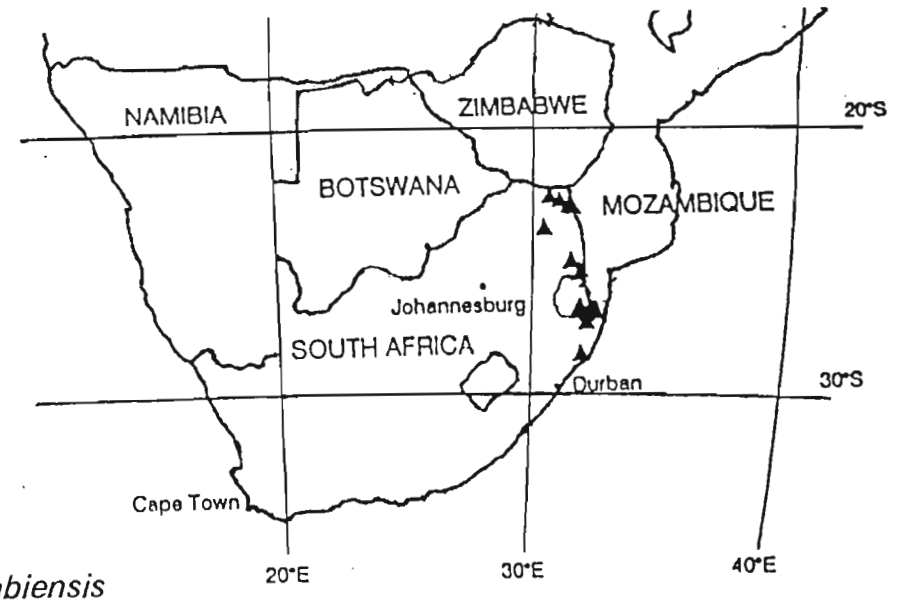
The availability of suitable breeding places influences the distribution of mosquitoes. Larvae of *An. quadriannulatus* are found in small, exposed, temporary pools (Gillies & Coetzee 1987). *Anopheles merus* is essentially a

coastal species (Mosha & Subra 1982) but it can be found at considerable distances from the coast usually in salt pans (Cross & Theron 1983). *Anopheles arabiensis* breeds in open, sunlit pools and may be found in both temporary and permanent habitats (Service 1977; Gillies & Coetzee 1987). The distributions of *An. arabiensis*, *An. merus* and *An. quadriannulatus* in South Africa have been compiled by Coetzee *et al.* (1993) from all published records and provide the most up to date distribution of these mosquito species (Figures 2.1).

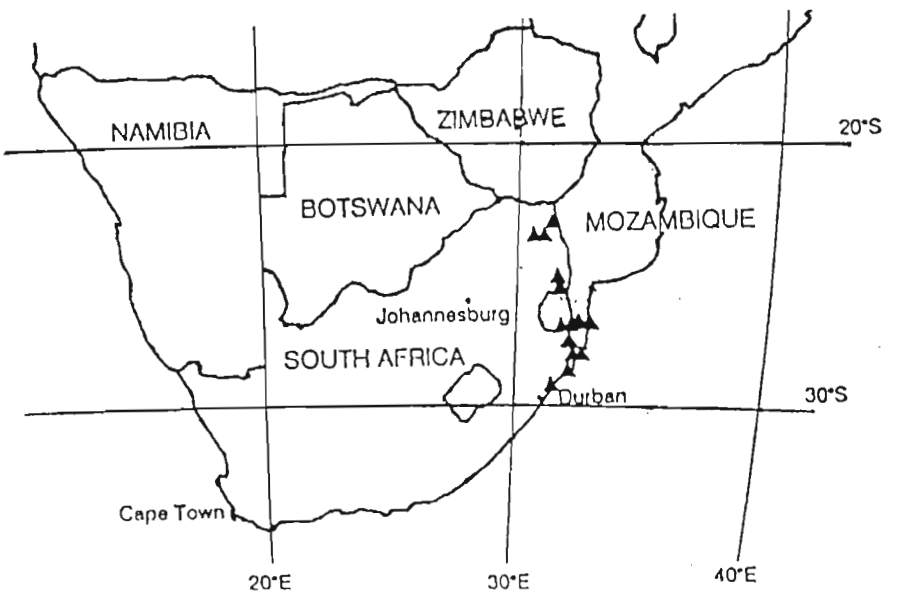
Since mosquitoes are very prone to desiccation, Muirhead-Thompson (1951) suggested that humidity also influences the distribution of mosquitoes since they occur only within certain saturation deficit limits. The fact that many species of *Anopheles* do not extend beyond the cooler and more temperate parts of the world, while the distribution of others is confined to warmer regions, suggest that temperature differences play an important part in determining their distribution (Muirhead-Thompson 1951).

2.3.5. **Larval Development**

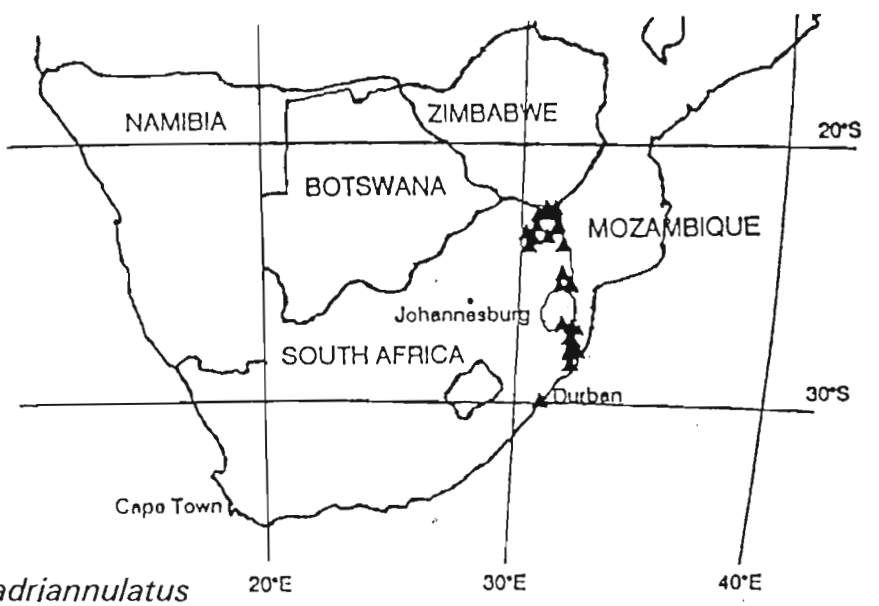
Insects, like most other poikilotherms, respond to changes in environmental temperature by passively conforming to the environment. Bar-Zeev (1958), Milby and Meyer (1986) and le Sueur (1991) have found that temperature influences the development of the immature stages of anopheline mosquitoes and Nayar (1972) found that the longevity of adult *Aedes taeniorhynchus* was temperature dependent. Bar-Zeev (1958) investigated the effects of temperature



(a) *An. arabiensis*



(b) *An. merus*



(c) *An. quadriannulatus*

Figure 2.1. The distribution of (a) *An. arabiensis*, (b) *An. merus* and (c) *An. quadriannulatus* in South Africa.

on the growth rate and survival of the immature stages of *Aedes aegypti* at constant temperatures from 14-38°C. She found that the later the instar, the lower the temperature at which growth is the most rapid. During this study on *Ae. aegypti* the highest temperature allowing development of newly hatched larvae to adult was 36°C. Milby and Meyer (1986) conducted field investigations to determine the effects of constant and fluctuating temperatures on the preimaginal development of *Culex tarsalis*. The average developmental time of immature stages ranged from eight days at 31°C to 16 days at 17°C. These authors concluded that there was no difference in the larval duration of *Cx. tarsalis* under constant or fluctuating temperatures, if the mean of the fluctuating temperatures was equivalent to that of the constant temperature. According to the model presented by le Sueur (1991), the growth of *An. merus* larvae ceases below 12.2°C and above 35°C. The duration of the larval instar also increases as temperature decreases.

2.3.6. **Sporozoite Development**

The temperature of the environment not only affects the mosquito directly, but may determine the part it plays in malaria transmission by its influence on the development of *Plasmodium* in the body of the female anopheline (Meyer *et al.* 1990). These authors found that exposure to a cooler thermal regimen lengthens the period of extrinsic incubation of the parasites within the infected mosquito. According to these authors, the duration of the extrinsic incubation period of a parasite in a mosquito vector is directly dependent upon the thermal environment experienced by the adult female. Chege and Beier (1990) suggested that the

effect of malaria parasites on the survival of their *Anopheles* host is potentially a limiting factor in malaria transmission. Decreased longevity of infected vectors would reduce the proportion of *Anopheles* that becomes infective and therefore their capacity to transmit malaria diminishes. Klein *et al.* (1982) found that the percentage mortality of *Anopheles dirus* was much greater when infected with *Plasmodium cynomolgi*. The percentage mortality of uninfected mosquitoes ranged from 1.71-33.01% while that of infected mosquitoes varied between 1.49-41.3%, over a period of 40 days. Here, mortality was found to occur as a result of deterioration of the mid-gut and salivary gland. Day *et al.* (1990) have suggested that body size may affect both the vector capacity and the vector potential of mosquitoes by influencing their ability to become infected and once infective, their survivorship and their potential to transmit malaria.

Day *et al.* (1990) found that large mosquitoes were more successful in obtaining blood than were small individuals. Larger, blood fed mosquitoes survived longer and were therefore more likely to become infective. Grimstad and Haramis (1984) reported that small adult *Aedes triseriatus* females transmitted the La Crosse virus at higher rates than medium- and large-sized adults. They proposed that small adults ingested a proportionally larger infectious blood meal than the medium and large-sized mosquitoes.

If temperature reduces the longevity of the female mosquito to such an extent that it dies before becoming infective, then the transmission of the *Plasmodium* parasite

is curtailed. The influence of temperature on the size, longevity and fitness of the mosquito may be of interest from a control point of view. Rueda *et al.* (1990) have stated that information on the effects of temperature on the rates of development and survival of the various stages of the mosquito vector are necessary in designing control strategies. According to Meyer *et al.* (1990), at high temperatures the survival of the mosquito may not exceed the minimum duration of the extrinsic incubation of the malaria parasite. Under these conditions it would not be economically feasible to implement control measures as malaria transmission cannot occur.

2.4. **Notification of Malaria Cases**

Malaria is a notifiable medical condition in terms of section 45 of the Health Act (Act No. 63 of 1977). The procedure for notification is described in regulation 19 of the Regulations relating to communicable diseases and the notification of notifiable medical conditions (Government Notice No. R. 2438 of 30 October 1987). This regulation requires all medical practitioners or any other person legally competent to diagnose and treat a person with regard to a notifiable condition to report notifiable medical conditions. The report must include the name, age, sex, population group, identity number (if not available, the date of birth), address, place of work or school, as well as the date of commencement of the notifiable medical condition and any available information concerning the probable place and source of infection (Department of National Health 1995).

The method of notification is shown in Figure 2.2. When malaria is diagnosed in a patient the diagnosis should be reported, as for other communicable diseases, without delay orally, and confirmed in writing on form within 24 hours to the specific local authority for the area. The local authority makes weekly notifications to its provincial Department of Health. The malaria cases diagnosed through the active detection method of the provincial malaria control programmes are also reported to the provincial information system. The reports are sent weekly and monthly. The provincial Department of Health collates all the notifications and computerises the notifications. The provincial Department of Health then makes weekly notifications to the Epidemiology section at the national Department of Health in Pretoria. These notifications are made via computer networks or on computer disks.

2.5. HISTORY OF MALARIA IN SOUTH AFRICA

Malaria has been a serious problem in South Africa until very recently. Louis Trichardt, one of the early pioneers, wrote of the trials and tribulations of his party on their journey from Pietersburg to Lourenço Marques (Maputo). Half of his party died after being stricken by malaria (Gear 1989). Ever since then the lowveld of the Northern Province, Mpumalanga, KwaZulu-Natal and Mozambique has been infamous for the threat posed by malaria (De Meillon 1986).

Malaria has been brought under control through a determined and sustained control programme. The history of malaria in South Africa and the endemicity of the disease before 1905 is unknown and records of the disease prior to 1920 is very scanty and

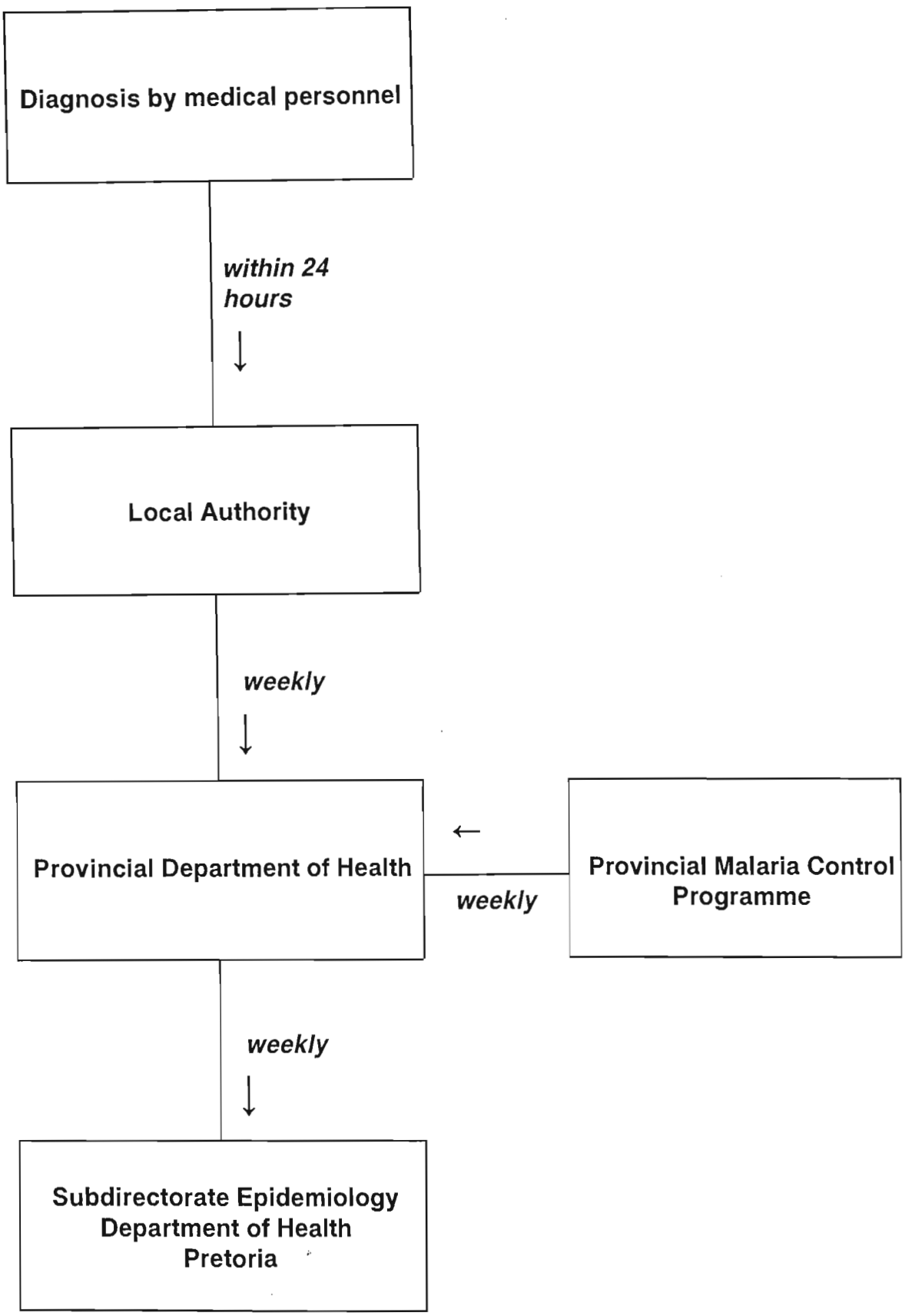


Figure 2.2. Procedure for the notification of malaria.

uncertain. The existing records for malaria are as follows:

1905 - A malaria epidemic was experienced in Durban during the summer of 1904/1905 (Hill & Haydon 1905). This epidemic was severe with 4177 cases and 42 deaths being reported in Durban. *Pyretophalis costalis* (*An. gambiae* s.l.) was incriminated as the vector.

1910 - Dr Park Ross (District surgeon at Nqutu) investigated an outbreak in northern KwaZulu-Natal and advised the use of quinine, screening of dwellings, bed nets and protective clothing.

1920 - There was a malaria epidemic in KwaZulu-Natal which radiated from the St. Lucia area (Department of Health Annual Report 1920).

1921 - The State Department of Health issued instructions regarding treatment and prophylaxis of the disease. This involved the use of quinine. Dr Park Ross carried out the first malaria survey of South Africa (Department of Health Annual Report 1921).

1923 - The Department of Health Annual Report (1923) stated that malaria retarded agricultural development in the Northern Province and Mpumalanga.

1924 - Irrigation areas near Pretoria were severely affected by malaria epidemics until 1924 when antilarval measures were enforced (Department of Health Annual Report 1924).

1925 - Antimalarial committees formed by farmers in KwaZulu-Natal to coordinate preventive measures (Department of Health Annual Report 1925).

1926 - Umfolosi Co-operative sugar mill began employing labourers tolerant of

malaria. During the construction of the Mtubatuba-Gollel railway, a heavy outbreak of malaria followed the importation of labourers (Swellengrebel 1931).

1927 - A mosquito survey of South Africa was started by Ingram and De Meillon and provided information on larval sites of anopheline mosquitoes (Ingram & De Meillon 1927).

1928 - A large number of employees in the sugar mills and plantations of KwaZulu-Natal were afflicted during a severe epidemic of malaria. Of an estimated population of 6 000 whites at risk, 7 died, of 20 000 Asians 151 died and of 215 000 Blacks 2 600 died (Nethercott 1974). The 1928 Department of Health Annual Report (1928) referred to a malaria epidemic extending from Zeerust to Nylstroom - 62% Europeans and 74% of Natives in Rustenburg and Nylstroom were found to be suffering from malaria.

1929 - From 1929-1933 extensive epidemics of malaria severely affected the population of KwaZulu-Natal. The first efforts at control of the disease were instituted. Ingram and De Meillon (1929) completed the mosquito survey of South Africa. *Anopheles gambiae* and *An. funestus* were implicated as the main malaria vectors.

1930 - The South African Government invited Sir Malcom Watson to report on the situation and he advised the application of antilarval measures (Watson 1930). Malaria outbreaks were reported as far south as Umzinto. Professor N.H. Swellengrebel of the University of Amsterdam visited KwaZulu-Natal to take part in an investigation of the malaria situation in South Africa as a whole

(le Sueur 1991). A study by De Meillon (1931, 1934) confirmed that the main vectors of malaria were *An. gambiae*, a pool breeder, and *An. funestus*, a stream breeder, and that both species were house-frequenting and anthropophilic. Female mosquitoes were found to rest indoors thus making them vulnerable to insecticidal sprays.

1931 - Professor Swellengrebel submitted his report to the State Secretary of Health. He recommended that the bionomics and ecology of the vectors should be thoroughly studied with a view to implementing `species sanitation' and that a malarial research station be established at Tzaneen for this purpose (Swellengrebel, Annecke and De Meillon 1931). He also recommended that no malaria control work be done in Ingwavuma, Ubombo and Hlabisa. The disease was extremely prevalent in these districts throughout the year and the indigenous population had consequently developed some immunity to the disease.

1932 - A malaria epidemic severely affected KwaZulu-Natal. The official estimate of the number of deaths was 10 000 (Department of Health Annual Report 1932) but calculations based on the deaths per magisterial district showed that there were actually 22 132 deaths as a result of malaria during this year (le Sueur *et al.* 1993). During the 1931/32 malaria season, Park Ross attempted to control malaria by fumigating huts using sulphur but this was unsuccessful as the fumigant rapidly diffused through the thatched roofs (le Sueur *et al.* 1993). Antilarval measures using oil and Paris Green were introduced and continued to be the best means of control until 1946 (Sharp *et al.* 1988).

1933 - A full scale trial was conducted by Dr Park Ross on the effect of indoor spraying with a kerosene-pyrethrum mixture on malaria incidence. This was started at Eshowe in KwaZulu-Natal, endemic *An. gambiae* country with a high rate of infestation. A field and research station was established at Tzaneen (Department of Health Annual Report 1933).

1934 - Pyrethrum was introduced as a knock-down insecticide in the houses. Spraying was repeated weekly during the main transmission season (Sharp *et al.* 1988).

1936 - Dr. Fred Soper, an American Epidemiologist, visited Eshowe and expressed scepticism at the great reduction in indoor resting *An. gambiae*. He was critical of the methods and suggested that for every mosquito that came indoors, there were hundreds outside that would be out of reach of insecticidal sprays. Nevertheless, the findings of the study in Eshowe were presented to the League of Nations (Park Ross 1936). The interruption of transmission was clearly demonstrated.

1937 - An extensive epidemic occurred from the Limpopo River south to Pongola with excessive mortality and morbidity among the Blacks (Department of Health Annual Report 1937).

1938 - Dr De Meillon remained doubtful of the efficacy of indoor residual spraying as he could find *An. gambiae* s.l. resting outdoors. The observation by Dr De Meillon is important as this was probably the first evidence indicating that *An. gambiae* was a complex of species (le Sueur *et al.* 1993). The mosquitoes that Dr De Meillon observed were probably *An. quadriannulatus*, a zoophilic

and largely exophilic member of the species complex.

1939 - An extensive outbreak developed as far south as Pretoria and west to Rustenburg. Among an estimated population of 1 018 800 a total of 9311 deaths was reported with mortality being highest in Potgietersrus - 2.7% of the residents (Department of Health Annual Report).

1946 - The use of pyrethrum was discontinued and was replaced by DDT, both for house spraying and larviciding (Sharp *et al.* 1988). The DDT campaign lead by Dr Siegfried Annecke was extremely successful and malaria was virtually eliminated from South Africa (De Meillon 1986).

1953 - DDT spraying was gradually extended to include Ingwavuma, Ubombo and Hlabisa, beginning with the spraying of all habitations within a 3 kilometre radius of all mission stations and police camps (Sharp *et al.* 1988).

1956 - Antilarval measures were abandoned. Malaria became a notifiable disease in South Africa (Government Notice no. 2081 of 1956).

1958 - DDT spraying south of the Tugela River was discontinued. Total coverage with a residual insecticide was achieved in the north for the first time in October 1958. In areas where they had discontinued antilarval measures, a system of vector surveillance was instituted.

1959 - Geographical reconnaissance of the entire malarious area of KwaZulu-Natal was undertaken. The various areas were classified into phases of control, in accordance with WHO specifications.

1961 - An expert committee of the WHO visited the Northern Province and recommended the use of DDT instead of benzene hexachloride (BHC). It

could not be determined when the use of BHC was initiated in the Northern Province, but BHC was used in the malaria control programme in this area probably because it was cheaper than DDT, as an agricultural formulation of BHC was used (C.F. Hansford 1995, pers. comm.[2])

1962 - Dr Paterson (Paterson & Paterson 1963) suggested that the *An. gambiae* was a complex of species.

1967 - Following six years of drought, there were heavy rainfalls followed by periods of bright sunshine thus creating favourable conditions for mosquito breeding. Mosquitoes flourished and a severe, though limited malaria epidemic resulted (De Meillon 1986).

1974 - Entomological investigations in KwaZulu-Natal, during or after malaria transmission in the area showed *An. arabiensis* to be the predominant species in the area (White 1974).

1975 - The existing malaria control programme was modernised through the training of field teams and the upgrading of laboratories and clinics (Kustner 1990). Active surveillance was introduced (Dept. of Health and Population Development 1988).

1976 - Heavy rains resulted in a vector build up and an increase in the overall parasite incidence. A laboratory was established at Jozini. Malaria control was delegated to homeland authorities, i.e. Venda, Lebowa, Gazankulu and Kangwane, over the districts for which they were responsible (Department of Health 1995).

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[2]Dr C.F. Hansford, Department of Health, Tzaneen, South Africa.

1977 - The KwaZulu Department of Health was established, separate from the Department of Health and Population Development of South Africa. There was an increase in the number of malaria cases, especially in the Hlabisa District of KwaZulu-Natal (Department of Health 1988). This increase in malaria cases may have been due to increased case detection as a result of active surveillance.

1978 - Favourable climatic conditions were responsible for an upsurge of malaria. Dr J.H. Pull of the WHO visited South Africa to review the problems relating to the malaria programme and to propose practical solutions. He found the existing structures to be adequate (Pull 1978). Intradomicilary spraying of DDT was reduced from two sprays per annum to one application of DDT every 12 months (Department of Health 1988).

1979 - Dr De Meillon visited Richards Bay to investigate the mosquito problem.

1984 - From 1984 - 1988, field surveys showed *An. arabiensis* to be widely distributed in Natal (le Sueur & Sharp 1988). Cyclone Demoina caused widespread heavy rains and this created favourable conditions for malaria transmission. Confirmed infections for 1984 and 1985 were the highest since the introduction of the malaria control programme (Uyirwoth 1994).

1985 - Chloroquine resistance was detected in KwaZulu (Herbst *et al.* 1985).

1987 - Heavy rains during the flood created ideal breeding places for malaria vectors and once again there was heavy transmission of malaria (Department of Health 1984). In KwaZulu-Natal, the increase in malaria cases was as a result of the influx of Mozambican refugees as well as the continued use of

chloroquine, resulting in the inadequate control of the parasite reservoir (D. le Sueur 1995, pers. comm.[3]).

1988 - The first policy on malaria was drawn up by the Department of Health (1988).

1993 - Contained epidemic of malaria occurred in northern KwaZulu-Natal (Uyirwoth 1994, 1995).

1994 - The Malaria Advisory Group was formed to advise on the drawing up of malaria policy, control measures and administering of prophylactics (Minutes of the first Malaria Advisory Group meeting)

1995 - The malaria control policy was revised (Department of Health 1995) and based on the recommendations made by the World Health Organisation (WHO 1992, 1994). The malaria control programmes of the former self governing states were incorporated into the malaria control programmes of the newly formed provinces.

2.6. EPIDEMIOLOGY OF MALARIA IN SOUTH AFRICA

All malaria cases notified to the Directorate of Epidemiology, Department of Health during the period January 1980 to December 1994, were analysed.

2.6.1. MALARIA IN SOUTH AFRICA (1980 - 1994)

In South Africa major gains have been made in containing malaria and efficient malaria control strategies are in operation. However, despite a

[3]Dr. D. le Sueur, Medical Research Council, Durban, South Africa.

spraying programme in the endemic areas, many cases of malaria still occur in these areas. Figure 2.3 indicates the long-term (15 years) trend of malaria transmission in South Africa.

After the outbreak of malaria in 1972, active surveillance was introduced on a large scale, in 1975 and hospitals, clinics and laboratories were upgraded with respect to diagnosis (Department of Health and Population Development 1988). This improved detection may have contributed to the remarkable increase (from hundreds to thousands of cases) in the annual total malaria cases reported. From Figure 2.3, it can be seen that outbreaks were reported in 1985-1986, 1987, 1988 and 1993.

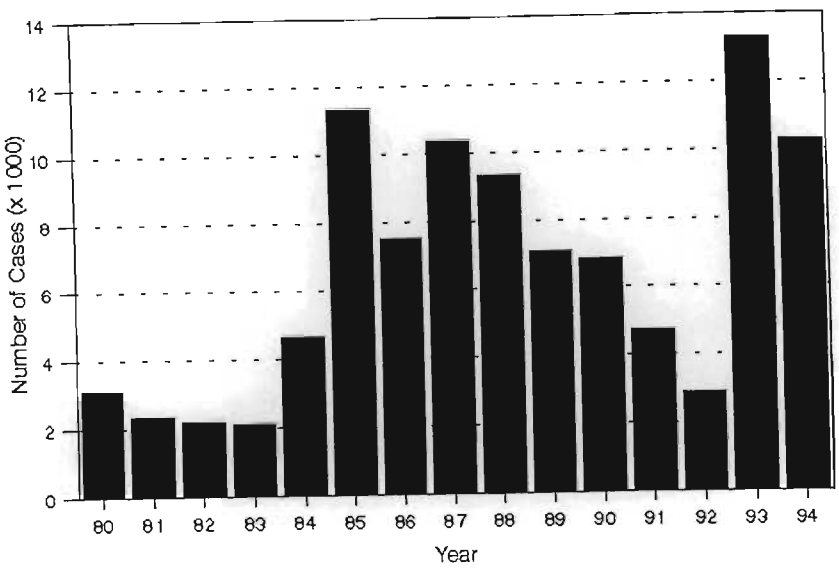

Figure 2.3. The incidence of malaria in South Africa (1980 - 1994).
Source: Department of Health, Pretoria.

2.6.1.1. Annual Incidence

The annual numbers of cases reported for 1980 to 1994 are given in Table 2.1. From 1980 to 1983 there was a steady decline in the annual incidence rate (Table 2.1).

Table 2.1. Annual malaria notifications in South Africa (1980 - 1994).

Year	Number of Notifications	Population (x 1000)	Incidence rate (per 100 000)
1980	3109	28062	11.1
1981	2343	29003	8.1
1982	2184	29975	7.3
1983	2130	30983	6.9
1984	4642	32285	14.4
1985	11358	33105	34.3
1986	7491	33928	22.1
1987	10374	34749	29.9
1988	9317	35566	26.2
1989	7055	36384	19.4
1990	6822	37213	18.3
1991	4693	38049	12.3
1992	2872	38892	7.4
1993	13285	39739	33.4
1994	10286	40715	25.3

There was a peak in the annual incidence rate in 1985 and a decline to a trough of 7.4 per 100 000 in 1992. In 1993 there was a sharp increase in the incidence of malaria. In the past 15 years the total number of malaria notifications exceeded 10 000 cases in 1985, 1987 and 1993. These were all

years in which epidemics occurred. The least number of cases was in 1992, a year of severe drought throughout southern Africa, when only 2 872 cases were reported.

2.6.1.2. Monthly Incidence

Table 2.2 shows the seasonality of malaria that is typical of malaria in South Africa. There is a peak in the number of cases from March to May. In 1987, 1988, 1993 and 1994 more than 1 000 cases were reported for each of these months. Usually the peak in the number of malaria cases occurs in April, but in 1987 it occurred in June with more than 1 000 cases beingreported each month for the period March to June. In 1993 the peak occurred in May. It is one of the objectives of the present study to determine the cause of this seasonal pattern of transmission.

2.6.2. DISTRIBUTION OF MALARIA NOTIFICATIONS

2.6.2.1. By Province

The number of cases reported from each of the nine provinces in South Africa are given in Table 2.3. The number of notifications ranges from zero to 5 268 with the largest number of cases being reported from Mpumalanga, Northern Province and KwaZulu-Natal - the endemic malaria areas in south Africa. Parts of the Northern Cape and North-West provinces experience sporadic malaria transmission during conditions favourable for transmission. Malaria cases reported from the other provinces are most probably due to imported

Table 2.2. The monthly incidence of malaria in South Africa (1980 - 1994).

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1980	245	194	630	1007	433	146	142	81	35	65	76	55
1981	175	169	303	589	368	154	71	67	49	104	170	124
1982	218	342	243	407	273	168	77	74	84	102	129	67
1983	131	111	272	305	338	198	86	64	52	151	115	307
1984	960	565	472	683	484	255	168	130	179	273	222	251
1985	718	603	974	1875	929	766	1113	856	630	814	1262	818
1986	991	968	942	869	893	538	544	373	337	292	281	463
1987	594	884	1103	1304	1223	1331	547	504	483	324	425	1652
1988	1395	1234	1159	1728	1258	604	393	347	437	273	245	244
1989	657	638	1151	1656	851	322	362	265	207	262	200	484
1990	793	798	1278	875	711	601	341	414	393	233	209	176
1991	647	436	589	648	544	305	260	252	271	340	230	171
1992	396	310	326	235	283	258	230	230	176	157	128	144
1993	1602	1010	1963	2062	2769	1359	361	417	242	402	555	543
1994	1122	1204	1441	1776	1306	1076	444	388	256	303	382	588

Table 2.3. The distribution of malaria in the different provinces for the period 1980 - 1994.

