THE ECOLOGY, OVER-WINTERING AND POPULATION DYNAMICS OF THE PRE-IMAGINAL STAGES OF THE *ANOPHELES GAMBIAE* GILES COMPLEX (DIPTERA: CULICIDAE) IN NORTHERN NATAL, SOUTH AFRICA

by

David le Sueur

Submitted in partial fulfilment of the
requirements for the degree of
Doctor of Philosophy
in the Department of Zoology and Entomology
University of Natal
1991

Pietermaritzburg
1991

PREFACE

The experimental work described in this thesis was carried out in the Department of Zoology and Entomology, University of Natal, Pietermaritzburg and the Research Institute for Diseases in a Tropical Environment of the Medical Research Council, Durban. The work was carried out over the period 1984 to 1990, under the supervision of Prof. C.C. Appleton

These studies represent original work by the author and have not been submitted in any form to another University. Where use was made of the work of others it has been duly acknowledged in the text.

The decimal notation used in this study is a comma, which conforms to that of the Republic of South Africa. Deviations from uniformity in layout may occur as a result of submission of certain portions for publication to different journals. These have however been kept to a minimum. Terminology relating to race in Chapter 1 is as reported in the original documents.

ACKNOWLEDGEMENTS

I would like to thank the following people;

My supervisor Prof. Chris Appleton for his patience, criticism of the manuscript and support. Dr. Brian Sharp for initiating my training in this area and constant encouragement, motivation and discussion. Mr. Colin Sapsford for insight and to whom I owe my interest and understanding of animal physiology. Mr. Sipho Ngxongo of the Department of Health KwaZulu and present malaria co-ordinator for Natal Province, for his enthusiasm, support and implementation of findings. Dr. R.H. Hunt for training me in chromosome cytology. Mrs. Colleen Fraser for help with the compilation of the final document. Mr's. David Mthembu and Joseph Shozi for assistance in the field and in the insectary. All the staff of the Medical Research Council, Durban for logistical support. The staff of the Killie Campbell Africana Library and the National Archives.

To my parents who made it all possible.

The Medical Research Council for funding this research.

ABSTRACT

This study investigated aspects of the breeding biology, eco-physiology, morphology, over-wintering and population dynamics of the pre-imaginal stages of members of the *Anopheles gambiae* complex in northern Natal, South Africa,

Investigation of the breeding biology, concentrated on breeding site utilisation by the different members of the *Anopheles gambiae* complex. Surveillance personnel were unable to locate the breeding sites of *Anopheles arabiensis* and postulated that location and description of these would offer a unique opportunity for control. The difficulty in locating the breeding sites of *An. arabiensis* at certain localities was found to be a product of their low density, presumably as a result of the intra-domiciliary, residual insecticide spray programme.

The effect of temperature on larval physiology and adult morphology was investigated. The findings are discussed in terms of their implications for anopheline taxonomy and the *Anopheles gambiae* complex.

The effect of temperature on larval growth rates was investigated in both the field and laboratory. The finding of this part of the study indicate that the larval stages play an important role in the over-wintering of populations within the region. The control implications of these findings and winter breeding site localisation are discussed. A theory for the so called 'late season transmission i.e. Apri-May, within

the province and southern Africa is proposed.

The population dynamics of *An. merus* were investigated, together with the effect of abiotic factors such as temperature, salinity and rainfall. The effect of sampling bias due to factors such as behavioural avoidance were studied.

CONTENTS

PAGE

CHAPTER 1

A HISTORICAL PERSPECTIVE OF MALARIA IN NATAL WITH PARTICULAR EMPHASIS ON THE PERIOD BETWEEN 1928 AND 1932.

The first publication concerning malaria in Natal Province was that of Hill & Haydon, which dealt with the outbreak of malaria in Durban in 1905. They incriminated *Pyretophalis costalis (Anopheles gambiae)* as the vector with a sporozoite rate of 16,2%. In the 1906 Annual Report for the City of Durban the following important observations were made.

> "The malaria carrying mosquito appears not to be a domestic one. It does not seem to be even seen in rooms unless it has overgorged itself during the night. It seems strictly attracted to business by entering houses, biting one of the inmates, and once more returning to the shelter of the bush. It is characteristically a night flying insect."

Thereafter there is little information available until the epidemics of the early 1930's. I have chosen to cover this period as this is when and where the modern day control strategies of indoor residual spraying had their origin. This early work played an extremely important role both in the province and subsequently in other areas of the world in terms of disease reduction, economics and decrease in human suffering and mortality.

The major source of information for this period has been the files of the Department of Health of the Union of South Africa. As a result much information has been extracted from some 432 memos, letters, telegrams and reports, as well as numerous newspaper cuttings. This results in the information

being somewhat "punctuated" but I have attempted to draw them together to give an overall picture of the malaria situation, its economic impact, the control strategies used and their results. Where pertinent I have reproduced relevant tables and figures. Only where I felt it necessary have I given the actual report (memo, telex, letter etc.) title, date, author and file number. As a result the reference list essentially covers the main sources utilised.

In 1910 Dr. Park Ross stationed at Nqutu (Figure 1.) as the district surgeon was requested by the Department of Native Affairs to undertake an investigation of what the Zulus called Isigwebedhla-Umkhulane, which was then ravaging northern Zululand (Ferguson, ?1982). This he proved was principally a result of malaria infection. In his report, he concluded that where there were no human carriers, there would be no infected *Anopheles*. He thus strongly advocated the extensive use of quinine in addition to other methods such as screening of dwellings and the use of bed nets and protective clothing.

In 1921 Park Ross carried out the first malaria survey of the Union, which resulted in the division of the country into five scheduled areas of malaria risk. These were geographically recorded on coloured contour maps:

Areas in which malaria is	continuous	-	Yellow areas
	serious	-	Red areas
	moderate	-	Blue areas
	occasional	-	Green areas
	absent	-	White areas

In 1922 in an address to the Medical Association of South

Africa on "The control of malaria", Park Ross stressed the economic chaos caused by the disease. He once again stressed the importance of human carriers and outlined the control strategies suggested in his 1910 report to the Department of Native Affairs.

On the 27 June 1928 Dr. Park Ross (Assistant Health Officer, Union of South Africa) wrote to the Secretary for Health, Sir Edward Thornton requesting that the area between the Mkhuze and Unyala rivers be designated a yellow zone (Figure 1). This was an area in which malaria was considered to be present all year round (i.e. government employees were receiving the maximum "malaria allowance"), and represented an extension of the area classified as such in 1921. This was the first indication of the gradually increasing case rate and the impending epidemic between 1930-32. Natal province, however, was not even mentioned in the annual report of the department. This was largely due to a more serious outbreak in the Transvaal where, in some areas (Rustenberg and Nylstroom) the infection rate was as high as 62% amongst europeans and 74,4% amongst natives, with respective mortalities of 1,1 and 0,54% (Annual Report, 1928).

Control measures were at this stage largely based on the use of quinine. However during this year a mosquito survey of the Union was published, which also covered the types of sites utilised by the various species and included suggestions for the reduction of such sites (Ingram and de Meillon, 1928). This document was to play an important role in the initiation of control strategies within the Union.

Figure 1. Map of the Province of Natal.

SCALE 1 : 1 000 000

Kilometres
0 5 10 20 30 40 50 60 70 80
0 5 10 20 30 40 50
Miles

Orange Free State
Transvaal
Lesotho
Cape
Transkei
Mocambique
Indian Ocean

NEWCASTLE
LADYSMITH
DUNDEE
VRYHEID
ESTCOURT
MQUTU
UNDERBERG
MOOI RIVER
GREYTOWN
PIETERMARITZBURG
NKANDLA
ULUNDI
MONGOMA
RICHMOND
IXOPO
MELMOTH
HLABISA
NKWALINI
ESHOWE
STANGER
TONGAAT
DURBAN
GINGINDLOVU
EMPANGENI
RICHARDS BAY
UMLAZI
PORT SHEPSTONE
LAKE SIBAYA
LAKE ST. LUCIA
MYALATZE

The 1929 season saw the onset of a severe epidemic which resulted in considerable delays in the sugar mills and plantations due to the majority of the labour force being infected. This impact is clearly illustrated by the hospital records of the Amatikulu sugar mill;

	Total admissions	Malaria cases
January	22	1
February	52	39
March	62	50
April	66	55
May 1-17	44	40

The chairman of the local planters association in Gingindlovu stated that most planters were unable to commence the seasonal work due to the severity of the epidemic. Huletts' representative had visited hundreds of planters whose average work force was 80, but on average only 3 were reporting for work. The Amatikulu mill was receiving only one truck-load per day instead of the expected 1500 tons.

Railway construction in the Stanger area came to a virtual standstill due to infection amongst the labour force (Annecke, 1929). Almost one million quinine tablets were handed out. Resistance to the taking of prophylaxis was, however, encountered amongst the native population. The reason for this was largely related to advice from Inyangas who stated that the taking of the tablets would result in death or sterility. The reason for this attitude has been largely attributed to the law (Section 98 of Act No. 13 of 1928) which restricted the activities of the Inyangas.

During this period the department's staff dispersed oil-soaked bagasse (sugar cane trash, after milling) in swamps and water bodies around human habitations. Park Ross suggested that this was preferable to the use of pure oil or paraffin as the "bagasse fragments tend to cling to stems and diffuse oil where it is most needed". I concur with this as sampling studies conducted by myself in rice paddies show that larvae tend to aggregate around the stems of the rice plants.

The mortality rate for the season was estimated to be 7 Europeans (popn. 6000), 151 Indians and Coloureds (popn. 19 800) and 2600 natives (popn. 215 000). The efficacy of the use of quinine was demonstrated by the example of one sugar estate which kept their workers on 10 grains per diem, from February to June. They only had 4 cases out of 80, as opposed to 90% infection on the neighbouring estates (Park Ross, 1929a). Park Ross also met Drs. Annecke and English in Eshowe. Dr. Annecke suggested the localities at which 23 distribution points were to be established to distribute quinine. Mr. Rodseth was seconded from the Department of Native Affairs to co-ordinate this (Park Ross 1929). In addition to the non-compliance by the native population to the taking of tablets, there was also a tendency towards the magistrates being reluctant to hand out tablets for fear that the expenditure might be "frowned upon by head office".

The severity and impact of this epidemic was highlighted by a letter from a missionary based in the Umvoti region and

addressed to Drs. Annecke and English of the Department of Health.

> "An epidemic of this kind is a big economic loss to the individuals concerned, the community as such and to the country in general. The aggregate of lost labour through the time lost through illness alone is tremendous, especially to agriculture. The aggregate loss through the larger number of deaths of course is much greater. Moreover it is to a large degree unnecessary and ought not to be allowed to repeat itself from time to time."

The Induna (tribal chief) in the above area pointed out a kraal where all five family members had died within six weeks. At one kraal visited, there was a corpse of an individual who had been dead two days but had not been buried, due to all the local inhabitants being down with malaria (Annecke and English, 1929).

The epidemic was one of the most serious for a long time and the Health Department did not have the means or infrastructure to cope with it. They also played down the seriousness of the situation and were slow to distribute pamphlets etc. In addition, quinine was not freely available as they stated because in most areas locals were still being made to pay for it. The pamphlets were written in English so that many of the locals were not aware of the drug's availability. These factors were compounded by the reluctance of locals to take these tablets and the inadequate number of distribution points. The visit by Drs. Annecke and English was an important one in that it exposed this weakness. They were however forced in their short 9 day survey to cover mainly regions of developed infrastructure such as the mills, towns, police stations, dipping points etc., and little coverage of the

native reserves was possible. However letters by missionaries such as the Rev. Abrahams pointed to the seriousness of the situation. The government authorities responsible initially refused to acknowledge the severity of the situation, however their slow acceptance is evident from the weekly notices issued by the Department of Public Health for the period of the 11 May 1929 to the 6 July. See excerpts below..lm 0.50"

a) Bulletin no 19, 1929 for the week ended 11 May, 1929. "Up to now there has been no special prevalence of malaria ....in any part of the Union, but during the past month or so what may be described as a mild epidemic....of about 1000 cases".

Compare this with one missionary who reported in the region of 1300 cases and 30 deaths in his area, approximate population 4000, up to the week ending the 14 May 1929.

"Ample supplies of quinine are available and large quantities have been issued free of charge in the affected areas"

This only included the areas which were developed or of some economic importance, compare this with the letter by H.A. Koch to the Secretrary for Health, dated 27 May 1929, " I noticed that you state there is an ample supply of quinine and can be got on application. I am sorry to state that when I enquired I was told to buy my own if I wanted any. I fail to see where this ample supply of quinine is to be found. I may also mention that I have spent and given 2000 5 grain tabloids to sick natives in the native reserve..."." You seem to be always ready to advise others what to do when you have neglected your duty here.....why don't you carry out your obligations.....natives are dying daily and practically everything is at a standstill. Thanks to the officials of the Health Department!"

b)Bulletin 20 1929, week ending 18 May. "The malaria position in Natal and the Zululand coastal belt has become more serious during the past week, and the epidemic now prevailing is in some localities the worst experienced in many years past. Some sugar planters report the greater part and in some instances the whole, of their labour force down with malaria, and some sugar mills have been unable to commence crushing. Hardly a European household in the areas chiefly affected has escaped..... In most of the native reserves in these districts the disease is exceptionally severe"

c) Bulletin 21, 1929, week ending 25 May. This bulletin reported the situation as unchanged and said that drug

distribution depots had been set up and that mill owners and planters were actively assisting. The notice indicates that the authorities were hoping for the onset of cooler weather to end the epidemic.

d) Bulletin 22, 1929, for week ending 1 June 1929. Authorities hoping for cooling of weather but says that the weather was still warm. Cerebral cases were common. In the Eshowe and Mtunzini area the position is stationary, with half of the Amatikulu mill still being ill. In this bulletin the authorities also admitted that they could not get reliable figures of native deaths due to the fact that these were not registered.

e) Bulletin 23, 1929, week ending 8 June 1929. Cases began to abate

f) Bulletin 24, 1929, week ending 15 June 1929. "The general position has improved along the coastal belt especially south of the Tugela river. In affected inland areas, especially along the Tugela, there has been little improvement. The weather is very wet but not yet cold enough to effect marked improvement."

g) Bulletin 25, 1929, week ending 22 June. "Still considerable prevalence in the Mtunzini, Stanger, and Eshowe districts..... Deaths still occurring amongst the natives, but the general situation is still greatly improved."

h) Bulletin 26, 1929, week ending 29 June. Situation improved but new infections were still occurring.

i) Bulletin 27, 1929, week ending 6 July. "A few new infections from the Mtunzini area, but elsewhere malaria is now disappearing."

j) Bulletin 28, 1929, week ending 13 July. "...the epidemic may now be considered to be ended."

The epidemic in Natal during 1929 was severe but occurred in a fairly circumscribed area. This comprised the coastal districts of northern Natal and Zululand and extended some distance up the Tugela river valley. The epidemic in 1930 was far more widespread, with cases occurring as far south as Umzinto and beyond, isolated outbreaks also occurred in Flagstaff and Libode in the Transkei. Cases occurred in regions of fairly high altitude (762 m and over) and extended north west to Weenen and Ladysmith (Figure 1). Considera-

ble extension also occurred up all the river valleys between the Umgeni and Umhlatuzi. Approximately four times the population was exposed than in the previous season, mortalities were however estimated to be 1 653.

Due to public pressure, Park Ross was granted a team of 25 specially trained staff consisting of a medical officer, four european health inspectors and about 20 native assistants. The health inspectors and medical officer then undertook a general survey of all local authorities and health inspectors were placed in these areas to co-ordinate the issuing of tablets and anti-larval measures, as well as to advise on the screening of houses. The native assistants were largely used to cover the areas such as the Native reserves. In some of these areas the chiefs were keen and strongly supported their efforts while in others they were either indifferent or hostile. The Railway's administration also organised a system of control by appointing sanitary inspectors who were responsible for taking anti-mosquito measures on either side of the line and ensuring that construction staff were given adequate protection/treatment using quinine.

The first mention of the impact of the epidemic on tourism in the Province was made in a letter from Park Ross (15 April 1930) to the Secretary for Public Health, expressing concern about the malaria outbreak on the South Coast down as far as Umzinto and beyond (Figure 1). He was worried that people might write to the Secretary saying that the bulletins he was issuing were scaring away tourists but said that he felt the

situation warranted this.

Following this a series of letters appeared in the newspapers;

> 19 May 1930. The Star, Jhb. Letter by R.L. Brodie to the editor stating that he had been on holiday on the South coast and he and his wife had contracted malaria. He further stated that it was all very well for the assistant health officer (Park Ross) to say that people should use mosquito nets, but these were not readily available at the resorts. Another letter to the editor of the Rand Daily Mail asking for a"plain statement of the position" as he was concerned about visiting tourists, signed anxious.

In the above mentioned communication by Park Ross to the Secretrary for Health, the first mention of Malaria Inspector Hamilton was made; he was to feature greatly later. Park Ross also suggested the appointment of inspector Parish as a malaria inspector as he spoke Afrikaans and came strongly recommended by Dr. Annecke. Enclosed was a copy of Parish's report stating that he had addressed meetings of doctors and various public gatherings. He also mentioned the initiation of cleanups in towns and the ordering of a Beauty duster, Paris green, pumps, Mosquito Smear and mosquito proofing for the Umzinto hospital.

During this transmission season the use of Paris Green as a larvicide was introduced and reported upon favourably. Sir Malcolm Watson also visited South Africa and was accompanied in his tour of the country by Dr. Botha de Meillon. Sir Malcolm Watson, during his visit, stressed his opinion that malaria control was the responsibility of the landowner and that the government's role was merely to act as advisors. This was to become a highly contentious issue in the Province

and costly in terms of loss of human life, especially amongst the Native population. During this year the government also secured the services of Prof. N. H. Swellengrebel to conduct a detailed study in the malarious areas of Natal and the Transvaal and advise on control. This investigation was to be carried out between October 1930 and April 1931. In January 1930 malaria was once again made a notifiable disease in the borough of Durban.

The 1931 season continued the 1930 trend of southwards extension, with cases being reported as far south as Port St Johns (32^{o} 30'E ; 39^{o} 42'S) and extending northwards, with incidence of the disease occurring in virtually every one of the larger river valleys. This was thought to be due to many of the rivers drying up in the exceptionally dry summer and thus providing suitable breeding puddles in the river beds. The lack of rain together with the initiation of organised control efforts probably played a role in the reduced incidence of the disease. The total deaths for the Province was estimated at some 844, 373 of these having occurred in the lower Tugela valley (popn. 46 000).

On the 17 February 1931, Dr. S. Annecke wrote a report to the Secretary for Public Health. During the 1930 season he had spent considerable time visiting farms, giving lectures and advising, mainly on drainage etc. The main complaint he received from both the sugar planters and farmers was that while they were being policed and were putting considerable effort into screening of houses, drainage etc., the govern-

ment was doing nothing in the adjoining native reserves, thus negating their efforts.

This report was followed shortly thereafter (27 February 1931) by the Government Notice 344, 446 Health, which gazetted malaria regulations. These are summarised as follows.

1. It was unlawful to have collections of water on your property which may act as breeding sites for mosquitoes, unless screened or treated regularly. This applied to all municipal areas, barracks, factories, mills etc.

2. Mills, factories , barracks etc., had to provide adequate storm water drainage, or screen/treat any water bodies resulting therefrom.

3.Borrow pits, quarries ,rail and road works situated within or within one mile of an urban boundary or 1/2 mile of an inhabited house had to be drained, screened, or filled up after use, or permanent drainage supplied.

4. All screening used should be of mesh with not less than 14 apertures per inch.

5. a) All water storage receptacles on a person's premises have to be constructed and maintained to exclude mosquitoes. b) All irrigation channels had to be maintained in such a condition as to not favour mosquito breeding. c) Roof gutters had to be kept free of stagnant water. d) All private property had to be kept free of all tins, bottles crockery etc. In addition all water collections resulting from seepage or rainfall, including hoof prints , surface depressions had to be kept free of water.

6. a) When upon inspection, a personal property was found to contain conditions favourable to mosquito breeding, written notice would be given to the owner to rectify this. b) Such notices would specify the property in question and the remedial action to be taken. c) The notice would require the owner to take whatever measures necessary within a specified time period. d) If the owner refused to do so the local authority would carry out such measures at his cost.

7. Any person who contravenes/fails to carry out the measures specified was liable on conviction to the penalties provided in section forty-five of the Public Health Act, No. 36 of 1919.

These measures thus followed the advice of Sir Malcolm Watson and placed the responsibility squarely on the shoulders of

the landowner.