Year	Eastern Cape	Mpumalanga	KwaZulu-Natal	Northern Cape	Northern Province	North-West	Free State	Gauteng	Western Cape	Unknown
1980	2	1609	981	2	437	6	3	62	3	4
1981	4	852	216	14	1167	6	2	63	6	13
1982	9	1060	144	0	838	2	4	57	8	62
1983	9	1164	309	3	407	4	9	213	11	5
1984	9	2320	1189	4	604	3	50	424	10	29
1985	7	3867	1619	4	5243	4	90	500	15	9
1986	14	2340	2225	2	2244	4	49	605	6	2
1987	13	1994	6757	5	1051	5	63	474	7	5
1988	16	2403	3937	17	2465	23	42	364	13	37
1989	9	1655	3021	67	1946	11	32	193	9	173
1990	7	1804	2769	8	1686	32	26	264	5	221
1991	4	1555	1855	2	809	41	42	204	5	176
1992	9	1850	318	4	385	41	14	179	11	61
1993	7	4179	3834	22	2200	148	60	616	17	258
1994	7	3086	4544	0	1940	140	35	260	36	238

malaria. Gauteng, a non-malarious area, reports more malaria cases than any of the other non-malarious area. This can be attributed to the higher percentage of labourers that work in this region but have their family homes in the malarious areas. Gauteng province has become the fastest developing region in the country and therefore attracts more people from the rural areas.

The malaria case rate can be examined in terms of the population density. KwaZulu-Natal has a population density of 93,5 people per square kilometre (km^2) whereas Mpumalanga has a density of 34,7 people per km^2 and Northern Province has a density of 42,8 people per km^2 (Department of Health 1994). Therefore, in a given area in the endemic malaria regions, more people in KwaZulu-Natal are exposed to the malarial parasite than in either of the other two provinces. In addition, an increase in density is likely to favour an increase in transmission.

2.6.2.2. By Population Group

The malaria case distribution by race group of the resident population in South Africa is given in Table 2.4. The occurrence of malaria among Blacks for this period is much higher than among any of the other population groups. This is due to the fact that Blacks make up the greatest proportion of the population in malaria endemic areas. Whites have the next highest number of malaria notifications, and whites make up the next largest percentage of the resident population. Malaria notifications among Asians and Coloureds were low with

Table 2.4. Malaria in the different population groups in South Africa.

Year	Asian	Black	Coloured	White	Unknown
1980	10	2963	3	131	2
1981	7	2145	11	177	3
1982	4	2034	5	136	5
1983	7	1951	12	156	4
1984	15	4383	7	222	15
1985	7	10990	3	353	5
1986	8	7290	3	188	2
1987	12	10145	9	207	1
1988	6	8976	24	307	4
1989	5	6822	19	206	3
1990	2	6574	7	236	3
1991	0	4488	8	159	38
1992	2	2638	6	184	42
1993	11	12188	13	850	223
1994	4	9793	12	437	40

less than 30 cases being reported for either population group in any given year.

Blacks are most affected by malaria because of their low socio-economic status. Most Blacks in the endemic malaria areas do not have access to water on tap, they cannot afford chemoprophylaxis and their low cost housing is of poor quality and allows easy entry to mosquitoes. Whites are exposed to malaria because of their agricultural and recreational activities. Most Asians and Coloureds live in non-malarious areas thus accounting for the low

malaria notifications among these population groups.

2.6.2.3. By Sex

Table 2.5 gives the distribution of malaria cases by sex. More males were affected by malaria than females. This is significant as there are fewer males (18 748 323) than females (19 195 654) in South Africa.

Table 2.5. The distribution of malaria by sex.

Year	Female	Male	Unknown
1980	1433	1663	13
1981	981	1359	3
1982	888	1295	1
1983	801	1326	3
1984	1896	2719	27
1985	5387	5950	21
1986	3188	4290	13
1987	4982	5383	9
1988	4381	4930	6
1989	3239	3795	21
1990	3047	3769	6
1991	2089	2590	14
1992	1058	1812	2
1993	5665	7591	29
1994	4251	6020	15

The highest male to female ratio was 1:0.93 in 1987 and the lowest was

1:0.43 in 1994. This discordance may be due to the high mobility of males into and out of malaria areas since they represent the country's main labour force. This mobility into and out of malaria areas would not result in any significant immunity being developed by these males, whereas the females spend more time in the malaria areas and will build up some immunity to malaria.

2.6.2.4. By Age

Data on the age demographic profile of the population in South Africa were obtained from the 1991 population census. The age distribution of malaria cases closely follows the age distribution of the total population of South Africa (Figure 2.4) for the period 1980 - 1994. The majority of the reported

Figure 2.4. Percent of malaria cases for age-specific categories in terms of the population structure.

malaria cases were accounted for by those under 25 years of age. The highest number of cases is reported from the 15 - 19 year age group. The trend for South Africa obtained in this study is similar to that obtained by Sharp *et al.* (1988) who looked at 10 years of data for KwaZulu-Natal, one of the endemic provinces.

2.6.3. Mortality

Between 1980 and 1994, there were 318 deaths out of 91 117 malaria cases, representing an average of 21 deaths per annum (Table 2.6). This gives a case fatality ratio of 0.35%. The annual case fatality ratio varied between 0.1% and 0.61%.

The highest number of deaths occurred in 1988 when 48 deaths were reported. During this year, 29 of the deaths occurred in KwaZulu-Natal. This was probably related to the continued use of chloroquine for treatment, resulting in complicated malaria and death. Due to the existence of chloroquine resistance in the malarial areas of South Africa, namely northern KwaZulu-Natal and Mpumalanga (Freese *et al.* 1994, Deacon *et al.* 1994), chloroquine was replaced with sulphadoxine/ pyrimethamine, in 1988, for the treatment of *P. falciparum* in the Ingwavuma and Ubombo districts of KwaZulu-Natal (Hansford 1989).

Table 2.6. The annual case fatality ratio (CFR) for South Africa.

Year	Dead	Live	Total	CFR
1980	10	3109	3119	0.32
1981	9	2343	2352	0.38
1982	13	2184	2197	0.59
1983	13	2130	2143	0.60
1984	19	4642	4661	0.41
1985	32	11358	11390	0.28
1986	20	7941	7511	0.27
1987	10	10374	10384	0.10
1988	48	9317	9365	0.51
1989	30	7055	7085	0.42
1990	35	6822	6857	0.51
1991	19	4693	4712	0.40
1992	14	2872	2886	0.48
1993	45	13285	13330	0.34
1994	12	10286	10298	0.12

Figure 2.5 shows the malaria mortality expressed as a percentage of the total mortality for the period 1980 - 1994.

Infant mortality is very low, constituting less than 1% of the total mortality. The highest mortality (11%) occurs in the 30-34 year age group, whereas this age group contributes only 9% to the total number of malaria cases reported. Individuals in this age group are a highly mobile segment of the population, due to employment obligations and are prone to developing severe and complicated malaria since they may go to areas where there are resistant

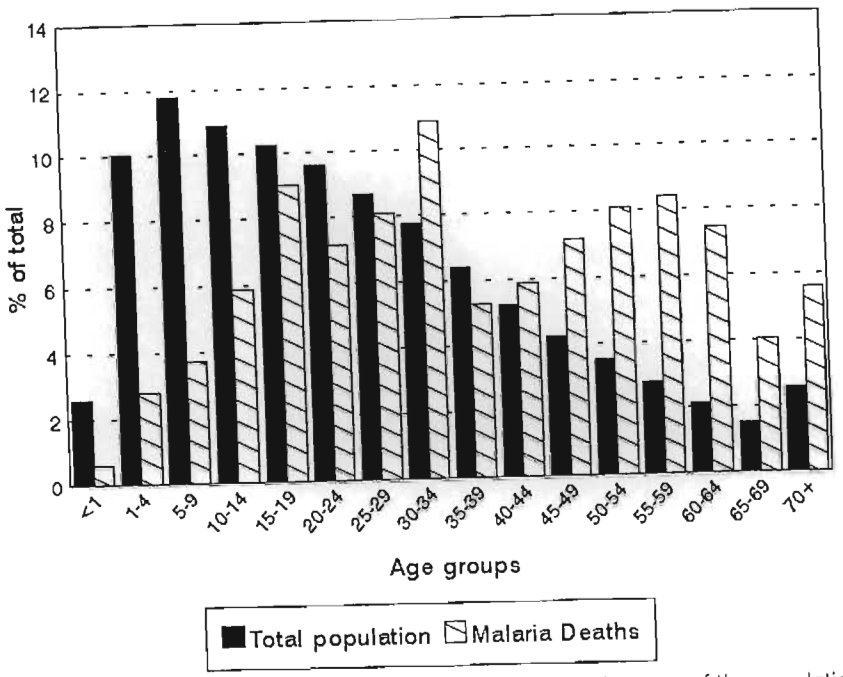

Figure 2.5. Percent of malaria mortality for age-specific categories in terms of the population structure.

strains of *P. falciparum*, or where malaria is misdiagnosed.

2.7. Conclusions

South Africa has a long history of malaria control and the current objective of the malaria control programme is to limit the ill effects of malaria through the reduction of malaria transmission. The malaria control programme has been successful in making large parts of the country malaria free and endemic malaria areas occur only in the northern and eastern borders regions of South Africa.

Although DDT has proven to be very effective in controlling adult vectors of malaria,

antipathy to DDT has developed because of pollution, danger to the environment, ineffectiveness against household pests and staining of walls. It is therefore necessary to find suitable alternatives to DDT that are cost-effective and have a similar long lasting insecticidal effect. However in our quest to replace DDT, the gains that have been made in malaria control over the past 50 years should not be jeopardised.

Although the notification system in place in the malaria control programme is very efficient, there is a need for improvement. The forms that are being used to gather information need to be standardised throughout the country to ensure that comparable data is collected from all areas. Due to the incorporation of the former self-governing states into the new provincial structures, different health administrations have to be amalgamated. The various health administrations had different forms for gathering information and this has caused numerous problems since the data gathered was not comparable. As soon as the amalgamation process is completed, the forms used must be standardised to collect uniform epidemiological data.

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CHAPTER 3

LIFE TABLE INFORMATION OF *ANOPHELES ARABIENSIS* UNDER SIMULATED CONDITIONS OF SEASONAL TEMPERATURE AND RELATIVE HUMIDITY

3.1. INTRODUCTION

The impact of temperature on insects was emphasised by Andrewartha (1971) who stated, "Temperature influences the speed of development, the duration of life, the fecundity, and behaviour of animals, especially poikilotherms". Life tables provide information on maximum expression of a species' genetic potential under existing conditions. Survivorship and reproductive strategies have been extensively studied in culicine mosquitoes (Crovello & Hacker 1972; Walter & Hacker 1974; Reisen *et al.* 1979), but complete adult life tables have been constructed for relatively few anophelines (Reisen & Mahmood 1980).

Age-specific horizontal life tables present a succinct tabular summary of mortality and reproductive schedules (Reisen & Mahmood 1980). When constructed under insectary conditions, with the requisites of life continually available, the values obtained may approach the maximum expression of the species' genetic potential and may be used to study inherent differences in the survivorship and reproductive strategies of populations evolving under different ecological regimes.

Since insects respond readily to changes in environmental temperature, numerous studies have been done on the effects of temperature on physiological systems in insects (Bailey & Gieke 1968; Reisen *et al.* 1984; Morsy *et al* . 1995). However, few studies have been carried out on the effects of temperature on life table parameters of mosquitoes (Beier 1990; Milby & Meyer 1986; Nayar 1972). The comparison of life table phenomena among natural populations within one species can help explain why certain strains survive only in particular environments. *Anopheles arabiensis* is generally considered to be the most important vector of human malaria in South Africa and indeed over much of southern Africa. Because of the interest in the ability of this species to survive long enough to become both infected and infective with *Plasmodium falciparum*, studies on longevity were conducted in the laboratory under conditions of fluctuating seasonal temperature and relative humidity.

The purpose of this study is therefore to describe the life table characteristics of South African *Anopheles arabiensis* under a range of simulated seasonal temperature and relative humidity conditions.

3.2. MATERIALS AND METHODS

The *An. arabiensis* used in these experiments were the F_2 progeny of wild-caught females collected from Dondotha in (28°34'S 31°56'E) northern KwaZulu-Natal (Figure 3.1). Fresh breeding stock was caught at the start of each set of experiments. Field collected females readily laid eggs under insectary conditions of temperature

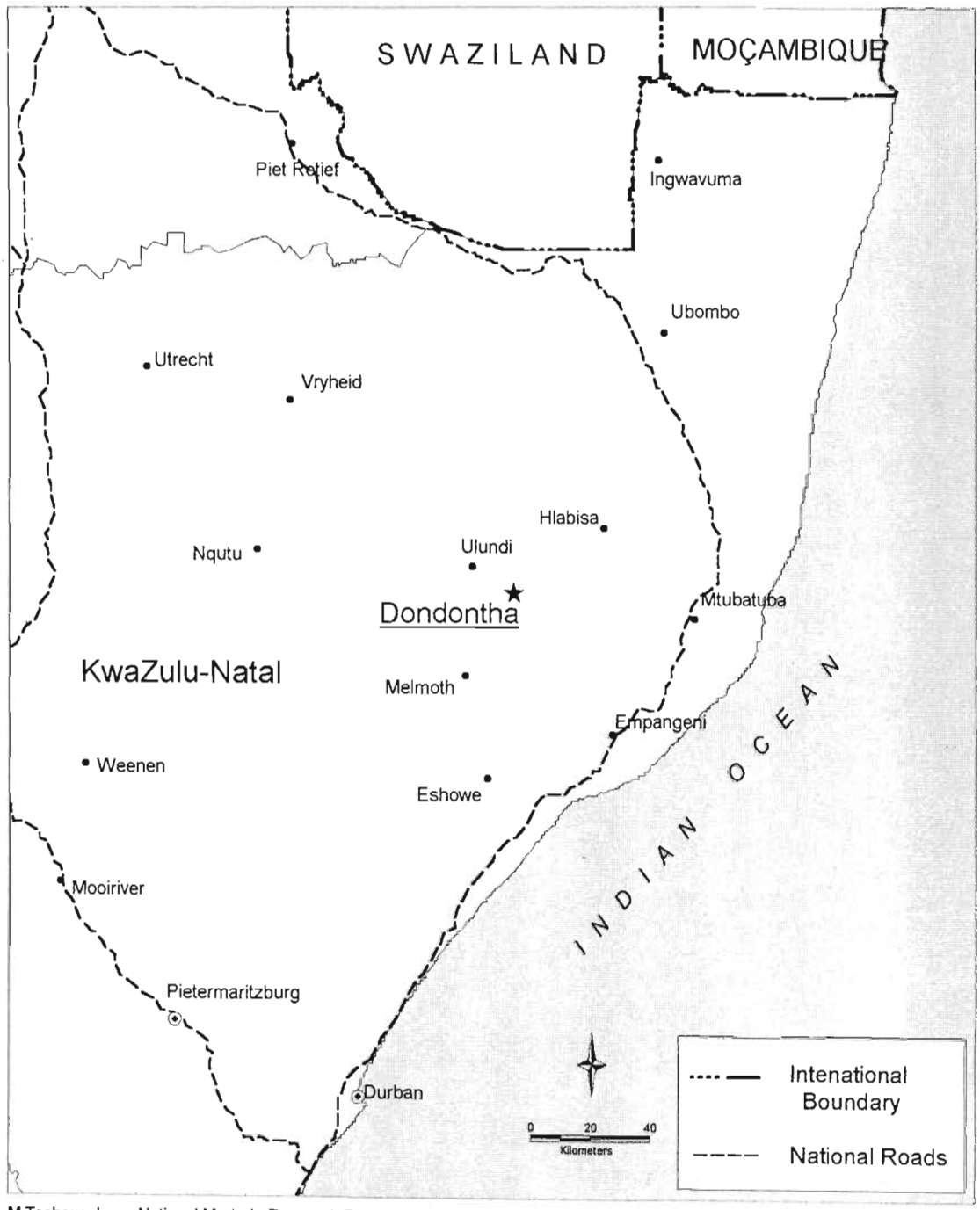


Figure 3.1. Map showing the location of Dondotha in KwaZulu-Natal.

(27 ± 2°C), relative humidity (70 ± 10%) and photoperiod (12L:12D with 1 hour simulated crepuscular period). Identification was obtained by using the Polymerase Chain Reaction (PCR) method on samples of the first larval instars of each female (Paskewitz & Collins 1990; Bredenkamp & Sharp 1993).

All experiments were conducted in a Specht Scientific programmable growth cabinet (model SFPGR066) fitted with a Dumo Dicon P temperature and humidity control unit. The development and survivorship of immature *An. arabiensis* was observed at four fluctuating temperatures with means of 17.9, 23.2, 26.1 and 21.4°C representing winter, spring, summer and autumn temperature profiles respectively. The temperature and humidity profiles were those obtained from field recordings by a MCS 200 data-logger fitted with a MCS 174-02 temperature and humidity probe. In South Africa, *An. arabiensis* has been found to feed indoors and rest outdoors (Sharp *et al.* 1993). Outdoor resting sites of *An. arabiensis* are not known, although restinig sites would theoretically be in a moist, shaded area probably near a water body. Therefore, temperature and relative humidity measurements were made under a tree at the edge of a large pool where *Anopheles* larvae were found.

Mean hourly temperature and humidity values were calculated for each season and programmed into the growth cabinet. For each temperature and humidity profile, 4 replicates of 100 first instar larvae were reared in plastic containers (35 x 25 x 10 cm) filled to a depth of 4 cm with deionised water. Larvae were fed on finely ground Epol® cat food. Pupae were separated daily and placed in 2ℓ buckets with screened tops.

Estimates of the duration of each larval stage were obtained by following ten replicates of 12 first instar larvae, placed individually in the wells of a microtitre plate.

Gonotrophic cycles: All adults that emerged were pooled together. The length of successive gonotrophic cycles was determined as the time from blood feeding to oviposition. Blood fed females that had spent the first three days of their lives with males were placed individually in breeding tubes. Breeding containers consisted of a mesh covered specimen bottle containing moist cotton wool and filter paper. Eggs were collected and counted daily. Females were allowed to re-feed on human blood at two day intervals.

Egg hatchability rates: Eggs from individual females were collected, counted and placed in individual containers containing distilled water. Hatched first instar larvae were removed and counted daily, up to four days after the first eggs hatched.

Life table characteristics: The adults that survived from the egg stage were counted and sexed, providing the egg to adult survival rate and sex ratio.

Once all the adults had died, life tables were drawn up. Four parameters were estimated for each season - mean male lifespan, mean female lifespan, net reproductive rate and the intrinsic rate of increase. The mean lifespans were those recorded during the experiments. The net reproductive rate (R_o) was calculated from the formula:

$$R_o = a \sum_{x=0}^{w} l_x m_x$$

where w is the last interval to which any female survived, a is the proportion of females that survive from egg to adult emergence, l_x is the proportion of adult females surviving to age x and m_x is the mean number of female progeny produced per female of age x. The value m_x was calculated using the formula:

$$m_x = E_x s$$

where E_x is the mean number of eggs produced per female of age x and s is the proportion of these eggs that are female.

The intrinsic rate of increase (r_m) was calculated using the following equation:

$$1 = a \sum_{x=0}^{w} l_x m_x e^{-r_m(x+D)}$$

where a, l_x and m_x are as above, e is the base of the natural log, r_m is the intrinsic rate of increase, x is the time interval, and D is the length of time for larval development from egg to the age of egg production. The mean generation time (G) was also calculated:

$$G = \ln R_o / r_m$$

G is an estimate of the time from mean oviposition in the present generation to oviposition in the offspring generation. Age-specific survivorship was also determined under seasonal temperature and humidity regimes.

For the purposes of this study, these parameters were considered sufficient to give an indication of the mosquitoes' potential to transmit malaria.

3.3. RESULTS

The results for the development of the immature stages are discussed separately from the life table parameters of the adult mosquitoes.

3.3.1. **Immature Development**

Seven developmental attributes were examined for the ten replicates of *An. arabiensis* reared under conditions of fluctuating seasonal temperature (Table 3.1). Survivorship from first instar to adult emergence was over 75% for all seasons. Development to pupation ($P_{\bar{x}}$) was fastest in summer (10.9 days). Survivorship from pupal stage was over 86% for all seasons. Adult emergence times ranged from 11 days in summer to 32 days in winter. Similar emergence times were observed for both males and females. Sex ratios were similar for all temperatures.

3.3.2. **Gonotrophic Cycle**

The length of time from blood-feeding to oviposition was determined for individual females maintained at the four fluctuating temperatures (Table 3.2). Only a few females laid eggs and the number of females ovipositing decreased over time. The duration of the first gonotrophic cycle ranged from 4 days at 26°C

Table 3.1. Developmental attributes of immature *An. arabiensis* reared under simulated seasonal conditions[1] ($\bar{x}$ ± SD)

ATTRIBUTE	WINTER	SPRING	SUMMER	AUTUMN
Survivorship, L1 to P* (%)	85.8 ± 4.3 A**	86.67 ± 4.2 A	84.2 ± 4.8 A	90.0 ± 4.2 B
$P_{\bar{x}}$	28.8 ± 2 A	17.6 ± 1.98 B	10.9 ± 0.77 C	16.43 ± 1.29 B
Survivorship, P to Ad (%)	88.5 ± 5.0 A	86.2 ± 3.8 A	92.1 ± 3.5 B	95.4 ± 2.6 B
$E_{\bar{x}}$				
♀♀	32.3 ± 2.01 A	19.72 ± 1.81 B	12.46 ± 0.69 C	18.82 ± 1.24 B
♂♂	32.02 ± 1.99 A	19.2 ± 1.58 B	11.76 ± 0.72 C	17.96 ± 1.1 B
Survivorship, L1 to Ad (%)	75.8 ± 4.3 A	76.5 ± 3.7 A	77.5 ± 2.6 A	85.8 ± 1.9 B
Sex ratio (♂:♀)	0.82:1	0.79:1	0.82:1	0.85:1

[1]Experiments for each season were started using 400 newly emerged first instar larvae.

* L1 = first instar larvae, P = pupae, Ad = adult, $P_{\bar{x}}$ = mean time (days) to pupation, $E_{\bar{x}}$ = mean time (days) to adult emergence. Analysis was performed using ANOVA with Duncan's Multiple Range Test and significance was determined at the 5% level of significance.

** Means with the same letter horizontally are not significantly different.

Table 3.2. Gonotrophic cycle for *An. arabiensis* during the different seasonal conditions

	GONOTROPHIC CYCLE (days) ($\bar{x}$ ± SD)					
SEASON	1	2	3	4	5	6
SPRING	5.2 ± 0.76	3.5 ± 0.5	3.3 ± 1.15	4.0 ± 1.15	3.75 ± 0.95	4.0 ± 0.1
n	87	67	59	36	20	7
SUMMER	4.0 ± 0.91	3.1 ± 0.88	3.0 ± 0.82	3.0 ± 0.04	3.7 ± 0.52	4.0 ± 0.01
n	79	59	54	40	30	5
AUTUMN	6.1 ± 1.11	5.1 ± 3.09	4.5 ± 1.91	5.0 ± 2.8		
n	54	25	14	7		

(summer) to 6 days at 21°C (autumn). Second and third gonotrophic cycles were shorter at each temperature but the duration of the gonotrophic cycles increased beyond the third cycle as the females aged. Females maintained under spring and summer conditions laid up to six batches of eggs. The number of egg batches ranged from 2 - 5 in spring (n = 87) and 3 - 6 in summer (n = 79). The number of egg batches laid in autumn ranged from 1 - 4 (n = 54). The last gonotrophic cycle was based on a maximum of two specimens, therefore accounting for the lack of variation in the values obtained.

During this experiment about ⅔ of all females oviposited and difficulties were encountered re-feeding some females. During the winter experiments, the females readily took a blood meal, became gravid but did not lay any eggs (see Appendix 3.1).

3.3.3. **Egg Hatch Rates**

Egg hatch rates were over 75% in summer and autumn but only 66% in spring (Table 3.3). Mean eclosion times were 3.7 days in spring, 2 days in summer and 3 days in autumn. Egg hatch rates for winter could not be ascertained as females became gravid but did not lay eggs under winter conditions.

3.3.4. **Adult Survivorship and Life Tables**

Life table parameters were determined at each temperature. The mean lifetimes for females were greater than for males at all temperatures (Table 3.4).

Table 3.3. Egg hatchability under conditions of seasonal temperature and humidity.

	n	Mean no. of Eggs produced	Mean % Hatch
WINTER			
SPRING	87	288.4	66.25
SUMMER	79	263	74.74
AUTUMN	54	159.3	82.5

Table 3.4. Life table characteristics for *An. arabiensis* under simulated seasonal conditions (n values are given in brackets).

SEX	WINTER	SPRING	SUMMER	AUTUMN
Ro		50.6 (76)	79.55 (112)	25.1 (64)
rm		0.09 (76)	0.17 (112)	0.08 (64)
G		43.6 (76)	25.7 (112)	40.3 (64)
Lifespan ♂	28.3 (136)	25.1 (134)	16.9 (139)	24.1 (157)
Lifespan ♀	43.2 (167)	32.0 (171)	21.4 (171)	34.3 (185)

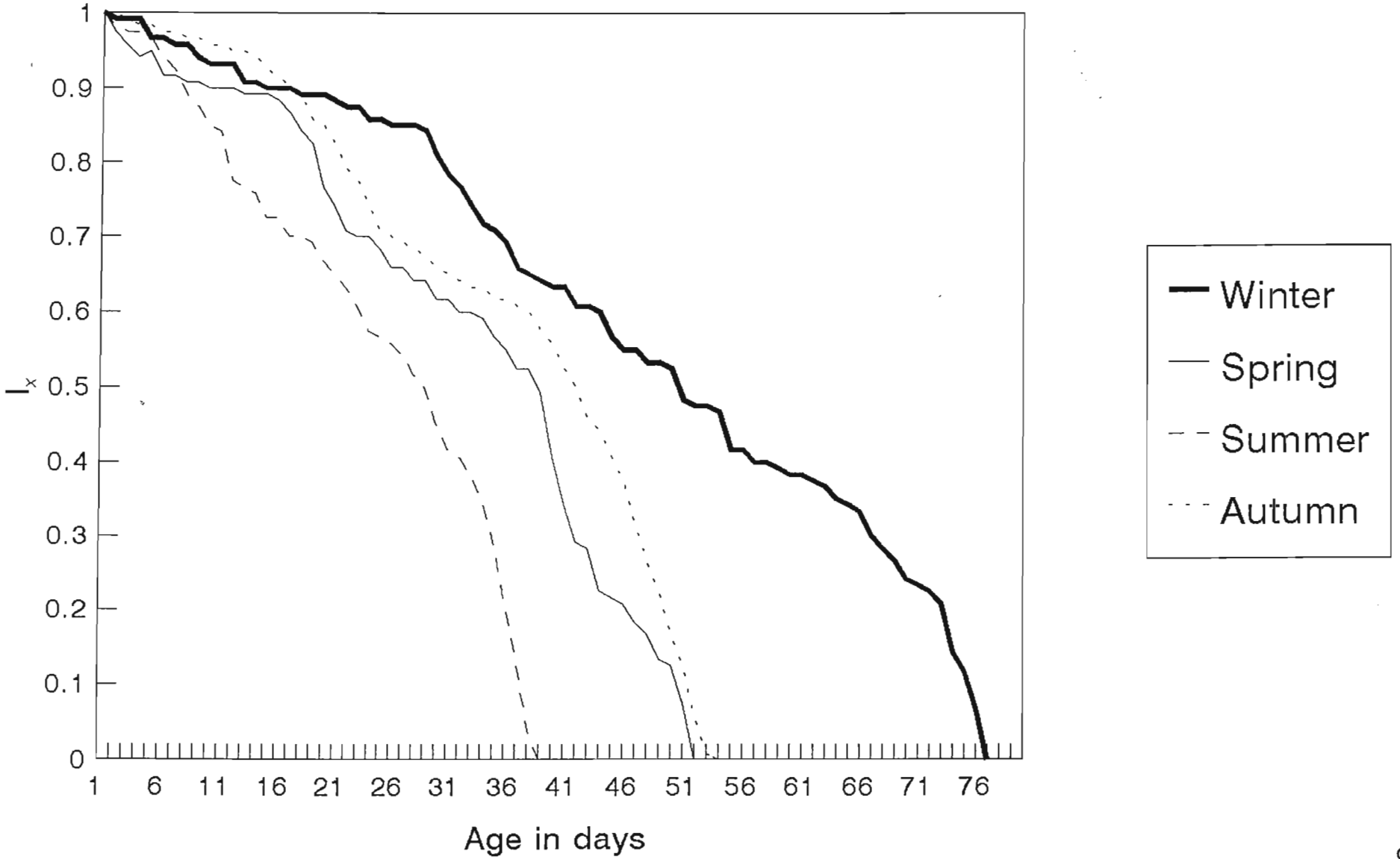
The lower the mean temperature and humidity, the longer the life span. The net reproductive rate was greater in summer (R_o = 79.55 females per female per generation). This R_o was three times that obtained in autumn and 1.5 times that obtained in spring. Due to the greater net reproductive rate and shorter generation time in summer, the intrinsic rate of increase (r_m) was greatest in

summer and lowest in autumn as a result of the low R_o and long generation time (43 days). The generation time (G) is also given in Table 3.4. Due to the fast rate of development at higher temperatures, the generation time during summer was the shortest. Age-specific survivorship was compared for the four seasonal profiles (Figure 3.2). In winter mortality was initially low and gradually increased over time, especially amongst the adults. In summer mortality was pronounced and the maximum life span was just over half that of winter reared adults. The mortality during spring and autumn followed almost similar curves and the maximum lifespan during the two seasons was not significantly different.

For all seasons, the mean female lifespan was significantly different from the mean male lifespan (t-test, $p << 0.01$). Both male and female lifespans were compared with those of the preceding season and highly significant differences were found (t-test, $p << 0.01$). The lifespans of both the male and female mosquitoes were found to increase as winter approach, but the increase in female lifespan was more marked than that of the males.

4. DISCUSSION

In these laboratory experiments *An. arabiensis* females did not lay eggs under winter conditions. Similarly, in a study in Sudan, Omer and Cloudsley-Thompson (1970) collected gravid females of *An. gambiae* during the dry season that continued to feed but ovarian development was retarded. *Anopheles gambiae* populations in tropical Africa increase rapidly in the early rainy season. In the laboratory, the females reared under winter conditions became gravid but did not lay eggs until the temperature increased. However le Sueur (1991) did find first instar larvae in winter. This implies



gure 3.2. Age-specific survivorship of *An. arabiensis* in individuals per day.
= proportion individuals surviving

that limited oviposition occurs in the field. In the laboratory, the females are continually exposed to the same cycle of temperatures whereas in the field, temperature fluctuates within a wide range of temperatures. In the field, mosquitoes may oviposit during winter when favourable temperatures are experienced. This may account for the sudden increase in mosquito abundance in early spring. This is remarkable as in some areas in Africa the dry season extends for six to nine months and under such conditions populations persist at low levels in a few, size limited larval habitats and in the adult stages (Omer & Cloudsley-Thompson 1968, 1970).

The interaction between longevity and the number of gonotrophic cycles for each season (Figure 3.3) shows that as longevity decreases as summer approaches, the number of gonotrophic cycles increase. It is also evident from Figure 3.3. that the greater the number of gonotrophic cycles the shorter the duration of each cycle. There are more egg batches laid in summer since the duration between each oviposition is shorter at higher temperatures (Figure 3.4). Field observations of longevity and gonotrophic activity, especially in winter, are necessary to confirm the results obtained in the laboratory. The mean number of eggs produced per season was greatest in spring and smallest in autumn. In terms of basic physiology, one would expect the number of eggs produced in autumn and spring to be similar. However, the low egg production in autumn may be due to the relatively small number of individual females (n = 54) used in these observations may have created a bias in the results. Although, on average, more eggs were produced during spring than in summer, the number of resulting adults was lower than in summer since there was greater egg mortality in

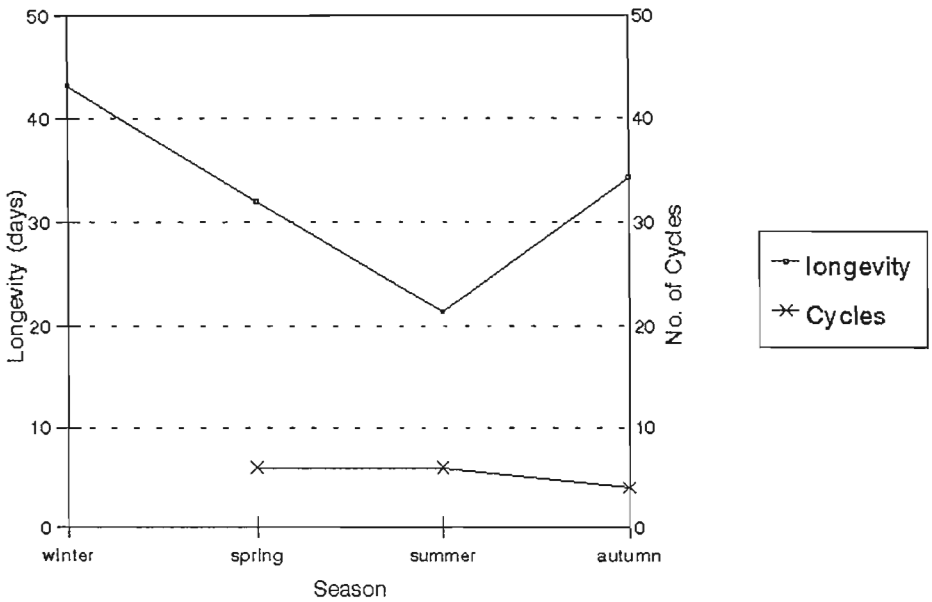


Figure 3.3. The relationship between longevity and the number of gonotrophic cycles.