Considerable impetus was given to control efforts with the release of Dr. Swellengrebel's report and the publication of these findings by Swellengrebel *et al.* (1931). The principle of Species Sanitation was proposed here. This was based on the fact that the breeding habits of the two main vectors *Anopheles costalis* (*An. gambiae*) and *Anopheles funestus* were fairly "definite". It was thus proposed that anti-larval work for malaria control "becomes a practicable measure, since it can be carried out on a vastly smaller scale than previously supposed". At this point it is important to return to the mosquito survey conducted by Ingram and de Meillon (1928) as at this stage they had already identified *An. funestus* as the other important vector and listed the breeding sites utilised by this species and *An. gambiae*. However little use was made of this information by government authorities. It took the commissioning of Prof. Swellengrebel and the publication (and report) of Swellengrebel *et al.*, (1931) before the expanded findings of Ingram and de Meillon were put to good use. Other recommendations by Swellengrebel *et al.* (1931), were the screening of houses, the killing of mosquitoes resting indoors every morning and the painting of walls (white) to facilitate this process. They also suggested that a division of malaria control similar to that already established in Natal by Park Ross, be instituted in the Transvaal.

The 1932 season proved to be the worst experienced in the documented history of Natal. The total numbers of natives

visited by the native assistants totalled 30 508 and they recorded some 2 620 deaths. The department eventually put the total numbers of deaths at 10 000 to take into account the portions of Natal and Zululand not included in the Reserves. " It can be quite definitely stated that the deaths did not exceed this number" (Annual Report, 1932). This figure was in striking contrast to those produced by the magistrates for the various districts:

Mortality as estimated by Magistrates during period November, 1931 to June, 1932

-----ooo----

Total Native Population 1931 Census	District	Europeans	Natives	Indians	Coloureds etc.	Remarks
48 188	Umzinto	3	110	6	-	-
32 078	Greytown	-	500	-		Total all races
51 139	Msinga	-	1 423	-	-	Figures for 2 more tribes still to be received
40 837	Verulam	15	750	310	-	-
18 264	Camperdown	-	300	-	-	Total all races
21 000	New hanover	-	199	3	-	-
21 174	Weenen	-	204	-	-	-
27 873	Harding	-	-	-	-	-
47 721	Vryheid	4	250	-	-	-
22 495	Richmond	-	200	3	-	-
32 008	Mapumulo	-	4 014	-	-	-
						574 reported by Native Malaria Assts. Balance from other sources
44 799	Durban	52	271	7	10	-
40 059	Estcourt	5	410	3	-	-
10 337	Helpmekaar	1	55	-	-	-
28 342	Dundee	-	167	-	-	-
	Babanango	2	90	-	-	-
32 783	Umgeni (P.M. Burg)	18	551	26	-	-
18 337	Bergville	1	65	-	-	-
19 080	Kranskop	-	846	-	-	-
40 459	Klip River	10	647	3	-	-
36 179	Pinetown	1	200	20	-	-
25 712	Ngotshe	7	160	-	-	-
	Ndwedwe	-	649	-	-	-
49 985	Ixopo	-	90	-	-	-
30 151	Stanger	8	1 250	450	-	-
34 657	Port Shepstone	1	13	2	-	-
9 641	Impandhle	-	20	-	-	-
	Mahlabatini	-	528	-	-	-
17 931	Melmoth	1	298	-	-	-
32 210	Nongoma	-	632	-	-	-
26 543	Ingwavuma	1	-	-	-	No estimate
15 602	Empangeni	2	562	26	-	-
17 903	Hlabisa	-	450	-	-	Total all races
15 716	Ubombo	2	25	-	-	-

Total Native Population 1931 Census	District	Europeans	Natives	Indians	Coloureds etc.	Remarks
29 365	Nqutu	-	480	-	-	-
19 021	Eshowe	-	1 560	-	-	-
23 662	Mtunzini	-	1 151	-	-	-
32 876	Nkandhla	-	2 000	-	-	-
985 385		134	21 122	859	17	
			GRAND TOTAL		22 132	

The magistrates figures became the subject of a contentious debate but were never made public. The estimated figure of 10 000 deaths officially released resulted in an enormous public debate and a special enquiry by the Department of Native Affairs. Park Ross' opinion is clearly summarised by the following quote taken from a letter to Sir Edward Thornton (Secretrary for Health);

> "Mr. Rodseth and I have gone over this district by district, and the only opinion I can pass on the most of it is that it is absolute and unadulterated tripe. Let us take the district of Mapumulo where 1/8th of the people are reported to have died in six months..... When we come to some other districts, it is interesting to consider Impendhle, high up on the slopes of the Drakensberg and under snow for a considerable period of the year, with 20, while Ubombo, our endemic yellow area debilitated from end to end with malaria, especially among the child population, has 25..... In only one point can I consider this thing helpful. It shows us that there is either malaria, or suspected malaria in every single district of Natal, with the one exception of Harding where , however, there were lots of cases last year, so that registration should apply to the whole country" (province).

Impendhle (29^{o} 50'E ; 29^{o} 38'S, altitude 1600 m) is however not in the Drakensberg and only occasionally experiences snow in winter. The cases in this region are also likely to have been imported. Two other factors are however also being ignored here: firstly that higher mortalities would be expected in regions not usually subject to the disease and in

which people have no acquired immunity; secondly that the reporting from the northern districts tended to be poor due to the almost total absence of infrastructure. Probably the most important point made by Park Ross here was as follows; "If any further plea was required as to the necessity for registration of Native deaths, this is sufficient". Over the preceding years he had repeatedly requested the passing of an ordinance to cover the registration of native births and deaths.

An important development during this period was the proposal for "malaria committees" to be established which would enforce the regulations of Government Notice 344 above. This was first proposed by Prof. Swellengrebel *et al*. in their 1931 report. Park Ross was strongly in favour of the establishment of such committees and motivated for a provincial ordinance to be promulgated. The South African Sugar Association were however against any such system. This was made apparent in a letter written by D.M. Eadie the secretary of the South African Sugar Association (SASA)to the Secretary for Health, E.N. Thornton (July, 1932). From this letter it became clear that their main concern was to attempt to circumvent the establishment of malaria committees which would levy taxes on them and ensure the implementation of Prof. Swellengrebel's recommendations. They were of the opinion that the bureaucracy resulting from these committees, their administration and policing activities, would exceed the cost of the actual control measures which were implemented as a result thereof. They proposed that Prof. Swellengrebel be

employed to provide fixed estimates of the costs and to decide on the necessity of committees within various regions. The aim here was obviously to avoid hidden and ongoing costs, especially administrative. Towards this end, they offered to pay half the cost with an immediate advance of 1000 pounds.

Sir Edward Thornton (Secretary for Health) replied along the following lines:

> a)That only if unlimited capital for drainage, filling etc., were available, could reasonable control be attained in a three-year period but even then there would be maintenance costs, i.e. stating that there was no short, cheap solution.
> b) That localised control could be attained using Prof. Swellengrebel's Species Sanitation, through anti-larval measures. He quoted an example of the Tongaat Sugar Company where these methods had brought about control in 12 of the 14 sections under their control. He concluded " Indeed, it was clearly shown that anti-larval work on the lines advocated by Prof. Swellengrebel can be relied on to localise the infection". He stated that the Zululand Milling Company, Umfolozi Co-operative Planters and Millers, Illovo Sugar Estates, Esperanza Sugar Estates and the Central Factory Verulam had all achieved similar success using these methods.
> c) He pointed out that the importance of the malaria committees lay in the lack of local government in rural areas to effect control. The committees would supply this infrastructure. He pointed out that Prof. Swellengrebel had in fact advised on the establishment of such committees, especially as certain estates were not taking adequate measures to prevent malaria.
> d) He concluded by saying that the Minister for Health was absent at present, but that he would not support the further contribution of funds towards engaging an expert as this had just been done and that he wished to see the establishment of the malaria committees as soon as possible as he saw these as being central to dealing with the malaria problem

There is little doubt retrospectively that these committees were essential to provide the necessary infrastructure for effective control. The SASA was looking at the problem purely in terms of their areas/situation and the minimising of cost. Thornton's outlook was a long term one for the province as a

whole.

During the course of the 1931/32 season Park Ross initiated an experiment using sulphur for the fumigation of huts (then used to control rodents on ships). This proved to be popular with locals due to the fact that it killed cockroaches, but was unsuccessful as it rapidly diffused through the grass construction of the huts. This was carried out in a region of the Tugela valley which had an extremely high incidence during the early part of the 1932 season "in an attempt to stem the rapidly spreading incidence of the disease". During the same period an experiment was carried out using "Pyagra" (liquid pyrethrum and kerosene) in the Msinga valley. These trials were carried out by Senior Malaria Inspector S. Hamilton. The results achieved were so striking that urgent requests for similar treatment were received from natives living outside the area (Cluver, 1939). The efficacy of pyrethrum and kerosene as a residual insecticide in the use against domestic flies had been demonstrated earlier by Ingram and de Meillon (1927).

In September 1932 the Secretrary for Health wrote to Park Ross and outlined some of the findings of Dr.'s Annecke and de Meillon, which were to be published in the Annual Report for 1932.

> "Work was also carried out on the habits and life histories of the mosquitoes known to be vectors of malaria. This has not yet reached a stage for reporting. The interesting fact, however, appears to have been established that the vectors are harmful because of a preference for confined, dark space -the ill-lit, dark, musty bedroom of the badly constructed dwelling. This was proved by means of Europeans sleeping in the very highly

> fever infected valley of Letsetele river. Two men slept in the open air camp beds protected by nets; the third slept under a similar net inide a patrol tent. Specimens were caught every two hours through the night; *Anopheles funestus* was caught only in the patrol tent. This tropism for confined dark spaces was again demonstrated by hanging a box with blackened inside walls in a dark native hut. It was highly favoured by the adult of the species and many were caught therein."

In October 1932 Inspector Hamilton wrote to Park Ross requesting the purchase of a "Prima" spray pump, 2 gallons of pyrethrum and 40 gallons of power paraffin for indoor spraying during the next summer in Msinga, Tugela and Mooiriver, in areas in which larval control had failed to contain the disease. During the same month it was also decided to carry out an experiment using Cyanogas, and other insecticides in the next season.

In summary the 1932 season was an important one, the economic devastation it caused and loss of human life prompted the passing of new laws, the development of new infrastructure and of new techniques to combat malaria. The severity of the economic impact is summarised by the following resolution adopted by the South African Federated Chamber of Commerce at its annual meeting in Durban in May, 1932.

> "This convention respectfuly urges upon the Government the need for continuous and effective action to eradicate the scourge of malaria which is having a serious effect upon Natal and the Eastern Transvaal, and reacting seriously upon the progress of industry, trade and agriculture in these provinces, and, through them upon the Union"

During the next year an extensive infrastructure for control was established in Natal by Park Ross for each district. This included the motivation and procurement of staff and equip-

ment. The former included new district surgeons, medical officers, sanitary inspectors and native assistants. Malaria committees were established throughout the Province and Malaria Inspectors were appointed by each committee. Regulations were promulgated covering the responsibilities of the committees and to cover other regions such as those adjacent to provincial roads and railway lines.

A special fund was created to cover the spraying of problem areas throughout the Province with Pyagra. Due to the efficacy of this method (Park Ross, 1936) and its cost effectiveness compared to larval control (de Meillon, 1936) its use gradually took over from larval control. By 1938 some 15 000 gallons of pyrethroid/kerosene was being used as opposed to approximately 10 000 gallons of malaria oil.

Focal larviciding remained an important tool despite the decrease of its usage. In October, 1938 Park Ross replied to a letter from F. L. Soper in Brazil, enquiring whether he (Park Ross) thought it would be possible to totally eliminate *Anopheles gambiae* from a defined region (Soper & Wilson, 1943).

> "It is not possible to give emphatic opinions because we do not know your terrain. We have no notion as to permanent rivers, perennial swamps, or whether your dry season is in cold weather, as ours is, but seeing you know the terrain here, a few statements relating to it may be of service to you even if they may not be orthodox.
>
> A. Winter control appears to us to be the key to the situation.... In the Mandini area of Zululand (which you know) we have know kept up winter control by spraying breeding places for some years. *Funestus* has know been absent for some years. Until we had winter control, fever had never been absent from the area, at least not

> for 50 years. The summer incidence of *gambiae* used to average 200-300 adults per hut. It has now dropped to a summer average of two to three per hut and that mainly in the vicinity of uncontrollable streams. We are sure that had it not been for these streams we could have got 100% control by our winter measures plus summer adult work."

Prior to the obtaining of this reference a similar conclusion regarding winter larviciding was reached as a result of data collected and experimentation conducted in the present study.

The impact of indoor residual spraying is shown in Figure 2, which is reproduced from an internal departmental report by Park Ross in 1935. It reflects the number of positive slides detected annually by the Government laboratories, Durban. The number of slides taken by the laboratory however increased markedly each year and thus masks to some extent the striking reduction in the number of cases annually due to indoor residual insecticide application. The efficacy of the method had however not been demonstrated scientifically despite the work of de Meillon (1935[6]) in which the trend of sporozoite reduction was evident but the sample size to small too be statistically significant.

de Meillon, however remained unconvinced as to the efficacy of the method and this is illustrated by a paper he presented at a congress in Lourenco Marques (Maputo), Mocambique in 1938.

> "Hopes have recently been raised high by the possibility of controlling malaria by killing adult mosquitoes in habitations with an insecticide. The method would appear to depend for its success on two peculiarities of our vectors, firstly their house-frequenting habit and secondly their reluctance to leave a shelter until their

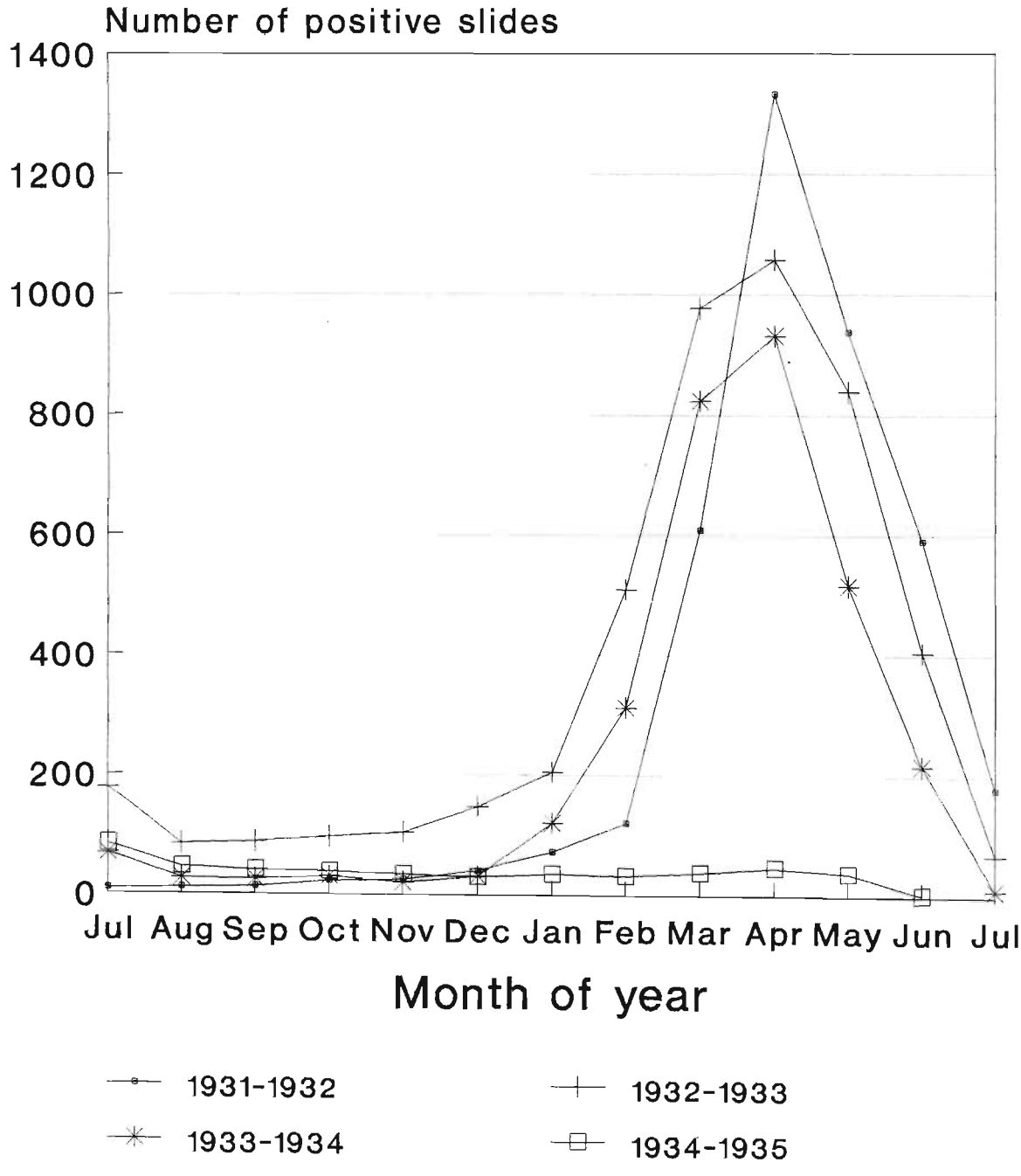


Figure 2. Graph showing monthly totals of positive malaria slides examined in Government Laboratory, Durban, from 1st July 1931 to 30th June 1935.

> eggs are fully developed. There is some evidence to show that under certain circumstances success can be obtained but I think that it is largely a special method, for special circumstances..... In Natal and Zululand weekly spraying was resorted to as a rural malaria measure after the epidemic of 1932 so that the great decline may simply have been a post epidemic phenomenon due to acquired resistance by the population and the restricted breeding of *gambiae* as a result of the return of normal climatic conditions."

The reasons for de Meillon coming to this conclusion were based on sound scientific reasoning as a result of the continued presence of *gambiae* within 'controlled' regions of Natal. Today we can conclude that this was probably due to the zoophilic and largely exophilic member of the complex, *Anopheles quadriannulatus*. This was probably the first evidence indicating that *An. gambiae* was a complex of species. To explain this outdoor population, Mastbaum (1954), working in Swaziland concluded that as a result of the indoor application of BHC, "It seems that *An. gambiae* having been discouraged from entering human habitations, has to a great extent lost its capabilities of transmitting malaria;....."

In 1946 pyrethrum was replaced as the insecticide of choice by DDT, which was used for both indoor residual application and larviciding. Until 1953 the northern districts of Ingwavuma, Hlabisa and Ubombo were not subject to any control measures. This was a result of advice from Prof. Swellengrebel who feared that this might destroy the natural immunity of the population living in this highly endemic region. In 1956 larvicidal measures were discontinued and by 1958 total coverage of the northern districts, which started two years previously, was achieved. During the 1970's the control

programme became increasingly more structured and the situation concerning malaria between 1976 and 1985 was documented by Sharp *et al.* (1988).

More recently as a result of work carried out in the present study and that by Sharp *et al.* (1990) and Sharp and le Sueur, (1991), focal larviciding has been re-introduced in regions where the annual application of DDT alone (to houses) has been inadequate in controlling disease transmission.

References

Annual reports of the Union of South Africa Health Department, 1928-1938.

Annual reports of the South African Institute for Medical Research 1933-1938.

Annecke, S. (1929). Inspection of railway construction works: Stanger - Tugela. Government Pathologist, report 3 June 1929.

Annecke, S. and English G.D. 11 June 1929. Report by Annecke (government pathologist) and G. D. English (assistant district surgeon) on their investigation of the malaria epidemic in Stanger, Mtunzini and Eshowe.

Cluver, F.W.P. (1939). Malaria control in Natal and Zululand. Paper presented at the South African Medical Congress, Port Elizabeth.

de Meillon, B. (1936). The control of malaria in South Africa by measures directed against the adult mosquitoes in habitations.-*Quart. Bull. Hlth. Org. L. of N.* 5, 134-137.

de Meillon, B. (1938). Remarks on the Entomology of malaria in the Tropics and Sub-tropics of Africa.-*Prim. Cong. Med.*, Lourenco Marques.

Ferguson, I. A. Booklet entitled "Park Ross of Natal". Date and publisher unknown. 16pp.

Hill, E. and Haydon, L.G. (1905). The epidemic of malarial fever in Natal, 1905.-*J. Hyg.* 5 (4), 467-484.

Ingram, A. and De Meillon. (1927). Efficacy of certain reagents as destructors of flies when used in the form of a spray.-*J. Med. Assoc. S. Afr.* **1**. 366-369.

Mastbaum, O. (1954). Observations of two epidemic malaria seasons (1946 and 1953)- before and after malaria control - in Swaziland.-*Trans. R. Soc. trop. Med. Hyg.* **48** (4), 325-331.

Park Ross (1929a) . Report on the malaria situation in the Province, following a tour of the region. File ref. 305/36/12.

Park Ross, G.A. (1936). Insecticide as a major measure in the control of malaria, being an account of the methods and organisations put into force in Natal and Zululand during the past six years.-*Quart. Bull. Hlth. Org. L. of N.* **5**, 114-133.

Sharp, B. L., le Sueur, D. and Bekker, P. (1990). effect of DDT on survival and blood feeding success of *Anopheles arabiensis* in northern KwaZulu, Republic of South Africa.-*J. Amer. Mosq. Contr. Assoc.* **6** (2), 197-201.

Sharp, B.L. and le Sueur, D. (1991). Behavioural variation of *Anopheles arabiensis* (Diptera: Culicidae) populations in Natal, South Africa.-*Bull. ent. Res.* **81**, 107-110.

Soper, F.L. and Wilson, D. B. (1943). *Anopheles gambiae* in Brazil. The Rockerfeller foundation, New York.

Swellengrebel, N.H., Annecke, S and De Meillon, B. (1931). Malaria investigation in some parts of the Transvaal and Zululand.-*Publ. sth. Afr. Inst. Med. Res.* **28** (4), 245-274.

CHAPTER2

BREEDING SITE CHARACTERISTICS

Note: The contents of this chapter have been published (excluding the sections on temperature and other anophelines), in the Bulletin of Entomological Research, 78, 549-560.

TITLE

The breeding requirements of three members of the *Anopheles gambiae* Giles complex (Diptera: Culicidae) in the endemic malaria area of Natal, South Africa.

ABSTRACT

The breeding sites used by three species of the *Anopheles gambiae* Giles complex were investigated in northern Natal, South Africa. Those used by the two freshwater species; *An. arabiensis* Patton and *An. quadriannulatus* (Theobald) were similar, immature stages of both being collected from the same pool at five out of seven localities from which *An. arabiensis* was recorded.. *An. quadriannulatus* was the most extensively distributed species of the complex and was found in association with *An. arabiensis* at only five of 49 localities. The difficulty in locating the breeding sites of *An. arabiensis* is a product of their low density, presumably as a result of the intra-domiciliary, residual insecticide spray programme. *An. merus* Donitz larvae and pupae were recorded only in water with a salinity greater than 5 p.p.t. The pH,

dissolved oxygen, pool size, turbidity, shade and association with vegetation of water bodies containing *An. gambiae s.l.* were also recorded and showed no difference between the three species. The importance of distinguishing between winter and summer breeding sites is discussed, as well as the need for entomological consultation prior to agricultural development in endemic malarious areas.

INTRODUCTION

Three species of the complex of *Anopheles gambiae* Giles commonly occur in the endemic malaria area of Natal, viz; *An. arabiensis*, *An. merus* and *An. quadriannulatus*. A single record exists for *An. gambiae s.s.* from Pelindaba (pt.no. 48, Figure 3), (Miles,1978), which acts as a control area within this and is not subjected to the intra-domicilary application of residual insecticide. The scarcity of *An. gambiae s.s.* throughout the endemic malaria area is probably a result of this spray programme and the endophilic and anthropophilic tendencies of this species (White, 1974).

Failure by local, malaria surveillance teams to locate the breeding sites of *An. arabiensis* led to speculation that these differed from those of *An. quadriannulatus*, which were easy to locate. The breeding sites of *An. arabiensis* elsewhere in Africa have been described (White & Rosen, 1973; Service, 1970, 1973, 1977; Gillies & Coetzee, 1987). Two records exist for *An. quadriannulatus*, in both cases it was collected from shallow pools in dry riverbeds (Paterson *et al.*, 1963 ;Gillies and Coetzee, 1987). Paterson *et al.* (1963) also collected *An. quadriannulatus* larvae from water filled

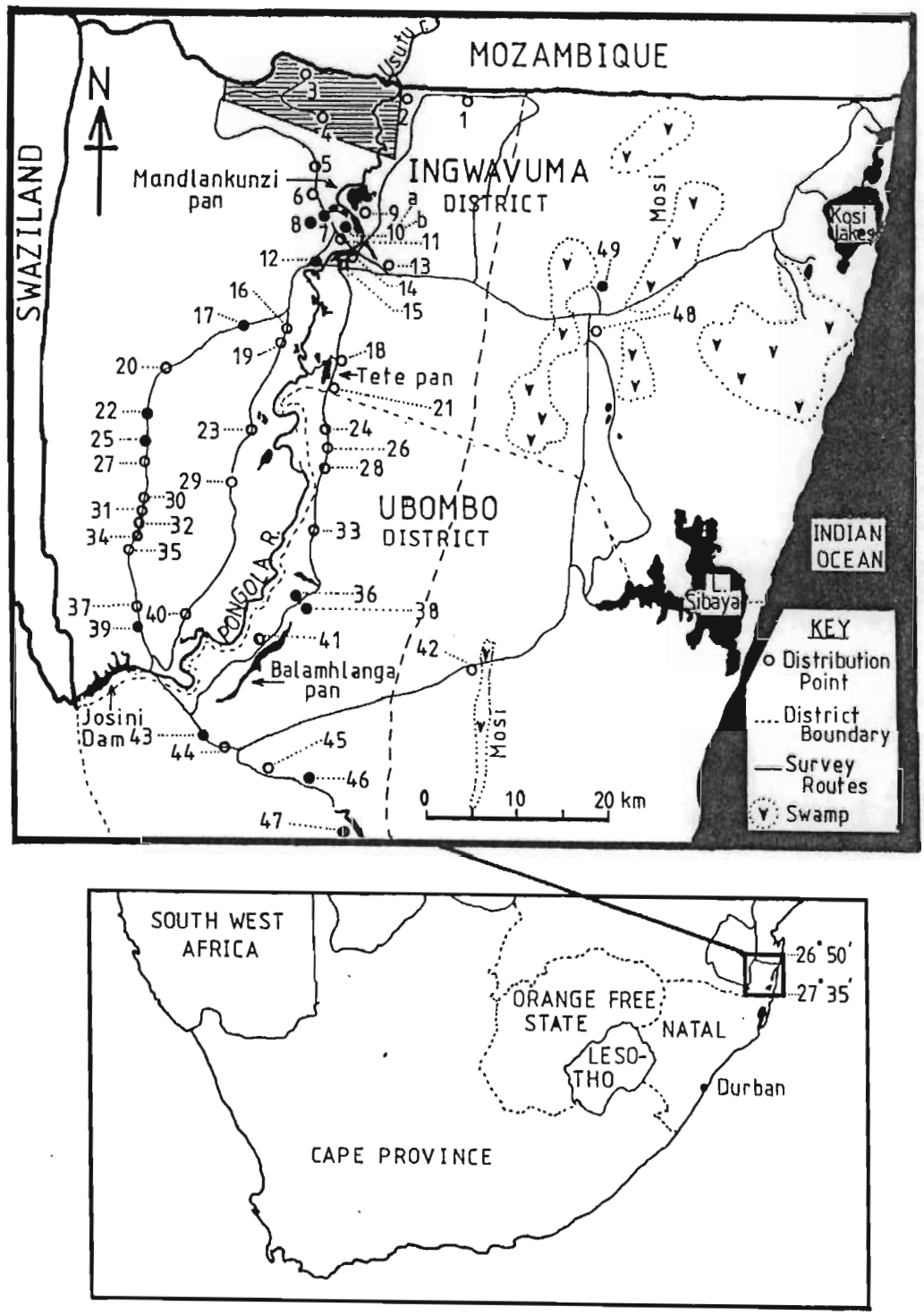


Figure 3. Map showing the sites at which *Anopheles quadriannulatus* was collected within northern Tongoland. Identifications were based on chromosomal plus electrophoretic (O) or electrophoretic alone (O).

rhino footprints. *An. merus* larvae have been collected from a large variety of waterbodies, including mangrove (*Avicennia marina*) swamps, ponds and small pools, all of which were saline (Gillies & De Meillon, 1968; Gillies & Coetzee, 1987). The present study was initiated to investigate the type and characteristics of breeding site used by *An. gambiae* complex members occurring in Natal.

MATERIALS AND METHODS

THE STUDY AREA

The endemic malaria area of Natal coincides well with the region known as Tongaland (32°E to Indian ocean; 26°50'S to 27°50'S, Figure 4) which forms part of the southernmost tip of the coastal plain, extending from Somalia in the north. This region has a largely tropical biota as a result of its low lying topography and the southward flowing, warm Mozambique and Agulhas currents (Bruton, 1980). It is encompassed within the 18°C Effective Temperature (ET) isoline that can be used as an indicator of the peripheral boundaries of a tropical climate (Stuckenberg, 1969). Seasonal malaria epidemics historically occurred to the south of this region, extending as far as Durban (29°52'S, 31°E in 1905) and Umzinto (30°49'S, 30°40'E in 1930),(Sharp *et al.*, 1988).

The coastal plains of Africa underwent a succession of marine inundations during Cretaceous, Miocene and Pliocene times (Maud, 1980). During the periods when Tongaland was inundated, the base of the Lebombo mountains formed the shoreline

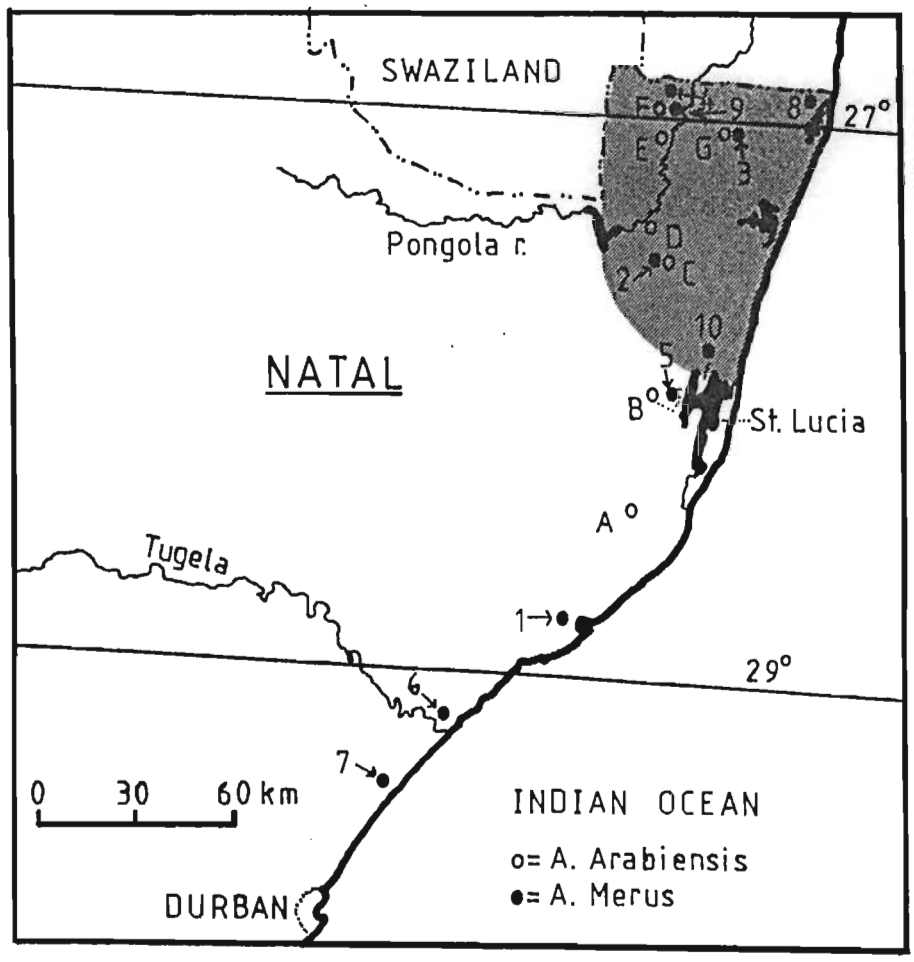


Figure 4. Map showing the sites at which *Anopheles arabiensis* and *An. merus* were collected, in northern Natal. The shaded area is the region known as Tongaland. The numbers and letters refer respectively to the sites from which *An. merus* and *An. arabiensis* were collected.

and marine cretaceous and tertiary deposits were laid down. Within Tongaland, these deposits are exposed at several points and are responsible directly, or by seepage for the saline nature of certain waterbodies within the area.

Lake St. Lucia and some of its associated marshes are also saline, but in this instance this is a direct result of sea water flow into the lake via the estuary. This flow occurs especially in times of drought when the lake level drops due to reduced input from rivers.

An important feature of the northern region of Tongaland is the floodplain of the Pongola river. The rapid change in gradient of the river, from 7,5 in 3000 (meters) to a slope of 1 in 3000, after the Josini dam (Figure 3), which results in a decrease in flow rate and the flooding of adjacent areas (Heeg *et al.*, 1982). It is this flooding process which acts as the major water supply to the approximately 90 lakes within the 13 000 ha floodplain. These lakes play an important role in the dynamics of *An. gambiae s.l.* populations within the region.

FIELD SPECIMEN COLLECTIONS

Monthly surveys were carried out in different regions of Tongaland from March 1984 to January 1987 (Figure 3). Occasional surveys were made to regions further south and account for the southerly points shown in Figure 4. The sites shown in Figures 3 and 4 represent localities which were positive for or more *An. gambiae* species. Surveys were conducted using the existing road infrastructure as a baseline from which all

observed waterbodies were then investigated. Permanent waterbodies in which no *An. gambiae* complex species were found in summer were re-investigated during winter to account for possible seasonal utilisation.

LABORATORY PROCESSING FOR IDENTIFICATION

The presence or absence of larvae was investigated using a white, 200 ml, plastic soup ladle. When larvae were found extensive collections were made and transported to the insectary in Durban. Larvae were raised using a mixture of dog pellets, activated yeast and brewers yeast (150:30:10 g), ground to a particle size of < 67 microns. Emergent adults were placed in a plastic cage constructed from a 6 litre plastic bucket and offered a 10% honey solution as food. From the fifth day, females were offered a blood meal daily.

The ovaries of half gravid females were removed and processed for subsequent chromosomal identification according to the methods of Coluzzi (1968) and Hunt (1973). Morphological slide preparation were made of all females from which ovaries were obtained, according to the method of Hunt & Coetzee (1986). The bodies of the same females were stored in liquid nitrogen for subsequent isoenzyme electrophoresis (Mahon *et al.*, 1976; Miles, 1978, 1979) When only a few emergent adults were obtained, identification was based on isoenzyme electrophoresis alone.

FIELD DATA COLLECTION

The pH, dissolved oxygen, salinity and temperature of a

number of pools from each site was measured, during winter and summer. The size of the pool and that of the whole site was recorded. Other features such as turbidity and the presence/absence of vegetation and shade were noted.

Salinity was measured using a standardised, temperature compensated optical salinometer, with a range of 0-160 ppt. pH was measured using a standardized portable Crison pH/mv-506 meter. Dissolved oxygen was measured using a standardized YSI model 58 dissolved oxygen meter. Temperature was initially measured using a mercury thermometer, but was later carried out using the probe on the dissolved oxygen meter. Temperature profiles were obtained using calibrated thermocouples linked to a Scientific Associates Data logger.

LABORATORY OVIPOSITION TRIALS

Oviposition selection trials were set up in a cage constructed from a 20 litre bucket. One hundred blood-fed females of *An. arabiensis* from a laboratory colony were placed in the cage with four oviposition bowls. Each bowl contained distilled water adjusted to a pH value of 3.5, 5.6, 7.3 or 11.0. The number of eggs laid in each bowl was counted daily. The trial was repeated.

RESULTS AND DISCUSSION

IDENTIFICATION

An. quadriannulatus identifications from 14 of the 49 localities (Figure 3), were based on both chromosomal and electrophoretic analysis. Identification at the remaining 35 locali-

ties was based on electromorph frequencies alone. All chromosomally confirmed *An. quadriannulatus* had the superoxide dimutase (SOD) and glucose oxalate transaminase (GOT) electromorphs at the 100/100, 100/100 and 95/95 loci, respectively.

Identifications of *An. arabiensis* at five of the seven localities was based on both chromosomal and electrophoretic analysis. Identification for the Nkunduse (near St. Lucia) and Ophansi samples (Fig 2. sites B & C) were based on electrophoretic analysis alone. The *An. arabiensis* SOD, GOT and octanol dehydrogenase (ODH) electromorphs occurred at the 100/100, 100/100 and 95/95 loci respectively.

Identification of *An. merus* at four of the ten localities (Figure 4, sites 1-4) was based on both chromosomal and electrophoretic analysis. Identification at the remaining six localities was based on the SOD enzyme system (Figure 4, sites 6 - 10). Chromosomally confirmed specimens had the SOD electromorph at the 95/95 locus.

BREEDING SITE CHARACTERISTICS

1. pH

The alkaline nature of the breeding sites in northern Natal is shown in Table 1. Mean pH values range between 8,2 and 8,7 for the three species. *An. arabiensis* and *An. merus* were never recorded at a pH of less than seven. *An. quadriannulatus* was recorded in pool water with a pH of less than 7,0 (6,7) in one instance.

Table 1. The mean dissolved oxygen, pH and salinity values of waterbodies containing immatures of one or more of the three species of the *Anopheles gambiae* complex occurring in Natal

		An. arabiensis	*An. merus*	*An. quadriannulatus*
Dissolved	**Mean**	9,4	9,7	8,2
oxygen	**s.d.**	1,5	1,4	2,8
(mg/litre)	**Range**	6,7-11,9	6,0-12,0	2,9-13,7
	n	15	12	43
pH	**Mean**	8,5	8,7	8,2
	s.d.	0,8	0,3	0,8
	Range	7,2-9,3	7,9-9,3	6,7-7,5
	n	18	68	43
Salinity	**Mean**	0	14,7	0,1
(p.p.t.)	**s.d.**	0	6,7	-
	Range	-	5-38	0-2
	n	22	137	38

The tendency for *An. gambiae s.l.* immatures to occur in alkaline waters has been documented by a number of authors (Hancock, 1934; Jepson *et al.*, 1947). The mineral springs in the Semiliki forest in Uganda, where *An. bwambae* is found locally, had pH values of 8,2 - 8,3 (White, 1973). However Pomeroy (1931) recorded *An. gambiae s.l.* immatures in water with a pH of 4.0. The importance of pH is thus difficult to assess, especially as values are usually only quoted for waterbodies from which immatures were actually collected. Thus the case recorded by Pomeroy (1931) may be a reflection

of the acidic nature of waters within the region and, conversely, that of Jepson *et al.*, (1947) of the alkaline nature. Improved assessment is, however, possible when the pH of a range of waterbodies within a region is noted and then related to the presence or absence of immatures (ie. where a choice exists). Such an assessment was carried out by Hancock (1934), who demonstrated that although the waters within the study area tended to be acidic, *An. gambiae s.l.* immatures occurred in those that were least acidic.

The effect of pH on utilization of waterbodies by *An. quadriannulatus* was investigated in northern Tongaland as waterbodies with a range of both acidic and alkaline pH values occurred together with fairly large populations of *An. quadriannulatus*. The *An. quadriannulatus* population within this region are highly zoophilic (Sharp *et al.*, 1984) and as a result appear to be little affected by the residual insecticide control programme. This may explain the extensive distribution of this species in Tongaland relative to the other *An. gambiae* (Figure 3).

The eastern sandveld (Figure 3 east of broken line) was extensively surveyed. Permanent waterbodies such as the northern Mosi swamp and Lake Sibaya were investigated during winter and summer, to investigate the possibility of seasonal differences. *An. quadriannulatus* was scarce being recorded at only three of the 58 localities surveyed in the eastern sandveld (Figure 3, sites 42, 48 & 49). Only localities positive for *An. quadriannulatus* are shown in Figure 3. The water

was acidic, with mean pH values of 5,1; 5,8 and 6,1 being recorded for inter-dune surface water, streams and lakes respectively. The three pools from which immatures were collected were newly formed, temporary and alkaline. These results indicate a tendency for *An. quadriannulatus* to be absent from the more acidic waterbodies within the eastern sandveld region. The absence may however be related to other characteristics of the waterbodies in question, such as their extremely high iron content in some instances (up to 42,0 mg/litre). Hancock (1934) suggested that the absence of anopheline mosquito immatures may be correlated with a high concentration of dissolved iron salts.