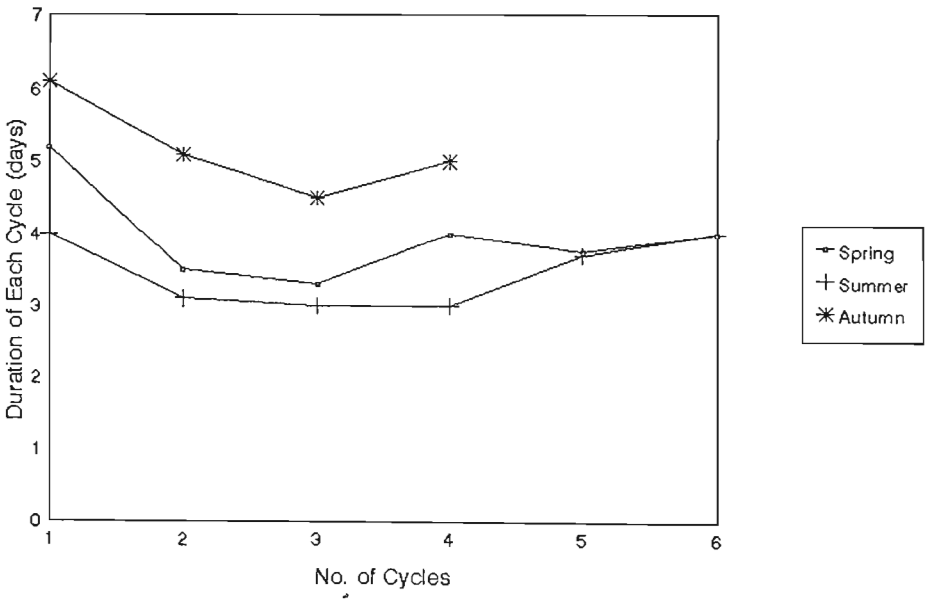


Figure 3.4. The duration of each gonotrophic cycle per season.

spring. From the n values in Table 2.2., it is apparent that fewer females are involved in egg production in successive gonotrophic cycles. This could be the result of a decrease in the reproductive ability with an increase in age. The times required for oogenesis and blood meal digestion are interrelated in most gonoactive blood-feeding insects (Roberts *et al.* 1983). Furthermore, the duration of oogenesis will determine the minimum time between blood meals for female mosquitoes. From the present experiments it can be seen that as temperature decreases the duration of each gonotrophic cycle becomes longer (Figure 3.4). This affects vector efficiency since the more often a blood meal is taken, the greater the potential for obtaining an infectious blood meal and transmitting disease. However, *An. arabiensis* has the capacity to undergo gonotrophic dissociation in winter, resulting in females feeding without laying eggs. This would not inhibit the transmission of malaria since the mosquitoes continue feeding. Reisen, Mahmood and Parveen (1982) found that the incidence of malaria reflects temporal variations in transmission rates directly attributable to vector feeding habits, abundance and survivorship.

It is well established that malaria is not transmitted uniformly throughout the year. Fluctuations in climatic conditions have a significant effect not only on the life expectancy of the mosquito but also on the development of sporogonic stages of malarial parasite within the mosquito's body (Rastogi, Pal & Sen 1987). To transmit *Plasmodium falciparum* the anopheline host must survive for about 12 days after taking an infective blood meal (with incubation at 27°C) (Macdonald 1957). Considering that initial host-feeding by *An. arabiensis* occurs on the second or third night after

emergence, the potentially infective portion of the population would consist of females not less than 14 - 15 days old. Based on the gonotrophic cycle length determined at 27°C, and a mean life span of 21 days, *An. arabiensis* could imbibe at least 2 blood meals after completing sporogony, if a *Plasmodium* infection was acquired during the first blood meal. Mosquitoes are ectotherms and during winter their energy requirements are limited. In this study it was found that mosquitoes reared under winter conditions in the laboratory did not oviposit. However, in the field female *An. arabiensis* were caught feeding on humans. This suggests that *An. arabiensis* does not develop large fat bodies through feeding on plant juices in autumn, but takes occasional blood meals in winter, developing their fat to some extent but not sufficiently to withstand long periods of fasting. The same feeding pattern was observed in *An. atroparvus* and *An. sacharovi* (Clements 1992).

The time required to become infective after consuming an infectious blood meal is directly dependent upon the thermal environment experienced by the adult female (Meyer *et al.* 1990). Stratman-Thomas (1940) found that as temperature increased, the development rate of *Plasmodium vivax* within *Anopheles quadrimaculatus* increased. This author found that *P. vivax* sporogonic cycle was shortest at 28-30°C (8 days) and longest at 22-23°C (14 days). Thus, one would expect the development of malaria within female *An. arabiensis* to be faster in summer than in winter. Sporogonic development during spring and autumn would probably be intermediate. These findings represent the result of laboratory experiments. In nature the extrinsic incubation period of an arbovirus is a function of the thermal conditions experienced by female mosquito

as a consequence of daily resting and movement patterns (Meyer *et al.* 1986) and seldom occurs within the realm described by laboratory experiments. This implies that field conditions are constantly changing and that the results obtained in the laboratory under controlled conditions may not reflect the situation in the field.

The mean life span of uninfected, laboratory reared mosquitoes does not provide a realistic duration for infected, free flying females. Klein *et al.* (1982) found that the longevity of *An. dirus* decreased when infected with *P. cynomolgi* and Hogg & Hurd (1995) found that the fecundity of *An. stephensi* decreased when infected with *P. yoelii.*

The slow development time of mosquitoes during winter may influence the survival of the adults. The life table parameters show that as temperature decreases, the larval developmental time increases and subsequently the adult lifespan increases. This may be an indication of the fitness of the adults since larvae developing slowly consume lessf nutrients per unit time (day) but more over the total larval duration, thus producing more robust adults. Adult fitness may be reflected in body size. Since mosquitoes are ectotherms, there is not much intra-specific competition in winter as there are relatively few over-wintering larvae and females do not readily lay eggs in winter. However, during conditions of high temperature there is increased intra-specific competition for food and space in the larval habitats (Lakhani & Service 1974). This results from females laying large numbers of eggs in a short space of time.

Although there are more breeding sites available during spring and summer, larval

densities in these sites would be high as a result of the larger number of eggs produced and the higher number of larvae produced as a result of the greater number of gonotrophic cycles (Tables 3.2.). Each larva does not obtain as much nutrient as winter-bred ones would (le Sueur 1991), and these larvae develop over a short time interval. Therefore summer and spring individuals are not as robust as winter-reared mosquitoes.

The microclimate is known to vary from habitat to habitat (Clements 1963), therefore the results obtained in the growth cabinet are analogous to that of a single population at a single breeding site. Based on the mean hatch rates per season, this study suggests that there would be higher larval densities during the cooler months than during the warm summer months. This concurs with the results obtained by le Sueur (1991) for *An. merus* under field conditions. However, due to its dependence on saline breeding sites, the distribution of *An. merus* is limited whereas that of *An. arabiensis* is not. Although, in the present study summer reared mosquitoes have a lower egg hatchability than autumn reared mosquitoes (difference of 7.76%), on average they produce more eggs than autumn reared mosquitoes (difference of 103.7 eggs)> Therefore the number of larvae produced in summer is almost twice the number produced in autumn.

From the age-specific survivorship curve and the gonotrophic cycles, it is evident that females lay a large number of eggs in several batches, especially during spring and summer. During these seasonal conditions, the females lay large numbers of eggs in

a short period since the females have a shorter lifespan compared to those reared under autumn and winter conditions. Spring- and summer-reared females take at least six blood meals over 6 (summer) to 20 (spring) days. Females reared under spring and summer temperature and humidity are capable of surviving the sporogonic period of development of *P. falciparum* (12 days under optimal conditions) (Vaughan *et al.* 1992). The autumn-bred females take fewer blood meals (determined by their gonotrophic cycles) over the same period as spring-reared females.

In the field, transmission of malaria is low during the spring months (September to November), gradually picking up during summer (December to February) and is greatest during autumn (March to May) (refer to Table 2.2.). Slow larval development in winter results in low population numbers in spring and therefore lower transmission. As the adult population increases, transmission increases. However, in autumn, transmission increases (as evidenced by the higher number of cases reported (Table 2.2.)) while population numbers are declining. This may be due to the fact that in autumn there is a longer period between blood meals, since the digestion of blood is slower because of lower temperatures (Day *et al.* 1990). Therefore, if we assume that each female has an infectious blood meal on the second night after emergence and if we take into account the mean lifespan of an individual, the sporogonic period of development of *P. falciparum* and the mean duration of each gonotrophic cycle, summer mosquitoes are, on average, capable of giving one infected bite during their lifetime whilst autumn mosquitoes can give at least two infected bites.

Although winter-bred females did not oviposit, the females did take a blood meal, on average every three days. From the survivorship curve it can be seen that winter reared mosquitoes survive the longest, with an adult lifespan of 27 days. Due to the decreased metabolic requirements of these ectotherms, the females only take an occasional blood meal. Due to the small overwintering female population and the low number of infected people found in the malaria areas in winter, the probability of these mosquitoes becoming infected and transmitting malaria is very low. Although winter-reared females do not lay eggs as a result of gonotrophic dissociation (Omer & Cloudsley-Thompson 1968, 1970), their long lifespan and their potential to oviposit during favourable conditions (Spring or the early rains) make them inportant targets from a control point of view. However, the low numbers of these overwintering females does not make control strategies against them economically feasible. This study therefore supports the conclusion by le Sueur (1991) that winter larviciding would be very effective in reducing malaria transmission during the succeeding seasons since larvae take 32 days to develop into adults in winter.

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APPENDIX 3.1[1]

EGG RETENTION BY ANOPHELES ARABIENSIS DURING THE DRY WINTER SEASON IN SOUTH AFRICA

R. Maharaj, D. le Sueur and C.C. Appleton

ABSTRACT: Anopheline mosquitoes are able to survive the cold, dry season as adults. Temperature plays an important role in egg retention by *An. arabiensis* since these females do not oviposit during the cold, dry winter months. During winter, egg are retained in the ovaries and when temperature increases, these eggs are then oviposited.

In some regions, particular species of *Anopheles* are capable of surviving the dry season as adult females (Clements 1963). During the dry season, Mnzava (1991) collected *Anopheles gambiae s.l.* in Tanzania, and le Sueur and Sharp (1991) collected *An. arabiensis* Patton and *An. merus* Dönitz in South Africa. In southern Africa, Leeson (1931) found gravid *An. gambiae* females taking blood meals. The sudden appearance of mosquito larvae with the first rains may be due to the survival of gravid females during the dry season. Anopheline females are capable of taking a blood meal without

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[1]A peer reviewed manuscript submitted to the *Journal of the American Mosquito Control Association*.

developing their ovaries (Clements 1992). Egg development appears to be under nervous control, since this process does not occur under adverse conditions. Mosquitoes oviposit shortly after the eggs have matured, unless oviposition is delayed or prevented by cold or the absence of appropriate larval habitats (Clements 1963). Little is known of the physiology of aestivating mosquitoes, but Omer and Cloudsley-Thompson (1970) suggest that aestivation involves gonotrophic dissociation.

This study reports on egg retention by *An. arabiensis* during the dry, winter season in South Africa. *Anopheles arabiensis* females were caught feeding in man-baited and window traps in the Dondotha district (28°34'S 31°56'E) of KwaZulu-Natal during June and July 1992. Of the 2300 anopheline females collected in this area, 8 were *An. quadriannulatus* and the rest were *An. arabiensis*. All identifications were made by the polymerase chain reaction method.

Field studies revealed that *An. arabiensis* was still actively searching for and feeding on humans. This is evident from the fact that mosquitoes were collected in man-baited and window traps. Of the 329 mosquitoes caught in exit traps, 44.2% of them had had a blood meal at some time after emerging. Only 17 (5.1%) of these were fully blood-fed. The remaining blood-fed females were either gravid or half-gravid.

In the field females were placed individually in breeding tubes (consisting of a gauze covered specimen bottle containing moist filter paper) and maintained in the field under prevailing environmental conditions. These individuals were offered a blood meal every

three days. Females that died after feeding were dissected and examined for ovarian development. Of the 65 females that were maintained under existing field conditions, seven females laid a single batch of eggs and subsequently died. At the end of six weeks, females that were still alive were killed and their ovaries were dissected out and examined. Of the 58 females that were examined 49 contained fully developed eggs and 9 were half gravid.

Other females collected in this area were taken back to the laboratory, 300 km away. In the insectary the wild-caught females were maintained at a constant temperature of 27°C and 75% RH with a photoperiod of 12L:12D. These females produced an average of 231 ± 103 eggs/female (n = 62). Eggs from these females were reared in a growth cabinet with fluctuating temperature ($\bar{x}$ = 17°C, range 12 - 25°C) and humidity ($\bar{x}$ = 68%, range 36 - 79%) and a fixed photoperiod (11L:13D with one hour simulated crepuscular period) to simulate winter environmental conditions. Fifty F_1 females were allowed to mate, were blood-fed and kept individually in breeding tubes. These females were also offered a blood meal every three days by placing a human arm over the breeding tube. Over a two month period, none of these females laid any eggs. A subsample of five females was removed from the growth cabinet and placed in the insectary. One of the females died, but the remaining four females laid an average of 123 ± 94 eggs/female over seven days starting on the third day after being placed in the insectary. The females in the growth cabinet that were still alive after two months were killed and their ovaries examined. Eleven of the remaining 17 females had well developed eggs (122 ± 12). Using the techniques outlined by the WHO (1975), it was

found that both the wild-caught females and the laboratory reared females had sperm in their spermathecae.

Thus, from the field and laboratory studies it appears that temperature plays a significant role in initiating egg retention. Although a few females did lay eggs under field conditions, this study has shown that mosquitoes maintained at simulated winter temperatures produce eggs but do not oviposit unless the temperature increases. Although oviposition sites (moist filter paper) was available to these mosquitoes, eggs were retained at low temperatures. These overwintering females are of great importance since they are potential vectors of malaria. They are readily attracted to man and they are capable of surviving the necessary incubation period (of *Plasmodium falciparum*) in order to become infective. From a control point of view, these females are important potential transmitters of malaria and they are responsible for the reintroduction of large numbers of mosquitoes during the rainy season.

We thank Rould Cibane and David Mtembu for their assistance with field work. This study formed part of a broader study funded by the Medical Research Council of South Africa.

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CHAPTER 4

THE EFFECT OF TEMPERATURE ON THE MORPHOLOGY OF *ANOPHELES ARABIENSIS*

4.1. INTRODUCTION

Variation in body size is a regular feature of natural mosquito populations and may reflect their vectorial capacity (Nasci 1986a). There is some evidence that small mosquitoes may be more susceptible to infection than larger individuals. However, field studies suggest that small mosquitoes may not survive long enough to serve as vectors of malaria (Day *et al.* 1990).

Although it is known that temperature influences mosquito body size, Walker *et al.* (1987) found no relationship between adult size and survivorship in either male or female *Ae. triseriatus*. It has been suggested (Haramis 1983) that large bodied mosquitoes are more successful in obtaining a blood meal in the field. This was corroborated by Kitthawee *et al.* (1990) who found that there were significant relationships between female body size and blood meal size. These authors found that larger females were capable of taking larger blood meals because their midguts were correspondingly larger. This suggests that blood meals may contain a correspondingly larger number of ingested pathogens. Longer survivorship of the female mosquito should then increase the probability of pathogen transmission since the female would be capable of surviving the necessary incubation period of the parasite and have more

time to transmit malaria. Temperature and photoperiod can have similar effects on insects (Hoffman 1985), perhaps because they are normally associated in particular ways in nature: low temperature is usually coupled with short photoperiod and high temperature with long photoperiod. Another insect characteristic influenced by temperature is longevity. Typically, mosquitoes survive better when reared under conditions of low temperature (Beier *et al.* 1987).

Anopheles arabiensis, a member of the *An. gambiae* Giles complex, is the main vector of *Plasmodium falciparum* in southern Africa. Work done by le Sueur (1991) has shown that temperature influences some morphological and biological characteristics of the larval cycle of both *An. arabiensis* and *An. merus* and has contributed greatly to the understanding of the bionomics of these species in South Africa. The influence of temperature on the size, longevity and fitness of the mosquito is of interest from a control point of view because information on the effects of temperature on the rates of development and survival of the various stages of the vectors are necessary in designing control strategies.

From her study on members of the *An. gambiae* complex, Coetzee (1986) concluded that the hind leg pale band at the junction of tarsomeres 3 and 4 is a very good character for grouping *An. gambiae/An. arabiensis* and *An. quadriannulatus/An. merus*. However, Coetzee (1986) cautioned that the reported measurements might only be applicable to the localities sampled and not to other areas of Africa. le Sueur & Sharp (1991) found that temperature influenced the size of morphological characteristics and they suggested that for morphological measurements to be used in species separation,

samples should be collected from different geographic areas and over different seasons.

Although le Sueur (1991) found that there is a linear relationship between wing length and tarsal length, his study also showed that there is no relationship between tarsal length and the pale band width. The pale band width was investigated in the current study to determine whether or not there is a seasonal change in the width of the hind leg pale band at the junction of tarsomere 3 and 4. Also, le Sueur (1991) concluded that there was a need to ascertain how "fixed" a particular character is under varying environmental conditions.

This chapter examines the effect of temperature (both controlled and natural) on the wing size and the hind leg pale band at the junction of tarsomeres 3 and 4 of adult *An. arabiensis*. These measurements will give an indication of the size of adults obtained at the various temperatures since body size is proportional to wing length (van den Heuvel 1963). These morphological measurements will therefore enable an investigation into the change in the average body size and pale band width of adult mosquitoes over a 12 month period in the field; as well as the changes occurring under simulated conditions of seasonal temperature.

4.2. MATERIALS AND METHODS

All the mosquito specimens used for this part of the study consisted of material collected from the field.

4.2.1. **Description of the Study Site**

The study site was situated at Dondotha (28°34'S 31°56'E) in northern KwaZulu-Natal. This is in the heart of the province's rural area where the main activities include stock farming and to a limited extent vegetables are grown for private use. The main sources of water in this area are three large, interconnected ponds fed by an underground spring. Water outlets from these ponds form a stream that flows into the agriculturally developed areas. Because of the presence of cattle, there are abundant breeding sites for anopheline mosquitoes produced by the trampling of cattle through the stream. The dwellings in this area consisted of simple mud and wood structures with thatched roofs. These structures had modern wooden framed, glass windows. There are 15 such structures within a distance of ±500 m of the water source. Of these structures, only five were selected for use during this study because the other dwellings were not occupied or were often smoke-filled and thus unsuitable.

4.2.2. **Collection of Climatic Data**

To investigate the effects of environmental temperature and relative humidity on adult body size, seasonal temperatures and relative humidity profiles were obtained from the field using a MCS 200 data logger fitted with a MCS 174-02 relative humidity and temperature probe. In South Africa, *An. arabiensis* has been found to feed indoors and rest outdoors (Sharp *et al.* 1993). Outdoor resting sites of *An. arabiensis* are not known, although resting sites would theoretically be in a moist, shaded area probably near a water body. Therefore, temperature and relative humidity measurements were made under a tree at the

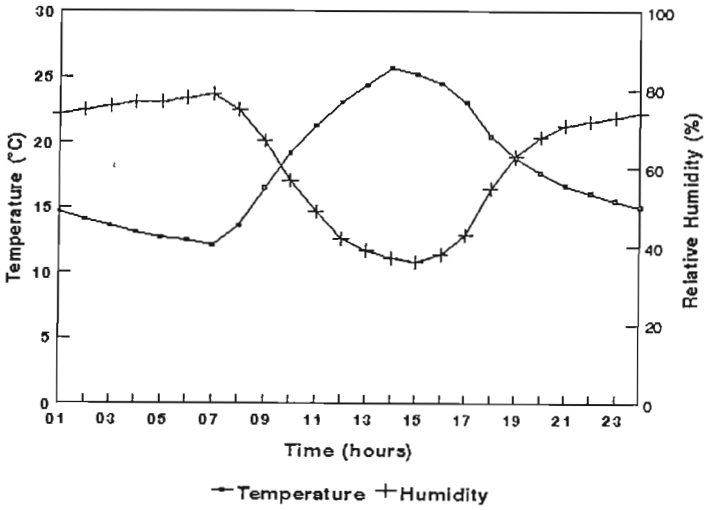
edge of a large pool where *Anopheles* larvae were found.

Seasonal profiles were obtained by collecting representative data for each season over a two month period as follows: winter (June - July), spring (September - October), summer (December - January) and autumn (March - April). The profiles obtained were then programmed into a growth cabinet (see chapter 3) to simulate the field conditions. Figure 4.1(a-d) represents the profiles for winter, spring, summer and autumn respectively. The photoperiod used was determined from the field as follows: winter (11L:13D), spring (12L:12D), summer (13L:11D) and autumn (12L:12D), each with a one hour simulated crepuscular period.

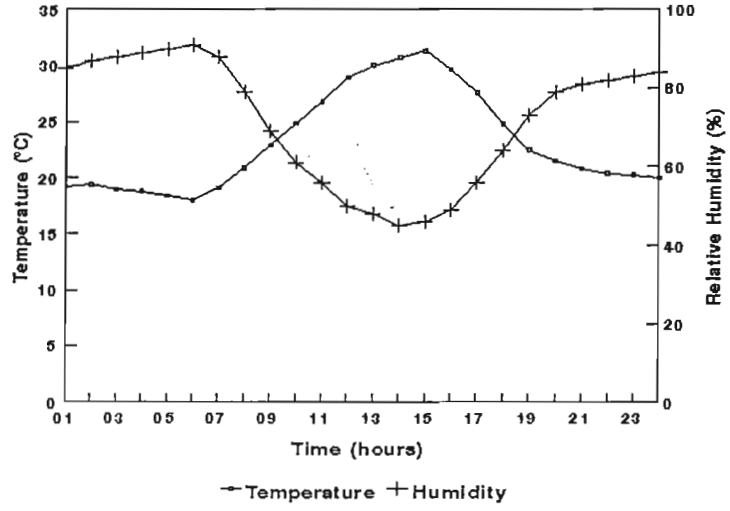
The mean monthly temperature and relative humidity profiles for the study area, for the duration of the study were obtained from the Weather Bureau and are illustrated in Figure 4.2.

4.2.3. **Field collection of specimens**

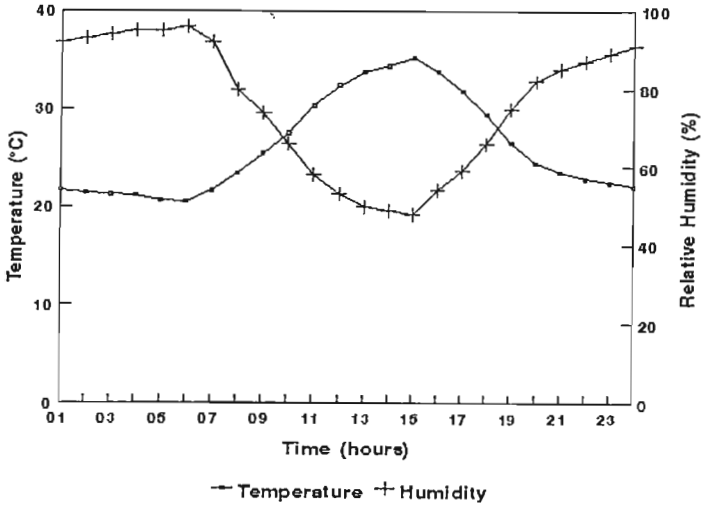
Monthly collections of mosquitoes were made at Dondotha from May 1992 to April 1993. The duration of each collecting trip lasted three to four days, depending on the abundance of mosquitoes. Mosquitoes were collected by employing window traps, human baited traps and landing catches.



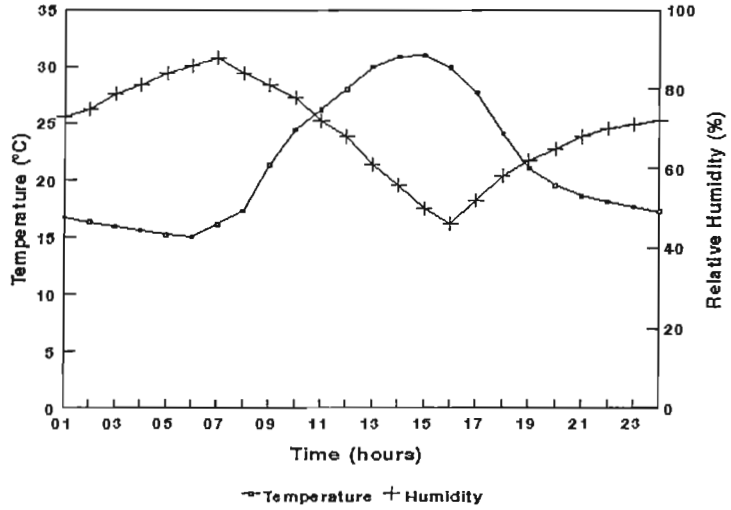
(a) Winter



(b) Spring

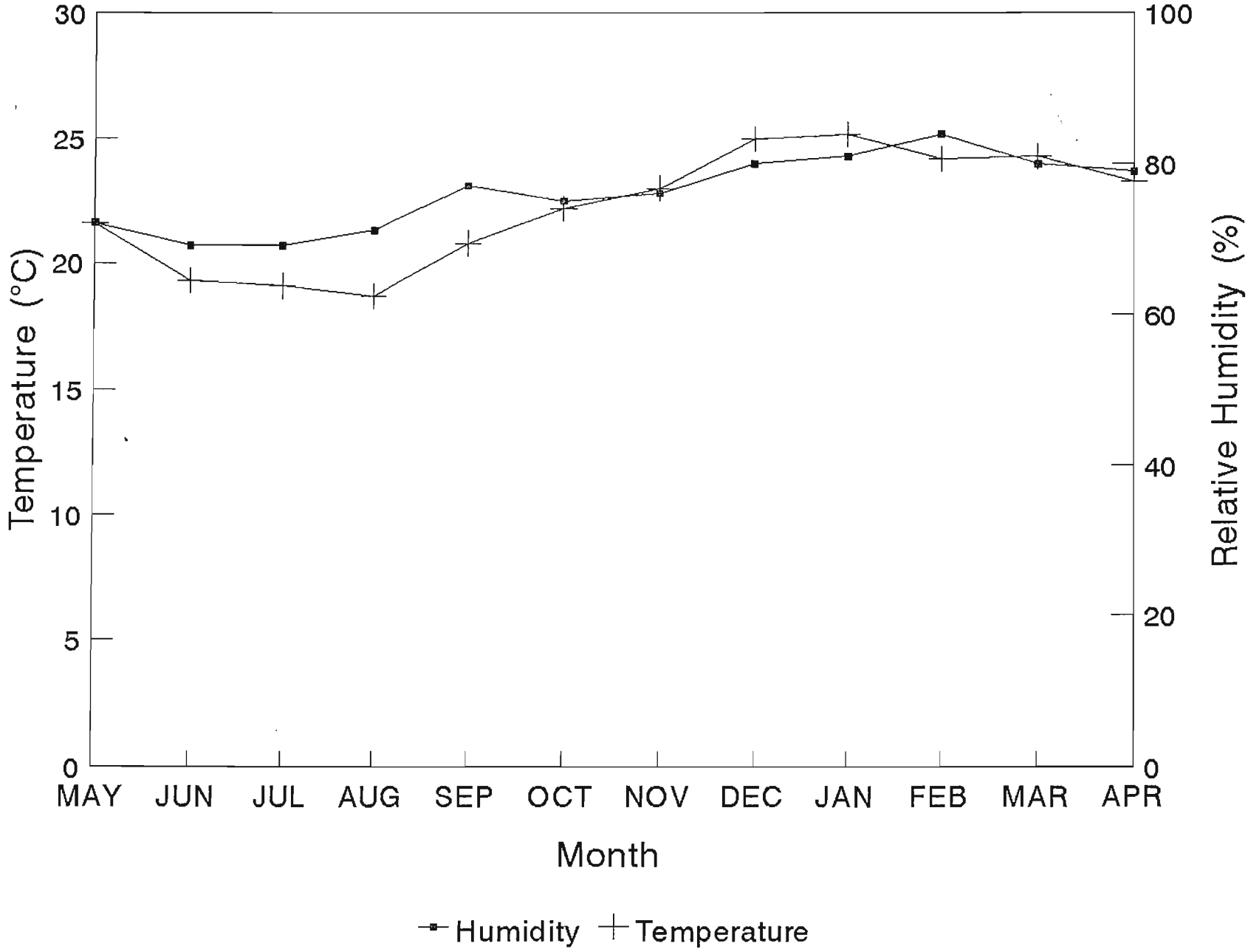


(c) Summer



(d) Autumn

Figure 4.1. The temperature and humidity profiles for the different seasons.



gure 4.2. The mean monthly temperature and relative humidity profile of Dondotha for the study period.

4.2.3.1. **Window Traps**

Window traps were fitted in five different houses within a distance of 500m from the water source. The traps were made of a metal frame covered with mosquito netting. On the inner side of the trap, a "funnel" was made which helped to guide mosquitos into the trap. The window was left open overnight and mosquitoes were caught as they attempted to leave the dwelling. All mosquitoes from these traps were collected at dawn the next day.

4.2.3.2. **Human Baited Traps**

Two collectors sat in a tent approximately 50m away from the water source. They were confined to the tent from 19h00 to 05h00 and captured all female mosquitoes that attempted to feed on them. Mosquitoes were captured using an aspirator and placed in a gauze covered cup.

4.2.3.3 **Surface catches**

Two collectors sat in a tent situated approximately 50m away from the breeding sites. Collections of resting female mosquitoes were made every 30 minutes between 19h00 and 05h00. Using battery powered flashlights, the collectors examined the fabric of the tent for resting mosquitoes. These were collected using an aspirator and these females were placed into a container until they were processed in the morning.

4.2.4. **Processing of Mosquitoes**

Each morning, the mosquitoes captured the previous night were processed after being fed on a volunteer's arm. Mosquitoes other than *Anopheles gambiae s.l.* were removed from the container and destroyed. All remaining mosquitoes were placed individually into breeding containers. Breeding containers consisted of a mesh-covered specimen bottle containing moist cottonwool. These mosquitoes were then transported to the insectary in Durban.

The field collected specimens were placed in the insectary and maintained at a constant temperature and relative humidity (27°C, 80% RH). Eggs laid by females in the breeding jars were removed and placed in separate plastic containers containing distilled water to a depth of 3cm. Once these eggs had hatched, it was possible to obtain correct identification by using the Polymerase Chain Reaction (PCR) method to identify the larvae (Bredenkamp & Sharp 1993) and hence the wild caught females.

Once the females had been identified, they were killed and the wings carefully removed and mounted, in preparation for being measured. Those females that did not lay eggs (i.e. the nulliparous females) were identified through performing PCR on the thorax and abdomen, once the wings and legs had been removed.

4.2.5. **Morphological Mounting and Measurements**

For microscopical examination, slide preparations of adults were made according

to the method of Hunt and Coetzee (1986).

Wing length measurements were taken between the axillary incision (Harbach and Knight 1980) and the wing tip in the region of vein 3 (Figure 4.3) (Gillies and Coetzee 1987). Measurements were carried out with a Wild M7A stereo microscope using a 10X eyepiece and 31X objective and an eyepiece micrometer with 120 divisions, each equal to 31 µm on the focal plane.

Measurements were taken of the pale band at the junction of tarsomeres 3 and 4 using a compound microscope (magnification X100) fitted with an eyepiece micrometer. The maximum and minimum length of the pale scaling were measured and the mean calculated.

4.2.6. **Sample size and statistical analysis**

The sample size is dependent upon the degree of precision required (Southwood 1978). For the purposes of this study, a standard error of 5% of the mean was considered to be satisfactory. Biologically significant differences in mean body size and body size range were determined from le Sueur (1991) and le Sueur (pers. comm.)[1]. According to calculations by Eleanor Gouws (Dept. of Biostatistics, Medical Research Council, Durban), to obtain a biologically significant difference between samples, a minimum sample size of 50 individuals would be sufficient. Accordingly, if n = 53, a precision similar to the data

[1]Dr D. le Sueur, Medical Research Council, Durban.