Such an investigation of pH was not possible for *An. arabiensis* as it was rarely encountered in sufficient numbers. Of the seven localities from which it was recorded, it was encountered at five in extremely low numbers relative to those of *An. quadriannulatus* (Figure 4, sites., B,C,E,F&G) and co-existing in the same pools. The infrequent occurrence of *An. arabiensis* within the region is probably a result of the control programme using residual insecticides and the anthropophilic and endophilic tendencies of this species.

One of the major problems associated with the investigation of breeding sites in the field is the inability to assess separately the importance of the different parameters being measured. The numbers of eggs laid in oviposition bowls containing alkaline, neutral or acidic water are shown in Table 2. Two separate trials were conducted and similar results obtained in both. Significantly more eggs were laid in

the acidic oviposition water than in the neutral or alkaline water ($p < 0,001$). The number of eggs laid in the neutral and alkaline bowls were not significantly different. The largest number of eggs (2861) was laid in the bowl with a pH of 5,6 and the lowest (1307) in the bowl with a pH value of 7,3. These results although not conclusive, support the hypothesis that the tendency for *An. gambiae s.l.* to occur in alkaline waters is a product of the availability of waterbodies and that the apparent avoidance of those with low pH is an indirect result of some other characteristic such as high organic or iron content. The limitations of such simulatory laboratory experiments and the use of colony material should however be borne in mind.

Table 2. Number of eggs laid by colonised *Anopheles arabiensis* females in water of different pH.

	pH 3,5	pH 5,6	pH 7,3	pH 11,0
Trial one	1174	1432	481	491
Trial two	1687	1836	826	1031
Total	2861	3268	1307	1522

2. DISSOLVED OXYGEN

Muirhead-Thomson (1942) suggested that low dissolved oxygen levels may be a limiting factor for *An. minimus* immatures and Reiter (1978) suggested that this might be due to the rapid outward diffusion of gas from the larvae. The mean dissolved oxygen values recorded for the habitats used by the three species of the *An. gambiae* complex in this study are given in Table 1. The values recorded for *An. arabiensis* and *An. merus*

are similar (9.4 and 9.7 mg/litre, respectively), while that for *An. quadriannulatus* is slightly lower. These are high when compared to those recorded for six anopheline species by Iyengar (1929-1930) and for *An. minimus* by Muirhead-Thomson (1942). Iyengar also noted that dissolved oxygen in water-bodies containing mosquito larvae ranged from 1 to 7 mg/litre, but those in the 1 to 4 mg/litre range contained the greatest number of immatures. He also showed that dissolved oxygen levels within a pool were high during the day and low at night, due mainly to the respective photosynthetic and respiratory action of unicellular algae. Reiter (1978) concluded that inorder for larvicides which rely on anoxia to be effective the dO_2 level would have to be considerably below saturation. He found that good larvicidal activity against *An. gambiae s.l.* was obtained when the dissolved oxygen levels dropped below 20% for about six hours of the night. The results in Figure 5 were obtained from six pools containing *An. arabiensis* larvae (Figure 4, site A) and show that dO_2 levels drop rapidly after midday (to 65% at midnight) and rise steeply with sunrise (to 127% at midday). Larvicides operating by means of anoxia would, however, not be effective under those conditions since Reiter (1978) has shown that *An. gambiae s.l.* larvae could remain submerged for approximately 7,5 h at dO_2 levels of 30%.

The high dissolved oxygen values recorded in this study are probably a result of two factors. Firstly all the breeding occurred in open sunlit pools and secondly the majority of these tended to be small and shallow. Other larger and deeper pools available during summer were not used.

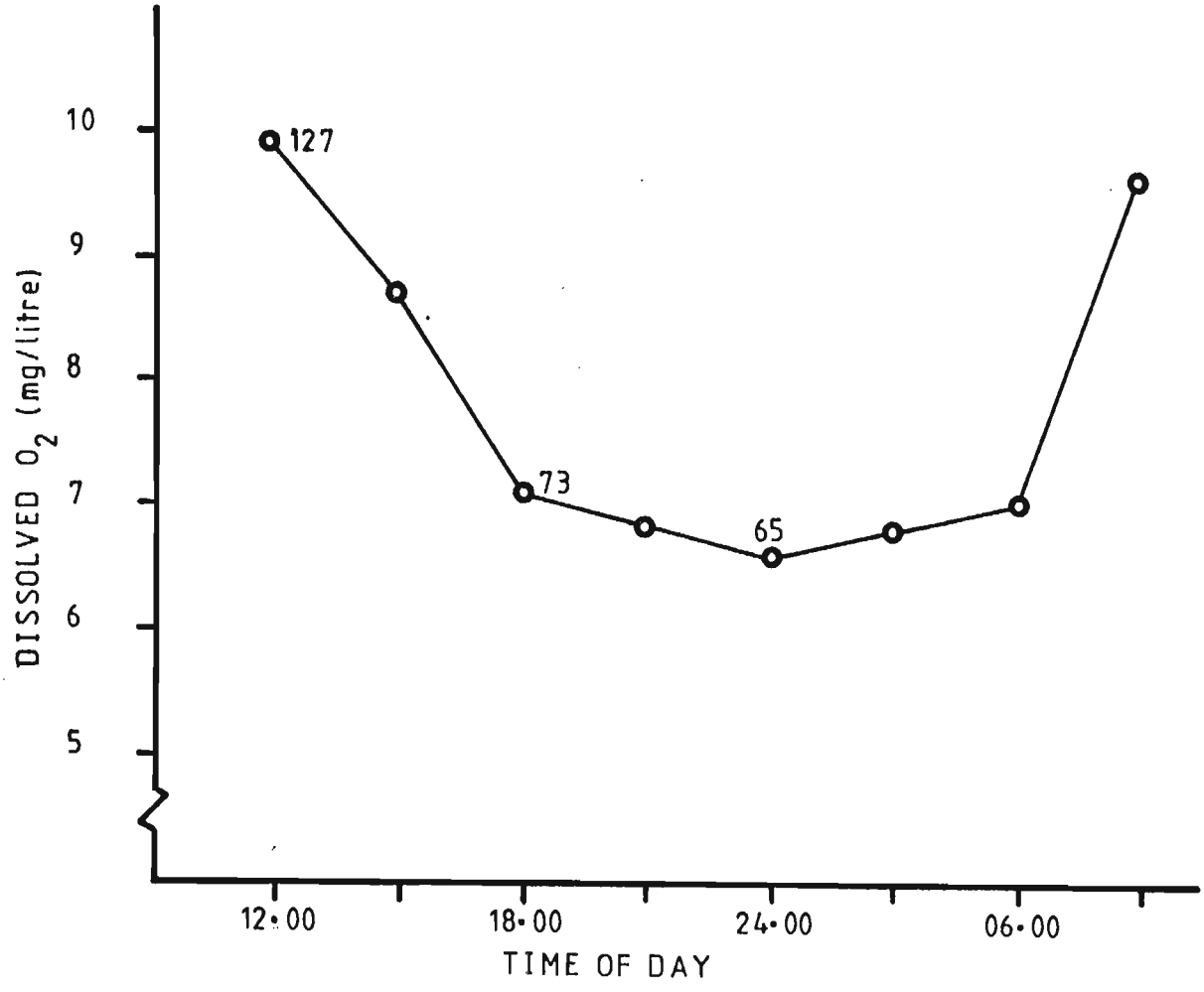


Figure 5. Daily fluctuation in dissolved oxygen levels in a waterbody containing *Anopheles arabiensis* immatures. Numerals adjacent to plotted points are the levels expressed as percentages of air saturation.

3. POOL SIZE

The mean pool size recorded for each species is given in Table 3.The winter breeding sites for *An. quadriannulatus* and *An. arabiensis*, as well as the rice fields, have been excluded and will be discussed later. On two occasions *An. quadriannulatus* larvae were recorded in larger pools, approximately 6 m in diameter. The results for all three species are similar and indicate that the pools used are characteristically small and shallow.

Table 3. The mean pool size (cm) recorded fro each of the species of the *Anopheles gambiae* complex.

Species	Length ± s.d.	Breadth ± s.d.	Depth ± s.d.
An. arabiensis	19,0 ± 9,2	14,0 ± 3,7	4,6 ± 1,1
An. merus	26,0 ± 8,4	18,0 ± 4,7	4,4 ± 1,8
An. quadriannulatus	27,4 ± 14,7	19,0 ± 12,9	3,1 ± 1,2

4. SALINITY

The mean salinity recorded for each species is given in Table 1. *An. arabiensis* immatures were found only in water which was totally fresh. *An. quadriannulatus* immatures were collected from waterbodies with a salinity greater than zero, but less than two p.p.t. or 5,7% sea water (SW) on three occasions. The mean salinity recorded for *An. merus* immatures was 14,7 p.p.t. (42% SW) and the maximum salinity 38 ppt (108% SW).

The highest field salinity recorded for what were presumably *An. merus* immatures is 205% SW (Jepson *et al.*, 1947). He recorded *An. gambiae s.l.* immatures at this salinity in a

salt lake in Mauritius but found no emergence from waters in excess of 185% SW. The most extensive study on the salinity tolerance and optima of *An. merus* was by Mosha & Mutero (1982). They demonstrated that adult populations within their study area peaked when the salinity of breeding sites was between 30 - 50% SW which compares well with the mean of 42% SW recorded in the present study. The maximum of 108% SW recorded in this study is slightly less than the 131% reported by Mosha & Mutero.

5. SHADE, VEGETATION AND TURBIDITY

Both the winter and summer breeding sites of *An. arabiensis* and *An. quadriannulatus* were totally unshaded or only received partial shade. The water was turbid in only 18% of the sites used by these two species. Gillies & De Meillon (1968) remarked that pools with suspended colloidal matter appear to be particularly favourable to *An.gambiae s.l.* This was also the case in Tongaland, but it is felt that the favourability was related to their temporary nature and the suspended colloidal matter purely a by-product of the substrate in which they had been formed. The suspended matter may be of nutritional value and thus enhance survival and growth.

The two freshwater members were only found in direct association with vegetation in the water, in winter sites. On three occasions they were however found in pools around the edge of which, grass (eg. *Cynodon dactylon*) was growing.

The water of all sites from which *An. merus* immatures were

collected was clear. Except for the saline marshes, the sites were also generally unshaded and free of vegetation.

6. ASSOCIATION WITH MAN

Gillies & De Meillon (1968) remarked on the association between the activities of man and the breeding sites of *An. gambiae s.l.* The sites investigated in this study were thus categorised into those which were due to the activities of man or of cattle and those which were natural. The results for the two freshwater member species are similar, (Table 4.) and many of the sites were formed as a result of the activities of man or his cattle. The sites used by *An. merus* showed a greater tendency towards being natural (64%), which may be a product of its saline breeding requirements and the consequent limitations placed on it.

Table 4. Classification of the breeding sites in the study by species and according to whether they were natural in origin or due to the activities of man or cattle.

Species	Number of localities	Natural (%)	Man (%)	Cattle (%)
An. arabiensis	6	37	26	37
An. merus	10	64	18	18
An. quadriannulatus	49	39	28	33

7. TEMPERATURE

Measurement of pool temperatures has little or no value unless correlated to season, time of day, pool size and shade. What is important however are the maximum and minimum temperatures and temperature profiles of breeding sites. The importance of these factors is their physiological effects in

terms of growth and mortality. The former is dealt with in Chapter 4.

Temperatures of all breeding sites were recorded (n = 197) to obtain an idea of the thermal tolerance of immatures of the complex and to ascertain whether it was a limiting factor within the region. The maximum pool temperature recorded in this study was 40°C at which no mortalities were observed. This observation concurs with that of Holstein (1954) in which larvae exposed to 41°C for six hours experienced the same mortality rate as a control maintained at 26°C. Significantly higher mortalities only occurred at temperatures of 42°C or more (exposure = 6 h). However exposure times of 12 hours did result in slightly higher mortalities even at temperatures < 42°C. In view of the fact that the pool temperature only remained at this level (40°C) for slightly in excess of one hour, it is not considered that thermal tolerance (maximum temperatures) posed a limiting factor within the study area. This does not take into account that these temperature result in extremely short generation times (Chapter 4) and may thus result in the production of small adults which have poor survivorship.

Some summer and winter temperature profile recordings are shown in Appendix 1. It is interesting to note that when the maximum temperature of 40°C was recorded, the shade, air temperature did not exceed 31.5°C. Also of interest is that the mid-summer/midday pool temperatures rarely exceeded 37°C. Although thermal tolerance to high temperature is not limit-

ing, the effect of these temperatures on evaporation rates, pool duration and thus larval mortalities is important. The two pools shown in Appendix 1 which dried out, both contained large numbers of larvae in all instars. The larvae remained in the drying mud at the bottom of the pool and many were still alive when water was added 5 hours later. de Meillon (1934) however showed that *An. gambiae s.s.* larvae in the Transvaal survived for less than 24 h on drying mud.

Mid-winter pool temperatures were rarely recorded below 13^{o}C, but often remained at 15^{o}C or less for more than 12 hours (Appendix 1). Holstein (1954) however demonstrated in the laboratory that *An. gambiae s.s.* larvae exposed to temperatures of 15^{o}C for 12 hours had higher mortality rates than those exposed for 1-6 hours (36% vs. 20%). It is however difficult to conclude anything from such studies conducted on insectary material as they ignore factors such as acclimation and the wider genetic spectrum available in field material. These studies were also carried out prior to the elucidation of the complex.

OTHER ANOPHELINES

The only other anopheline found in the same waterbodies as *An. merus* was *An. tenebrosus*. Of the sites utilised by *An. merus*, these two species only occurred sympatrically, in the saline marshes (section 2.3.4). The only other anopheline found in sympatry with the two freshwater members in temporary pools was *An. pretoriensis*. This sympatric occurrence was however rare and only recorded from 8% of the sites. In addition all these sites were located on the survey route

between points 39 and 17 on Figure 3. and during a two day survey conducted during January 1987. The fact that *An. pretoriensis* also favours temporary pools in this region was first noted by Swellengrebel *et.al.* (1931).

In the more permanent sites such as the winter sites and the irrigation overflow, the freshwater members were found in association with a number of other anophelines. A list of these is provided below;

Rice paddies	**Floodplain pans**	**Irrigation overflow**
An. coustani group	*An. pharoensis*	*An. pharoensis*
An. ziemanni	*An. coustani group*	*An. rufipes*
An. squamosus		
An. rufipes		

SITE DESCRIPTIONS

An. quadriannulatus occurred in the greatest range of sites within the study area (Table 5). This was probably related to the fact that it was the most common species within this region.

An. merus was found predominantly in association with exposed marine cretaceous deposits or with saline marsh areas. Where the deposits were exposed, the addition of rain water resulted in the formation of suitably saline pools. At certain localities (eg. Figure 3, site 2), the deposits were not exposed but horizontal leaching from the areas surrounding the lakes resulted in the accumulation of saline water in cattle

hoofprints at its edge. At some localities (Figure 4, site 4) this leaching process had resulted in the main waterbody of the lake being saline.

The saline marsh area studied was situated on the western shores of Lake St. Lucia (Figure 4, site 5) and the origin of the saline water was the lake itself. The predominant plant species within the marsh were *Phragmites australis, Juncus kraussi* and *Scirpus littoralis*.

Table 5. Description of the waterbodies used by the three species of the *Anopheles gambiae* complex occurring in Natal.

Site	Pool	An. arabiensis	An. quadriannulatus	An. merus
1. Natural pan	Main waterbody associated with aquatic macrophytes.	X	X	
	Cattle hoof-prints at edge.	X	X	X
	Seepage area at edge and hoof-prints.			X
2. Borrow pits	Cattle hoof-prints at edge.	X	X	
3. Riverbed	Sandy or rocky, pools formed as river dries up.		X	
4. Irrigation overflow	Natural depressions and cattle hoof-prints.	X	X	
5. Roadside pools	Runoff inhibited due to road construction.		X	
6. Natural springs			X	
7. Road	Vehicle tracks.	X	X	
8. Man-made wells			X	
9. River	Cattle hoof-prints at edge.	X	X	
10. Rice paddies			X	
11. Swamps	Swamp itself.			X
12. Seashore	Upper, inter-tidal zone, rock pools.			X
13. Exposed marine deposits	Natural water-filled depresssions.			X
14. Rain pools	Natural, temporary	X	X	

An. merus was also found breeding in rock pools in the upper inter-tidal zone (Figure 4, site 7). The pools were filled with sea water at spring high tide and then diluted by fresh-

water runoff from the coastal dune against which they were situated.

Sharp *et al.*,(1984) investigated a large *An. quadriannulatus* population at Mamfene (Figure 4, site D) breeding in overflow water from an irrigation scheme retention dam. They remarked on the absence of *An. arabiensis* at the time but pointed out that the two species occurred sympatrically at other localities within the endemic malaria area. In 1986 a large population of *An. arabiensis* arose at this locality, which was the site of the most intense *An. arabiensis* breeding encountered during the course of the present study. This region was subsequently responsible for the largest number of malaria cases recorded from any area within Natal, during 1987.

Situated approximately four km from Mamfene is a 35 ha experimental rice project. Only *An. quadriannulatus* immatures have been found in the rice field to date. The extensive use of rice fields by *An. arabiensis* in Kisumu, Kenya, has been documented (Service, 1973; Surtees *et al.*,1970). The absence of this species is thought to be due to the fact that the period when the rice fields are most suitable for breeding coincides with the time when populations are not well established. The seasonal increase in populations occurs when the rice fields are already densely vegetated. This situation may however be altered by the introduction of a second crop each season. (Note: This was the case at the time of publication (1988) however *An. arabiensis* are now found in the experimental rice paddies. The degree of utilisation is presently

being assessed).

SEASONAL SITE DIFFERENCES

When undertaking a study of this nature it is necessary to distinguish between wet and dry season sites. During the dry winter season, the temporary pools formed on exposed marine cretaceous deposits dry up and *An. merus* is confined to the more permanent sites such as the edge of pans and saline marshes. Thus breeding may be confined to sites which are less conducive to the completion of the development cycle. Similarly, the fresh water members are concentrated in the floodplain pans and disused borrow-pits. The formation of pools in the beds of receeding annual rivers, also play a role during early winter.

De Meillon, (1934) distinguished between regions of the Transvaal in which *An. gambiae s.l.* occurred all year round and those in which they only occurred during the summer months. A similar expansion and contraction of *An. gambiae s.l.* populations occurs in Natal. Within Tongaland, populations are present throughout the year and expansion and contraction occurs on a more localized basis. During the summer months *An. arabiensis* and *An. quadriannulatus* can be found in many of the temporary sites listed in Table 5. During winter the temporary sites dry up and breeding is confined to more permanent, marginal sites such as the floodplain lakes and disused borrow-pits.

The disused borrow-pits provide waterbodies which retain water throughout the dry winter season, and immatures occur

in water filled cattle hoofprints around the edge. These borrow-pits occur at regular intervals next to roads and thus facilitate the winter distribution of *An. gambiae* complex members, within the region. The use of the floodplain lakes was investigated at Namanini Lake (Figure 3, site 7). During the winter months, *An. quadriannulatus* and *An. arabiensis* immatures were found in association with dense macrophyte growth in the lake. The predominant plant species found was *Potamageton crispus*. Other macrophytes were *Cynodon dactylon* and *Ludwigia stolonifera*.

The growth pattern of *P. crispus* together with rainfall data and run of wind are shown in Figure 6. The growth of *P. crispus* and the decrease in wind result in the stabilization of the lake surface. This, together with the formation of suitable "pools" (Appendix 2, plate 5) within the dense macrophyte growth provide suitable winter sites for *An. quadriannulatus* and *An. arabiensis*. Thus as the temporary breeding sites within the region dry up, a suitable breeding habitat becomes available in the lakes. Conversely, *P. crispus* dissapears at the onset of rains, when the temporary breeding sites reappear. The main waterbody of the lake is not used during the summer.

These winter sites may be considered marginal and merely serve as a reservoir for the species until more suitable waterbodies become available with the onset of rains. De Meillon (1938) demonstrated similar usage of water cisterns by the *An. gambiae s.l.* in Mocambique. Swellengrebel *et al.* (1931) conducted a study in the same region and classified

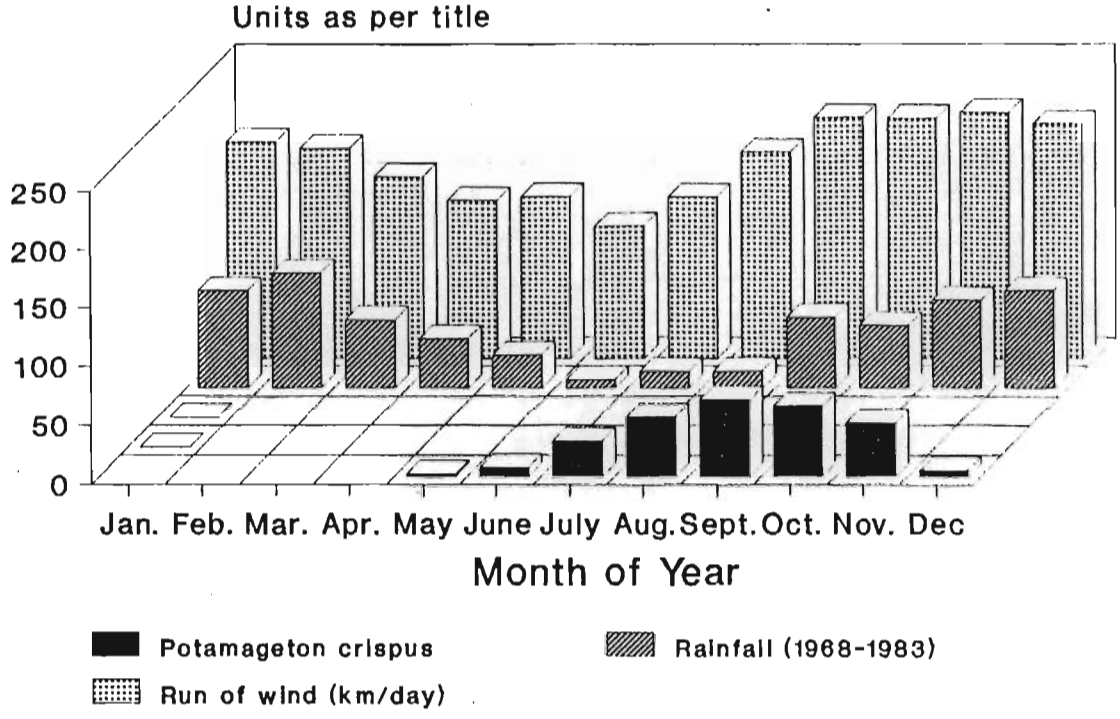


Figure 6. The mean monthly growth of *Potamageton crispus* (1986/87), at Namanini lake. Annual rainfall (mm) and run of wind (km/day) for the period 1968-1983.

'An. gambiae (costalis)' as a "puddle breeder". This study was however carried out in summer and thus excluded seasonal site differences.

CONCLUSIONS

The following conclusions may be drawn from this study;

1. Within the limitations placed by factors which are inimical to the development of immatures, species of the *An. gambiae* complex in Tongaland are highly opportunistic and use a wide range of waterbodies as breeding sites.

2. The summer sites are characteristically small, sun-lit, free of vegetation and may or may not be turbid.

3. That although the temporary nature of many of the sites may be advantageous in terms of reduced predation, their temporary nature may reduce survivorship due to their limited duration.

4. The saline marshes used by *An. merus* are an exception to 2 above.

5. The tendency for species of the *An. gambiae* complex to occur in alkaline waters is probably a product of what is available and their apparent avoidance of acidic waterbodies a result of some other factor.

6. Water with a high iron content appears to be inimical to the freshwater species in the study area.

7. Larvicides operating by means of anoxia would be ineffective against *An. gambiae s.l.* immatures in the majority of the breeding sites investigated.

8. The requirements of the two freshwater species is

essentially the same, and the difficulty with the location of *An. arabiensis* breeding sites is a product of the residual insecticide control programme.

9. The difference in breeding sites between *An. merus* and the freshwater species is largely a result of its saline requirements.

10. The optimal salinity for *An. merus* development is approximately 14 p.p.t. (40% sea water).

11. The occurrence of *An. gambiae s.l.* immatures in water with a salinity of 5 p.p.t. (14% sea water) or more in Natal indicates with a high degree of certainty that they are *An. merus*.

12. It is important to distinguish between summer (wet) and winter (dry) season sites.

13. The floodplain lakes and disused borrow-pits play an important role in maintaining a winter reservoir of the two freshwater members.

14. Entomological consultation should go hand in hand with agricultural development in malarious areas.

REFERENCES

Bruton, M.N. (1980). Introduction.-pp. xvii-xix in Bruton, M.N. & Cooper, K.H. (Eds.). The ecology of Maputaland. 560 pp. Rhodes University and the Natal Branch f the Wildlife Society. Cape Town.

Coluzzi, M. (1968). Cromosomi plitineci delle cellule nutrici ovariche nel complesso *gambiae* del genere *Anopheles*. -*Parassitologia*. **10**, 179-183.

De Meillon, B. (1934). Observations on *Anopheles funestus* and *Anopheles gambiae* in the Transvaal.-*Publ. S. Afr. Inst. Med. Res.* **6**, 195.

De Meillon, B. (1938). A note on *Anopheles gambiae* and *Anopheles coustani* var. *tenebrosus* Donitz from South African.-*Publ. S. Afr. Inst. Med. Res.* **12**, 648.

Gillies, M.T. & De Meillon, B. (1968). The Anophelinae of Africa south of the Sahara.-*Publ. S. Afr. Inst. Med. Res.* **no. 54**, 343 pp.

Gillies, M.T. & Coetzee, M. (1987). A supplement to the Anophelinae of Africa south of the Sahara.-*Publ. S. Afr. Inst. Med. Res.* **no. 55**, 143 pp.

Hancock, G.L.R. (1934). The mosquitoes of Namanve Swamp, Uganda.-*J. Anim. Ecol.* **3**, 204-222.

Heeg, J. & Breen, C.M. (1982). Man and the Pongola floodplain.-South African National Scientific Programme Report. **no. 56**, 117 pp.

Holstein, M.H. (1954). Biology of *Anopheles gambiae*, Research in French West Africa.-World Health Organisation, Geneva. 172pp.

Hunt, R.H. (1973). A cytological technique for the study of

the *Anopheles gambiae* complex.-*Parassitologia.* 15, 137-139.

Hunt, R.H. & Coetzee, M. (1986). Field sampling of *Anopheles* mosquitos for correlated cytogenetic, electrophoretic and morphological studies.-*Bull. Wld Hlth Org.* **64**, 897-900.

Iyengar, M.O.T. (1929-1930). Dissolved oxygen in relation to *Anopheles* breeding.-*Ind. J. Med. Res.* 17, 1171-1186.

Jepson, W.F., Moutia, A. & Courtois, C. (1947). The malaria problem in Mauritius: the bionomics of Mauritian *Anopheles*.-*Bull. ent. Res.* **38**, 177-208.

Mahon, R.J., Green, C.A. & Hunt, R.H. (1976). Diagnostic allo zymes for routine identification of adults of the *Anopheles gambiae* complex (Diptera:Culicidae).-*Bull. ent. Res.* **66**, 25-31.

Maud, R.R. (1980). The climate and geology of Maputaland.-pp. 1-7 in Bruton, M.N. & Cooper, K.H. (Eds.). The ecology of Maputaland.-560 pp. Rhodes University and the Natal branch of the Wildlife Society of Southern African. Cape Town.

Miles, S.J. (1978). Enzyme variation in the *Anopheles gambiae* Giles group of species (Diptera: Culicidae).-*Bull. ent. Res.* **68**, 85-96.

Miles, S.J. (1979). A biochemical key to adult members of the *Anopheles gambiae* group of species (Diptera: Culicidae).-*J. Med. ent.* **15**, 297-299.

Mosha, F.W. & Mutero, C.M. (1982). The influence of salinity on larval development and population dynamics of *Anopheles merus* Donitz (Diptera: Culicidae).-*Bull. ent. Res.*

72, 119- 128.

Muirhead-Thomson, R.C. (1942) Studies on the behaviour of *Anopheles minimus*. Part VII. Further studies on the composition of the water in breeding places and the influence of organic pollution.-*J. Mal. Inst. Ind.* **4**, 595-6

Paterson, H.E., Paterson, S.J. & van Eeden, G.J. (1963). A new member of the *Anopheles gambiae* complex. A preliminary report.-*Med. Proc.* (Med. Bydraes). **9**, 414-418.

Pomeroy, A.W.J. (1931). A report on the mosquito and tsetse problem at Takordi, 1930-1931. in Report of the Medical Department, Gold Coast, Accra, 1930-1931. 101 pp.

Reiter, P. (1978). The influence of dissolved oxygen content on the survival of submerged mosquito larvae.-*Mosq News*. **38**, 334-337.

Service, M.W. (1970). Ecological notes on species A and B of the *Anopheles gambiae* complex in the Kisumu area of Kenya.-*Bull. ent. Res.* **60**, 169-180.

Service, M.W. (1973). Mortalities of the larvae of the *Anopheles gambiae* Giles complex and detection of the predators by the precipitin test.-*Bull. ent. Res.* **62**, 350-369.

Service, M.W. (1977). Mortalities of the immature stages of species B of the *Anopheles gambiae* complex in Kenya: comparison between rice fields and temporary pools, identification of predators, and effects of insecticidal spraying.-*J. Med. ent.* **13**, 535-545.

Sharp, B.L., Quicke, F.C. & Jansen, E.J. (1984). Aspects of the behaviour of five anopheline species in the endemic malaria area of Natal.-*J. ent. Soc. S. Afr.* **47**, 251-258.

Sharp, B.L., Ngxongo, S., Botha, M.J., Ridl, F.C. & le Sueur, D. (1988). An analysis of 10 years of retrospective malaria data from the KwaZulu areas of Natal.-*S. Afr. J. Sci.* **84**, 102-106.

Stuckenberg, B.R. (1969). Effective temperature as an ecological factor in Southern African.-*Zoologica Africana.* **4** (2), 145-197.

Surtees, G., Simpson, D.I.H., Bowen, E.T.W. & Grainger, W.E. (1970). Rice field development and arbovirus epidemiology, Kano plains, Kenya.-*Trans. R. Soc. trop. Med. Hyg.* **64**, 511-518.

Swellengrebel, N.H., Annecke, S. & De Meillon, B. (1931). Malaria investigations in some parts of the Transvaal and Zululand.-*Publ. S. Afr. Inst. Med. Res.* IV, 245-274

White, G.B. (1973). Comparative studies on sibling species of the *Anopheles gambiae* Giles complex (Dipt., Culicidae). III. The distribution, ecology, behaviour and vectorial importance of species D in Bwamba county, Uganda, with an analysis of biological, ecological, morphological and cytogenetical relationships of Ugandan species D.-*Bull. ent. Res.* **63**, 65-97.

White, G.B. (1974). *Anopheles gambiae* complex and disease transmission in African.-*Trans. R. Soc. trop. Med. Hyg.* **68**, 278-301.

White, G.B. & Rosen, P. (1973). Comparative studies on sibling species of the *Anopheles gambiae* Giles complex (Dipt., Culicidae). II. Ecology of species A and B in savannah around Kaduna, Nigeria, during transition from wet to dry season.-*Bull. ent. Res.* **62**, 613-625.

APPENDIX 1

Temperatures recorded at Mamfene November 1984

Time	Shade Temp. (Air)	Pool 1 Surface	Bottom	S.	B.	S.	B.	S.	B.
05h30	23	23	23.5	24	24.5				
06h00	23	25	23.5	24	24.5	23	23.5	23	24
07h00	24	24	24	25.5	25.5	24.5	24.5	24	24
08h00	25	26	25.5	27	27	27.5	27	27	26
09h00	29	29	28.5	30.5	30	32	31	31.5	28
10h00	30	30.5	29.5	33	32.5	33	33	33.5	30.
11h00	30.5	33	32	39.5	38.5	37	36	38	34
12h00	31.5	34	33.5	39.5	39.5	pool dry		37	35
13h00	31.5	33	33	40	40			38	36.
14h00	31.5	pool dry		40	40			38	37.
15h00	31.5			38	38			37	36.
16h00	31			35.5	35.5			35	35
17h00	29.5			33	33			33	33.
18h00	28			31	31			31	32

Pools one and two were open pools with no shade and no vegetation. Pool three had grass growing in it. Pool four was slightly shaded. The pools were temporary pools formed in vehicle tracks and the larvae were identified as *An. quadriannulatus.*

Maximum pool temperatures recorded during mid-summer surveys

Time	Temperature	Locality	Pool Type	Photo no.
		13th January 1986		
12h35	32.5	pt.35 fig.1	Rocky riverbed	
14h10	32	pt.32 fig.1	Semi- continuous } edge of small pan }	3 & 4
	37.6	"	Small pool at edge}	
15h30	32.5	pt.31 fig.1	Hoofprints at edge of borrow pit	5
		14th January 1986		
12h20	35.4	pt.27 fig.1	Hoofprints at edge of borrow pit	-
13h30	35.8	pt.25 fig.1	Hoofprints at edge of small stream	-
14h45	33.2	pt.22 fig.1	Sandy riverbed, shallow edge of stream	6 & 7
16h10	36.7	pt.17 fig.1	Hoofprints at edge of stream	-

Winter temperature profiles recorded at Ophansi July 1986

Time	Day 1		Day 2		Day 3		Day 4		Day 5	
	air	pool	air	pool	air	pool	air	pool	air	pool
08h00	12.1	13.7	18.4	12.3	17.4	14.0	17.2	15.6	15.7	14.1
09h00	*14.5*	*14.3*	*17.7*	*17.7*	*19.2*	*15.9*	*18.5*	*15.6*	*19.8*	*15.5*
10h00	*14.7*	*13.7*	*18.4*	*15.7*	*20.0*	*17.1*	*19.4*	*17.6*	*21.3*	*17.6*
11h00	*13.5*	*14.1*	*19.1*	*15.4*	*20.5*	*17.7*	*20.8*	*18.3*	*21.9*	*20.4*
12h00	*13.5*	*14.4*	*18.5*	*15.8*	*20.3*	*18.4*	*20.3*	*18.8*	*23.3*	*21.2*
13h00	*15.0*	*14.7*	*17.7*	*15.4*	*20.0*	*18.4*	*20.9*	*18.5*	*23.2*	*21.4*
14h00	*15.0*	*14.5*	*17.4*	*16.1*	*19.5*	*17.5*	*20.6*	*18.8*	*24.0*	*21.5*
15h00	*15.7*	*14.5*	*16.1*	*15.5*	*19.5*	*17.9*	*20.4*	*17.9*	*24.7*	*21.0*
16h00	*15.4*	*13.8*	*17.7*	*15.5*	*18.2*	*17.2*	*19.8*	*17.0*	*22.6*	*20.4*
17h00	*15.4*	*12.6*	*16.9*	*14.2*	*18.4*	*15.6*	*18.8*	*17.0*	*21.6*	*18.8*
18h00	*15.0*	*12.6*	*16.7*	*14.5*	*16.6*	*16.2*	*18.6*	*16.0*	*18.7*	*18.3*
19h00	*15.0*	*12.9*	*16.6*	*13.7*	*16.1*	*15.7*	*17.5*	*16.2*	*17.9*	*17.2*
20h00	*14.7*	*12.1*	*16.2*	*14.0*	*16.3*	*15.3*	*17.5*	*15.5*	18.1	17.7
21h00	14.9	12.1	15.8	14.0	16.0	15.0	17.5	16.1	16.0	16.6
22h00	14.9	13.1	15.2	14.3	15.3	15.6	16.1	15.8	15.3	17.2
23h00	15.6	12.5	14.5	13.9	14.4	14.9	15.8	15.0	15.0	16.1
24h00	14.8	12.0	14.7	13.5	14.6	15.1	14.8	14.7	14.3	15.4
01h00	15.2	12.4	14.4	13.8	15.5	15.5	14.5	13.3	13.6	15.6
02h00	15.8	12.4	15.0	13.4	15.5	14.8	14.7	14.9	**16.6**	**16.3**
03h00	15.8	12.4	15.2	13.1	16.1	15.4	14.9	15.5	**15.6**	**15.9**
04h00	15.5	11.7	15.2	13.7	15.8	15.0	13.4	14.8	**15.9**	**15.5**
05h00	14.5	12.4	15.2	13.3	16.6	15.0	13.0	13.9	**15.5**	**16.2**
06h00	15.5	12.3	15.5	13.3	16.6	15.0	11.5	13.8	**15.5**	**15.7**
07h00	17.7	14.7	19.2	15.9	18.5	15.6	19.8	15.5	**15.4**	**16.0**

The pool temperatures are those from one of the typical pools of the breeding site shown in Appendix 2 (plate 18). The air temperature represents the shade temperature. The increase in temperatures (in bold) at 02h00 on day five were due to the wind dropping and the formation of cloud cover. The temperatures shown in italics represent a period when cloud cover was continuously present, which resulted in temperature stabilisation.

APPENDIX 2

Pictorial record of the type of sites utilised by freshwater members (*Anopheles arabiensis* & *Anopheles quadriannulatus*)

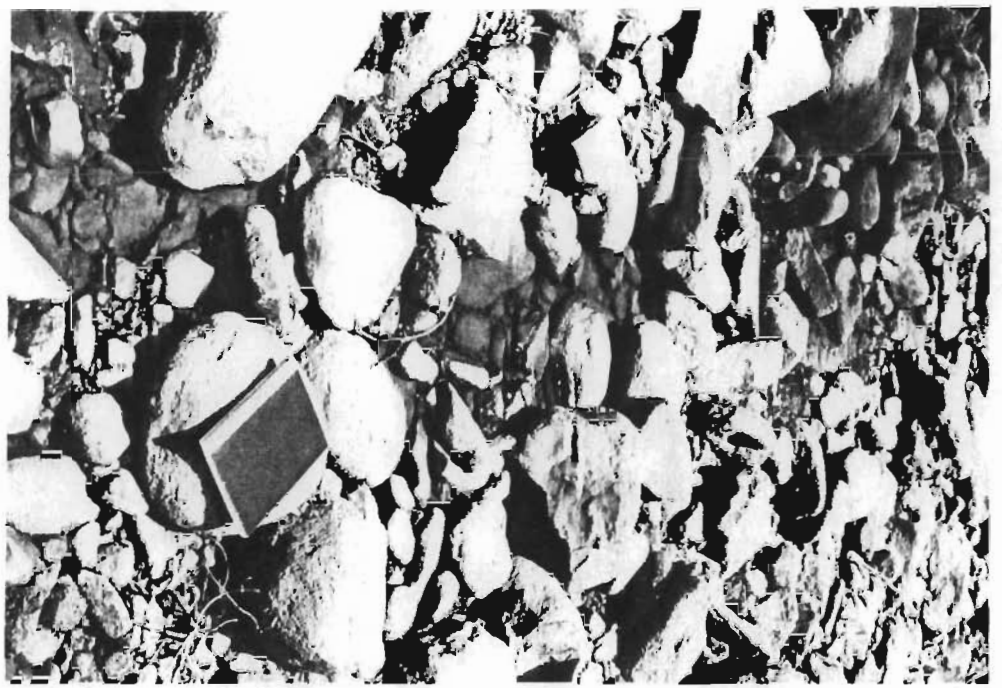
SUMMER SITES

Plate 1.

Rocky river bed. Larvae were collected from temproary pools formed due to collection of water in depressions on flat rock.

Plate 2.

Close-up picture of same river bed show edge of stream, where water flow was minimal and larvae were present.



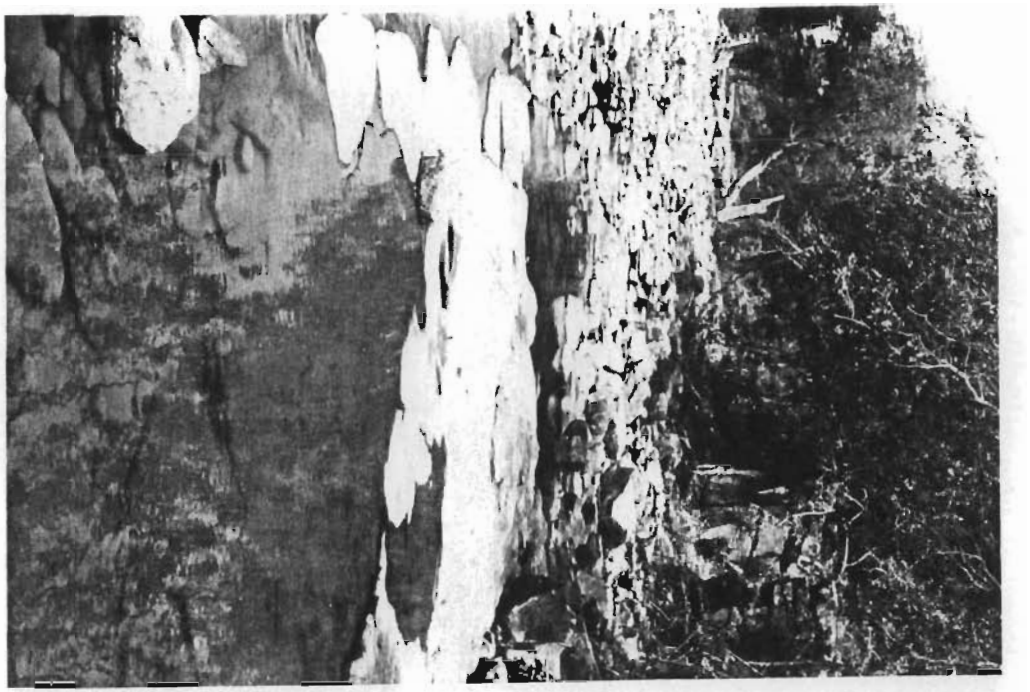


Plate 3 & 4

Small temporary lake. Larvae were found in pools formed at the edge of the lake, due to the activity of cattle. The pools were either seperate from or semi-continous with the main waterbody. This site was unusual in that the pan edge had extensive grass growth. The edge of the majority of such lakes were free from vegetation, as shown in plate 9.