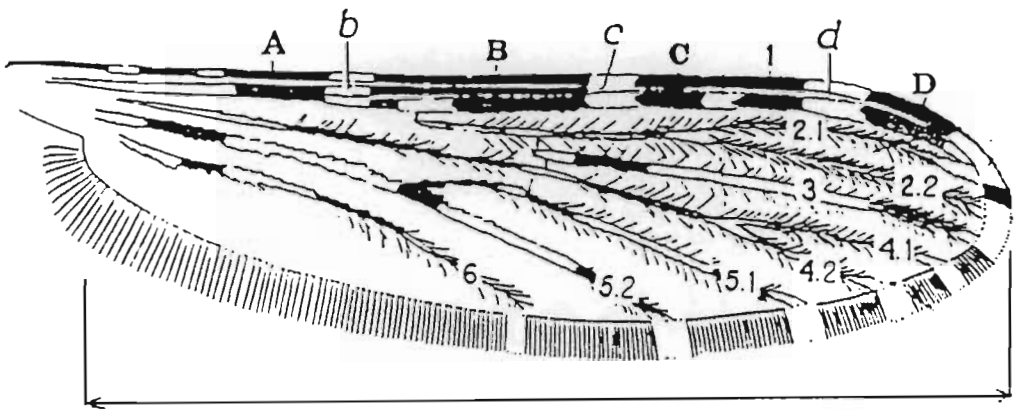


Figure 4.3. *Anophels* wing showing wing spots and area used for morphometric measurements (From Gillies & Coetzee 1987).

presented by le Sueur (1991) could be anticipated. During this study, the sample size was always greater than 50 individuals.

Statistical analysis of the data was carried out using Duncan's Multiple Range Test (Walker & Duncan 1969), Pearsons Correlation and Stepwise Multiple Regression Analysis (SAS Institute Inc. 1985). The simple statistics for the data used in this chapter are given in Appendix 4.1.

4.3. RESULTS

4.3.1. **The Influence of Climatic Factors on Wing Length of *An. arabiensis***

4.3.1.1. **Monthly in the Field**

Pearson's Correlation Coeffients were calculated to test for association between the mean wing-length, temperature and relative humidity. There was a negative correlation between the mean wing length and temperature ($r=-0.395$) as well as between mean wing length and relative humidity ($r=-0.331$, $p<<0.01$). Therefore, as temperature increased the mean body size decreased. Analysis of variance was used to compare the mean wing length for each of the 12 months. Significant differences were found between the temperature ($df = 11$, $p<<0.01$), relative humidity ($df = 11$, $p<<0.01$) and mean wing length ($F=42.82$, $df=11$, $p<<0.01$) for the different months. In order to compare all the months with each other, Duncan's Multiple Range test was used and the results are presented in Table 4. 1.

Table 4.1. Comparisons of mean wing length for each month[1].

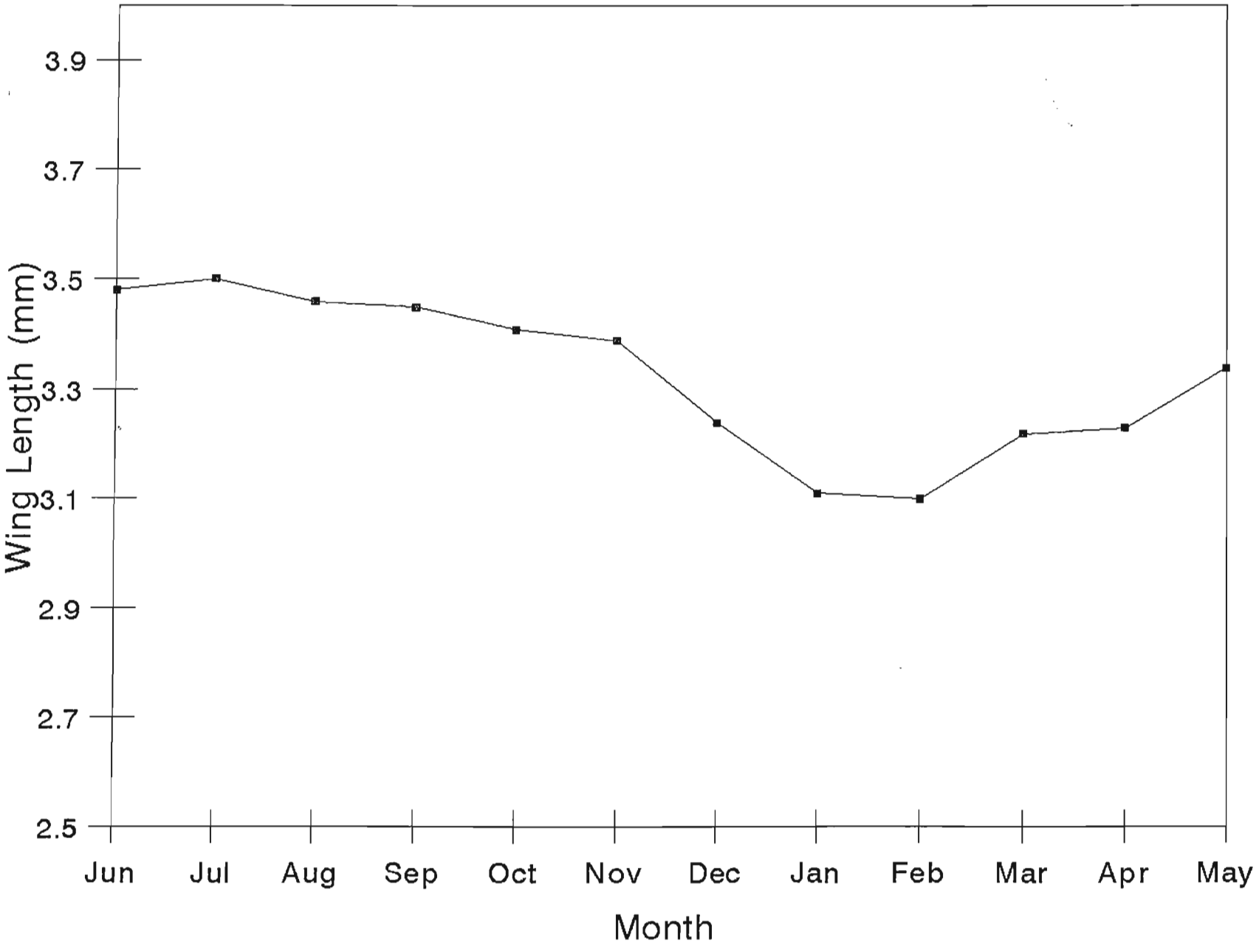
MONTH	n	MEAN ± S.D.	DUNCAN GROUPING
July	64	3.50 ± 0.229	a
June	104	3.48 ± 0.227	a b
August	69	3.46 ± 0.250	a b
September	66	3.45 ± 0.283	a b
October	58	3.41 ± 0.180	a b c
November	74	3.39 ± 0.199	b c
May	76	3.34 ± 0.430	c
December	77	3.24 ± 0.331	d
April	64	3.23 ± 0.183	d e
March	67	3.22 ± 0.415	e f
January	59	3.11 ± 0.139	f
February	80	3.10 ± 0.139	f

[1]Means with the same letters are not significantly different.

The mean wing lengths for the winter and spring months were significantly different for the summer and autumn months of the year. There was a trend for the larger wing size to occur during the cooler months of the year and the smaller wing size to occur in the warmer months of the year (Figure 4.4). The only disruption in this trend was presented by the data collected during April. There was a 13% increase in the mean wing size from February to July.

4.3.1.2. **Seasonally in the Laboratory**

An analysis of variance, performed in order to determine the effect of season on wing length, showed that there were significant differences (F=4.27, df=3,



gure 4.4. Wing length measurements for field collected *An. arabiensis*.

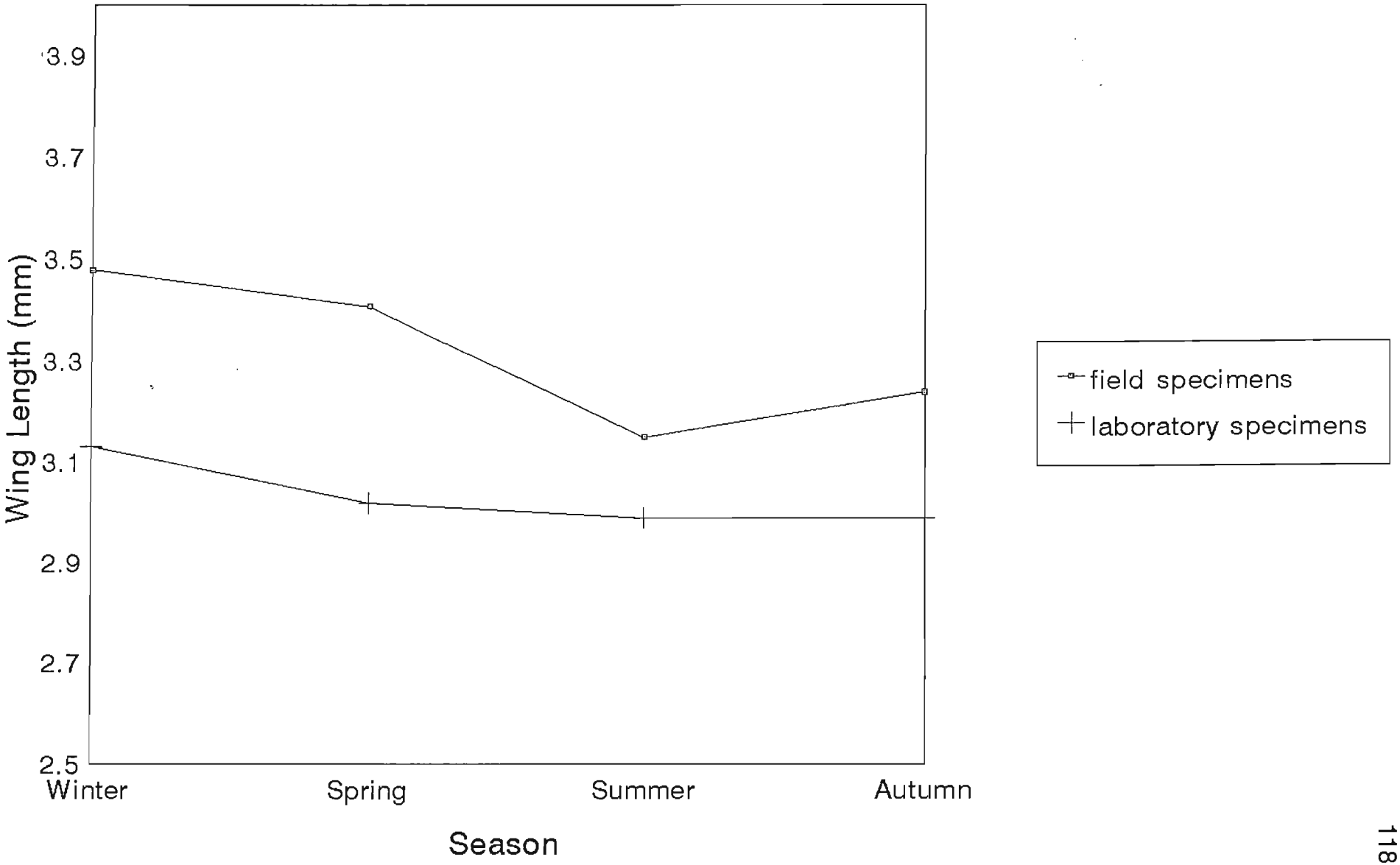
p<<0.01) between seasons. From Table 4.2 it can be seen that the mean wing length obtained in winter was significantly different from that in the other seasons. There were no differences between wing lengths obtained in spring, autumn and summer. This trend also fits well with the trend obtained for the monthly data. There was a 5% difference in wing size between winter and summer and also autumn. A 3.5% decrease in wing size occurred when moving from spring to summer. Therefore winter-reared *An. arabiensis* have the largest body size and summer-reared mosquitoes are small compared to winter-reared mosquitoes.

Table 4.2. Comparisons of mean wing length for each season[1].

SEASON	n	MEAN ± S.D.	DUNCAN GROUPING
Winter	110	3.13 ± 0.470	a
Spring	124	3.02 ± 0.176	b
Summer	216	2.99 ± 0.343	b
Autumn	176	2.99 ± 0.349	b

[1]Means with the same letters are not significantly different.

To determine whether or not temperature and relative humidity played an exclusive role in influencing body size, wing lengths from the laboratory reared mosquitoes were compared with the wing lengths of the field caught specimens (Figure 4.5). There was a significant difference ($p < 0.01$) between the field caught mosquitoes and the laboratory reared mosquitoes. The laboratory reared mosquitoes were all smaller than their field caught counterparts. The decrease



gure 4.5. Comparison of wing length measurements for field and laboratory reared mosquitoes.

in wing size between winter and summer is very apparent in field collected specimens.

4.3.2. **The Association between Temperature, Wing Length and Wing Spot Size**

4.3.2.1. **In the Field**

As has already been shown, mean wing length is negatively correlated to temperature and relative humidity. Pearson's correlation shows that costa B has a positive correlation with mean wing length ($r = 0.512$, $p << 0.01$). All other wing spots were weakly correlated with mean wing length. Temperature and relative humidity were tested against all the wing spots to determine if there was any association between these variables. Both had very weak correlations with all wing spots except costa B ($r = -0.581$, $p << 0.01$), costa C ($r = -0.372$, $p << 0.01$) and costa D ($r = -0.534$, $p << 0.01$). An analysis of these wing spots over the 12 month period showed that there were significant differences between these variables from month to month (ANOVA, $df = 11$, $p << 0.01$). A pair-wise comparison of the values for costa B, C and D was performed (i.e. all the months were compared with each other) and the results are presented in Tables 4.3 - 4.5.

Stepwise multiple regression analysis was performed on the data for each of the wing spots to determine which of the variables (wing length, temperature and month) affect it (Table 4.6). From the total R^2 values given in Table 4.6 it can be seen that costa B produces a highly significant value. Therefore 43.27% of the

Table 4.3. Duncan's grouping for costa B

MONTH	n	MEAN ± S.D.	DUNCAN'S GROUPING
August	69	0.867 ± 0.076	a
July	64	0.830 ± 0.084	b
June	104	0.827 ± 0.071	b
September	66	0.823 ± 0.074	b
May	76	0.796 ± 0.061	c
November	58	0.775 ± 0.092	c d
October	74	0.757 ± 0.066	d e
April	64	0.740 ± 0.085	e f
March	67	0.725 ± 0.062	f g
December	77	0.721 ± 0.064	f g
January	59	0.704 ± 0.059	g h
February	80	0.686 ± 0.062	h

variation in costa B can be explained by variation in wing length, temperature and month. From the partial R^2 values for costa B (Table 4.6), it can be determined that 33.7% of this variation can be attributed to variations in wing length. Costa D is influenced by temperature and month and 29.49% of the variation can be explained in terms of variation in these variables, of which 28.7% is due to variations in temperature.

Table 4.4. Duncan's grouping for costa C

MONTH	n	MEAN ± S.D.	DUNCAN GROUPING
July	64	0.608 ± 0.227	a
June	104	0.581 ± 0.082	a b
August	69	0.556 ± 0.085	b
September	66	0.545 ± 0.069	b c
May	76	0.511 ± 0.069	c d
April	64	0.502 ± 0.061	d
October	74	0.498 ± 0.062	d e
November	58	0.491 ± 0.075	d e f
December	77	0.485 ± 0.190	d e f
March	67	0.474 ± 0.064	d e f
January	59	0.461 ± 0.065	e f
February	80	0.453 ± 0.063	f

Table 4.5. Duncan's grouping for costa D

MONTH	n	MEAN ± S.D.	DUNCAN GROUPING
August	69	0.316 ± 0.031	a
June	104	0.302 ± 0.049	a b
July	64	0.301 ± 0.046	a b
September	66	0.294 ± 0.049	b
May	76	0.292 ± 0.037	b
November	58	0.267 ± 0.051	c
January	59	0.251 ± 0.043	d
April	64	0.248 ± 0.048	d
December	77	0.247 ± 0.039	d
October	74	0.243 ± 0.040	d
March	67	0.229 ± 0.038	e
February	80	0.219 ± 0.032	e

4.3.2.2. **Under Simulated Conditions In The Laboratory**

Since significant differences were found in mean wing length when comparing seasons, the individual wing spots were investigated to determine whether or not season played a role in influencing their size.

Table 4.6. Stepwise Multiple Regression analysis to determine the combined effect of the variables on wing spot size.

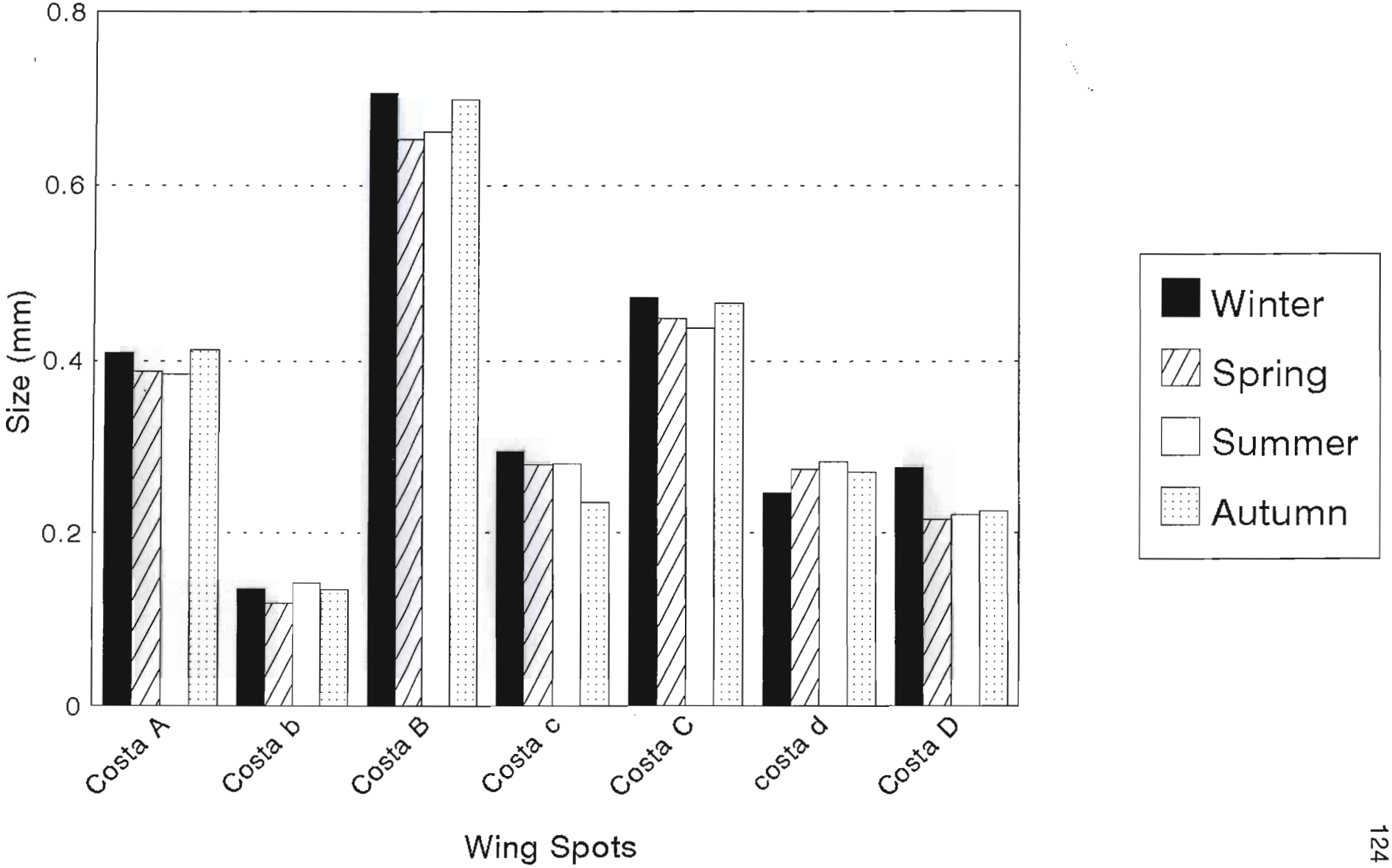
WING SPOT	SIGNIFICANT VARIABLES	p - VALUE	PARTIAL R^2	TOTAL R^2
Costa A	wing length	0.0001	0.031	0.1213
	temperature	0.0001	0.078	
	month	0.0006	0.012	
Costa b	wing length	0.0251	0.016	0.0234
	month	0.0367	0.005	
Costa B	wing length	0.0001	0.337	0.4327
	temperature	0.0001	0.095	
	month	0.0041	0.006	
Costa c	wing length	0.0001	0.045	0.0612
	month	0.0002	0.016	
Costa C	wing length	0.0001	0.016	0.1539
	temperature	0.0001	0.138	
Costa d	wing length	0.0001	0.024	0.0418
	temperature	0.0034	0.009	
	month	0.0490	0.009	
Costa D	temperature	0.0001	0.287	0.2949
	month	0.0018	0.008	

There were significant differences between seasons for all wing spots (ANOVA, df = 3, $p < 0.01$) including costa d (ANOVA, df = 3, $p = 0.05$). For each wing spot Duncan's Multiple Range test was used to compare the four seasons. Generally, winter and/or autumn were significantly different from spring and summer (Figure 4.6). From Figure 4.6 it is evident that the size of the wing spots was noticeably larger during winter than in summer. This may be attributed to the larger wing lengths obtained during the cooler seasons.

Correlation coefficients were calculated to test for association between mean wing lengths for each season and the mean wing spot size for that season (Table 4.7). For mean winter wing length there was a strong correlation with costae A and B and a moderate correlation with costae b and C. In autumn wing length was strongly correlated with costae A, C and D and there was little correlation with the remaining wing spots. Spring wing lengths were moderately correlated with costae A, B and C. Mean wing lengths for summer were strongly correlated with costa B, and moderately correlated with all remaining wing spots except costa b. Thus, it can be seen that temperature influences wing length which in turn influences the size of the wing spot. The only non-significant correlations were those for the pale wing spots in spring.

4.3.3. **Comparison Of Wing Spots For Field And Laboratory Reared Mosquitoes**

A comparison of each wing spot was performed for laboratory reared and field collected mosquitoes for both winter and summer specimens. In summer, the



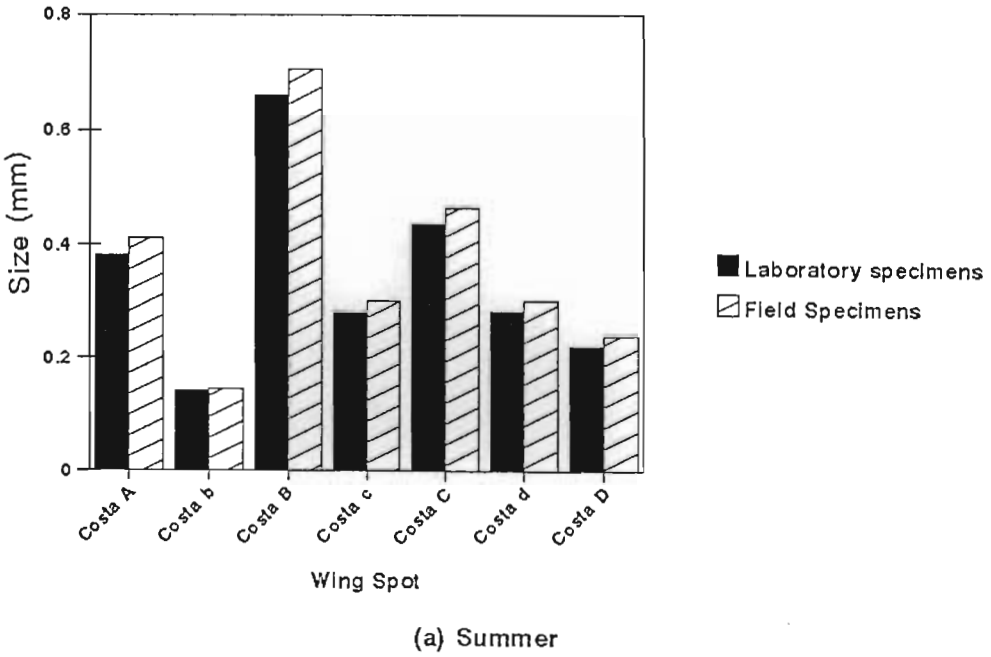
gure 4.6. Size of wing spots for laboratory reared *An. arabiensis*.

field collected mosquitoes had longer wings and the wing spots were consequently larger in field specimens (Figure 4.7(a)) than laboratory reared mosquitoes (t-test, $p<0.01$).

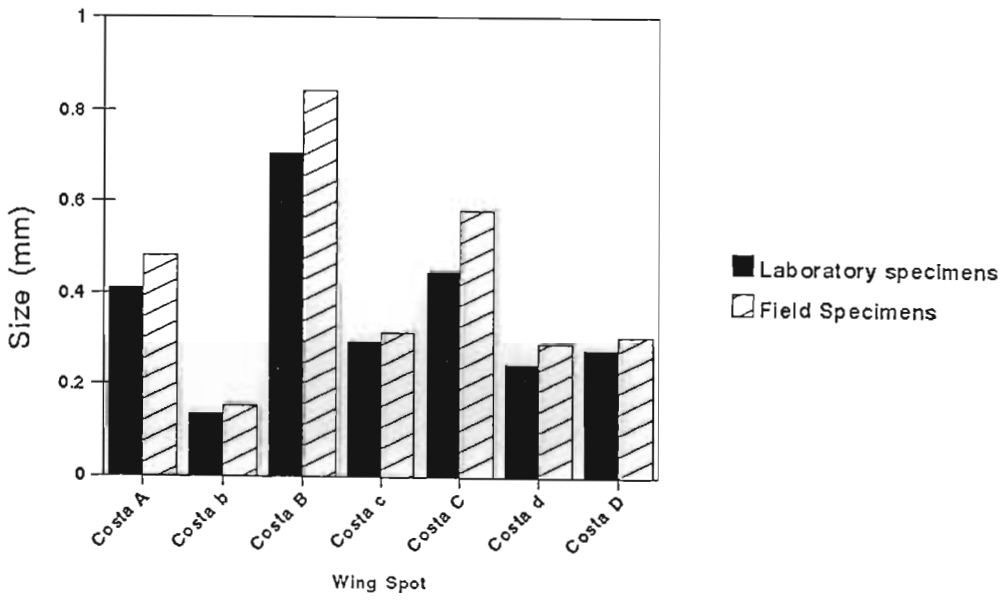
Table 4.7. Univariate Correlation analysis of wing length with the wing spots during the different seasons. Values reported are correlation coefficients (r) and p-values.

		correlation coefficients			
WING SPOT		WINTER	SPRING	SUMMER	AUTUMN
Costa A	r	0.793	0.563	0.686	0.669
	p	0.0001	0.0001	0.0001	0.0001
Costa b	r	0.549	0.102	0.397	0.387
	p	0.0001	0.2605	0.0001	0.0001
Costa B	r	0.895	0.553	0.837	0.843
	p	0.0001	0.0001	0.0001	0.0001
Costa c	r	0.368	0.050	0.546	0.447
	p	0.0001	0.5848	0.0001	0.0001
Costa C	r	0.674	0.561	0.547	0.658
	p	0.0001	0.0001	0.0001	0.0001
Costa d	r	0.370	0.073	0.543	0.292
	p	0.0001	0.4224	0.0001	0.0001
Costa D	r	0.273	0.390	0.519	0.618
	p	0.0040	0.0001	0.0001	0.0001

The wing length of field specimens collected in winter was greater and the resulting wing spots were larger (Figure 4.7(b)) than those on the wings of mosquitoes reared in the laboratory under simulated winter conditions (t-test, $p<0.01$). Therefore, wing length and wing spots of free flying mosquitoes were larger than those of laboratory reared mosquitoes.



(a) Summer



(b) Winter

Figure 4.7. Comparison of the wing spots for field and laboratory reared specimens.

4.3.4. **The Width of the Pale Bands on the Hind Tarsomeres**

4.3.4.1. **Field Collected Mosquitoes**

An analysis of variance shows that there are significant differences ($f = 15.25$, $p << 0.001$) between the widths of the pale bands for the 12 months during which specimens were collected from the field. To examine the differences between the length of the pale bands for each month, Duncan's Multiple Range test was performed on the data. The results of this test are shown in Table 4.8 and the mean length for each month is illustrated in Figure 4.8. The width of the pale band at the junction of tarsomeres 3 and 4 is influenced by temperature since these widths are larger during the cooler months and smaller during the warmer months of the year.

Table 4.8. Duncan's grouping for the monthly pale band widths.

MONTH	n	MEAN ± S.D.	DUNCAN GROUPING
June	72	0.088333 ± 0.00766	a
July	72	0.084167 ± 0.00317	a b
May	79	0.083734 ± 0.00843	a b
April	67	0.083507 ± 0.00747	b c
February	52	0.079423 ± 0.00618	b c d
March	64	0.078984 ± 0.00728	c d
August	63	0.077222 ± 0.00972	d e
September	62	0.073065 ± 0.00669	e f
October	62	0.072742 ± 0.00629	e f
December	69	0.072681 ± 0.00342	e f
November	65	0.071769 ± 0.00611	f
January	70	0.070000 ± 0.00599	f

4.3.4.2. **Under Simulated Conditions in the Laboratory**

Significant differences ($F = 22.47$, $p << 0.001$) were found between the

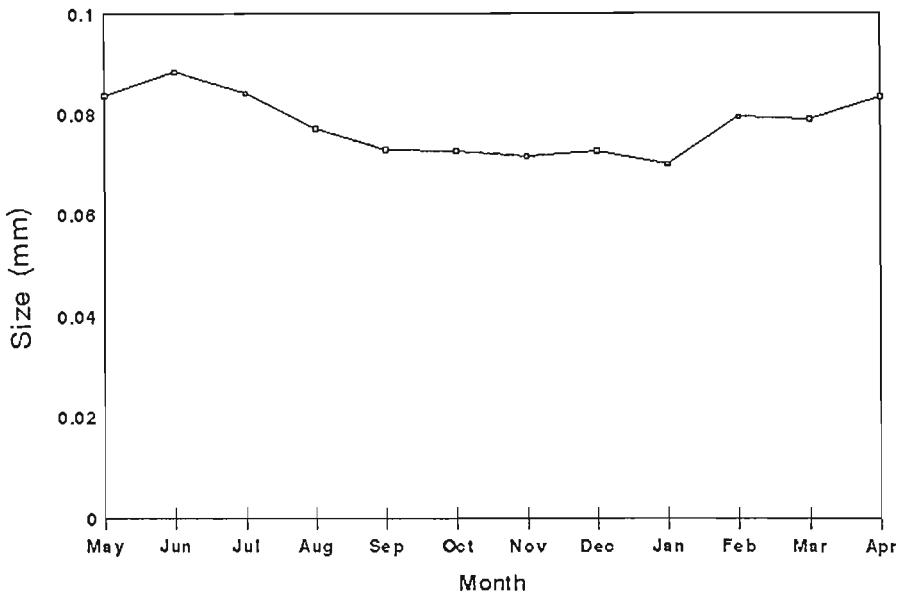


Figure 4.8. The pale band width for field collected *An. arabiensis*.

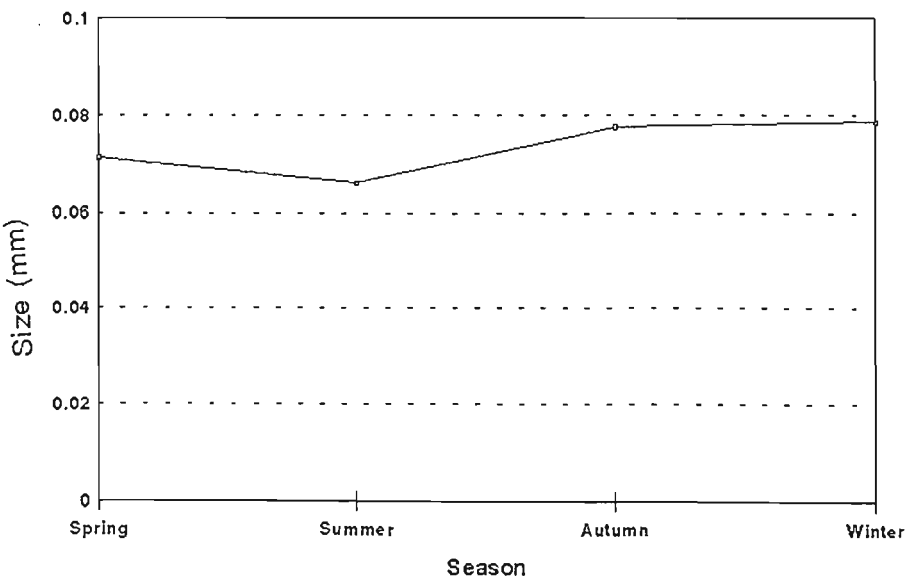


Figure 4.9. The pale band measurements of *An. arabiensis* reared in the laboratory under the different seasonal conditions.