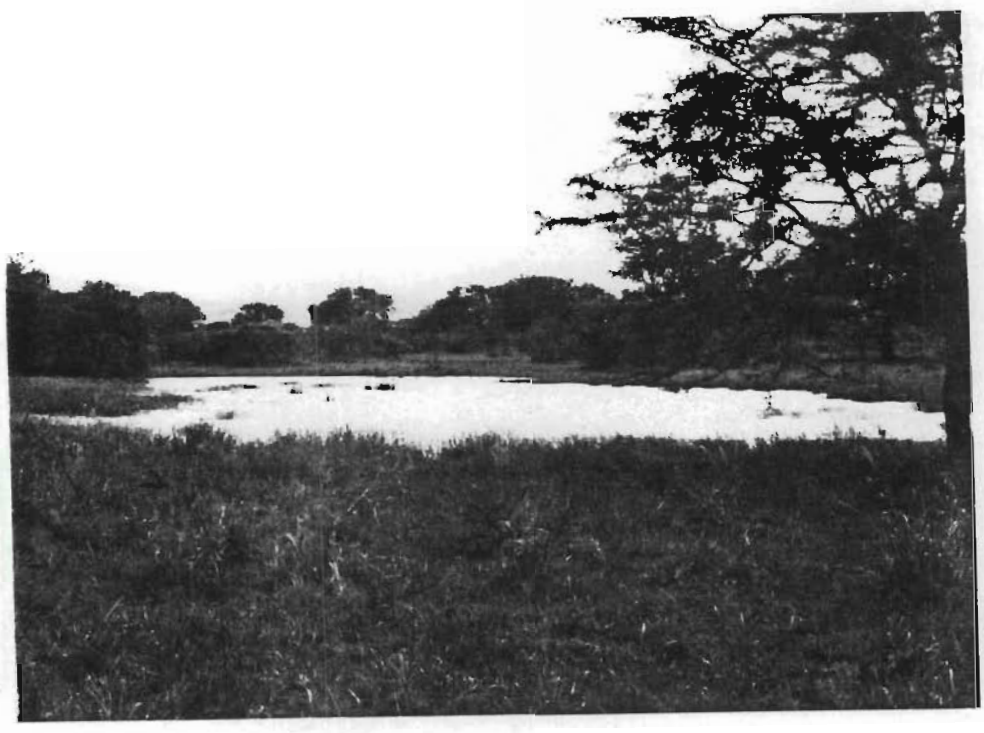


Plate 5

Dis-used borrow pit. Larvae were collected from hoofprints located at the edge of the water. These site could be classified as temoprary or permanent, depending on how much water collected in them and for how long they persisted.

Plate 6 & 7.

Small stream with sandy bed. Larvae were collected form the shallow edges which had a depth of approximately one centimetre and where water flow was almost non-existent.

Plate 8.

Roadside pools. These were fairly variable in appearance, but were usually formed due to road construction having inhibited the natural run off of water. The activity of cattle often resulted in these being broken into numerous small pools.

Plate 9.

Typical small temporary lake with edges free of vegetation. These sites were common and large numbers of larvae were often encountered in hoofprints at the waters edge.

Plate 10.

Vehicle tracks. Once again smaller "edge" pools were often formed by cattle hooves.

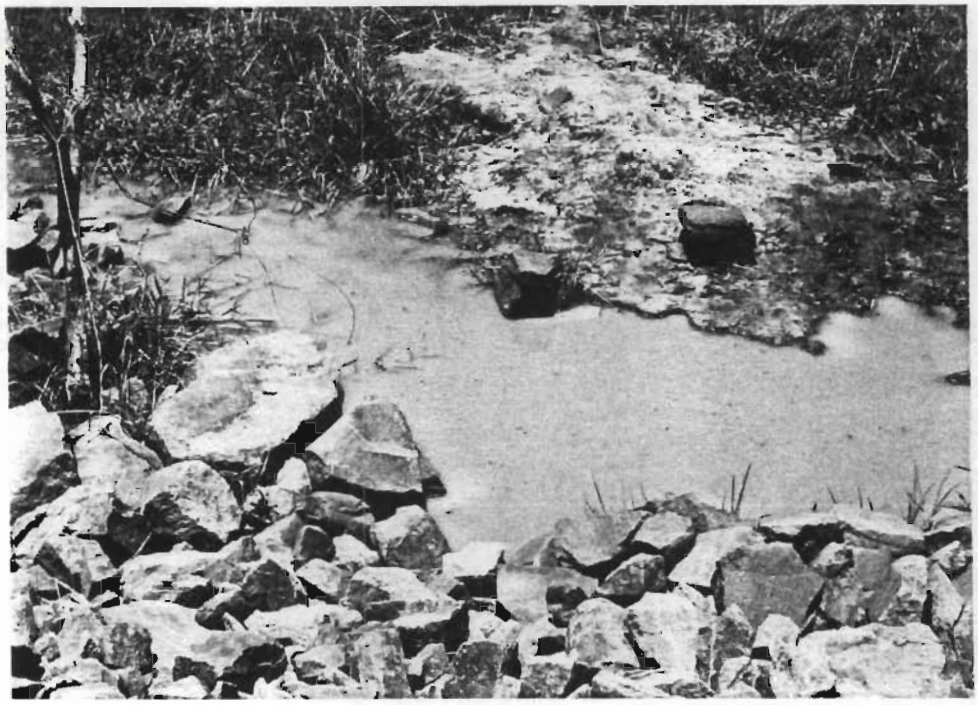


Plate 11 & 12

Small temporary stream, with muddy substrate. Larvae were collected from the shallows and small pools located at the edge.

Plate 13.

Rice paddies. The number of larvae present was fairly high during the early stages when the plants were small and the water fairly exposed. The occurrence of *An. gambiae* complex larvae was however almost negligible once the plants had reached an advanced stage of growth as shown here. A single crop was planted in November and reaped in March.

Plate 14.

Small natural spring, approximately one metre in diameter. Larvae were collected from the main pool.

WINTER SITES

Dis-used borrow pits (plate 5) were also included amongst these.

Plate 15.

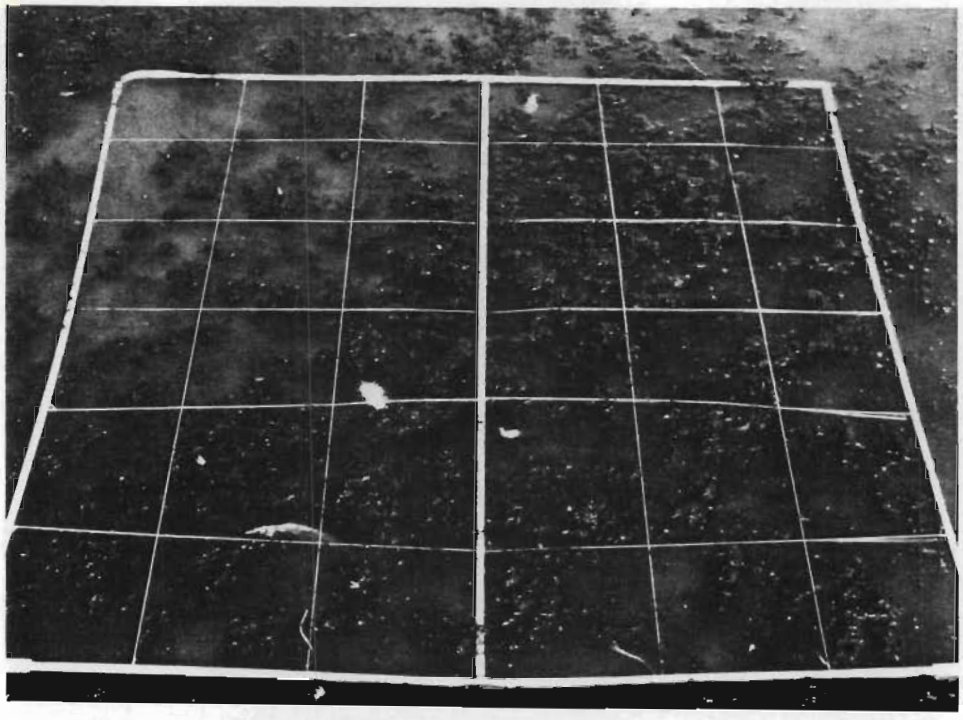
Photograph showing dense growth of Potamageton crispus which occurs in non-saline pans during winter months. The grid shown was used to estimate plant growth and site utilisation. Note the small pools formed within the macrophyte growth. This photograph was taken at Namanini pan, which has a surface area of approximately 2 kilometres^2.

Plate 16.

Man made wells. These wells are dug by local people to provide water.

Plate 17.

Irrigation overflow. Overflow from an irrigation retention dam results in flooding of low lying areas adjacent to a natural stream. This overflow occurs periodically and results in the formation of temmporary pools such as those shown here.



Pictorial record of the sites utilised by *An. merus*.

Plate 18.

Seepage area at edge of pan. Numerous small pools have been formed by cattle hooves. This site is a permanent site and was used in the overwintering an survivorship study reported in chapter 5.

Plate 19.

This photograph shows an area where the pools have dried out and the saline nature of the pool waters is evident from the deposited salt.

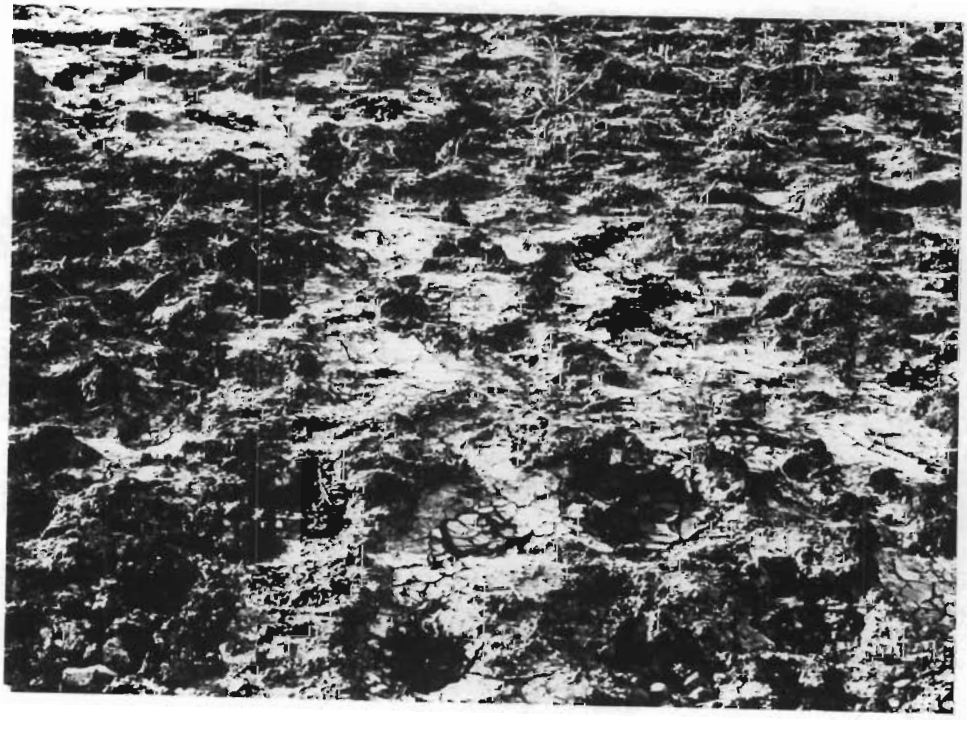


Plate 20.

Exposed marine cretaceous deposits which are saline in nature. The addition of rainwater to these results in the formation of saline pools. The pools in the foreground were formed in vehicle tracks.

Plate 21.

Saline marsh. Extensive breeding of *An. merus* occurs within these marshes, which may persist throughout the winter period.

CHAPTER 3

LARVAL MORPHOMETRICS

INTRODUCTION

The inclusion of the morphometrics of mosquito larvae in this study was considered important for reasons relating to;

1. The accurate separation of larval instars.
2. The possible identification of morphological criteria separating individual member species of the complex.

3.1 LARVAL INSTAR SEPARATION

Note: The contents of this section (3.1) comprise a manuscript submitted for publication.

TITLE.

Separation of larval instars in three members of the *Anopheles gambiae* Giles Complex in Southern Africa.

ABSTRACT

Criteria for the accurate separation of the larval instars of three species of the *Anopheles gambiae* Giles complex were determined. The three species are; *An. arabiensis, An. quadriannulatus* and *An. merus*. The findings were applicable to areas in which the mean annual range in temperature did not exceed $8^{o}C$. In areas where this range is exceeded, overlap

between instars may occur and reliability be reduced. The importance of accurate instar separation is discussed.

INTRODUCTION

The ability to distinguish the four larval instars of mosquitoes is important for a number of reasons. Identification of larval instars forms the basis of studies on larval population dynamics since certain techniques such as the estimation of survivorship are dependent upon it (Service 1970, 1973 & 1977). Accurate instar determination is also necessary to calculate the precise larvicide dosages needed during toxicological experiments. Sharp (1983) extended the first instar salinity tolerance test developed by Muirhead-Thomson (1951) to later instars. The increasing physiological salinity tolerance of instars II - IV which he demonstrated, however, necessitates accurate larval instar separation. Larval keys are based on fourth instar larvae (Gillies & Coetzee, 1987) and to avoid possible confusion it is important that these are reliably distinguished from earlier instars. Discussion with local entomologists elucidated the fact that identifications were often based upon relative body size.

A review of the literature concerning the methods of larval instars separation for the *Anopheles gambiae* Giles complex revealed only two reports: the first was conducted by Soper & Wilson (1943) and is based on the extent of branching of the palmate hairs of the fifth abdominal segment; the second is a report by Christie (1954) in which he states that the head capsule can be reliably used. However both these studies lack

specific identification and neither state the accuracy and limits of the respective techniques. Le Sueur & Sharp, (1991) have shown that the mean head capsule width of *An. merus*, Donitz within instars is significantly different for samples collected in winter and summer, in Natal, South Africa. The World Health Organisation (W.H.O.) manual of practical entomology (1975), in reference to larval instar separation, refers to a figure in which it shows the heads of the four instars, labels the egg breaker (1st. instar) and collar. However no further explanation is given in the text or figure. The present study reports on criteria investigated for the separation of the larval instars on *An. merus*, *An. arabiensis* Patton and *An. quadriannulatus*.

MATERIALS AND METHODS

ORIGIN AND COLLECTION OF MATERIAL

The origin of the material utilised was as follows;

1) *An. quadriannulatus* colony, South African Institute Medical Research (SAIMR) - Constantia, N.E. Transvaal ($23^{o}35'S$, $30^{o}35'E$).

2) *An. arabiensis* colony, (RIDTE) - Kanyemba, N. Zimbabwe ($15^{o}40'S$ $30^{o}20'E$).

3) *An. arabiensis*, field material - Dondotha, Natal ($28^{o}34'S$ $31^{o}56'E$).

4) *An. merus* colony (RIDTE) and field material - Ophansi, Natal ($27^{o}36'S$ $32^{o}16'E$).

The *An. merus* field material was collected from pools located at the edge of Nceswana pan (Ophansi). Larvae were col-

lected in waters with salinity ranging between 8 and 38 ppt (salinity of sea water = 34,5ppt). Over a 12 month collection period , no other anopheline besides *An. merus* was found breeding in these pools. Identifications were carried out using cytogenetic (Coluzzi, 1968; Coluzzi & Sabatini, 1969; Green, 1972; Green & Hunt, 1980; Hunt, 1973), electrophoretic (Mahon *et al.*, 1976; Miles, 1978), and salinity tolerance methods (Muirhead-Thomson, 1951).

The *An. merus* colony was established from gravid females collected from cattle kraals and pit shelters in the immediate vicinity of Nceswana pan. The individual family groups were identified by means of salinity tolerance tests and confirmed electrophoretically before consolidation. Larvae were initially maintained in distilled water for a period of approximately two years and subsequently in water made up to a salinity of 10ppt using NaCl. The colony material used in this study had been colonised for 74 generations. It was maintained at a constant temperature of 27^{o}C and a relative humidity greater than 80%.

The *An. arabiensis* field sample was from 16 family groups, with not more than 5 F1 individuals of each instar being used from each. Identifications were based on electromorph frequencies, and seven of these were confirmed chromosomally.

A priori separation of instars was as follows: Colony material was separated on the presence of larval skins after moulting, individuals having been maintained in individual wells

of a micro-titer plate. Field material was separated using the characteristics of the palmate hairs on the 5th abdominal segment.

EXPLANATION OF MEASUREMENTS.

The measurements utilised in this study are given below. The terminology (Fig 5.) follows Harbach and Knight (1980).

1) Head capsule width = distance between the extremities of the lateralia, when viewed either dorsally or ventrally.

2) Head capsule length = distance between the median labral plate and the base of the coronal gap.

3) Abdomen length = distance between the anterior extremity of the prothorax and the base of the ventral brush.

4) Thorax width = the distance across the extremities of the mesothorax.

5) Collar width = the width of the ventral aspect of the collar in line with the hypocranial ecdysial line.

RESULTS

HEAD MORPHOLOGY

The head morphology of *An. merus* larvae is depicted in Figure 7. Immediately after moulting the collar was totally absent in the first three instars. Fourth instar larvae had a rudimentary collar, which was visible immediately after moulting. The collar then grew progressively throughout the instar

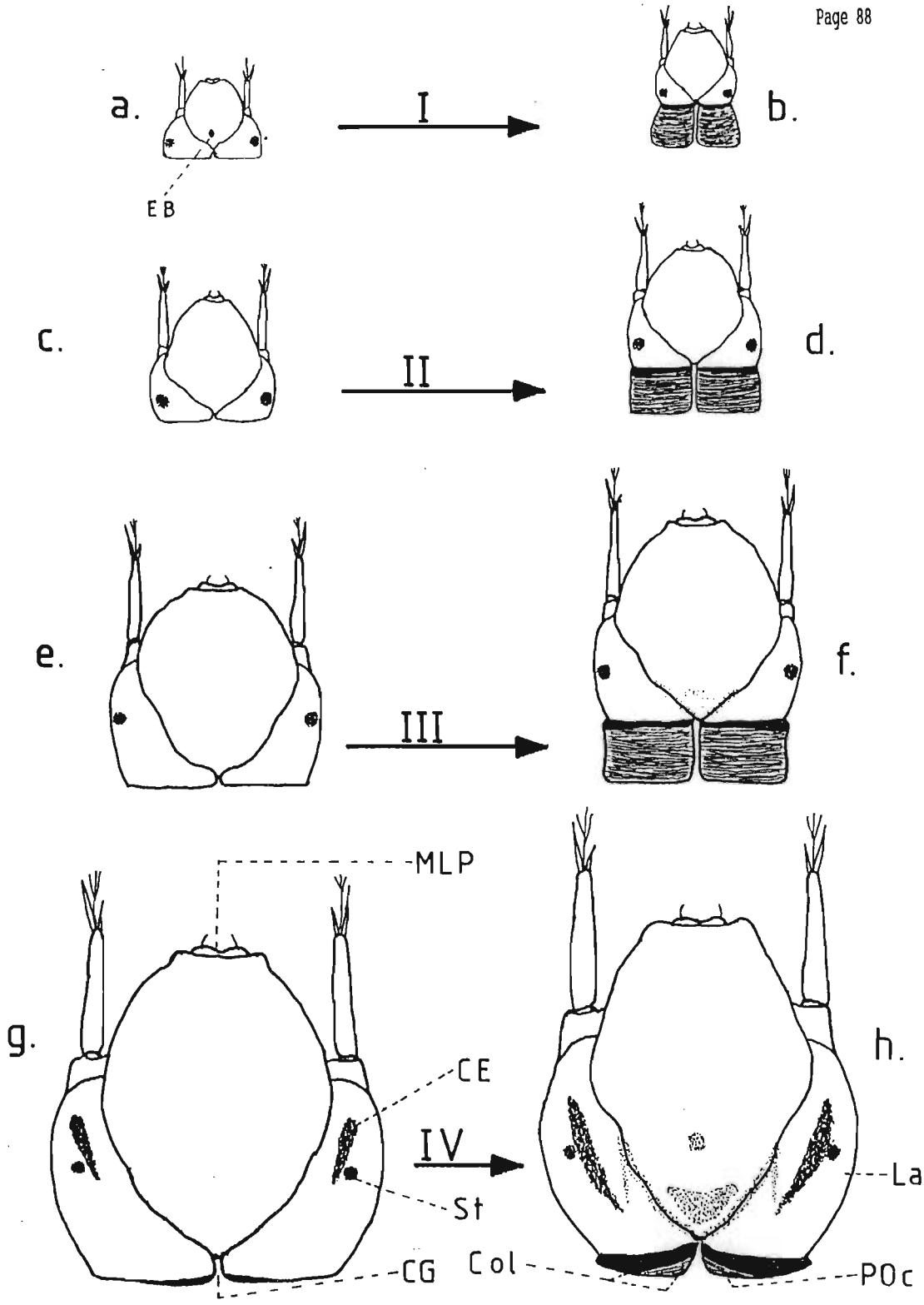


Figure 7. *An. merus* head capsule development for instars I to IV.

a,c,e & g = head capsule just after hatching/moulting
b,d,f = head capsule just prior to moulting
h = head capsule just prior to pupation

CE = Compound eye
MLP = Median labral plate
St = Stemma
CG = Coronal Gap
Lat = Lateralia
P = Pigmentation
Col = Collar
POc = Postocciptal region of colla
EB = Egg breaker

duration and the terminal appearance of the collar in each stage prior to moulting is shown (Figure 7). In fourth instar larvae the postoccipital region was rudimentary and did not extend around the sides of the collar.