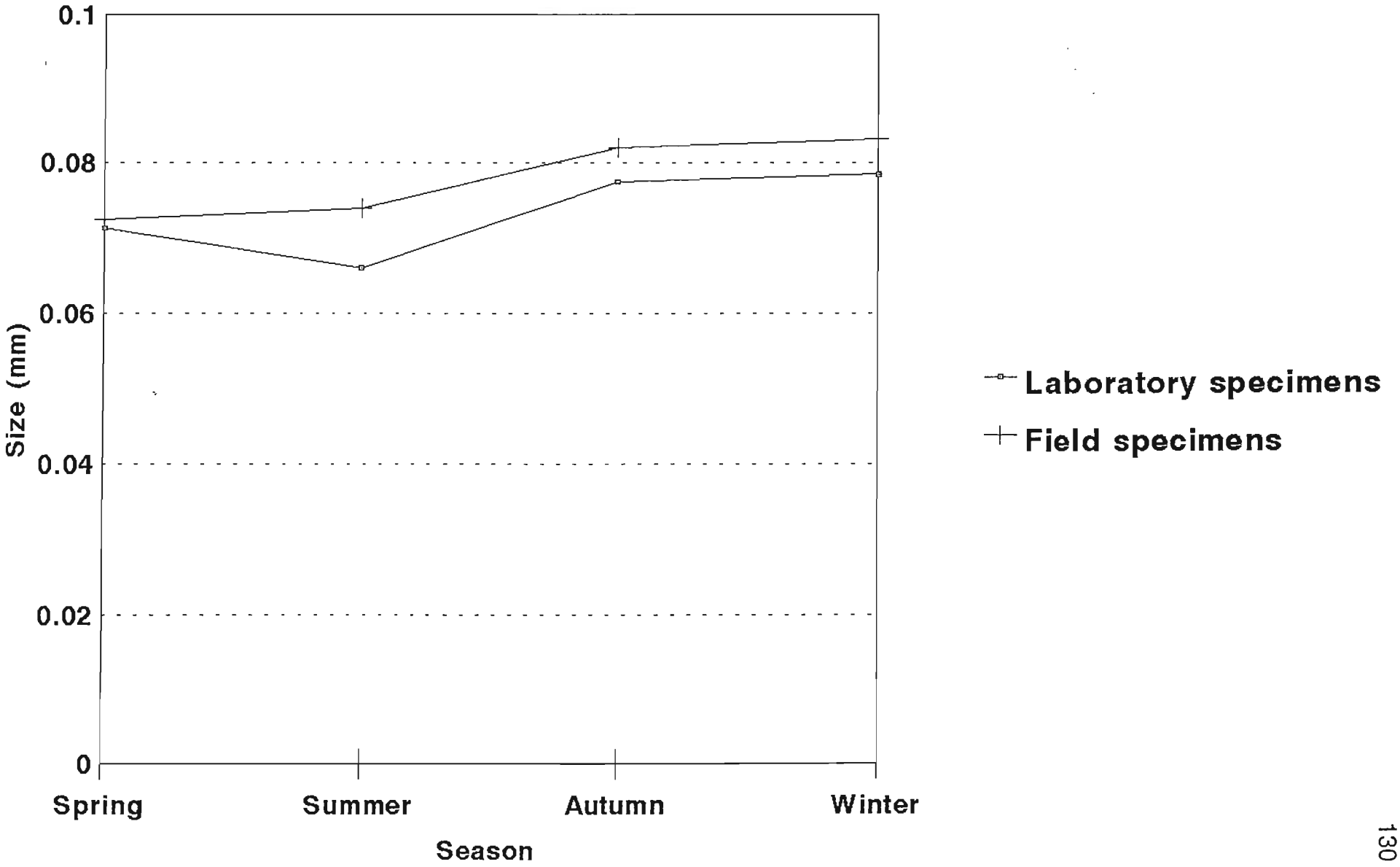
length of the pale band markings in mosquitoes reared under seasonal conditions. Duncan's Multiple Range test yielded a comparison of the seasons (Table 4.9). Under conditions of simulated seasonal change, the widths of the pale band at the junction of tarsomeres 3 and 4 were greater for mosquitoes reared under winter conditions than for those mosquitoes reared under summer conditions (Figure 4.9).

The pale band measurements obtained for specimens reared under simulated seasonal conditions were compared to the corresponding monthly field specimens.

The results of this comparison are illustrated in Figure 4.10. The width of the pale band was found to be significantly larger in field specimens for all seasons (t-test, $p < 0.05$) except spring.

Table 4.9. Duncan's grouping for the pale band widths under simulated conditions.

SEASON	n	MEAN ± S.D.	DUNCAN GROUPING
Winter	80	0.078500 ± 0.00381	a
Autumn	80	0.077500 ± 0.00472	a
Spring	80	0.071250 ± 0.00624	b
Summer	80	0.066000 ± 0.00229	c



gure 4.10. Seasonal pale bands measurements for field and laboratory reared specimens.

DISCUSSION

In mosquitoes, wing length remains constant throughout life (Christophers 1960). Since adult body size is proportional to wing length (van den Heuvel 1963), this study has shown that there is a variation in body size from month to month, as well as from one season to the next. It was found that there is an inverse relationship between temperature and body size since it was determined that wing length decreased as temperature increased. Although relative humidity is negatively correlated with body size, it is unlikely that it would play a role in determining the wing length in adult mosquitoes since the immature stages are restricted to an aquatic environment. As indicated by the field recorded temperature and humidity profiles (Figures 4.1 [a-d]), it is largely an indirect correlation as a result of the association between these two parameters. Generally the body size (as indicated by the wing length) was larger during the cooler season than during the warmer season. Thus winter reared mosquitoes have the largest body size and summer reared *An. arabiensis* are small compared to winter reared specimens. Although the body sizes of both field-collected and laboratory-reared were influenced by temperature, field-caught specimens were larger than laboratory-reared mosquitoes. This may be due to the wide range of temperatures that the larvae developing in the field are exposed to, whereas in the laboratory the larvae are exposed to the same cycle of temperatures over their entire developmental period. This may also be due to improved nutrition in the field since larvae in the laboratory are fed on an artificial diet that may not resemble the normal diet of larvae in the field.

The work by le Sueur (1991) is the only other study investigating the effects of temperature on the morphology of members of the *An. gambiae* complex in South

Africa. However, le Sueur (1991) studied these effects on *An. merus*. Based on the assumption that temperature would influence the morphology of both sibling species, the results of this study on *An. arabiensis* were compared with that of le Sueur's (1991) study on *An. merus* (Figure 4.11). Although the specimens were collected from different geographic areas, with different mean monthly temperatures, the general trend in wing length remains the same. The wing lengths are smaller during the warmer months of the year and longer during the cooler months. In the comparison (Figure 4.11), *An. merus* has a greater wing length than *An. arabiensis*. This may however be due to the differences in mean monthly temperatures of the locality where the *An. merus* specimens were collected.

This study showed that the width of the pale band at the junction of hind tarsomeres 3 and 4 is highly variable. The width of this pale band was found to have an inverse relationship with temperature - as temperature increased, the width of the pale band decreased. The measurements of the pale band were larger during periods of lower temperature such as autumn and winter under both field and laboratory conditions. The mosquitoes reared under the simulated seasonal conditions were all reared under the same conditions except for the change in the seasonal temperature and humidity profiles. The changes in the seasonal temperature and humidity profiles resulted in a change in the pale band measurement. This suggests that temperature influences the pale band measurements. Although the seasonal profile of pale band measurements for both field collected and laboratory reared mosquitoes followed the same trend (Figure 4.10) the pale band measurements were found to be larger in field collected specimens than in laboratory reared specimens. The larger pale band measurement

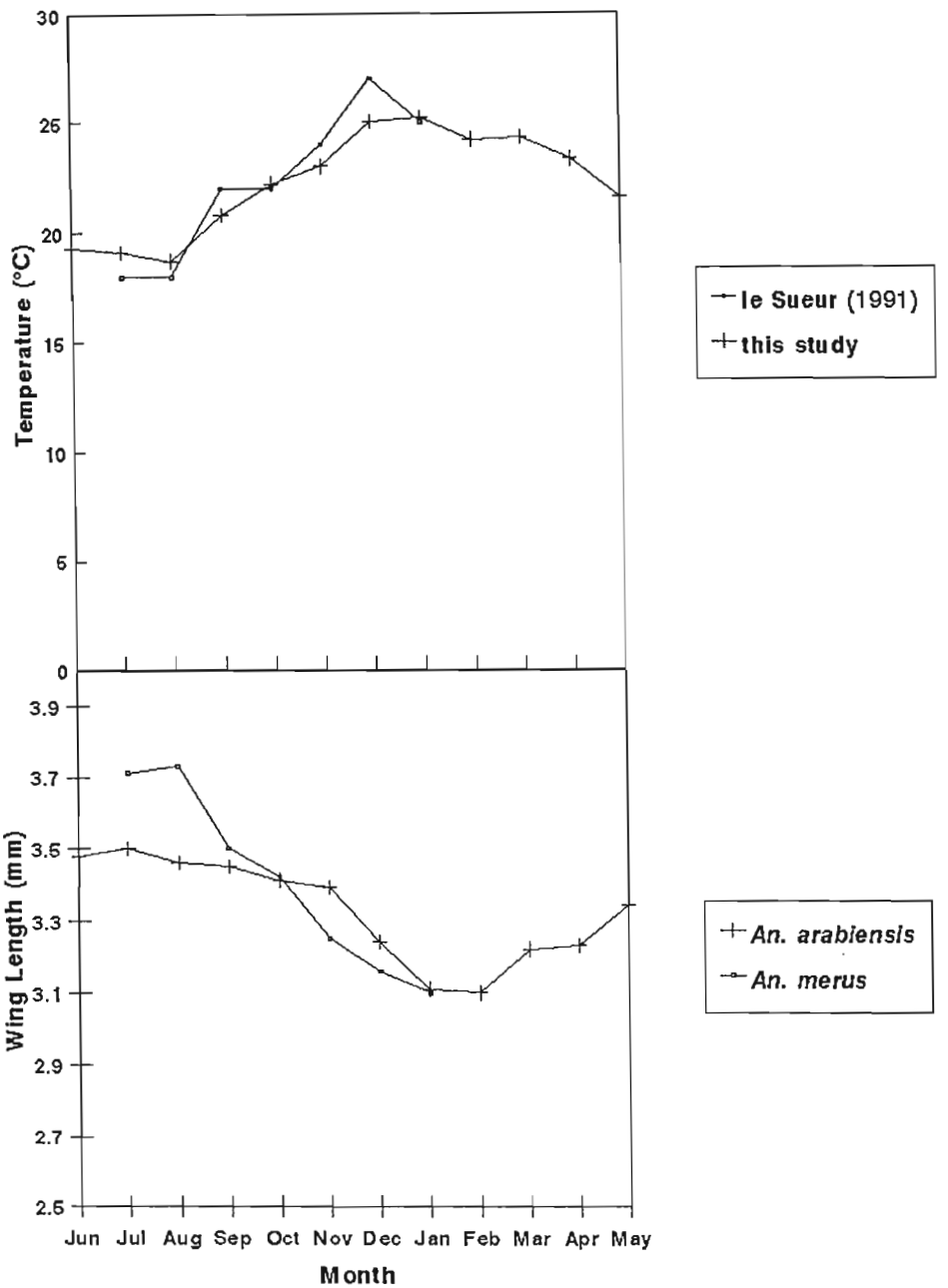


Figure 4.11. Wing length measurement of An. arabiensis (this study) and *An. merus* (le Sueur 1991), with their associated temperature profiles.

in field collected mosquitoes may be attributed to the fact that mosquitoes in the field are subjected to a range of temperatures that may influence development whereas laboratory reared mosquitoes are subject to the same 24 hour cycle of temperature and humidity throughout their development.

The width of the pale band at the junction of the hind tarsomeres 3 and 4 for *An. arabiensis* specimens collected from the field was compared to the values obtained for *An. arabiensis* in studies by le Sueur (1991) and Coetzee (1986). All three studies showed that there is a peak in the pale band width within the range 0.06 - 0.08 mm (Figure 4.12). The present study and le Sueur's (1991) study shows a peak at 0.07 mm. The specimens for both these studies were collected from the same locality, Dondotha in KwaZulu-Natal. The specimens used in Coetzee's (1986) study were collected from various localities in southern Africa and shows a peak at 0.06 mm. This might be due to (i) the measuring intervals that she used, i.e. 0.02 whereas le Sueur (1991) and the present study used 0.01 and (ii) her specimens being collected at a different time of the year. This supports the conclusion by le Sueur and Sharp (1991) that morphometric characteristics vary over geographic localities. From these studies it is apparent that the width of the pale band on the hind tarsomeres of *An. arabiensis* ranges from 0.02 - 0.14 mm, and that the actual width measured is influenced by the climatic variations of the geographic locality from which the specimens are collected. Figure 4.13 shows that season has a marked influence on the width of the pale band at the junction of hind tarsomeres 3 and 4 of *An. arabiensis* in summer and winter. The peak width in winter was 0.01 mm greater than the peak width in summer. The hindleg pale band at the unction of tarsomere 3 and 4 is thought to be a good character for

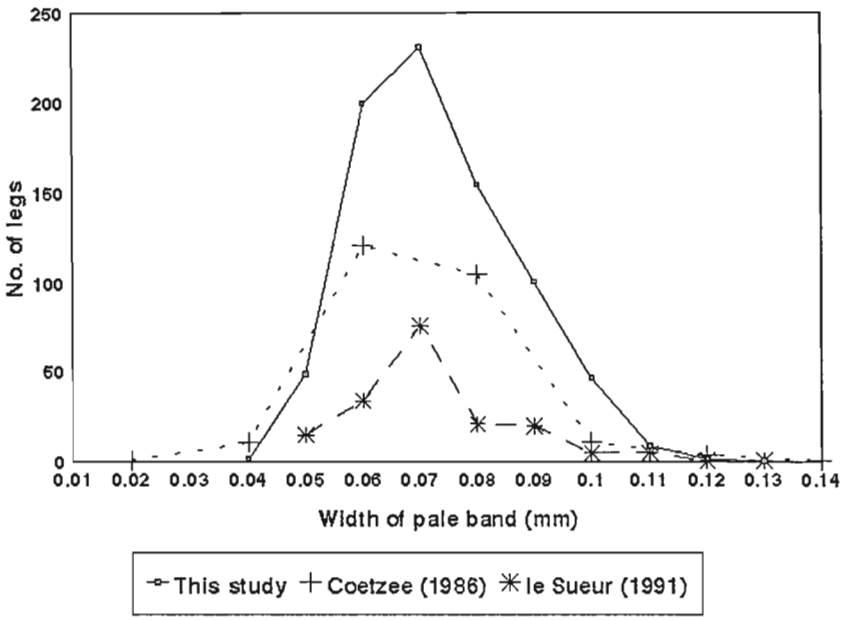


Figure 4.12. Comparison of leg band widths for *An. arabiensis* in three studies.

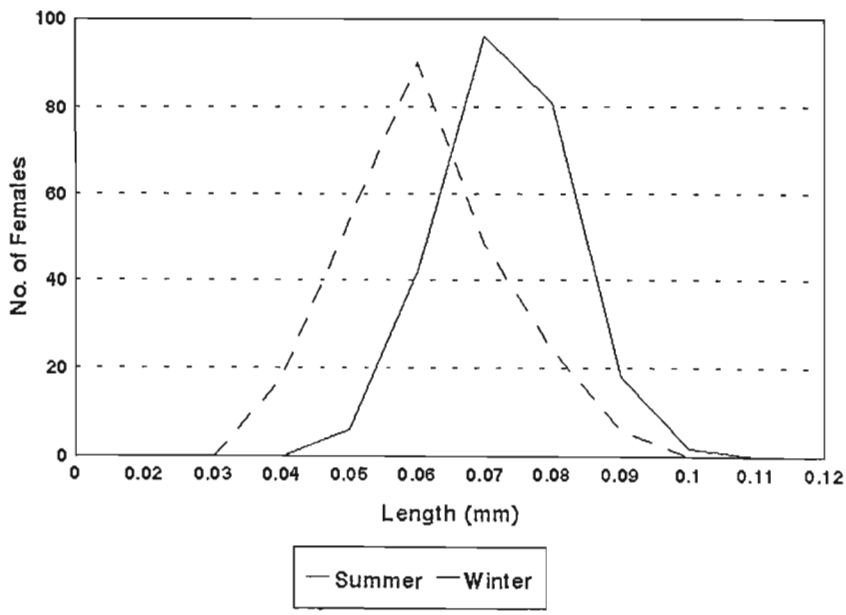
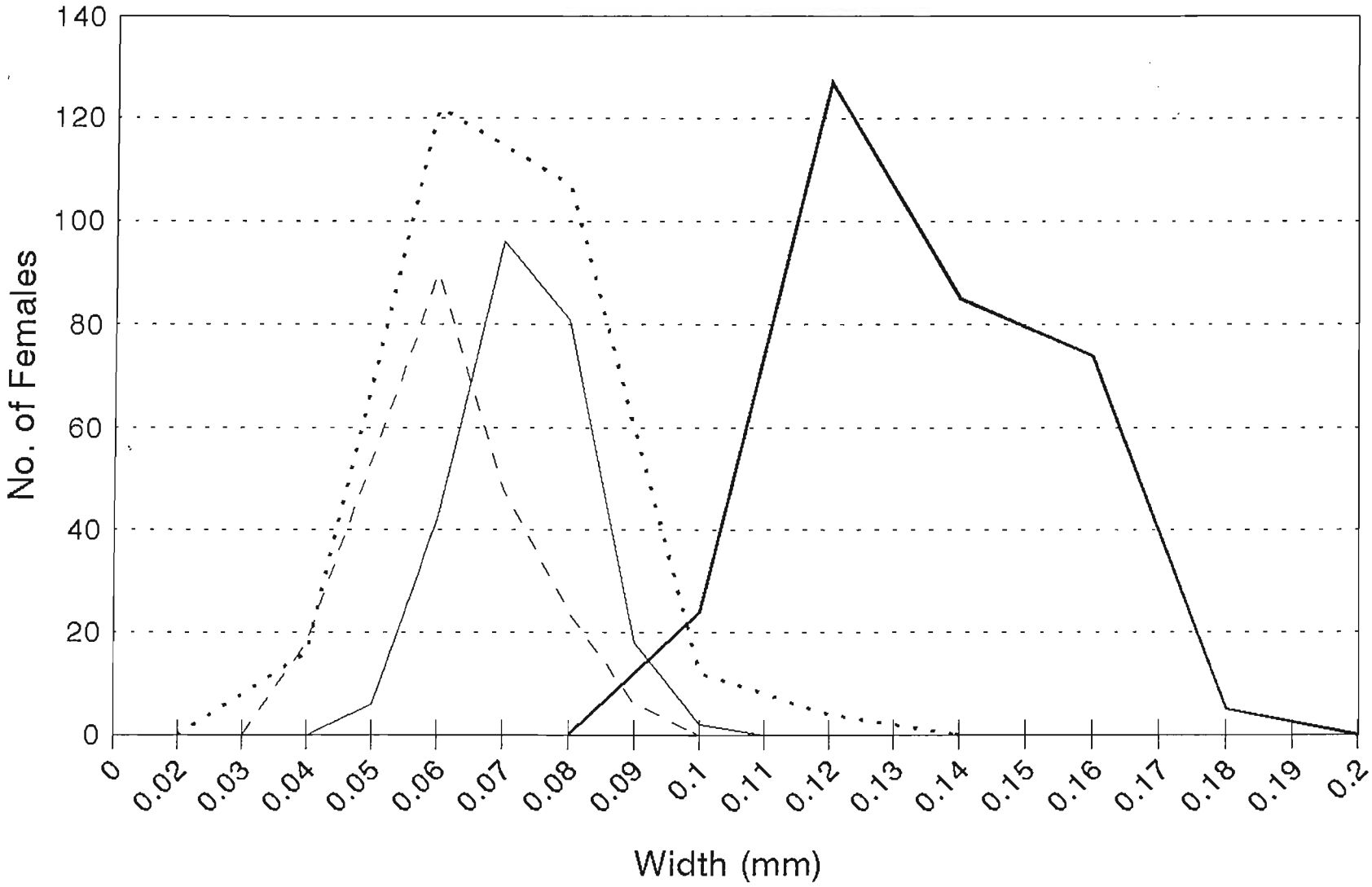


Figure 4.13. The distribution of the leg banding measurements of *An. arabiensis* in summer and winter.

grouping *An. gambiae/An. arabiensis* and *An. quadriannulatus/An. merus* (Coetzee *et al.* 1982) although *An. arabiensis* is known to be variable in this respect (Sharp *et al.* 1989). The method of Coetzee (1986) has shown that the pale bands in the major malaria vectors *An. gambiae* and *An. arabiensis* were generally narrower than those of the sibling species *An. merus* and *An. quadriannulatus*.

Since temperature influences the width of the pale band on the hindleg tarsomeres, the validity of Coetzee's (1986) species separation graph was tested by comparing it to the winter and summer distribution of the pale bands of *An. arabiensis* (Figure 4.14). The distribution of the pale bands given by Coetzee (1986) encompasses the distribution of the pale bands for both winter and summer. Although the peak in the pale band of *An. arabiensis* increases by 0.01 mm in winter, seasonality does not decrease the sensitivity of the technique since the peak in the distribution of the width of the pale band is still less than the 0.09 mm that Coetzee (1986) recommended as the cut-off point between the two species groups. Therefore the species separation graph appears to take into account seasonal differences. Coetzee (1986) collected specimens from numerous localities in KwaZulu-Natal, Northern Province and Mpumalanga, at various times during the year. This comparison reinforces le Sueur's (1991) conclusion that morphological variation should be assessed seasonally throughout the species distribution, if mosquito taxonomic keys are to include absolute size criteria. Figure 4.13 illustrates the difference in morphological measurement that can be obtained at different times of the year. Studies of a morphological nature should be broad-based to give a holistic overview of occurrences in nature.



igure 4.14. The distribution of the leg banding measurements of An. arabiensis in summer and winter
s well as Anopheles gambiae/arabiensis and merus/quardiannulatus.

The sizes of the wing spots on the costae of the wings of *An. arabiensis* are also influenced by temperature. Therefore, since temperature influences wing length, wing length in turn influences the size of the wing spots. It was found that there was a strong association between the length of the wing and the length of some of the wing spots. Measurements of the wing spots of mosquitoes reared under the different seasonal temperatures and humidities showed that there was an inverse relationship between temperature and the dark wing spots especially costae B, C and D. In accordance with the variation in wing length found from month to month and between seasons, there is a corresponding variation in the sizes of costae B, C and D. The sizes of the wing spots are generally larger during the cooler seasons. Since the sizes of the wing spots are temperature dependent, the use of wing spots in taxonomic identification should be avoided.

Under field conditions, the size of the dark wing spots increased proportionally as wing lengths increased and there was a small, non-proportional increase in the size of the pale spots as well. Under simulated seasonal conditions in the laboratory, there was a moderate to strong correlation between the size of the dark wing spot and wing length and a weak to moderate correlation between the size of the pale wing spot and wing length. The non-significant correlations obtained for the pale wing spots in spring are a reflection of the wide range of temperatures (20 - 32°C) experienced during this season. The year in which this seasonal profile was recorded experienced an unusually warm spring (b = 24°C) compared with a ten year average of 22°C. The spring temperature was high enough to produce wing lengths that were not significantly different from those produced during summer. The mean temperature in spring was

lower than that of summer and promoted greater melanin deposits since melanin production was found to be inversely related to temperature (Ford 1945). Therefore, unlike the other seasons, in spring all the increase in wing length occurred in the dark wing spots since the dark spots in the wing pattern are a reflection of melanin deposits. Once again, the discrepancies observed in the relationship between wing spot size and wing length for laboratory reared and field collected mosquitoes may be attributed to the fact that mosquitoes in the field are subjected to a range of temperatures that may influence development whereas laboratory reared mosquitoes are subject to the same 24 hour cycle of temperature and humidity throughout their development.

The reason(s) for the inverse relationship between temperature and body size are not clear but Laudien (1973) suggested that it may be the result of rapid development at higher temperatures. Although the growth rate may increase with temperature, this increase is out-weighed by a decrease in the time available for growth, and the resulting mosquitoes are smaller. The decrease in wing size may be partially explained by the possibility that at high summer temperatures the metabolic requirements may exceed the rate at which food can be gathered by the larvae (le Sueur 1991). Therefore at high temperatures, the developing larvae have a shorter time to take up nutrients thus producing smaller adults with shorter wings. This was corroborated by Shelton (1973) who found that as temperature increased, the average body weight of adults decreased.

In the field, females have a higher reproductive rate during periods of high temperature (summer) than during periods of low temperature such as the winter months (refer to Chapter 3). A high egg production and hatchability would result in high larval densities

and according to Kitthawee *et al.* (1992), larvae in crowded conditions appear to feed less efficiently and the resulting adults are likely to have a reduced body size, survivorship and reproductive potential. During these experiments, both wild-caught mosquitoes and the laboratory-reared mosquitoes showed the same trend in wing length and wing spot size even though the field- caught and laboratory mosquitoes were reared under different densities and nutritional conditions. Laboratory-reared mosquitoes were reared under conditions of uniform density and were provided with abundant nutrients, yet variations in body size were obtained at different temperatures. Although size variation can be genetically influenced (Greenough *et al.* 1971), all specimens used in this study were collected from a single site, representing a single gene pool, thus indicating that size variation was environmentally influenced. Day *et al.* (1990) found that the body size of field collected *Culex nigripalpus* showed a consistent pattern of seasonal variation; mosquitoes were largest during the cool winter and spring and smallest during summer and autumn. The results presented in this study followed a similar trend.

Larger bodied adults, produced mainly during the cooler season, survive much longer than the smaller adults produced during the warmer seasons (see Chapter 3). Therefore the longevity of mosquitoes is influenced by temperature and hence body size. Kitthawee *et al.* (1990) have suggested that the longer survival among larger adults may be related to protein accumulation during the immature stages. Van Handel and Day (1989) also determined that protein content generally increased with wing length. Body size is thus an indication of fitness. Investigations into the capabilities of adult mosquitoes in the field have shown that large bodied females (as indicated by

wing lengths) exhibit higher fitness qualities than do small-bodied females. Larger *Aedes triseriatus* (Say) females are more frequently parous (Haramis 1983); larger *Culex tarsalis* Coquillett have higher fecundity (Bock & Milby 1981) and larger *Anopheles* have higher survivorship rates (Hu *et al* . 1993). Studies on *An. arabiensis* (Chapter 3) have shown that larger mosquitoes (those reared under winter conditions and those having longer wings) have the longest survival period, are capable of having numerous blood meals and can become gravid. However, these large bodied females undergo gonotrophic dissociation with the onset of winter and overwinter as adults.

Temperature has a significant effect on the development of parasites in the mosquito. *Plasmodium falciparum* cannot complete its development in the mosquito below a mean temperature of 16°C, although the parasite survives to continue its development when the temperature rises above this critical point (Gear *et al.* 1988). At a temperature of 17.5°C, *P. falciparum* takes more than 23 days to develop (Gear *et al.* 1988). From Chapter 3 it can be seen that winter-reared *An. arabiensis* are capable of surviving the necessary incubation period of the main malaria parasite *P. falciparum*. In South Africa the winters are generally warm with an average temperature of 18°C (range of 10 - 27°C). Therefore, under winter conditions in the field, the development of *P. falciparum* will be retarded but not to the extent suggested by Gear *et al.* (1988). van den Heuvel (1963) noted that body size can influence the size of the blood meal that is consumed and Briegel (1990) found that the feeding capacity on vertebrate hosts was more than doubled between small and large females. Therefore large bodied females may contribute more to the maintenance and amplification of malaria transmission than small bodied individuals. In the field Nasci (1986a) established that larger individuals were

more successful at obtaining blood meals. The reason for this is not known but Nasci (1986b) suggested that small individuals may fly for a shorter time or distance, or are not as persistent or may become exhausted more quickly when compared with large individuals.

There has been a lot of interest in variation in mosquito body size and its possible influence on disease transmission. Many authors (e.g. Day *et al.* 1990; Kitthawee *et al.* 1990, 1992) have suggested that size may affect both the vector capacity and the vector potential of mosquitoes by influencing their ability to become infected and once infected, their survivorship and potential to transmit the parasite to other susceptible hosts. Thus, large *An. arabiensis* that develop during winter are capable of taking larger blood meals since they have a larger mid-gut. They can therefore take up a larger parasite load if fed on an infected individual. Since these mosquitoes are ectothermic, they do not travel long distances in search of a blood meal. They usually rest in close proximity to their hosts (Ramsdale & Wilkes 1985). Resting sites serve as refuges from adverse environmental conditions. Voluntary flights will be performed only if environmental factors permit (Bidlingmayer 1985).

In South Africa, the number of reported cases of malaria is very low and it is thought that these cases are mainly asymptomatic cases detected through active surveillance by the malaria control personnel. The large adults produced during the cooler seasons do not play an important role in the transmission of malaria. It is hypothesised that these large-bodied mosquitoes become infected and serve as reservoirs of the parasite that contribute towards the transmission of malaria in spring. These mosquitoes probably

become infected late in autumn or early in winter and due to the decrease in temperatures, the parasite development is slowed. When these mosquitoes take a blood meal they are unlikely to transmit malaria due to the slowed development of the parasite. Since the numbers of blood meals required in winter are very few, infectious mosquitoes may account for a very small proportion of the malaria cases reported. The large body size enables the mosquitoes to survive the adverse environmental conditions. When conditions improve in spring, these mosquitoes oviposit thereby contributing to the increase in population density. With an increase in temperature the parasites within the mosquitoes complete their development and contribute towards the amplification of malaria transmission.

This study has therefore shown that temperature does influence the body size of *An. arabiensis* as well as the longevity and overall fitness of these mosquitoes. The results reported in this chapter corroborate the findings of le Sueur (1991) in that morphological characteristics used in taxonomic identification are affected by temperature. The width of the pale band at the junction of tarsomeres 3 and 4 as well as wing length and the resulting wing spots are much larger in field collected specimens than in laboratory reared mosquitoes. The results in this chapter therefore demonstrate a limitation to the use of insectary reared mosquitoes in taxonomic studies. Material to be used in morphological studies, or in studies involving temperature differences, should be collected from various geographic localities as well as at different times of the year so that a truly representative sample of the wild population is obtained.

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APPENDIX 4.1

Simple statistics for the data used in Chapter 4.

Month: JANUARY

Wing Spot	n	Mean	S.D.	Minimum	Maximum
Costa A	59	0.3981	0.0519	0.29	0.55
Costa b	59	0.1495	0.0257	0.08	0.18
Costa B	59	0.7044	0.0595	0.57	0.84
Costa c	59	0.3085	0.0504	0.18	0.41
Costa C	59	0.4612	0.0647	0.29	0.58
Costa d	59	0.3000	0.0504	0.12	0.41
Costa D	59	0.2505	0.0426	0.18	0.34
Length	59	3.1133	0.1391	0.26	3.54

Month: FEBRUARY

Wing Spot	n	Mean	S.D.	Minimum	Maximum
Costa A	80	0.4088	0.0482	0.31	0.54
Costa b	80	0.1392	0.0236	0.07	0.18
Costa B	80	0.6861	0.06277	0.54	0.83
Costa c	80	0.2991	0.0443	0.20	0.44
Costa C	80	0.4533	0.0635	0.25	0.55
Costa d	80	0.2986	0.0435	0.15	0.39
Costa D	80	0.2191	0.0327	0.15	0.31
Length	80	3.1042	0.1391	2.73	3.54

Month: MARCH

Wing Spot	n	Mean	S.D.	Minimum	Maximum
Costa A	67	0.4177	0.0424	0.33	0.50
Costa b	67	0.1465	0.0216	0.10	0.20
Costa B	67	0.7252	0.0626	0.58	0.83
Costa c	67	0.3002	0.0581	0.15	0.46
Costa C	67	0.4744	0.0646	0.23	0.60
Costa d	67	0.3188	0.0949	0.15	0.96
Costa D	67	0.2285	0.0383	0.15	0.31
Length	67	3.2219	0.4151	2.87	3.63

Month: APRIL

Wing Spot	n	Mean	S.D.	Minimum	Maximum
Costa A	64	0.4267	0.0501	0.34	0.60
Costa b	64	0.1150	0.0365	0.03	0.18
Costa B	64	0.7395	0.0848	0.60	0.97
Costa c	64	0.2512	0.0783	0.12	0.42
Costa C	64	0.5017	0.0618	0.37	0.63
Costa d	64	0.2287	0.0658	0.15	0.37
Costa D	64	0.2482	0.0483	0.15	0.36
Length	64	3.2318	0.1833	2.73	3.86

Month: MAY

Wing Spot	n	Mean	S.D.	Minimum	Maximum
Costa A	76	0.4430	0.0483	0.31	0.54
Costa b	76	0.1501	0.0246	0.08	0.18
Costa B	76	0.7956	0.0618	0.65	0.96
Costa c	76	0.3082	0.0545	0.21	0.50
Costa C	76	0.5109	0.0695	0.34	0.65
Costa d	76	0.3007	0.0442	0.15	0.44
Costa D	76	0.2919	0.0374	0.18	0.37
Length	76	3.3438	0.4300	2.95	3.73

Month: JUNE

Wing Spot	n	Mean	S.D.	Minimum	Maximum
Costa A	104	0.4820	0.1693	0.31	2.07
Costa b	104	0.1491	0.0384	0.06	0.29
Costa B	104	0.8267	0.0717	0.63	1.04
Costa c	104	0.3104	0.0783	0.08	0.67
Costa C	104	0.5808	0.0833	0.36	0.81
Costa d	104	0.2975	0.1969	0.12	1.94
Costa D	104	0.3023	0.0497	0.18	0.47
Length	104	3.4808	0.2265	2.76	3.99

Month: JULY

Wing Spot	n	Mean	S.D.	Minimum	Maximum
Costa A	64	0.4706	0.0649	0.34	0.65
Costa b	64	0.1548	0.0385	0.06	0.25
Costa B	64	0.8304	0.0843	0.67	1.05
Costa c	64	0.2978	0.0686	0.05	0.47
Costa C	64	0.6087	0.2279	0.37	2.28
Costa d	64	0.2760	0.0546	0.13	0.39
Costa D	64	0.3018	0.0460	0.17	0.41
Length	64	3.5028	0.2297	2.89	3.86

Month: AUGUST

Wing Spot	n	Mean	S.D.	Minimum	Maximum
Costa A	69	0.4895	0.0529	0.34	0.63
Costa b	69	0.1500	0.0369	0.08	0.21
Costa B	69	0.8672	0.0766	0.70	1.10
Costa c	69	0.3311	0.0685	0.12	0.48
Costa C	69	0.5563	0.0854	0.41	0.76
Costa d	69	0.3028	0.0409	0.18	0.39
Costa D	69	0.3156	0.0317	0.25	0.42
Length	69	3.4594	0.2500	2.92	3.84

Month: SEPTEMBER

Wing Spot	n	Mean	S.D.	Minimum	Maximum
Costa A	66	0.4896	0.0394	0.41	0.57
Costa b	66	0.1616	0.0710	0.10	0.54
Costa B	66	0.8231	0.0747	0.67	1.00
Costa c	66	0.3283	0.0577	0.25	0.52
Costa C	66	0.5446	0.0695	0.42	0.65
Costa d	66	0.3283	0.0557	0.23	0.47
Costa D	66	0.2942	0.0493	0.20	0.41
Length	66	3.4466	0.2833	2.41	3.81

Month: OCTOBER

Wing Spot	n	Mean	S.D.	Minimum	Maximum
Costa A	74	0.4550	0.1262	0.31	1.45
Costa b	74	0.1471	0.0278	0.08	0.21
Costa B	74	0.7568	0.0669	0.57	0.89
Costa c	74	0.3405	0.0593	0.25	0.50
Costa C	74	0.4982	0.0629	0.37	0.68
Costa d	74	0.3571	0.1925	0.17	0.92
Costa D	74	0.2431	0.0400	0.18	0.34
Length	74	3.4072	0.1803	2.92	3.76

Month: NOVEMBER

Wing Spot	n	Mean	S.D.	Minimum	Maximum
Costa A	58	0.4603	0.0781	0.34	0.77
Costa b	58	0.1525	0.0310	0.08	0.21
Costa B	58	0.7750	0.0927	0.60	0.99
Costa c	58	0.3381	0.0528	0.25	0.47
Costa C	58	0.4910	0.0753	0.34	0.65
Costa d	58	0.3410	0.0487	0.25	0.41
Costa D	58	0.2672	0.0517	0.18	0.37
Length	58	3.3914	0.1998	2.76	3.70

Month: DECEMBER

Wing Spot	n	Mean	S.D.	Minimum	Maximum
Costa A	77	0.4314	0.0853	0.31	0.99
Costa b	77	0.1463	0.0326	0.08	0.21
Costa B	77	0.7214	0.0642	0.60	0.92
Costa c	77	0.3037	0.0586	0.15	0.47
Costa C	77	0.4849	0.1901	0.28	2.02
Costa d	77	0.3042	0.0545	0.10	0.46
Costa D	77	0.2466	0.0391	0.18	0.31
Length	77	3.2350	0.3308	2.76	5.76

Season: SPRING

Wing Spot	n	Mean	S.D.	Minimum	Maximum
Costa A	124	0.3883	0.0471	0.31	0.50
Costa b	124	0.1350	0.0290	0.08	0.21
Costa B	124	0.6529	0.0672	0.50	0.83
Costa c	124	0.2787	0.0553	0.15	0.54
Costa C	124	0.4725	0.0749	0..25	0.63
Costa d	124	0.2738	0.1262	0.18	1.58
Costa D	124	0.2256	0.0396	0.15	0.31
Length	124	3.0193	0.1756	2.57	3.37

Season: SUMMER

Wing Spot	n	Mean	S.D.	Minimum	Maximum
Costa A	176	0.3837	0.0594	0.19	0.54
Costa b	176	0.1434	0.0316	0.06	0.25
Costa B	176	0.6616	0.0971	0.33	0.96
Costa c	176	0.2803	0.0714	0.36	0.50
Costa C	176	0.4378	0.0934	0.25	0.67
Costa d	176	0.2828	0.0622	0.25	0.44
Costa D	176	0.2213	0.0519	0.16	0.37
Length	176	2.99	0.3429	2.37	3.60

Season: AUTUMN

Wing Spot	n	Mean	S.D.	Minimum	Maximum
Costa A	216	0.4217	0.0714	0.11	0.63
Costa b	216	0.1196	0.0321	0.03	0.18
Costa B	216	0.6988	0.0956	0.43	0.92
Costa c	216	0.2355	0.0693	0.14	0.41
Costa C	216	0.4662	0.0774	0.26	0.63
Costa d	216	0.2707	0.1250	0.19	1.92
Costa D	216	0.2159	0.0467	0.13	0.31
Length	216	2.9949	0.3485	2.31	3.67

Season: WINTER

Wing Spot	n	Mean	S.D.	Minimum	Maximum
Costa A	110	0.4100	0.0822	0.26	0.63
Costa b	110	0.1357	0.0334	0.07	0.21
Costa B	110	0.7061	0.1205	0.45	0.96
Costa c	110	0.2945	0.0943	0.15	0.47
Costa C	110	0.4487	0.1065	0.28	0.73
Costa d	110	0.2473	0.0807	0.18	0.47
Costa D	110	0.2763	0.1559	0.19	1.76
Length	110	3.1269	0.4704	2.45	3.67

CHAPTER 5

THE EFFECTS OF SIMULATED SEASONAL CONDITIONS ON THE BODY SIZE OF THREE *ANOPHELES* SPECIES

5.1. INTRODUCTION

Adult mosquito size may be influenced by several factors during larval development. Density dependent conditions include the accumulation of metabolites, food depletion and growth retardant factors associated with overcrowding (Moore & Fisher 1969, Ikeshoji & Mulla 1970). Temperature is a density independent factor that influences the rate of larval development (Bock & Milby 1981). Vectorial capacity is partially reflected by body size which is obtained from measurements of wing lengths (van den Heuvel 1963). Numerous authors have studied the effects of temperature on the body size of anopheline mosquitoes, viz., *An. merus* (le Sueur 1991; le Sueur & Sharp 1991)

Variations in adult body size are known to occur in field populations of several mosquito species (Feinsod & Spielman 1980; Bock & Milby 1981; Haramis 1983). Investigations into the capabilities of adult mosquitoes have shown that relatively large bodied females (as indicated by longer wing lengths) exhibit higher fitness qualities than do small-bodied females (Nasci 1986a,b). le Sueur *et al.* (1992) found that during cooler months of the year adult mosquitoes were darker and they had longer wings. The amount of pale and dark scales in the wing patterns is a reflection of melanin deposition (Ford 1945). In insects it is well known that melanin production is inversely related to

temperature since high temperatures limit the deposition of tyrosine, the precursor of melanin (Ford 1945).

Among mosquitoes belonging to the *Anopheles gambiae* complex, two of the sibling species *An. gambiae* and *An. arabiensis* are widespread vectors of human malaria in Africa. A third member, *An. merus* is also a malaria vector but is not as notorious as the afore-mentioned species. *Anopheles arabiensis* is the major vector of malaria in South Africa and *An. merus* may be a minor vector of *Plasmodium* (White 1974; Braack *et al.* 1994). Due to a highly effective malaria eradication campaign in the early 1950s, *An. gambiae* has been eliminated from this country although it is still an efficient vector in the rest of Africa (Gillies & De Meillon 1968).

It is known that body size influences the vectorial potential of mosquitoes. Longevity is also influenced by body size (Kitthawee *et al.* 1990). The present study constitutes an attempt to measure the potential vectorial capability of *An. arabiensis* under conditions found typically in the malaria endemic areas of KwaZulu-Natal. Since body size reflects the fitness of mosquitoes, the body size of *An. arabiensis, An. gambiae* and *An. merus* was obtained by measuring wing length. The potential vectorial capacity of each species was evaluated and subsequently compared to determine which of these mosquito species were competent vectors of malaria.

5.2. MATERIALS AND METHODS

All experiments were carried out on *An. arabiensis*, *An. gambiae* and *An. merus* and

were done in the laboratory at the Medical Research Council in Durban.

5.2.1. **Specimens Used**

The three species of the *An. gambiae* complex used were obtained from different sources. The *An. arabiensis* used were progeny of wild caught females from Dondotha in KwaZulu-Natal, the *An. merus* were obtained from a colony maintained at the South African Institute of Medical Research in Johannesburg and the *An. gambiae* were obtained from a colony maintained at the School of Hygiene and Public Health, Johns Hopkins University, Baltimore, USA. The polymerase chain reaction method was used to obtain correct identifications of the mosquito species used and to determine whether or not they were contaminated.

The different generations of *An. arabiensis* used in determining the effect of temperature on insectary reared specimens were as follows:

1. F_{200} progeny of *An. arabiensis* KGB which had been maintained in the insectary for more than 16 years.
2. F_{45} progeny of *An. arabiensis* collected from Dondotha that had been reared in the insectary for three years.
3. F_2 progeny of wild *An. arabiensis* collected from Dondotha in late summer.

5.2.2. **Seasonal Climatic Profiles**

All experiments were conducted under simulated conditions of winter and

summer. Experiments were conducted under controlled conditions of temperature and humidity in a programmable growth cabinet (see Chapter 3). The winter and summer temperature and humidity profiles used were those obtained from the field as described in Chapter 4. Figures 4.1. (a) and 4.1.(c) in Chapter 4 showed the profiles for winter and summer respectively. The photoperiod used was determined from the field as follows: winter (11L:13D) and summer (13L:11D) each with one hour simulated crepuscular period.

5.2.3. **The Experimental Methods**

For each species of mosquito and for each season, 4 replicates of 100 first instar larvae were reared in plastic containers (35 x 25 x 10 cm) filled to a depth of 4 cm with deionised water. Larvae were fed on finely ground Epol® cat food. Pupae were separated daily and placed in 2ℓ buckets with screened tops.

5.2.4. **Mounting and Measurement**

Once the adults had emerged, they were killed and their wings were carefully removed and mounted on glass slides according to the method of Hunt and Coetzee (1986). The wing length measurements were taken between the axillary incision (Harbach and Knight 1980) and the wing tip, excluding the fringe, in the region of vein 3 (Gillies and Coetzee 1987). Measurements were carried out with a Wild M7A Stereo microscope using a 10X eyepiece and 31X objective and an eyepiece micrometer with 120 divisions, each equal to 31 µm on the focal plane.

5.2.5. **Statistical Analysis**

Duncan's Multiple Range Test was used to analyse the data. The simple statistics for the data used are given in Appendix 5.1.

5.3. RESULTS

It was found that wing length and the resulting wing spots were influenced by the simulated seasonal temperature and humidity regimens.

5.3.1. **Effect of Temperature on Insectary Reared Mosquitoes**

A comparison between the mean wing lengths obtained in winter and summer for the three generations of insectary reared mosquitoes showed that wing length was consistently larger in winter than in summer (Table 5.1).

Table 5.1. Mean wing lengths (mm) obtained in winter and summer for the three generations of *An. arabiensis*

GENERATION	WINTER ($\bar{x}$ ± S.D.)	n	SUMMER ($\bar{x}$ ± S.D.)	n
F_{200} (approx.)	3.32 ± 0.158	98	3.09± 0.165	100
F_{45}	3.18 ± 0.389	99	3.08 ± 0.321	98
F_2	3.12 ± 0.470	110	2.99 ± 0.343	176

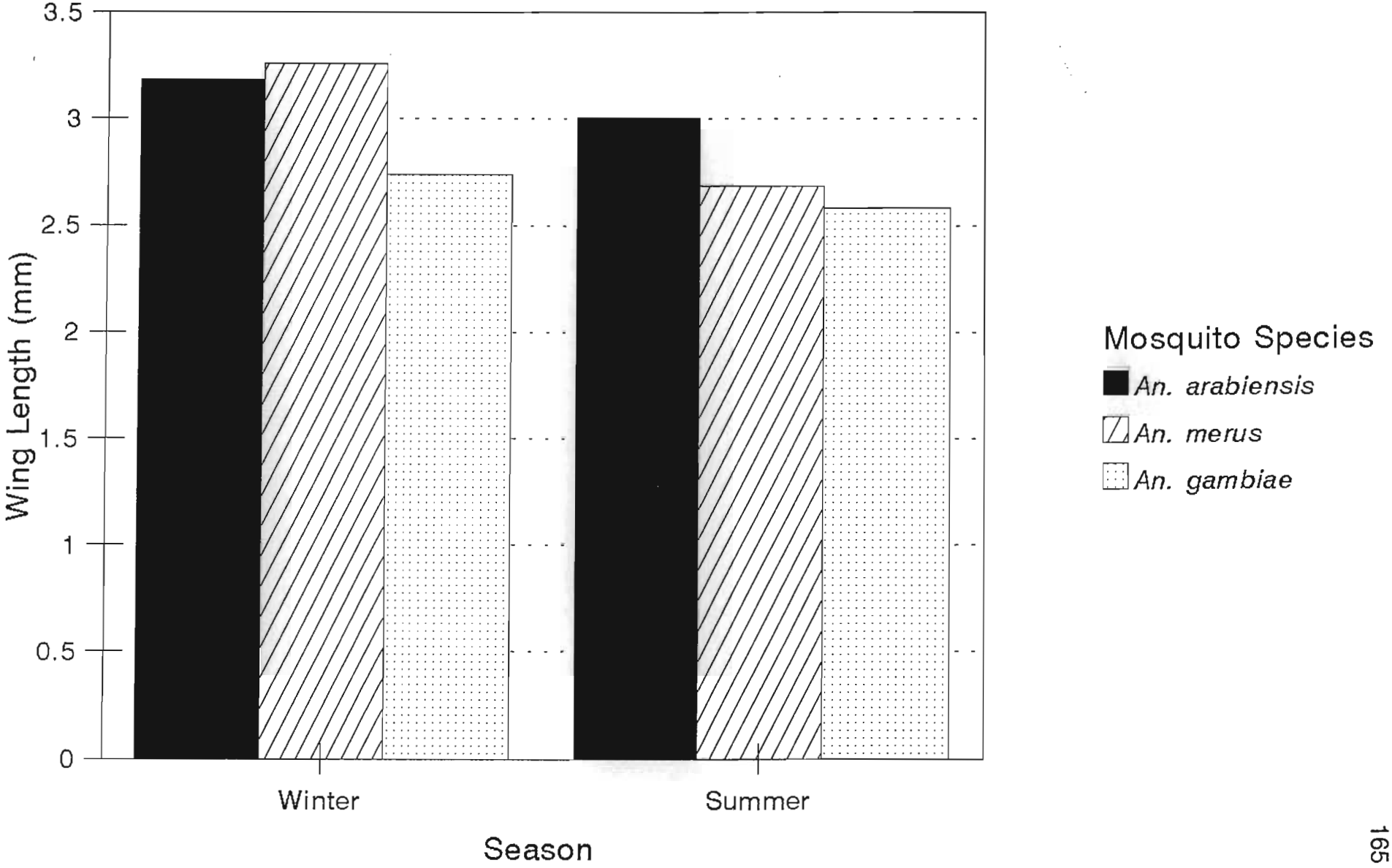
The size difference in wing length between winter and summer bred mosquitoes is most marked in the KGB (F_{200}) population where a difference of 0.23 mm was

found. Analysis of the data showed that as the number of generations spent in the insectary increased, the wing length obtained under simulated seasonal conditions also increased. The difference in mean wing lengthsbetween the F_2 and the F_{45} generations was smaller in winter. In summer the difference between the F_{200} and F_{45} generations was only 0.01rnm. The$_2$F progeny of the field collected mosquitoes was least affected by the winter temperatures and most affected by the summer temperatures.

5.3.2. **Interaction between Season, Wing Length and Species**

A 2-way analysis of variance was used to compare the wing lengths of adult mosquitoes across seasons and species and to look at the season-species interaction. There was a distinct difference between the wing lengths obtained during the two seasons ($F = 159.0$, $p<<0.01$). The wing lengths obtained under winter conditions were always greater than those obtained under simulated summer conditions (Figure 5.1). In winter the wing lengths obtained for *An. merus* were largest and those for *An. gambiae* were smallest. Under summer conditions, the wing lengths of *An. gambiae* were smallest and those of *An. arabiensis* the largest.

Since the *An. merus* and the *An. gambiae* used in these experiments were all colony material, the size differences could have been an artifact of prolonged rearing in the insectary at constant temperature and relative humidity. Therefore the percentage change in wing length was calculated between winter and



gure 5.1. The effect of seasonal temperature on wing length of the three species.

summer reared individuals. All three species showed an increase in wing size when moving from summer conditions to winter conditions. The percent change for *An. arabiensis* was 4.5%, for *An. merus* 21.3% and for *An. gambiae* 6%. It is apparent that *An. merus* was most affected by changes in environmental temperature and *An. arabiensis* was least affected. Once again the percent change in wing length may be influenced by the number of generations for which *An. merus* and *An. gambiae* had been reared in the insectary.

A comparison of wing length between the three species showed that there was a highly significant difference ($p<<0.01$, Duncan's Multiple Range Test). Overall, *An. arabiensis* had the greatest wing length followed by *An. merus*. Once again, *An. gambiae* had the shortest wing length.

An analysis of the species-season interaction on the wing length of adult mosquitoes reared under similar conditions showed that both season and the species of mosquito had a significant effect on wing length ($p<<0.01$). From Figure 5.1 it can be seen that the species-season interaction has the greatest influence on *An. merus*. *Anopheles arabiensis* was the least affected by the changing seasonal conditions.

5.3.3. **Influence of Season and Species on the Wing Spot Size**

Since the size of the wing spots of *An. arabiensis* are influenced by wing length (see Chapter 4), wing spot size was compared for all three species across the

two seasonal conditions, summer and winter, using a 2-way analysis of variance. From Table 5.2 it can be seen that there was always a significant difference between the size of the wing spots in winter and summer for all except costa d. There was a highly significant difference in the mean wing spot size between all three anopheline species ($p<<0.01$). The season-species interaction has a highly significant influence ($p<<0.01$) on the size of all wing spots except costae b, c and D.

Table 5.2. A summary of the ANOVA used to compare the seasons and species.

		p - values		
WING SPOT	F-VALUE	SEASON	SPECIES	SEASON-SPECIES INTERACTION
Costa A	32.8	0.0001	0.0001	0.0001
Costa b	64.0	0.0424	0.0001	0.0904
Costa B	106.9	0.0001	0.0001	0.0001
Costa c	49.7	0.0068	0.0001	0.5608
Costa C	37.9	0.0001	0.0001	0.0001
Costa d	53.28	0.1778	0.0001	0.0001
Costa D	32.02	0.0001	0.0001	0.0868

Duncan's Multiple Range test was used to determine which of the inter-species differences were significant. The results are presented in Tables 5.3 and 5.4. During winter, the sizes of the wing spots were different for all species but some

of the wing spots were not significantly different in two of the three species (Table 5.3). For costa A, *An. gambiae* differed from the measurements obtained for *An. arabiensis* and *An. merus*. In adults of *An. merus* the mean size of costa C was significantly different from the other two species. With regards to the size of costa d, in *An. arabiensis* it was significantly larger than that of its two sibling species.

Similarly in summer (Table 5.4), costa A was significantly larger in *An. arabiensis* and costa C was significantly smaller in *An. gambiae*. *Anopheles arabiensis* was significantly different from the other two species when comparing costa D. Overall, the size of the wing spots produced by the three anopheline species tended to follow the same trend as that obtained when rearing the mosquitoes under the simulated winter conditions.

5.3.4. **Influence of Wing length on Wing Spot Size**

Temperature is known to influence wing length as well as wing spot size. Pearson's correlation coefficients were calculated to examine the association between wing length and wing spot size. This has already been done for wild caught *An. arabiensis* (Chapter 4). For *An. gambiae*, wing length was moderately correlated with costae B, C and D ($r = 0.64$, 0.51 and 0.4 respectively) and there was a weak correlation between wing length and the rest of the wing spots.

Table 5.3. Duncan's grouping for winter wing spots

WING SPOT	SEASON	SPECIES	n	MEAN ± S.D.	DUNCANS GROUPING
Costa A	Winter	*An. arabiensis*	108	0.418 ± 0.061	a
		An. merus	65	0.441 ± 0.032	a
		An. gambiae	82	0.370 ± 0.041	b
Costa b	Winter	*An. arabiensis*	108	0.138 ± 0.028	a
		An. merus	65	0.115 ± 0.027	b
		An. gambiae	82	0.090 ± 0.031	c
Costa B	Winter	*An. arabiensis*	108	0.719 ± 0.072	a
		An. merus	65	0.824 ± 0.035	b
		An. gambiae	82	0.633 ± 0.061	c
Costa c	Winter	*An. arabiensis*	108	0.300 ± 0.086	a
		An. merus	65	0.257 ± 0.039	b
		An. gambiae	82	0.195 ± 0.053	c
Costa C	Winter	*An. arabiensis*	108	0.457 ± 0.087	a
		An. merus	65	0.568 ± 0.082	b
		An. gambiae	82	0.481 ± 0.050	a
Costa d	Winter	*An. arabiensis*	108	0.252 ± 0.073	a
		An. merus	65	0.223 ± 0.043	b
		An. gambiae	82	0.203 ± 0.034	b
Costa D	Winter	*An. arabiensis*	108	0.281 ± 0.152	a
		An. merus	65	0.257 ± 0.039	b
		An. gambiae	82	0.209 ± 0.034	c

Table 5.4. Duncan's grouping for wing spots in summer.

WING SPOT	SEASON	SPECIES	n	MEAN ± S.D.	DUNCANS GROUPING
Costa A	Summer	*An. arabiensis*	175	0.386 ± 0.051	a
		An. merus	57	0.357 ± 0.039	b
		An. gambiae	85	0.367 ± 0.047	b
Costa b	Summer	*An. arabiensis*	175	0.144 ± 0.029	a
		An. merus	57	0.128 ± 0.027	b
		An. gambiae	85	0.087 ± 0.040	c
Costa B	Summer	*An. arabiensis*	175	0.665 ± 0.083	a
		An. merus	57	0.625 ± 0.054	b
		An. gambiae	85	0.593 ± 0.063	c
Costa c	Summer	*An. arabiensis*	175	0.282 ± 0.068	a
		An. merus	57	0.235 ± 0.043	b
		An. gambiae	85	0.188 ± 0.048	c
Costa C	Summer	*An. arabiensis*	175	0.443 ± 0.081	a
		An. merus	57	0.435 ± 0.079	a
		An. gambiae	85	0.410 ± 0.060	b
Costa d	Summer	*An. arabiensis*	175	0.286 ± 0.054	a
		An. merus	57	0.184 ± 0.035	b
		An. gambiae	85	0.227 ± 0.039	c
Costa D	Summer	*An. arabiensis*	175	0.224 ± 0.046	a
		An. merus	57	0.173 ± 0.023	b
		An. gambiae	85	0.165 ± 0.025	b

Pearson's correlation coefficients show that the wing lengths for *An. merus* was strongly correlated with costae A, B, C and D (r = 0.82, 0.89, 0.82 and 0.82 respectively). This species has a moderate association with costae c and d (r = 0.55 and 0.64 respectively).

The ratio of wing spot to wing length was calculated for *An. arabiensis, An. merus* and *An. gambiae* for both winter and summer reared mosquitoes. From Table 5.2 it can be seen that , except for costa d, there was always a significant difference between the wing spots in winter and summer reared mosquitoes. Duncan's Multiple Range test was used to determine whether there was inter-specific variation in the ratio of wing spot to wing length. For the winter ratios, there were significant differences between all three sppecies for costae B and c (Table 5.5). For summer reared mosquitoes, there were significant differences in the ratios of costae A and d (Table 5.6). All other ratios were not significantly different.

5.4. DISCUSSION

Body size as determined by wing length, was always greater when the mosquitoes were reared under winter conditions. During the experiments to determine the effects of temperature on insectary reared mosquitoes, it was found that the longer mosquitoes were maintained in insectary colonies, the greater was their response to low temperatures. The *An. arabiensis* KGB strain, which had been maintained at 27°C and 70% RH for over 16 years, was most affected by exposure to winter temperatures. This

Table 5.5. Duncan's grouping for the ratio of winter wing spots to wing length.

WING SPOT	SEASON	SPECIES	n	MEAN ± S.D.	DUNCANS GROUPING
Costa A	Winter	*An. arabiensis*	108	0.1313 ± 0.0144	a
		An. merus	65	0.1354 ± 0.0001	b
		An. gambiae	82	0.1336 ± 0.0002	a b
Costa b	Winter	*An. arabiensis*	108	0.0445 ± 0.0001	a
		An. merus	65	0.0345 ± 0.0001	b
		An. gambiae	82	0.0319 ± 0.0001	b
Costa B	Winter	*An. arabiensis*	108	0.2256 ± 0.0003	a
		An. merus	65	0.2502 ± 0.0002	b
		An. gambiae	82	0.2325 ± 0.0003	c
Costa c	Winter	*An. arabiensis*	108	0.0945 ± 0.0008	a
		An. merus	65	0.0794 ± 0.0002	b
		An. gambiae	82	0.0713 ± 0.0004	c
Costa C	Winter	*An. arabiensis*	108	0.1428 ± 0.0006	a
		An. merus	65	0.1794 ± 0.0003	b
		An. gambiae	82	0.1757 ± 0.0004	b
Costa d	Winter	*An. arabiensis*	108	0.0795 ± 0.0006	a
		An. merus	65	0.0689 ± 0.0001	b
		An. gambiae	82	0.0835 ± 0.0049	a
Costa D	Winter	*An. arabiensis*	108	0.0842 ± 0.0003	a
		An. merus	65	0.0765 ± 0.0001	b
		An. gambiae	82	0.0869 ± 0.0084	a b

Table 5.6. Duncan's grouping for the ratio of summer wing spots to wing length.

WING SPOT	SEASON	SPECIES	n	MEAN ± S.D.	DUNCANS GROUPING
Costa A	Summer	*An. arabiensis*	175	0.1286 ± 0.0002	a
		An. merus	57	0.1325 ± 0.0002	b
		An. gambiae	85	0.1419 ± 0.0003	c
Costa b	Summer	*An. arabiensis*	175	0.0481 ± 0.0001	a
		An. merus	57	0.0470 ± 0.0001	a
		An. gambiae	85	0.0335 ± 0.0001	b
Costa B	Summer	*An. arabiensis*	175	0.2209 ± 0.0003	a
		An. merus	57	0.2332 ± 0.0002	b
		An. gambiae	85	0.2297 ± 0.0004	b
Costa c	Summer	*An. arabiensis*	175	0.0912 ± 0.0006	a
		An. merus	57	0.0877 ± 0.0002	a
		An. gambiae	85	0.0728 ± 0.0003	b
Costa C	Summer	*An. arabiensis*	175	0.1469 ± 0.0006	a
		An. merus	57	0.1617 ± 0.0006	b
		An. gambiae	85	0.1587 ± 0.0005	b
Costa d	Summer	*An. arabiensis*	175	0.0938 ± 0.0004	a
		An. merus	57	0.0691 ± 0.0002	b
		An. gambiae	85	0.0880 ± 0.0002	c
Costa D	Summer	*An. arabiensis*	175	0.0746 ± 0.0002	a
		An. merus	57	0.0642 ± 0.0001	b
		An. gambiae	85	0.0642 ± 0.0001	b

is illustrated by the longer wings produced by the KGB strain under these conditions. The F_{45} generation of *An. arabiensis* from Dondotha had been maintained in the insectary for three years and produced intermediate results. The F_2 progeny of *An. arabiensis* that had spent a month in the insectary were least affected by exposure to low temperatures. This suggests that mosquitoes become acclimatised to living at a constant temperature in the insectary but retain their capacity to respond to changes in environmental temperature. The longer a mosquito population is maintained at a high constant temperature, the greater is the response of the next generation to a decrease in temperature.

Low temperatures are known to decrease the rate of development in mosquitoes thereby producing larger bodied individuals (refer to Chapter 3). Although insectary-reared mosquitoes retain their ability to respond to changes in temperature, a fast development rate may be selected for under constant high temperatures in the insectary. Thus when these mosquitoes are exposed to a lower temperature, the developmental time increases and development is further hindered by the selection to development at high temperatures. This would ensure that the larval duration was further protracted and may explain why mosquitoes such as *An. arabiensis* KGB strain that have spent many generations in the insectary have a slower developmental rate than the F_{45} and F_2 generation *An. arabiensis* under conditions of low temperature.

When exposed to simulated summer conditions, the *An. arabiensis* KGB strain has the longest wings and the F_2 progeny of females collected from Dondotha the shortest

wings. Once again, the insectary colonised mosquitoes were being exposed to a lower temperature (25°C) than that of the insectary (27°C) to which they have become acclimatised.

When the mosquitoes were reared under such conditions, *An. merus* was the most robust followed by *An. arabiensis*. Due to the higher mean temperature during simulated summer conditions, larval development was fastest and the mosquitoes produced were smaller in size than those produced under simulated winter conditions. When reared under summer conditions, *An. arabiensis* had the greatest wing length and hence the largest body size. Irrespective of the seasonal conditions under which the larvae were reared, *An. gambiae* always had the smallest body size. A comparison between the wing lengths of the three mosquito species showed that *An. arabiensis* was the most robust species. However, it cannot be concluded that wing size can be used in the taxonomic separation of species since only insectary reared specimens of *An. merus* and *An. gambiae* were used in this study.

le Sueur *et al.* (1992) found that the dark scaled areas on the wings of *Anopheles* mosquitoes were selectively affected by temperature related size variation. As a result of the decrease in wing length when moving from winter to summer, there was a corresponding decrease in the size of the wing spots. For all three mosquito species the increase in wing length occurred in the dark scaled areas, namely costae A, B, C and D. This was consistent with the results obtained by le Sueur *et al.* (1992) for *An. arabiensis* and *An. merus*.

Zahar *et al.* (1970) reported on the use of wing spot ratios in species separation. In view of the fact that dark wing spots increase in size during winter, while pale spots remain constant, the validity of the use of this ratio is questionable. Since the ratio varies from winter to summer, this characteristic is not "fixed" under varying environmental conditions. Therefore, it cannot be used to separate out species from a specific locality at a given time of the year since variation should be assessed seasonally.

For *An. merus*, the results of the present study were compared with those of le Sueur (1991). For mosquitoes exposed to winter temperatures, it was found that the results obtained were very similar (Figure 5.2). *Anopheles merus* exposed to summer conditions in the present study and in that of le Sueur (1991), followed the same trend (Figure 5.3) except that the sizes of the pale wing spots in the present study were smaller than that obtained by le Sueur (1991). For both seasons, the sizes of costae A and B obtained in the present study were larger than those obtained in le Sueur's (1991) study.