The head capsule was initially translucent after hatching or change of instar. However it gradually darkened and certain areas became heavily pigmented (Figure 7h). The pigmentation pattern was extremely variable and the areas shown in Figure 7h are those which were most commonly affected. Pigmentation occurred in all fourth instar larvae, but was progressively less common from third to first instar. The compound eye of fourth instar larvae became increasingly pigmented with age, being present in earlier instars but was totally lacking in pigmentation. The stemma was clearly visible in all stages. Chaetotaxy has been excluded from Figure 7. The egg breaker which was visible in first stage larvae shortly after hatching, was black in colour and contrasted with the translucent head capsule;it became less obvious as the head capsule darkened.

MORPHOMETRICS OF INSTAR SEPARATION

The age-related increase in thorax width, abdomen length and collar width for *An. merus*, instars I-IV over time is shown in Figure 8. The overlap in thorax width and abdomen length between successive instars is demarcated by the shaded areas. The growth of a new collar as well as the overlap in values for collar width in each instar is evident. The greatest terminal (=just prior to moulting) collar width was attained in the third instar. The terminal shape of the collar for

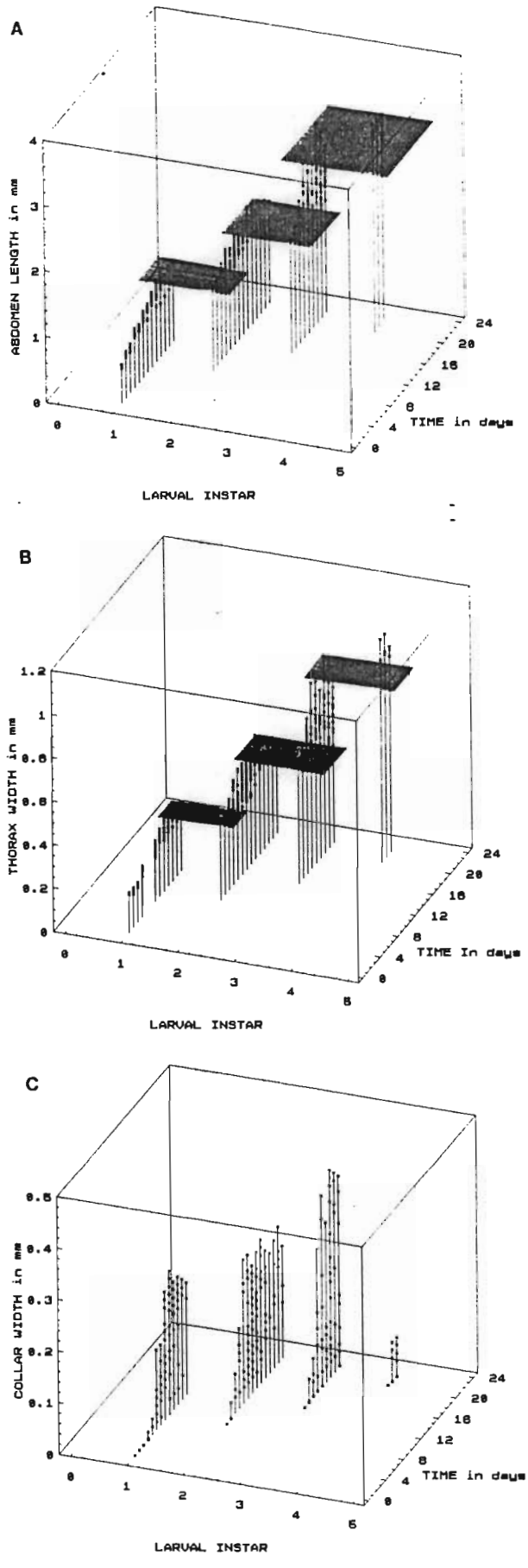


Figure 8. Three dimensional plot of the increase in: A, abdomen length, B, thorax width and C, collar width against larval instar and time.

first instar larvae is shown schematically in Figure 7b and was characteristic, as was that of fourth instar larvae (Figure 7h). The measurements of abdomen, thorax and collar, shown for the fourth instar were not at termination of the instar as the trial was discontinued prematurely due to infection with a parasitic fungus.

Scatterplots of abdomen length versus head capsule width are given (Figure 9) for field and colony material of *An. arabiensis* and *An. merus*. The *An. merus* field sample included material collected in winter and summer months. The mean larval density per pool during winter and summer was 221 and 80 respectively (total sample, n= 19 904). This seasonal difference in pool density was significant, ($P < 0,001$). The vertical lines illustrate the separation of instars based on head capsule width, larval skins and palmate hairs. The overlap in abdomen length for successive instars of *An. merus* and *An. arabiensis* is evident. Head capsule width measurements of field material showed greater variability than those from colony specimens. The *An. merus* 1st., 3rd. and 4th.,instar colony material gave low values of abdomen length relative to those for wild material, however, the head capsule width values were similar. Separation of *An. quadriannulatus* larvae into instars on the basis of head capsule width is shown in Figure 10. Changes in the values for *An. merus* head capsule width with time are shown in Figure 11 and it is evident that no increase occurred during instars. The instar differentiation values for the three species are given below (Table 6). The common values reflect the means between the maximum and minimum values of the

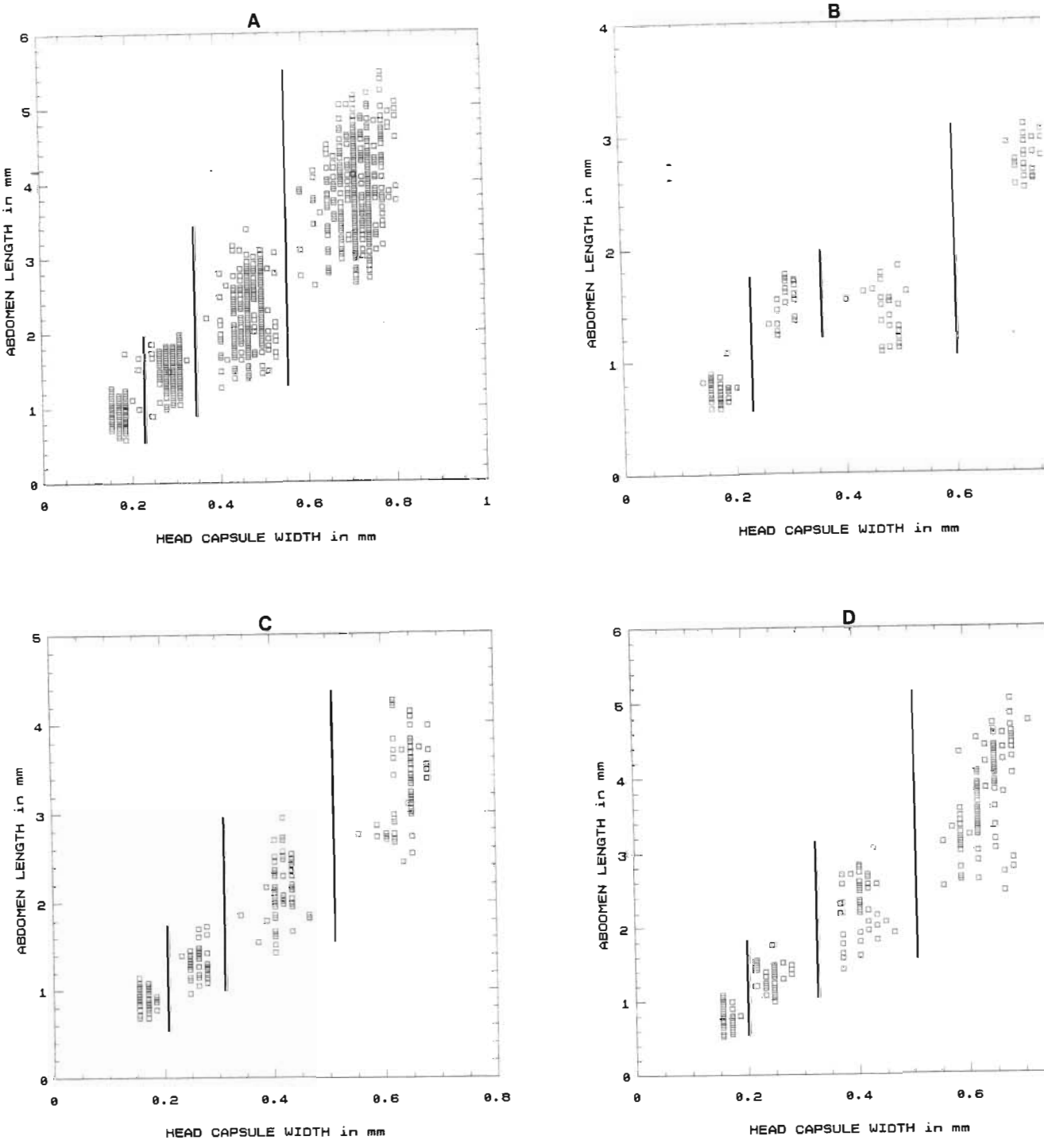


Figure 9. Scatterplots of abdomen length against head capsule width for: A, *An. merus* wild caught, B, *An. merus* colony, C, *An. arabiensis* wild caught and D, *An. arabiensis* colony.

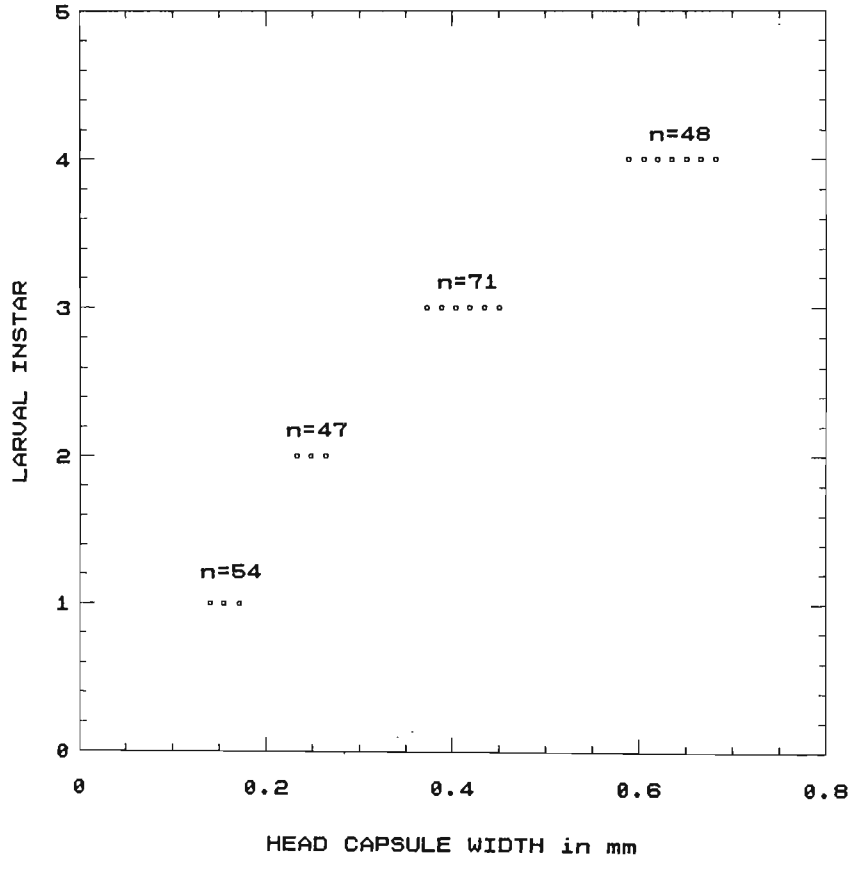


Figure 10. Plot of larval instar against head capsule width for *An. quadriannulatus*.

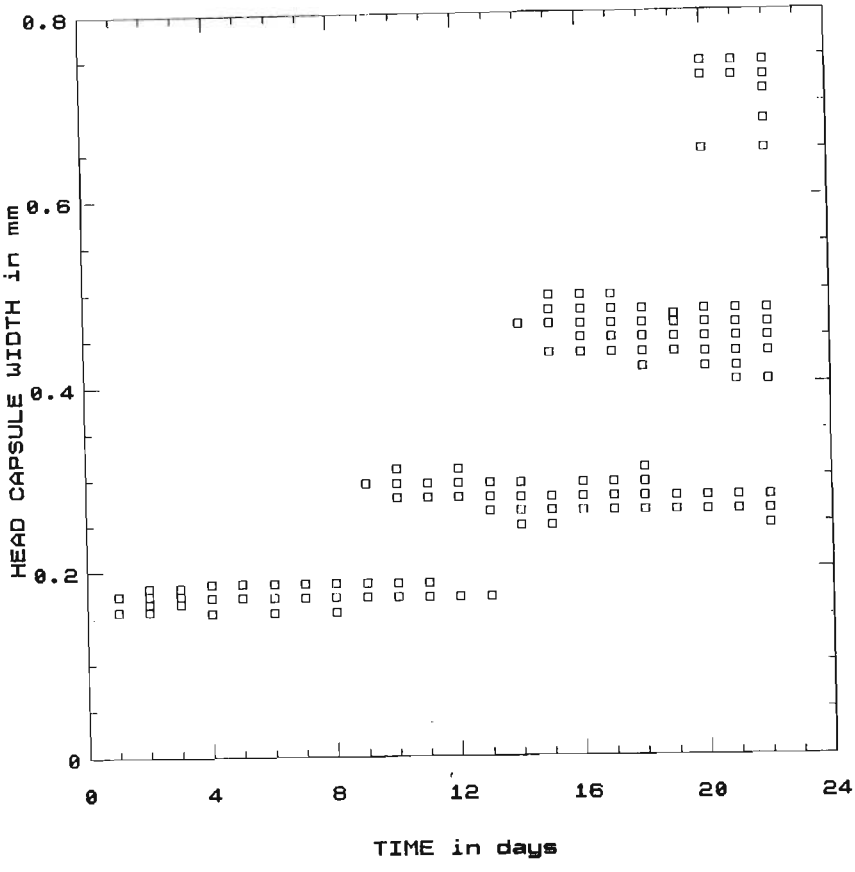


Figure 11. Plot of the head capsule width of colony *An. merus* against time, for larvae raised simulated winter temperature and light cycles.

respective instars .

Table 6. Optimal instar differentiation values for individual species, as well as common values for all three species

Species	Source	Differentiation values between instars in millimetres		
		I & II	II & III	III & IV
Anopheles merus	Colony	0.242	0.361	0.608
	Wild	0.233	0.350	0.551
Anopheles arabiensis	Colony	0.200	0.328	0.515
	Wild	0.220	0.312	0.517
Anopheles quadriannulatus	Colony	0.200	0.321	0.518
Common values for all three species		0.218	0.335	0.540

The values recorded for the two freshwater members are similar and slightly lower than those for *An. merus*. Values allowing the separation of all instars for the three species occurring in Natal are presented. A plot of abdomen length versus head capsule length for *An. arabiensis* is shown in Figure 12. The overlap in head capsule length in successive instars is evident.

DISCUSSION

The head morphology and growth of structures such as the collar have been described for other anophelines (*An. maculipennis* Meigen, Mitrofanova, 1929; *An. quadrimaculatus* Say, Jones, 1953). No such studies have been carried out for members of the *An. gambiae* complex. In addition, the function of the collar is unknown (Clements, 1963). The present study showed that the head capsule size does not increase during each instar. However, the head capsule did increase in size

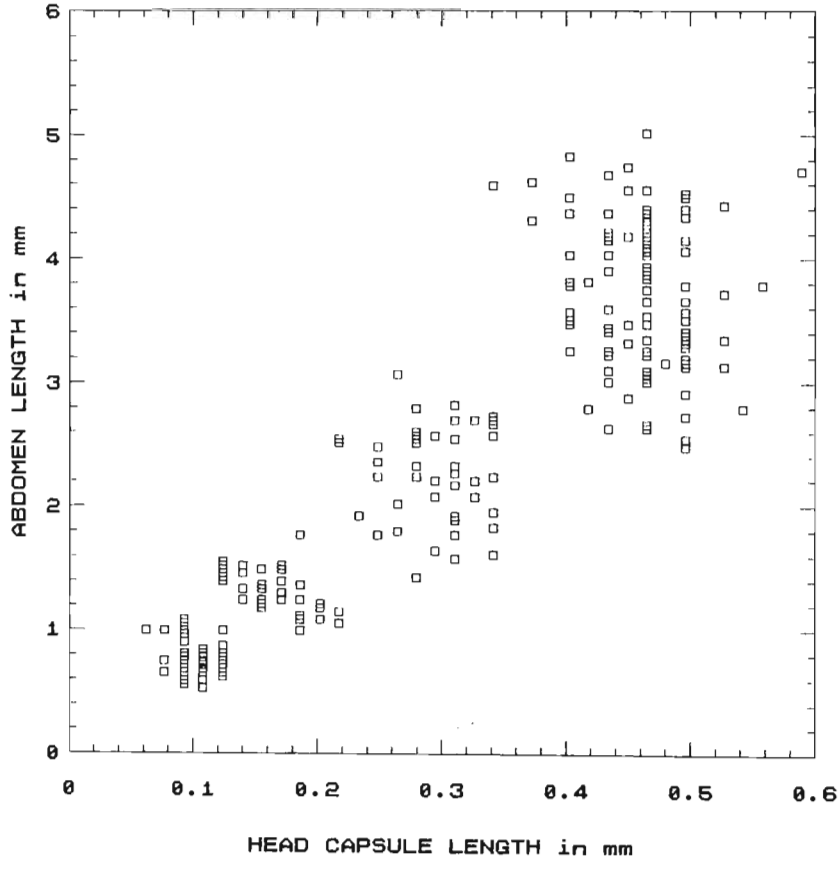


Figure 12. Plot of abdomen length against head capsule length for colony *An. arabiensis*

by a constant factor between successive instars; this is known as Dyar's rule (le Sueur, unpublished data). It is suggested therefore that the growth of the collar may enable the development of the underlying new head capsule such that its full size is attained shortly after moulting. This would have to be confirmed histologically.

Separation of larval instars according to the W.H.O. Manual of Practical Entomology (1975) results in confusion due to the a lack of explanation of the illustration showing the heads of the four larval instars; furthermore no mention is made that:

1) The collars shown represent their appearance just prior to moulting in each instar;
2) Except for a rudimentary collar in the fourth in star, no collar is present at the beginning of each instar; and
3) The increase in head size is diagnostic and facilitates the separation of the various instars.

In addition the terminal shape of the first instar larval collar is incorrectly presented and the same applies to the illustrated terminal size of the second instar collar. The above-mentioned omissions are corrected for in Figure 7. The shape of the first and fourth instar collar was important as it was diagnostic for these instars during their later stages. No further use could be made of the collar in the separation of instars as larvae in various intermediate, overlapping stages of collar growth were observed in the

samples studied.