Coetzee (1986) examined the size of the wing spots in *An. arabiensis, An. gambiae, An. merus* and *An. quadriannulatus* and these were compared to those obtained for *An. arabiensis, An. gambiae* and *An. merus* in the present study. The comparison for *An. arabiensis* showed that results of both studies were very similar (Figure 5.4). The same was true for *An. gambiae*, except that the size of costa c was markedly smaller in the present study (Figure 5.5). Although the results of both studies on *An. merus* followed a similar pattern (Figure 5.6), the results were highly variable. The dark bands

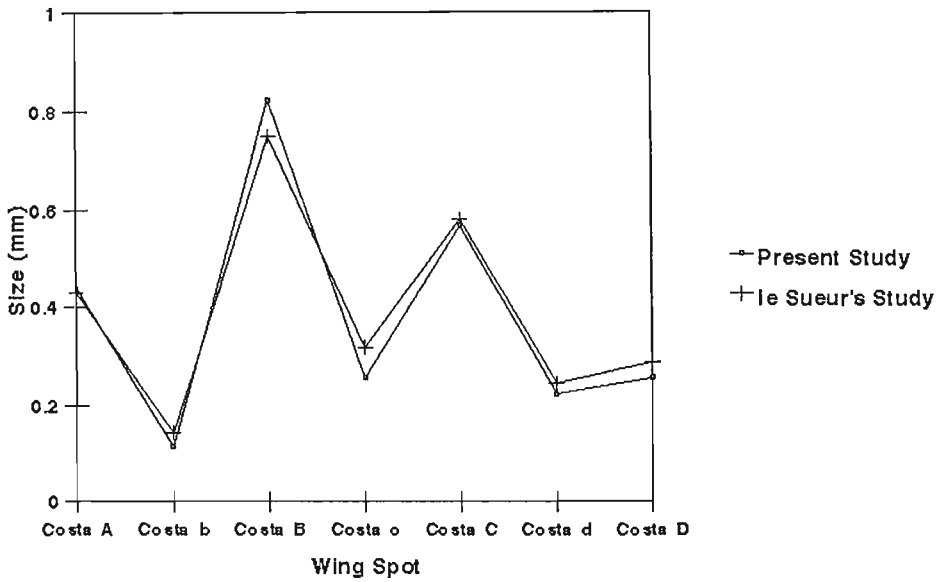


Figure 5.2. Comparison of wing spot size of *An. merus* for this study and that of le Sueur (1991), reared under winter temperatures.

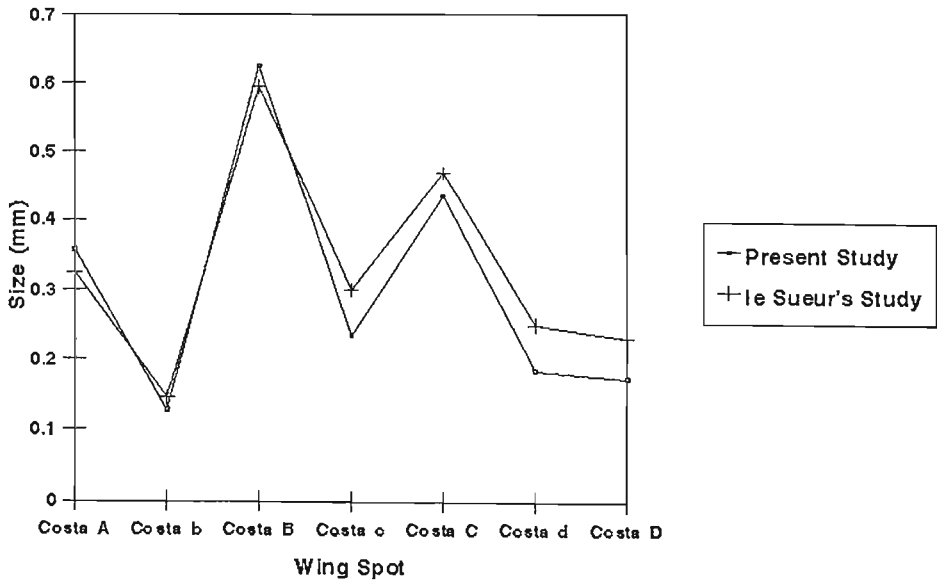
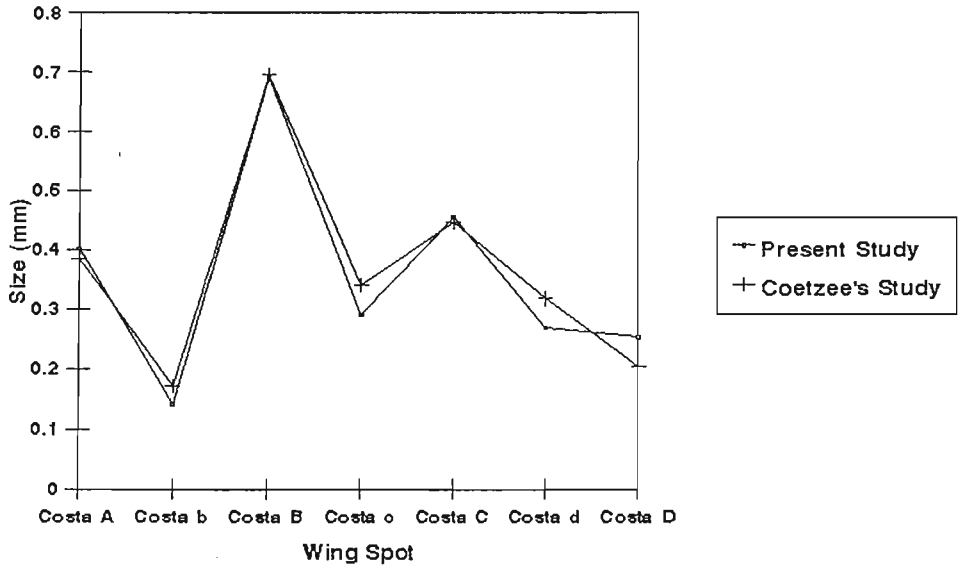
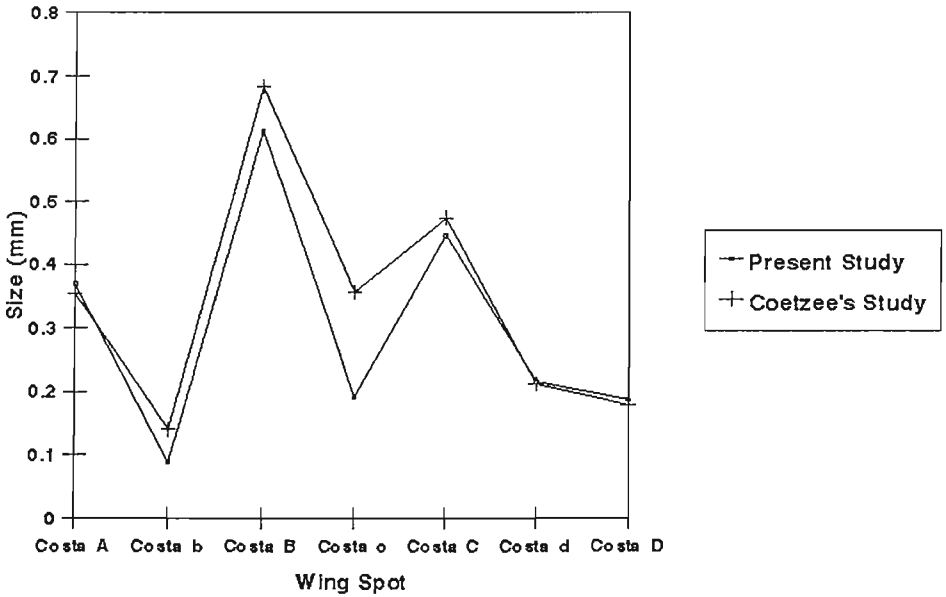


Figure 5.3. Comparison of wing spot size of *An. merus* for this study and that of le Sueur (1991), reared under summer temperatures.



ure 5.4. Comparison of wing spot size of An. arabiensis for this study and that of Coetzee (1986).



ıre 5.5. Comparison of wing spot size of An. gambiae for this study and that of Coetzee (1986).

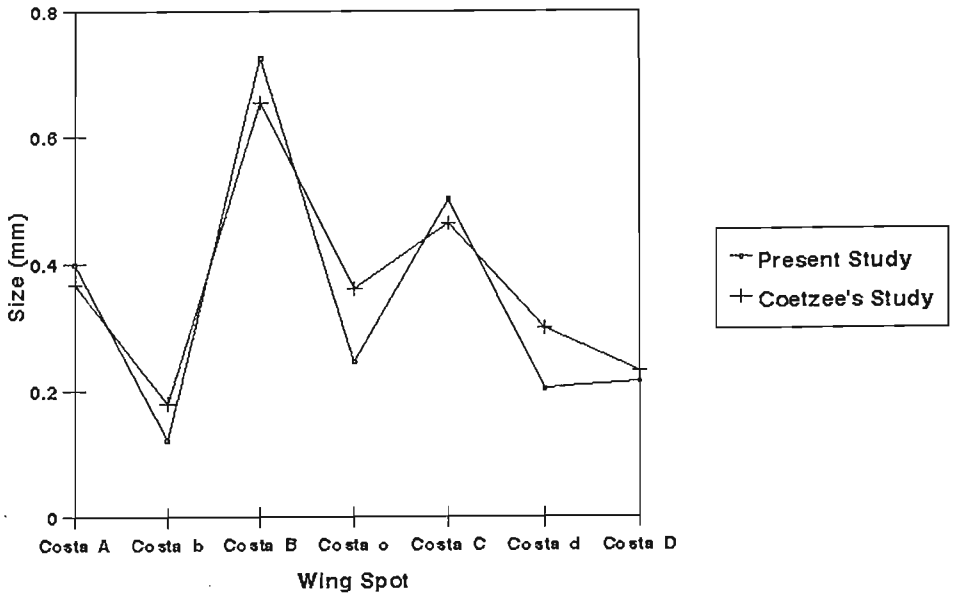


Figure 5.6. Comparison of wing spot size of An. merus for this study and that of Coetzee (1986).

on the wings of *An. merus* were larger in the present study than in Coetzee's (1986) study. The difference in results in these studies may be due to the fact that Coetzee (1986) collected material throughout the year and in different geographical localities throughout southern Africa, whereas the results of the present study were based on measurements obtained from laboratory reared material.

This study serves to reinforce the conclusions reached by Coetzee (1986) and le Sueur *et al.* (1992) regarding the use of wing spot measurements in taxonomic identification of members of the *An. gambiae* complex. Although the mean size of several wing spots showed significant differences when compared between species (Tables 5.3 and 5.4), these were not sufficiently large to be of any use for easy identification purposes. The degree of overlap in the distribution of the wing spot measurements for all three species was large for all wing spots (see Appendix 5.2).

Nutrient carry-over from the larval stage is important during the first few days of adult life (Day *et al.* 1980). Larger mosquitoes contain more energy reserves at emergence (Nayar & Pierce 1977) and thus have more flight energy to expend searching for a suitable host. Body size would influence the flight range of adults since larger adults would have more strongly developed flight muscles and adults emerging during winter would have greater protein accumulation. In a study conducted in the Kruger National Park, South Africa, Braack *et al.* (1994) found that the greater numbers of *An. arabiensis* were collected feeding away from the breeding site than at the site of larval development. These authors also found that *An. arabiensis* abundance did not show

a linear decline at increasing distances from the breeding site. *Anopheles arabiensis* is thus capable of hunting for blood meals at considerable distances from breeding sites.

According to Clements (1992), blood meals are converted to glycogen and triacylglycerol. The energy for flight comes from glycogen. Since larger females have a larger midgut and can take a correspondingly larger blood meal (Kitthawee *et al.* (1990), it is reasonable to expect these mosquitoes to produce more glycogen. Since Nayar and Sauerman (1973) found that there is a linear relationship between flight speed and metabolic rate as measured by glycogen consumption, larger females would have the potential for increased flight speed or duration. Therefore a large body size would allow mosquitoes to migrate further in search of hosts.

It has already been established that the body size of *An. arabiensis*, *An. gambiae* and *An. merus* showed regular seasonal variation - mosquitoes were largest during the cool winter and smallest during the hot summer months. Although the material used in this study was colony material that had been maintained in the insectary for various lengths of time, *An. gambiae* (25 years), *An. merus* (17 years) and *An. arabiensis* (1 month), significant differences were found between mean wing lengths obtained under summer and winter conditions. Temperature therefore has a direct effect on the rate of larval development. The size of emerging adult mosquitoes also depends on the amount and quality of larval food (Carpenter 1983). The length of time required for a mosquito to complete larval development is temperature-dependent with development being rapid

at higher temperatures (Nayar 1968a,b). The duration of the larval stages affects the size of the emerging adults (le Sueur 1991). During winter *An. merus* has a slower developmental rate than *An. arabiensis* but during summer *An. arabiensis* develops more slowly than *An. merus*. The duration of the pre-imaginal stages of *An. merus* was 38 days in winter and nine days in summer at temperatures of 17.5°C and 25°C respectively (le Sueur 1991). The duration of the immature stages of *An. arabiensis* was 32 days in winter, temperature of 17°C, and 11 days in summer, temperature 25°C (Chapter 3). Therefore, when reared at a fluctuating temperature with a mean of 17°C, *An. merus* would have a slower larval developmental rate than *An. arabiensis* which would account for *An. merus* having a larger body size when reared under simulated winter conditions. The difference between the summer and winter wing lengths for *An. merus* was 20%. In summer the developmental time of *An. arabiensis* was slower than that of *An. merus* by two days and the adult *An. arabiensis* subsequently produced were larger. The larger size of the *An. merus* produced in winter by le Sueur (1991) was due to the fact that the winter temperatures were lower than those used in the present study.

Of the three species used in these experiments, *An gambiae* has the smallest body size (as determined by wing length). Although *An. gambiae* has been maintained under insectary conditions for 25 years, the small body size obtained, relative to the other two species, was not unexpected. An examination of the distribution of *An. gambiae* showed that this species occurs in the warmer areas of Africa and not in the cooler regions. Therefore one would expect *An. gambiae* to have a relatively small body size. Although this implies that *An. gambiae* should have a poor vectorial capacity due to its

small size, we know that this is not true. Iit is well known that in the rest of Africa *An. gambiae* is a very efficient vector of malaria. This may be due to the fact that *An. gambiae* is more susceptible to infection than its sibling species.

It has long been known that *P. falciparum* can only be transmitted by mosquitoes of the genus *Anopheles*. However not all species of *Anopheles* are equally susceptible to infection. In theory, vector susceptibility can range from total susceptibility, where all individuals support sporogonic development, to total refractoriness where no individuals support development. In reality, vector susceptibility is usually somewhere inbetween. Thus susceptibility is a relative attribute ascribed to a species based upon comparisons with other species (Vaughan *et al.* 1994). From unpublished laboratory studies by Dr Charles Pumpuni (1993, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, USA) and myself, it was determined that *An. gambiae* was more susceptible to infection by *in vitro* cultured *P. falciparum* than was *An. arabiensis*. Also, Beier *et al.* (1992) found that *An. gambiae* transmitted, on average, more than twice as many sporozoites as *An. freeborni* thereby showing that it is not realistic to generalise that all vectors have an equal potential for sporozoite transmission.

Since *An. gambiae* is anthrophilic and endophilic, it is very vulnerable to control measures utilizing residual insecticides. It is this particular trait that has lead to the elimination of *An. gambiae* in South Africa by the use of intradomiciliary spraying of DDT. It is more difficult to control *An. arabiensis* effectively since Sharp *et al.* (1993) found that this species of mosquito rests outdoors after feeding.

Changes in climatic conditions across seasons also result in changes in the morphology of the adult mosquito since there is temperature induced variation in the wing length and banding pattern of the wing scales. Once again, this study serves to reinforce the observations made by le Sueur & Sharp (1991) that it is not feasible to use morphometric measurements in species identification without taking into account seasonality, geographic location and climatic conditions at the locality where the specimens were collected.

The results of this study have shown that *An. merus* is the larger species in winter and *An. arabiensis* is the larger species in summer. However according to the study by Sharp (1987), *An. merus* feeds predominantly on cattle. This suggests that *An. merus*, although capable of transmitting malaria, is not a major vector of malaria in South Africa. Furthermore, if one looks at the distribution of *An. merus* and *An. arabiensis* in South Africa, it becomes apparent that *An. arabiensis* is more widely distributed in the endemic malarious areas and *An. merus* is restricted to the coastal plains and salt pans (Cross & Theron 1983). *Anopheles arabiensis*, due to its adaptability to temperature variations, its size and distribution is the main vector of *Plasmodium* during both winter and summer. It is therefore apparent that size is not the only criterion that determines the vector status of mosquito species, as size merely determines the ability of the mosquito to transmit malaria once it has been infected.

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APPENDIX 5.1

The simple statistics used in the analysis of the data for chapter 5.

Season: SUMMER **Species:** *An. arabiensis*

Wing Spot	n	Mean	S.D.	Minimum	Maximum
Costa A	176	0.3837	0.0594	0.19	0.54
Costa b	176	0.1434	0.0316	0.06	0.25
Costa B	176	0.6616	0.0971	0.33	0.96
Costa c	176	0.2803	0.0714	0.36	0.50
Costa C	176	0.4378	0.0934	0.25	0.67
Costa d	176	0.2828	0.0622	0.25	0.44
Costa D	176	0.2213	0.0519	0.16	0.37
Length	176	2.99	0.3429	2.37	3.60

Season: SUMMER **Species:** *An. gambiae*

Wing Spot	n	Mean	S.E.	Minimum	Maximum
Costa A	85	0.3672	0.0478	0.28	0.50
Costa b	85	0.0872	0.0402	0.02	0.21
Costa B	85	0.5934	0.0635	0.44	0.73
Costa c	85	0.1882	0.0484	0.12	0.34
Costa C	85	0.4097	0.0601	0.21	0.57
Costa d	85	0.2267	0.0391	0.15	0.31
Costa D	85	0.1645	0.0259	0.12	0.25
Length	85	2.5852	0.1245	2.28	2.83

Season: SUMMER **Species:** *An. merus*

Wing Spot	n	Mean	S.E.	Minimum	Maximum
Costa A	57	0.3577	0.0398	0.25	0.47
Costa b	57	0.1280	0.0274	0.05	0.18
Costa B	57	0.6249	0.0545	0.50	0.79
Costa c	57	0.2349	0.0432	0.15	0.34
Costa C	57	0.4350	0.0796	0.21	0.60
Costa d	57	0.1836	0.0355	0.12	0.25
Costa D	57	0.1728	0.0234	0.12	0.25
Length	57	2.6863	0.1742	2.34	3.37

Season: WINTER **Species:** *An. arabiensis*

Wing Spot	n	Mean	S.D.	Minimum	Maximum
Costa A	110	0.4100	0.0822	0.26	0.63
Costa b	110	0.1357	0.0334	0.07	0.21
Costa B	110	0.7061	0.1205	0.45	0.96
Costa c	110	0.2945	0.0943	0.15	0.47
Costa C	110	0.4487	0.1065	0.28	0.73
Costa d	110	0.2473	0.0807	0.18	0.47
Costa D	110	0.2763	0.1559	0.19	1.76
Length	110	3.1269	0.4704	2.45	3.67

Season: WINTER **Species:** *An. gambiae*

Wing Spot	n	Mean	S.E.	Minimum	Maximum
Costa A	82	0.3697	0.0410	0.31	0.47
Costa b	82	0.0898	0.0315	0.02	0.15
Costa B	82	0.6334	0.0611	0.50	0.79
Costa c	82	0.1951	0.0530	0.08	0.31
Costa C	82	0.4809	0.0508	0.37	0.57
Costa d	82	0.2028	0.0337	0.12	0.28
Costa D	82	0.2092	0.0339	0.15	0.34
Length	82	2.7393	0.1202	2.57	3.05

Season: WINTER **Species:** *An. merus*

Wing Spot	n	Mean	S.E.	Minimum	Maximum
Costa A	65	0.4410	0.0321	0.37	0.54
Costa b	65	0.1146	0.0271	0.05	0.18
Costa B	65	0.8240	0.0352	0.76	0.89
Costa c	65	0.2569	0.0584	0.15	0.37
Costa C	65	0.5680	0.0820	0.44	0.70
Costa d	65	0.2230	0.0427	0.15	0.31
Costa D	65	0.2566	0.0395	0.18	0.34
Length	65	3.2595	0.1983	2.99	3.63

APPENDIX 5.2

The distribution of the measurements obtained for the wing spots of *An. arabiensis, An. merus* and *An. gambiae.*

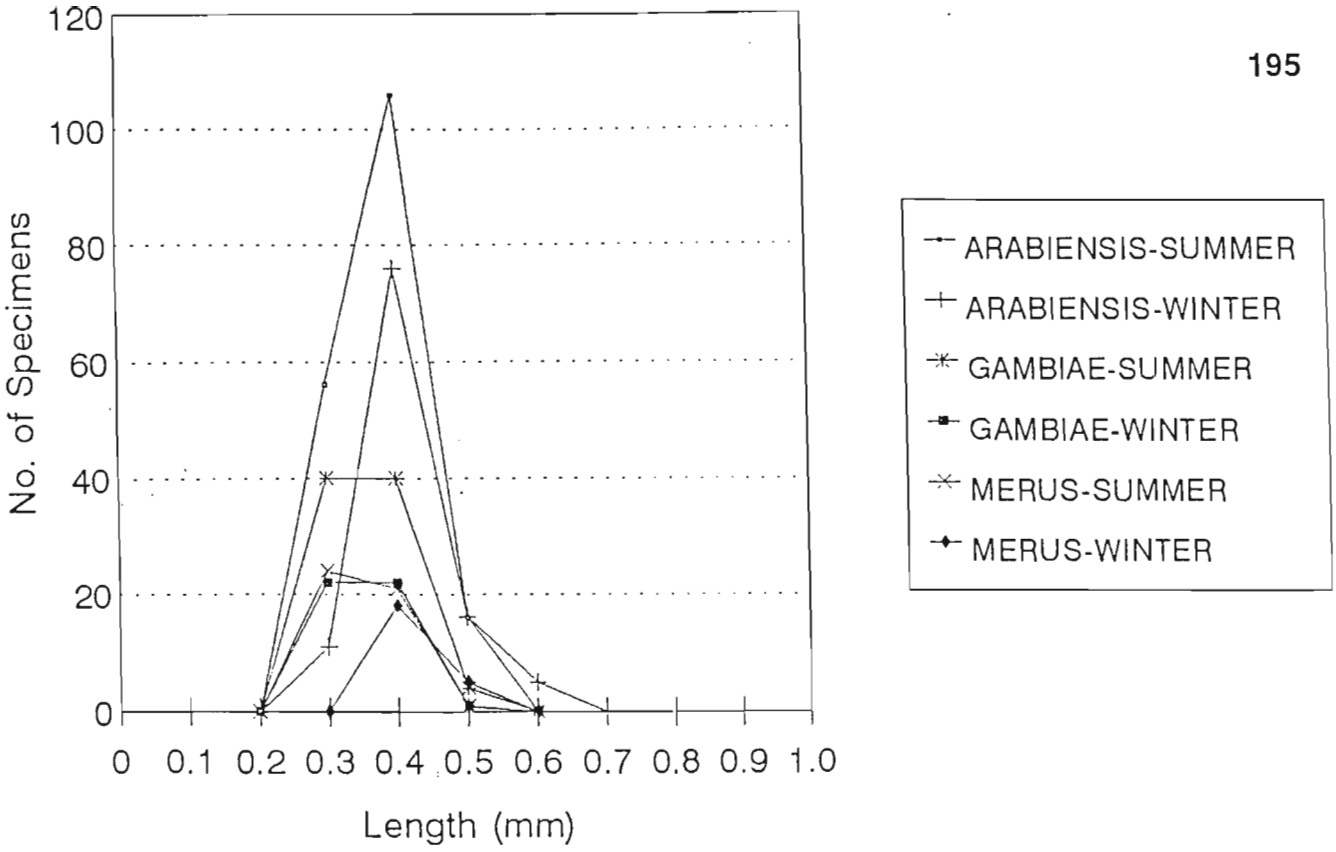


Figure 1. Distribution of the size of costa A for *An. arabiensis, An. gambiae* and *An. merus*.

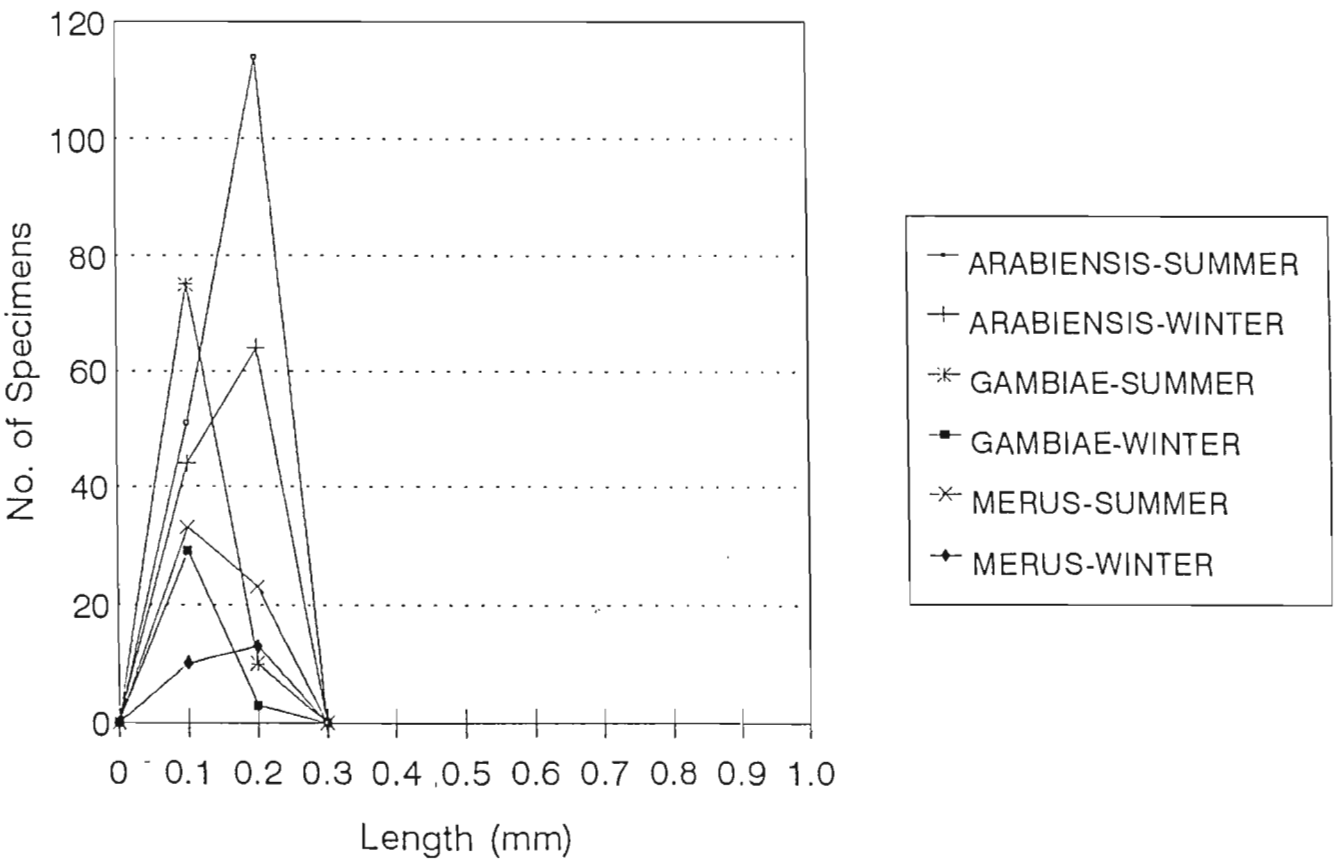


Figure 2. Distribution of the size of costa b for *An.*

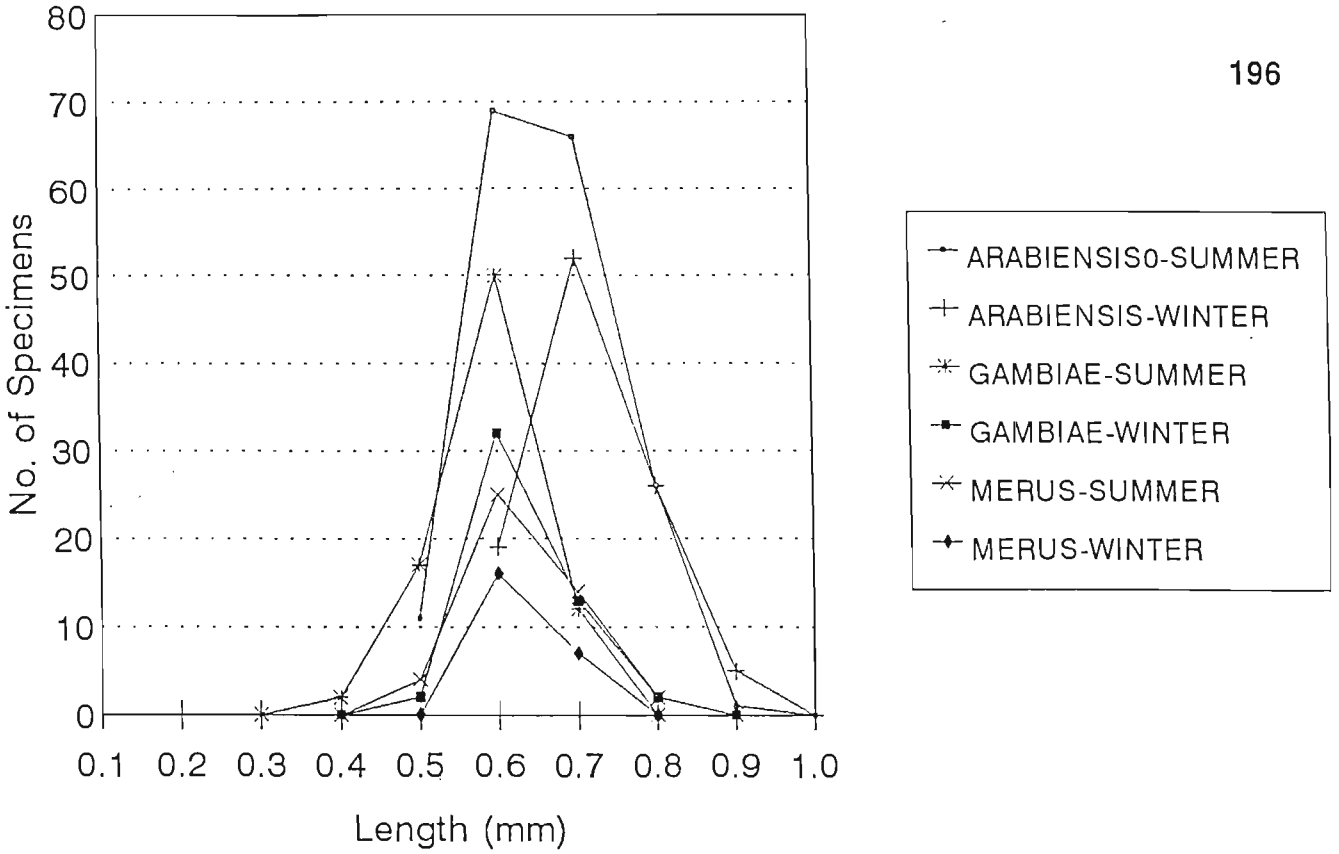


Figure 3. Distribution of the size of costa B for *An. arabiensis*, *An. gambiae* and *An. merus*.

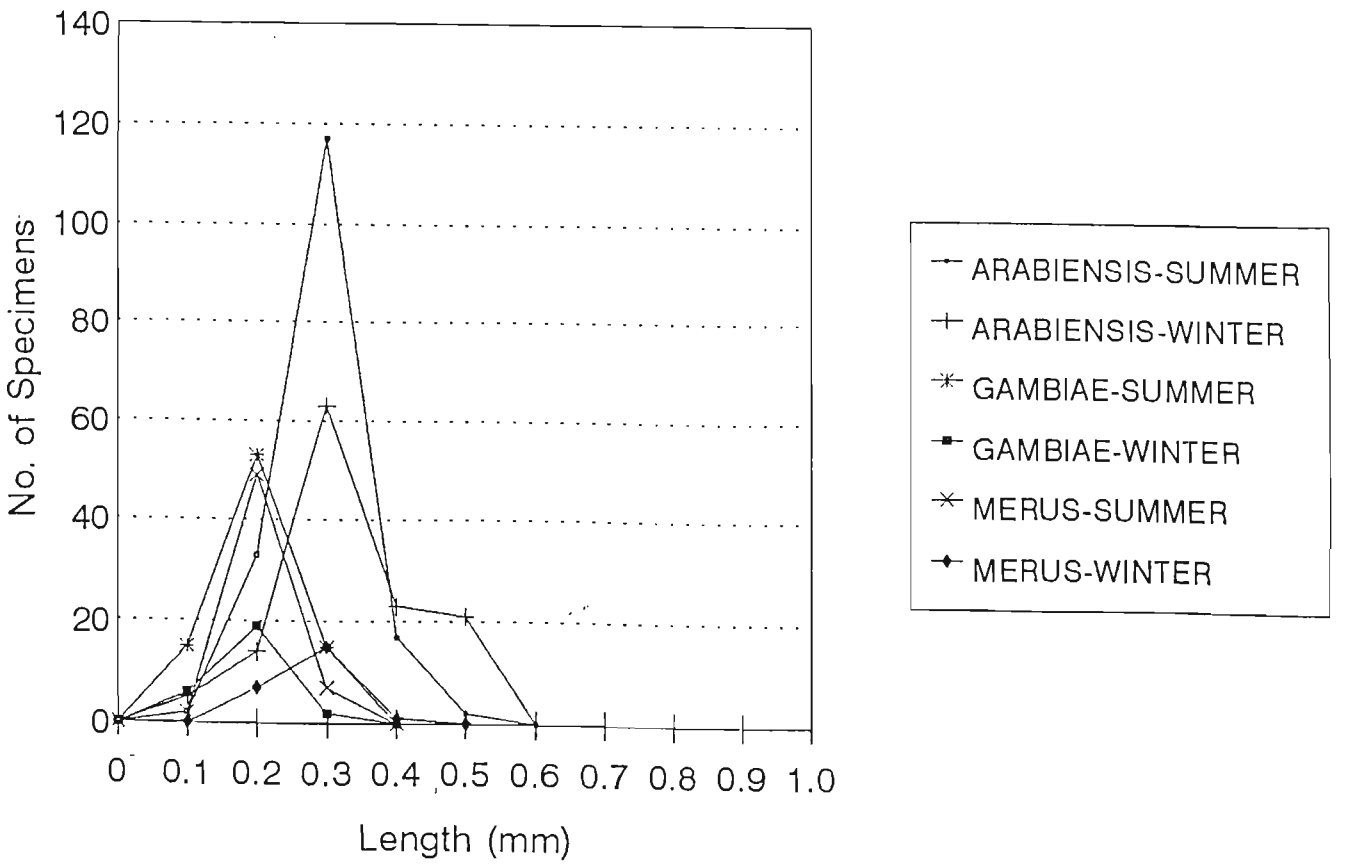


Figure 4. Distribution of the size of

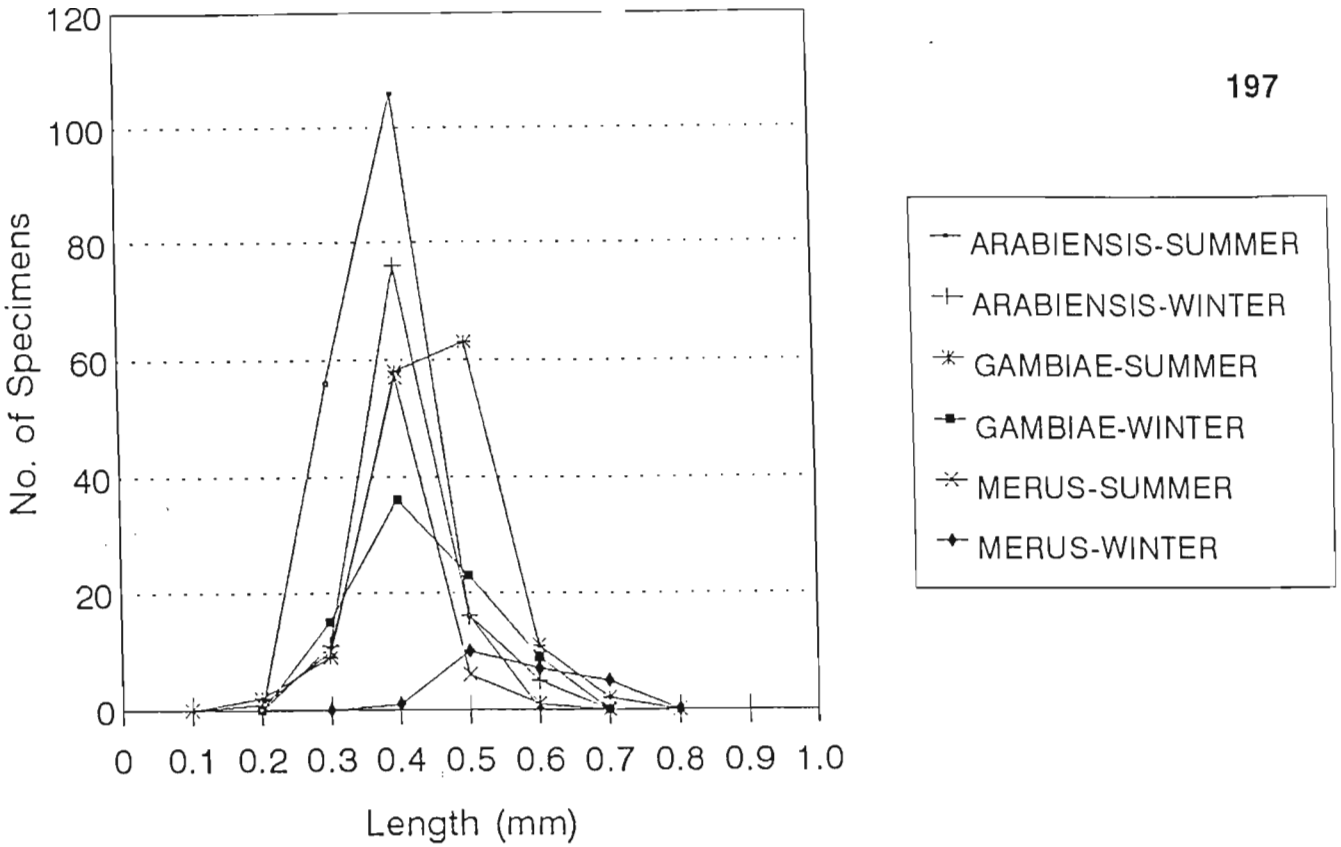


Figure 5. Distribution of the size of costa C for *An. arabiensis*, *An. gambiae* and *An. merus*.

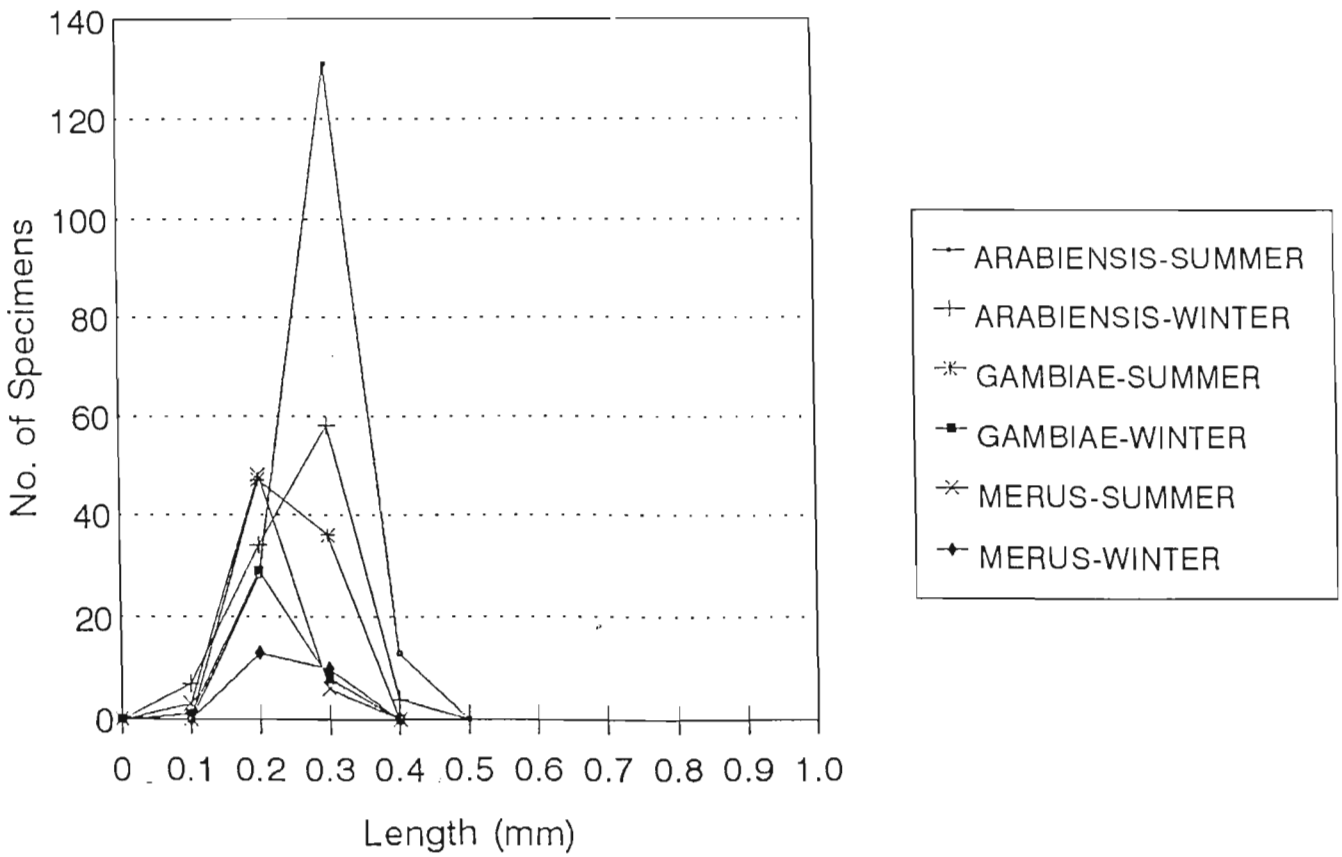


Figure 6 Distribution of the size of [illegible]

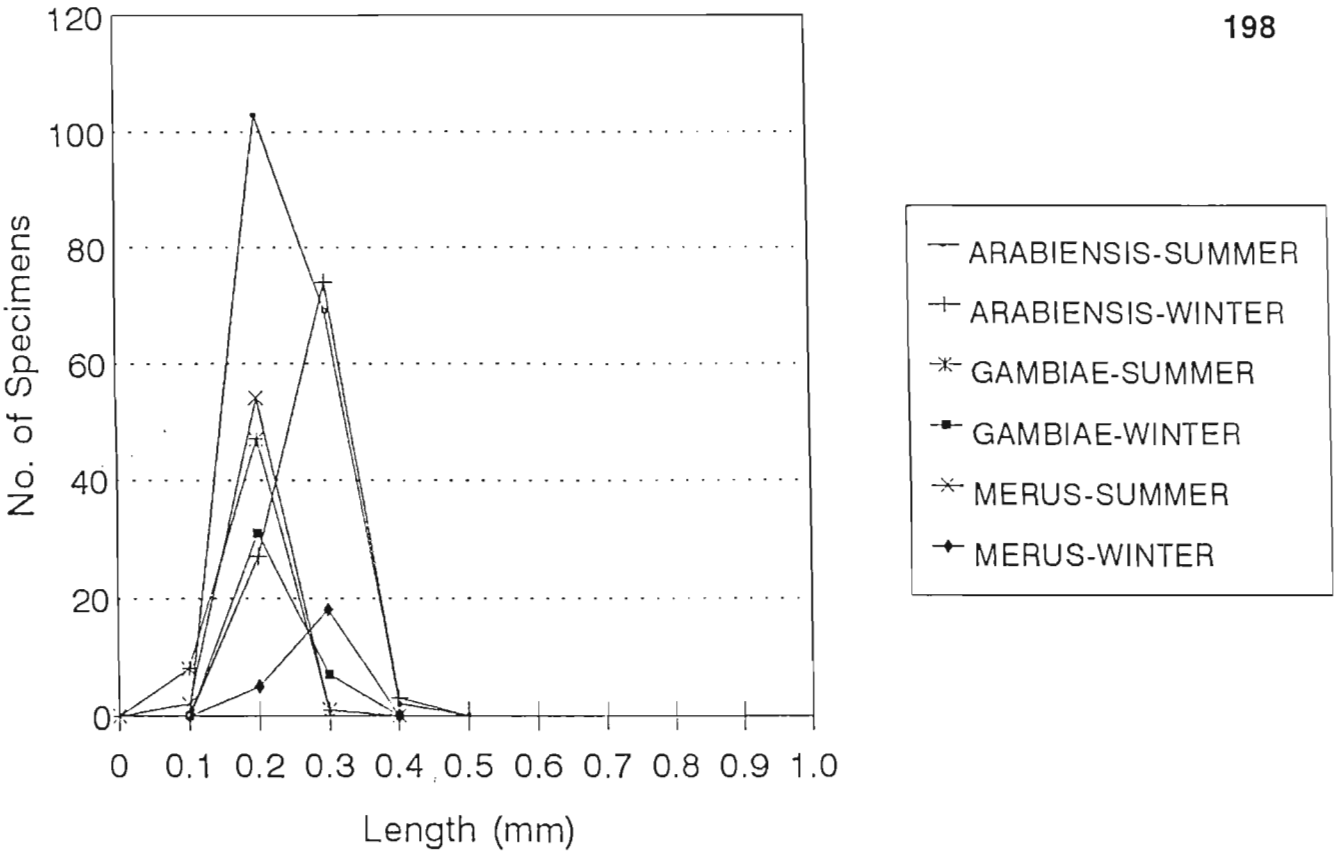


Figure 7. Distribution of the size of costa D for *An. arabiensis*, *An. gambiae* and *An. merus*.

CHAPTER 6

IMPLICATIONS FOR TRANSMISSION AND CONTROL

6.1. THE EFFECTS OF SEASONALITY ON THE MORPHOLOGY AND BIONOMICS OF *ANOPHELES ARABIENSIS*

This study has demonstrated that during its life cycle, *An. arabiensis* is affected by the seasonality of the environment, in terms of temperature and humidity. Temperature influences aspects of the mosquitoes physiology such as the longevity of the adults, the duration of the gonotrophic cycle, egg production and the generation time. Temperature also influences the larval developmental time. It was established that the lower the temperature, the slower the developmental rate of the larvae and hence the larger the resulting adults. Consequently, the larger the adults the more robust they are since they have the capacity for greater protein accumulation and glycogen production, thereby increasing their longevity and flight range. However, low temperatures also result in longer gonotrophic cycles, lower egg production (gonotrophic disassociation in the laboratory and limited oviposition in the field), longer generation times and also increases the duration of the sporogonic development of the malaria parasite.

Together these factors influence the density of mosquito populations and so influence their potential for malaria transmission. The longevity of female mosquitoes determines their capacity to become infected and hence their potential to become infectious. Long-lived females have a greater potential to transmit malaria since they can take more blood meals after they become infectious. However, a major factor influencing the

transmission of malaria is the susceptibility of the *Anopheles* mosquito to infection by *P. falciparum*. Although *An. arabiensis*, was found to be susceptible to infection in the laboratory, field tests need to be conducted to determine the proportion of the wild population that is infected with the malaria parasite, in order to determine its true susceptibility under field conditions.

An inverse relationship was found between temperature and body size for *An. arabiensis, An. merus* and *An. gambiae*. *Anopheles* mosquitoes reared under conditions of low temperature resulted in large bodied adults. In *An. arabiensis*, body size was found to influence the longevity of the adult mosquito. Due to the influence of temperature on the morphology of adult mosquitoes, the use of absolute size criteria for the purpose of species separation is not recommended unless specimens used are collected throughout the year. Where morphological measurements are used in taxonomic separation, specimens should be collected from as many localities as possible to negate differences arising from a narrow range of temperatures in individual habitats. However, if possible, alternative methods of identification should be used for *Anopheles* species since there is some degree of overlap in morphological measurements between species.

6.2. VECTOR POTENTIAL AND MALARIA TRANSMISSION

In Chapter 3 it was concluded that temperature influences wing length and that wing length (as a measure of body size) in turn influences the longevity of adult mosquitoes. The relationship between wing length and longevity is described by the linear function

Y = -111 + 43.77X which has a correlation coefficient of 0.7. From this equation, the longevity of mosquitoes in the field can be ascertained by using the wing length data from field collected specimens.

The number of mosquitoes caught biting man, their wing length and longevity were used to calculate parameters of malaria transmission such as vector potential, the number of infectious mosquitoes biting an individual, and the number of infective bites that an individual might receive during an entire season.

The vector potential (v) was calculated from the formula

$$v = l_x p_x$$

where l_x is the mean longevity of female mosquitoes and p is the mean number of mosquitoes caught feeding on an individual per night per season.

Assuming that all mosquitoes have an infectious blood meal on the second night after emergence, the estimated number of infected mosquitoes feeding on an individual (i), per day for each season was calculated as follows:

$$i = \frac{(v - 2p_x) - p_x s}{l_x}$$

where v, l_x and p are as above and s is the average duration of sporogonic

development.

Taking into account the time available for mosquitoes to transmit malaria, the mean number of infective bites (*b*) that an individual can receive each season was calculated using the following equation:

$$b = {}^{i}/_{g}\, l_x$$

where *i* is as above and *g* is the average duration of the gonotrophic cycle.

The results of these calculations are presented in Table 6.1.

Table 6.1. Parameters of malaria transmission.

	l_x	p_x	s^*	g	v	i	b
winter	41	11			451		
spring	37	13	12	4	481	8	74
summer	27	22	10	3	594	12	108
autumn	31	20	23	5	620	4	25

*Source of data: Gear *et al.* (1988)

These calculations provide information on the transmission potential of the mosquito vector based on information obtained from both the field and laboratory. The data presented in this table take into account the feeding potential of mosquitoes on a single individual. *i* represents the total number number of infectious mosquitoes that have the

potential to feed on an individual each day for an entire season. *b* represents the total number of infectious bites that a person can receive for the duration of a season.

Vector potential is influenced by longevity and population numbers per season. A greater longevity and a small population size result in a low vector potential. The number of infected mosquitoes biting an individual per season is largely determined by the duration of the sporogonic cycle. A low vector potential coupled with a long sporogonic cycle results in few infected mosquitoes biting an individual each day during the entire season. The maximum number of infectious bites that an individual is likely to receive during each season is determined by the length of the gonotrophic cycle of the infectious mosquitoes. A low number of infectious mosquitoes, each with a long gonotrophic cycle, results in a low number of infectious bites per individual.

Although the calculated vector potential reflects the expected number of mosquitoes that can feed on an individual over an entire season, this information can be extrapolated to determine the number of mosquitoes that are feeding on a community of a specific size. This can be achieved by multiplying the vector potential by the host population. Vector potential can therefore be used to estimate malaria transmission since the greater the vector potential, the greater is the potential for transmission.

In winter the metabolic requirements of the female mosquito are very low, since mosquitoes are ectothermic. Also the development of the parasite within the mosquitoes is severely retarded during conditions of low temperature. As indicated by

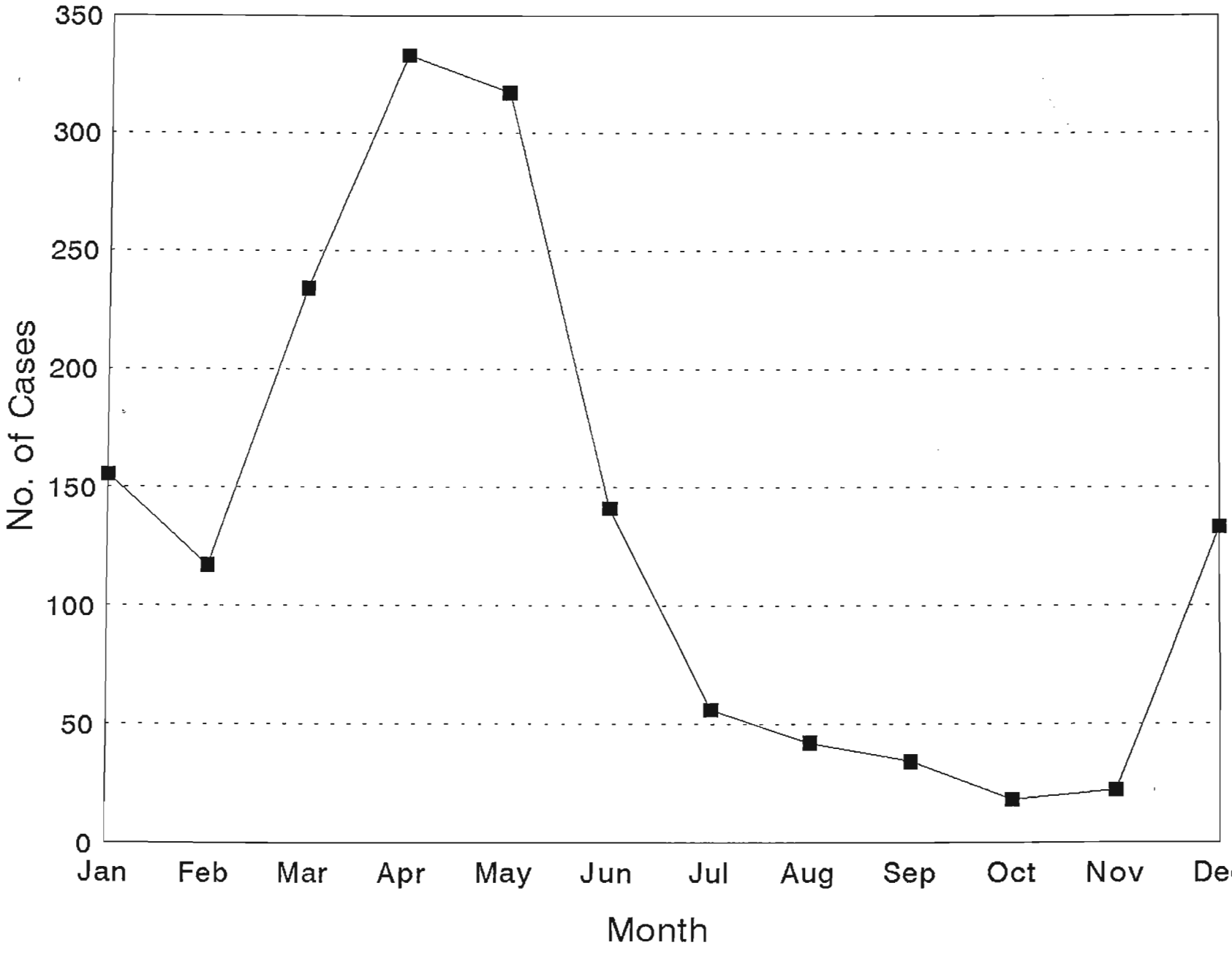
the mean number of mosquitoes caught feeding on man, the population density of mosquitoes, during winter, is very low. Judging by the low level of transmission during winter (Figure 6.1), there are not many infectious mosquitoes feeding on man.

The vector potential during spring is only slightly greater than that in winter (Table 6.1). This may be due to the low population numbers caught feeding on man during the collection period. The rise in temperatures during spring also increases the sporogonic development of the malaria parasite within mosquitoes and these mosquitoes become capable of giving a high number of infectious bites per person during the entire season.

Although the longevity of mosquitoes is shortest during summer, the mosquito population numbers are highest and therefore the vector potential is also high. The duration of sporogonic development and the gonotrophic cycle are therefore short resulting in a high number of infectious mosquitoes giving a high number of infectious bites to an individual over an entire season (Table 6.1).

The vector potential is greatest in autumn since both the population numbers and the longevity are high at this time (Table 6.1). However, the duration of sporogonic development and the gonotrophic cycle are longer resulting in a small number of infectious mosquitoes and a small number of infectious bites per person.

These conclusions are limited by the assumption that all mosquitoes have an infected blood meal on the second night after emergence. Discrepancies in the vector potential



gure 6.1. The mean monthly malaria notifications for KwaZulu-Natal (1980-1994).

may arise as a result of discrepancies in the field collection of specimens. Environmental factors such as wind and rain affected the collection of mosquitoes in the field. The greatest weakness in the above calculations is the lack of information on the duration of sporogonic development. It is therefore necessary to determine the proportion of infectious or infected mosquitoes in wild mosquito populations.

Figure 6.2. shows the absolute number of mosquitoes biting an individual each month, as well as the vector potential associated with these months. This figure shows clearly the association between high population numbers and vector potential. The index of transmission for these months was calculated as the product of the vector potential and the absolute number of mosquitoes biting an individual. From Figure 6.3. it can be seen that the trend for the transmission index closely follows that of the number of notified malaria cases. The greater the transmission index, the greater the number of malaria cases. Therefore vector potential is a reliable indicator for determining the rate of malaria transmission.

6.3. THE LATE SEASON PEAK IN MALARIA TRANSMISSION

The peak in malaria transmission that occurs in autumn (March to May) in KwaZulu-Natal (Figure 6.1) may be due to environmental factors such as temperature. From Table 6.1 it can be seen that the highest vector potential occurs during autumn. This indicates that the mosquito population is sufficiently large and the longevity of mosquitoes during this period would be conducive to malaria transmission.

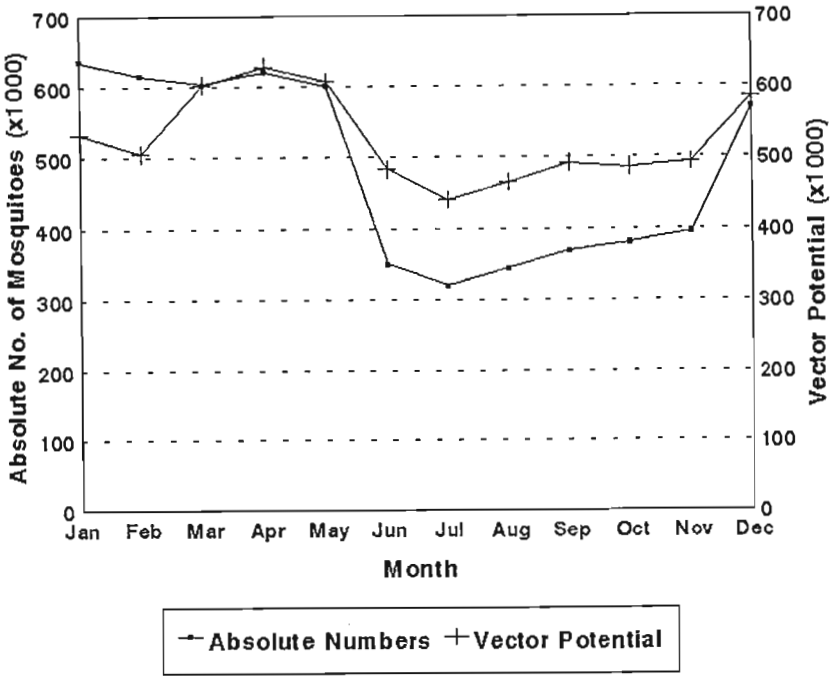


Figure 6.2. The monthly mosquito population and vector potential.

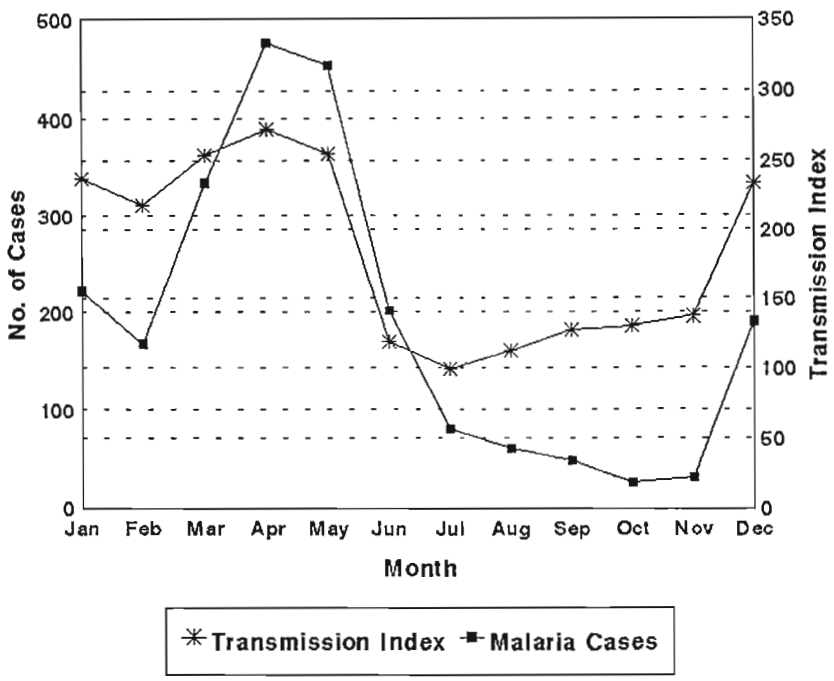


Figure 6.3. The mean monthly malaria notifications and the transmission index.

The transmission of malaria is dependent on the sporogonic development of *Plasmodium* within female mosquitoes and the survival of the mosquito until it becomes infectious. Plasmodial sporogony involves a complex continuum of events, and disruptions at any point in the sequence may affect the sporozoite transmission potential (Vaughan *et al.* 1992). Therefore, unfavourable climatic conditions such as very high or very low temperatures could inhibit sporogony. Vanderberg and Yoeli (1966) found that *P. falciparum* could only complete its development in the mosquito within a temperature range of 18°C to 30°C. In summer the daytime temperature in KwaZulu-Natal fluctuates between 22°C and 38°C and the night temperature between 18°C and 22°C. Although these conditions are ideal for mosquito propagation, such high temperatures may adversely affect plasmodial sporogony. Large numbers of mosquitoes may imbibe infected blood but sporogony may not be completed in all of them. During autumn temperatures are lower (day 20°C - 30°C, night 15°C - 20°C) and may not inhibit sporogony as much as summer temperatures since Vanderberg and Yoeli (1966) found that malaria parasites were more readily damaged by unfavourably high temperatures than unfavourably low ones.

Under ideal conditions (27°C and 70% RH) *P. falciparum* completes sporogony in 12 - 14 days (Boudin *et al.* 1991, Vaughan *et al.* 1992). Thus to transmit malaria, mosquitoes need to be at least 12 - 14 days old, assuming a blood meal is taken on the first night after emerging. Thus mosquito longevity would also influence malaria transmission. The longevity of autumn reared mosquitoes is greater than that of summer reared ones and therefore has greater potential to become infected and hence

infectious. This is reinforced by the greater vector potential during autumn.

The peak in the number of cases in autumn may therefore be entirely due to environmental factors that affect mosquito physiology but further investigations have to be carried out to ascertain the effects of temperature on the sporogonic development of *P. falciparum* within *An. arabiensis* in the field.

6.4. MALARIA CONTROL

The malaria control programme in South Africa has been successful in decreasing the size of the malaria areas and malaria occurs only in a limited area of the country. Although the control programme which makes use of intradomiciliary spraying of DDT and larviciding using temephos has been very successful, further success in controlling malaria will only be possible through the refinement of the procedures currently in use.

The introduction of winter larviciding has helped to reduce the population density of adult mosquitoes in spring. However, the value of this winter larviciding can be increased by:

i) Locating and mapping all permanent water bodies by geographical survey as well as surveying malaria cases (le Sueur *et al* . 1995). Child cases are more indicative of focal areas of transmission than adult cases, since children are confined spatially to a particular locality. Mapping the permanent water bodies would facilitate easier larviciding of these habitats since the spraying teams will know precisely where these water bodies are situated and will not have to spend time locating them during

their spraying programme. This would decrease the spraying time and increase the coverage of larval control.

ii) Treating permanent water bodies with larvicide at least every four weeks, since larval development is slow during winter. Treatment of water bodies associated with high human densities should be carried out regularly, especially during winter when the number of breeding sites is restricted.

iii) Involving the local community with larval control measures such as environmental control through proper agricultural practices as well as the destruction of suitable breeding sites. This would also increase the cost effectiveness of larval control and it would draw malaria control closer towards the primary health care approach.

Since *An. arabiensis* was found to feed indoors and rest outdoors, the value of intradomiciliary spraying with DDT or pyrethroids is greatly reduced. Alternative methods of controlling the adult stages is required. Therefore, a concerted effort should be made to determine the outdoor resting sites of *An. arabiensis.* If the outdoor resting sites can be determined, the spraying of these sites in winter with a synthetic pyrethroid would reduce the number of over-wintering females, further decreasing winter oviposition and it may also serve to destroy infectious females that may serve as reservoirs of the malaria parasite. Winter larviciding coupled with the spraying of potential resting sites in winter would serve to decrease both the vector population and the reservoir of infection. This would contribute to reducing malaria transmission during the rainy season.

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