The use of relative body size of larvae also resulted in incorrect classification as it was a function of two body measurements, abdomen length and thorax width. These two measurements increased linearly and both showed overlap between successive instars.

The width of the head capsule, however, was successfully used and no overlap was found between any instar in the three species of the *An. gambiae* complex investigated in this study, irrespective of seasonally induced changes in size. It should be noted that this applied to areas where the mean annual range in temperature does not exceed 8^{o}C. Increased variation (and overlap between instars) in this characteristic will occur with a greater mean annual range in temperature.

The relationship between temperature and size, in mosquitoes, has been shown to affect morphometric measures in taxonomy (le Sueur & Sharp, 1991). In the case of the *An. merus* field material, no overlap existed between instars in either winter or summer collections despite the 276% increase in larval pool density in winter. This indicates that head capsule width remains reliable irrespective of the density/nutritional status of the instars.

It could be expected that the use of head capsule length should also separate the various instars. However, overlap

was shown to occur. This overlap was a result of the head of larvae (especially dead), drooping slightly relative to the rest of the body. The measurement of head length was thus underestimated resulting in subsequent overlap with the previous instar.

It is suggested that the following features of the four instars be used to facilitate their separation:

First instar

1) The presence of the egg breaker.
2) The terminal shape of the collar.
3) A head capsule width of < 0.218 mm.

Second instar

1) A head capsule width >0.218 and <0.335 mm.

Third instar

1) A head capsule width >0.335 and <0.540 mm.

Fourth instar

1) The presence of the pigmented compound eye.
2) The terminal shape of the collar.
3) A head capsule width >0.540 mm

The majority of larvae could be easily separated into instars by a visual comparison of the relative size of their head capsules. Defining the limits of each instar, however, prevented confusion between individuals lying at the extremities of each group, which could not be separated visually (especially between third and fourth). The chaetotaxic characteristics as defined by Soper and Wilson (1943) were also valid. These, however, were less easily visualised, especially in earlier instars and resulted in a much slower sorting rate.

The use of head capsule width provided a criterion which showed limited variation in mosquitoes raised under different climatic and nutritional conditions and provided clear and rapid instar separation for all three species occurring in Natal. Similar results have been obtained for *An. quadrimaculatus* (Abdel-Malek & Goulding, 1948; Jones, 1953) and *Aedes vigilax* Skuse (Shinkarenko et al., 1986). There is overlap in the head capsule width of the larval stages of *Ae. vigilax*, but a 99,4% correct classification is still possible. The overlap in body length between successive instars is similar to that found during this study and, using this criterion, only 88% of instar determinations were correct. The low values of abdomen length shown for the *An. merus* were colony were purely a result of young individuals of each instar being selected and fell within the lower limits recorded for field material.

3.2 MORPHOMETRICS IN SPECIES SEPARATION

3.2.1 SEASONALITY AND RELATIVE BODY SIZE

In section 3.1 it was demonstrated that the use of larval head capsule measurements provided an accurate tool for separation of the instars. During the analysis it was however noted that although no overlap occurred between successive instars in any of the three species, the head capsule width of the salt water member of the complex (*An. merus*) was significantly larger than that of the two freshwater members (*An. arabiensis* and *An. quadriannulatus*). When dealing with

species complexes there is a tendency to seize upon any apparent morphological difference as a species difference. At first this difference 'apparent' species difference in head capsule was perceived as being a potentially, rapid method for identification of *An. merus* larvae in the field. Subsequently it was found that it was in fact a function of seasonality and the time of the year at which the larvae were collected from the field.

The following publication describes this temperature-related difference and highlights the importance of considering the effect of the environment on the genotypic expression of phenotype.

Note: The contents of this section 3.2.1 have been published in Medical and Veterinary Entomology (1991) 5, 55-62.

TITLE

Temperature-dependent variation in *Anopheles merus* larval head capsule width and adult wing length: implications for anopheline taxonomy.

ABSTRACT

Seasonal variation in mosquito larval head capsule width and adult wing length was investigated in a field population of Anopheles merus Donit zat Nceswana Lake, Ophansi, which is within the endemic malaria area of Natal, South Africa. An inverse relationship was detected between both these morphological characters and seasonal fluctuations in air/water temperatures. Mean head capsule width in all instars de-

creased by 4.8 to 7.9% in summer, while mean wing length decreased by 19.6%. These changes are discussed in relation to the annual range in mean air temperature in southern Africa and the distribution of *An. merus*. Implications for the use of such morphological characteristics in existing taxonomic keys is discussed.

INTRODUCTION

Since the initial work of Evans (1931) on "Anopheles gambiae var. melas" in relation to the occurrence of the four-banded palp morphology, considerable effort has been expended on the attempted morphometric separation of the members of the *An. gambiae* complex. Initial studies included the use of morphological criteria such as egg size and larval pecten shape to separate the saltwater species *An. melas* Theobald (Ribbands, 1944) from freshwater members. Other criteria such as the maxillary index (Holstein, 1954) were shown to be enviromentally-dependent (Gillies & Shute, 1954).

During the elucidation of the complex, numerous studies have been conducted in the quest for morphological criteria which would allow visual separation of individual species, but with limited success (eg. Bushrod, 1981; Coetzee et al., 1982; Coetzee, 1986a&b; Coluzzi, 1964; Green, 1971; White & Muniss, 1972 and Zahar et al., 1970). An important omission in some of these studies is a consideration of the possible effect of environmental factors on the criteria being investigated, particularly in relation to species distribution. Gillies and Shute (1954) stated that their work made "it clear that, unless adults are reared under standard condi-

tions, no valid comparisons can be made of the maxillary indicies of population derived from different localities or breeding sites" Sharp et al., (1989) provide evidence that a measured change in the mean leg band width at the junction of tarsomeres 3 and 4, for an *An. arabiensis* population is correlated to the application of residual insecticide. As stated by Green (1981), "morphological variation is complex, very rarely understood genetically, and very probably involves varying combinations of genetic components and environmental influences". Rattanarithikul & Green (1986) point out that only when progeny broods are raised under standard conditions can the assumption be made that observed morphological differences reflect genetic variation. More importantly they raise the question of whether there exist in nature conditions that will mask the genetic component observed in the laboratory.

This paper reports an investigation of the effects of envionental temperature, on the size of *An. merus* female wing length and larval head capsule width, with implications for the taxonomy of other Afrotropical anophelines.

MATERIALS AND METHODS

The study was conducted on *An. merus* from Nceswana Lake, Ophansi ($27^{o}34'S$, $32^{o}17'E$- see Figure 3), northern Natal, Republic of South Africa. Monthly larval collections were made from a breeding site situated at the edge of the lake. A salinity gradient existed between pools at the waters edge (7 p.p.t.) and those at the top of the site (33 to 70 p.p.t or

94 to 200% sea water). The lake contained freshwater (0 p.p.t) and Anopheles merus larvae were not found in the lake or in pools with a salinity greater than 39 p.p.t (111% sea water). *An. merus* adults were collected from a pit shelter (Muirhead -Thomson, 1948) situated approximately 200 meters from the breeding site.

IDENTIFICATION

Identifications were based on the salinity tolerance test (Muirhead-Thomson, 1951), isoenzyme electrophoresis (Mahon et al., 1976; Miles, 1979) and chromosome cytology (Coluzzi & Sabatini, 1969; Hunt, 1987). *An. merus* was the only anopheline species recorded from the saline breeding site.

MORPHOLOGICAL MOUNTING AND MEASUREMENTS

For microscopical examination, slide preparations of adults were made according to the method of Hunt & Coetzee (1986). The wing length measurements were taken between the axillary incision (Harbach & Knight, 1980) and the wing tip in the region of vein 3 (Gillies & Coetzee, 1987).

Larval samples were stored in a 4% formalin solution. Larval head capsule width was measured between the extremities of the lateralia (Harbach & Knight, 1980), when viewed either dorsally or ventrally.

Measurements were carried out with a Wild M7A Stereomicroscope using a 10X eyepiece and 31X objective and an eyepiece micrometer with 120 divisions, each equal to 31.0 um on the focal plane.

RESULTS

Comparison of mean head capsule width for first to fourth instar *An. merus* larvae, during winter and summer are shown in Figure 13 and Table 7. The mean head capsule width of all instars was significantly greater in winter (July-August) than in summer (December-January). The percentage increases in mean head capsule width between January and July for larval instars I-IV was 4.2%, 5.0%, 7.9% and 7.1% respectively.

Table 7. Comparisons of mean head capsule width of *An. merus* larval instars I-IV (cf. Figure 13).

Instar	Season	n	t-Value	P
First	Winter	50	2,81	<0,005
	v. Summer	50		
Second	Winter	50	4,59	<0,001
	v. Summer	50		
Third	Winter	49	9,96	<0,001
	v. Summer	51		
Fourth	Winter	50	7,48	<0,001
	v. Summer	50		

The mean wing length for *An. merus* adult females are shown in Figure 14 for the months of July 1984 to January 1985, together with mean monthly temperature for the same period. The inverse relationship between wing length and temperature is described by the linear function; $Y = 5.01 - 0.071X$, which has a correlation coefficient of -0.97. Excluding the months of July and August, mean wing length decreased significantly each month as the summer approached (Table 8). The increase in mean wing length between the months of January and that of July was 19,6%.

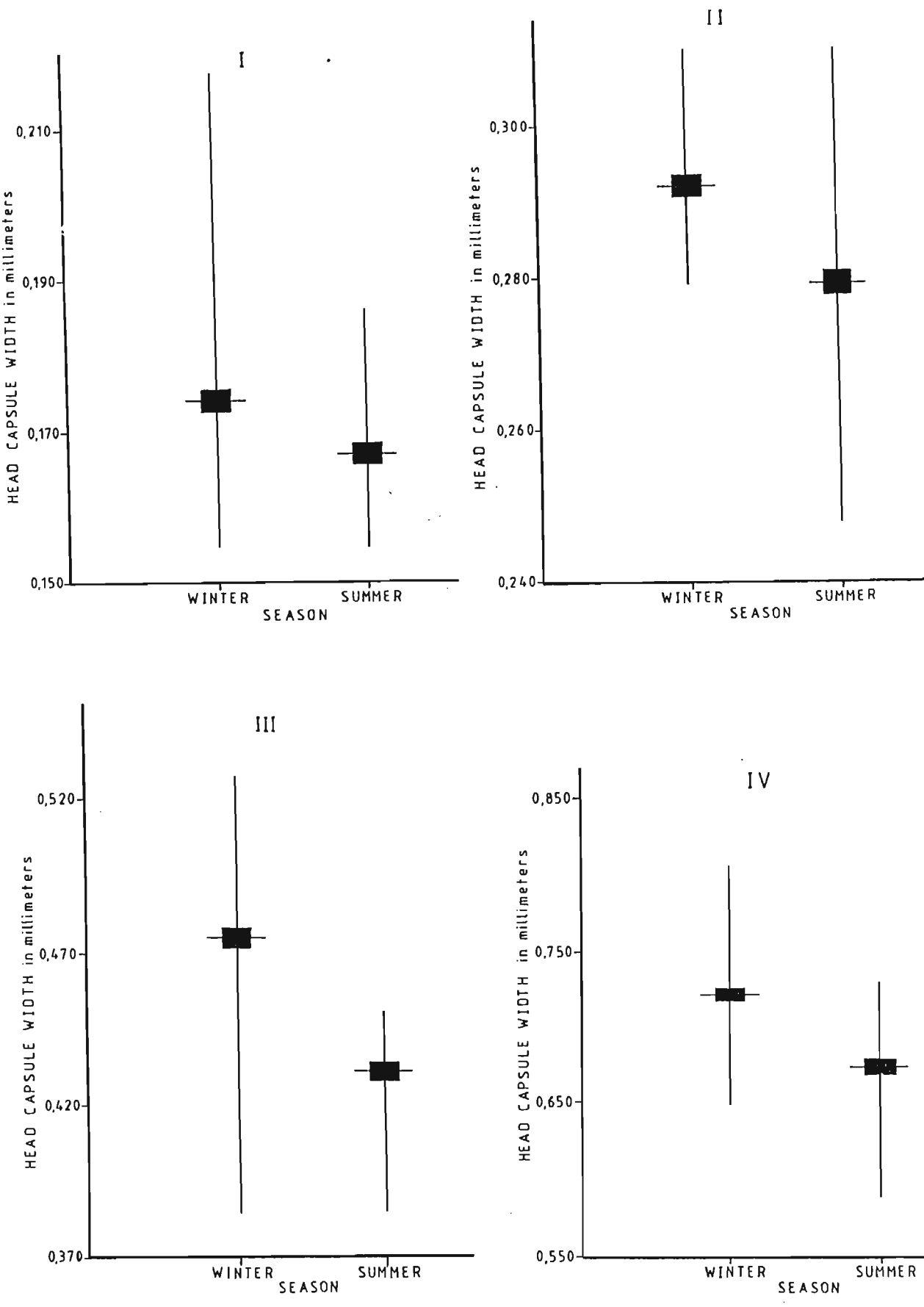


Figure 13. Seasonal comparisons of mean head capsule width for *An. merus* larvae, during winter and summer. I = first instar, II = second, II = third and IV = fourth. The horizontal line is the mean, the vertical the range and the bar twice the standard error of the mean

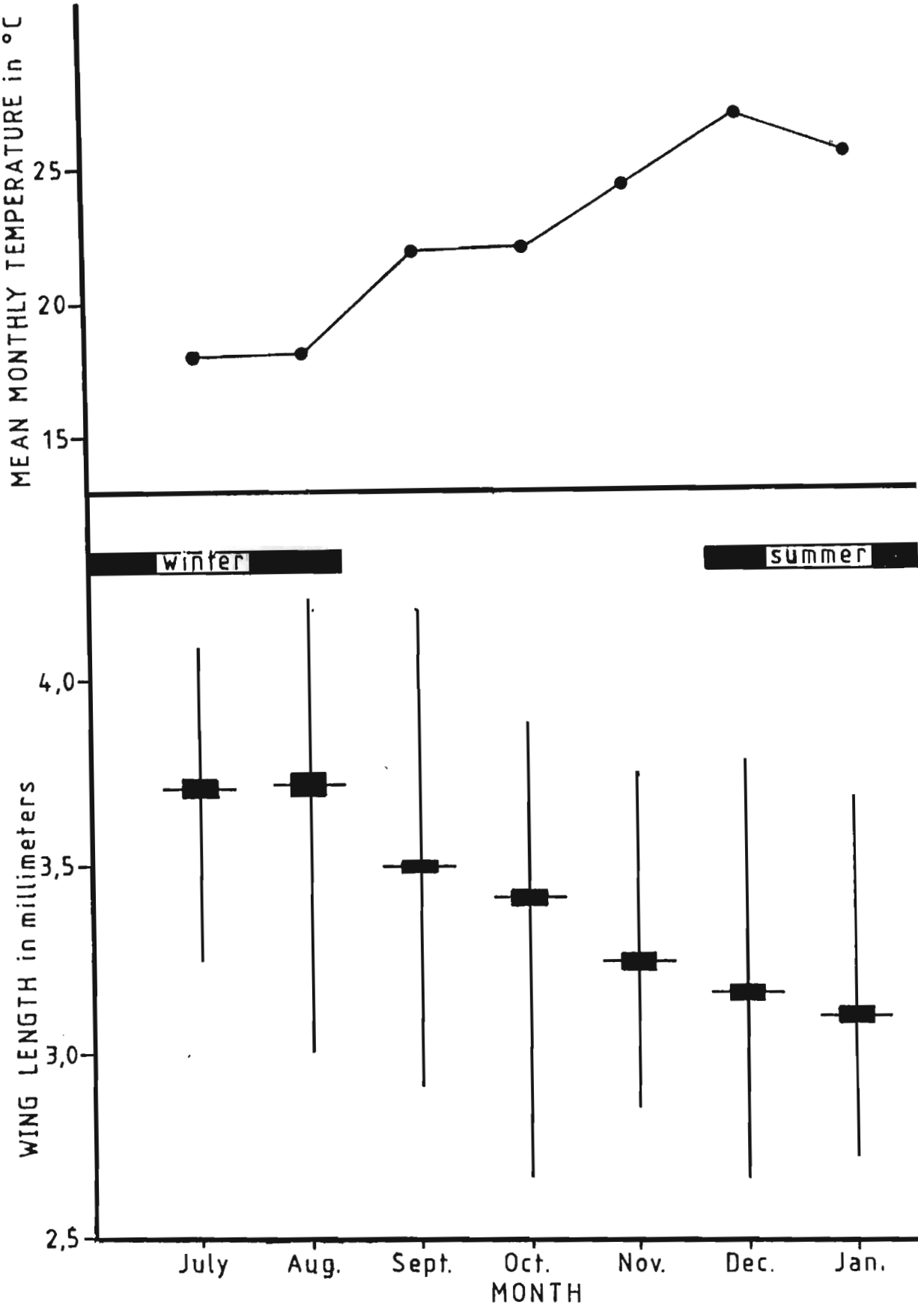


Figure 14. Mean monthly wing length of *An. merus* females, with mean monthly air temperature for the same period.

Table 8. Monthly temperature-related statistics for decrease in wing length, of *An. merus* females (*cf.* Figure 14).

MONTH	n	t-VALUE	P
July vs August	42 40	-0.38	< 0.699
August vs September	40 139	7.06	< 0.001
September vs October	139 69	5.68	< 0.001
October vs November	69 43	2.95	< 0.005
November vs December	43 53	2,80	< 0.005
December vs January	53 53	-2.7	< 0.05

DISCUSSION

Ray (1960) reviewed the way that insects and other poikilotherms generally grow bigger and slower at lower temperatures. Accordingly the size of some major body components (wing length, larval head capsule width) of *An. merus* are shown in this study to be negatively related to temperature. Relationships between temperature and the growth and size of mosquitoes, when food is not limiting, are discussed in detail by Clements (1963). The decrease in wing size under summer conditions in this study may be partially explained by the possibility that at high summer temperatures the requirements may exceed the rate at which food can be gathered by larvae. This is reflected in the decrease in size of all

instars under summer conditions, as indicated by the mean values for head capsule width. However it is interesting to note that the difference is only in the region of 7% in the fourth instar, when food gathering and growth ceases. This contrasts with the, approximately 20% decrease in wing length. Van den Heuval (1963) concluded that the effect of temperature on wing length of *Aedes aegypti* (L.) was greatest in the pupal stage and the results of this study support a similar conclusion for *An. merus*. This effect may be a result of temperature related metabolism, available energy reserves and the fact that the pupal stage does not feed.

The implications of using such morphological characters in taxonomic procedures are of practical significance. Phenotypic variations in colour, scaling, size, egg morphology larval setal structure and maxillary index, due to abiotic factors such as temperature, density, salinity and nutrition have been documented (Deane & Causey, 1943; Clements, 1963). The importance of such variation to the systematist was pointed out by Clements (1963), but appears to have been largely ignored in studies conducted on the *An. gambiae* complex.

Clarke (1971) used spermatheca size, corrected for variation in body size (using wing length as an index of the latter) to separate *An. gambiae s.s.* from *An. arabiensis* using colonised material from Nigeria. White & Muniss (1972) tested the method on East African material, which also included *An. merus*. They found that the differences reported by Clarke (1971) in colonised material were not as pronounced in wild material as to be of any taxonomic value. These observations

support the conclusion made by Rattanarithikul & Green (1986) that laboratory-observed variation could be masked under natural conditions. If taxonomic criteria are first identified in laboratory material, their validity should then be assessed under natural conditions and throughout the distribution of the species in question. This is more easily said than done, but is essential if taxonomic keys are to be accurate and incorrect identification avoided.

Data generated in this study demonstrate that wing length and head capsule width of *An. merus* will vary significantly where the annual range of mean daily temperature is large. Figure 15 shows the annual range of mean temperature for southern Africa (Knoch & Schulze, 1956) and the known distribution of *An. merus* (Gillies & De Meillon, 1968; Gillies & Coetzee, 1987; le Sueur & Sharp, 1988).

At the study locality, the mean temperature range during the study months of July to January was 9^{o}C (Figure 14), similar to the value of 8^{o}C reported for the region by Knoch & Schulze (1956). Figure 15 shows how this decreases at latitudes closer to the equator. This is more clearly seen in central Africa than in the coastal regions where temperatures are stabilised by the ocean. The size of *An. merus* as measured from head capsule width and wing length, will vary little with season in areas where it occurs close to the equator, such as Tanzania, Kenya and Somalia, but more in areas further from the equtor such as Zimbabwe and Zambia (Figure 15).

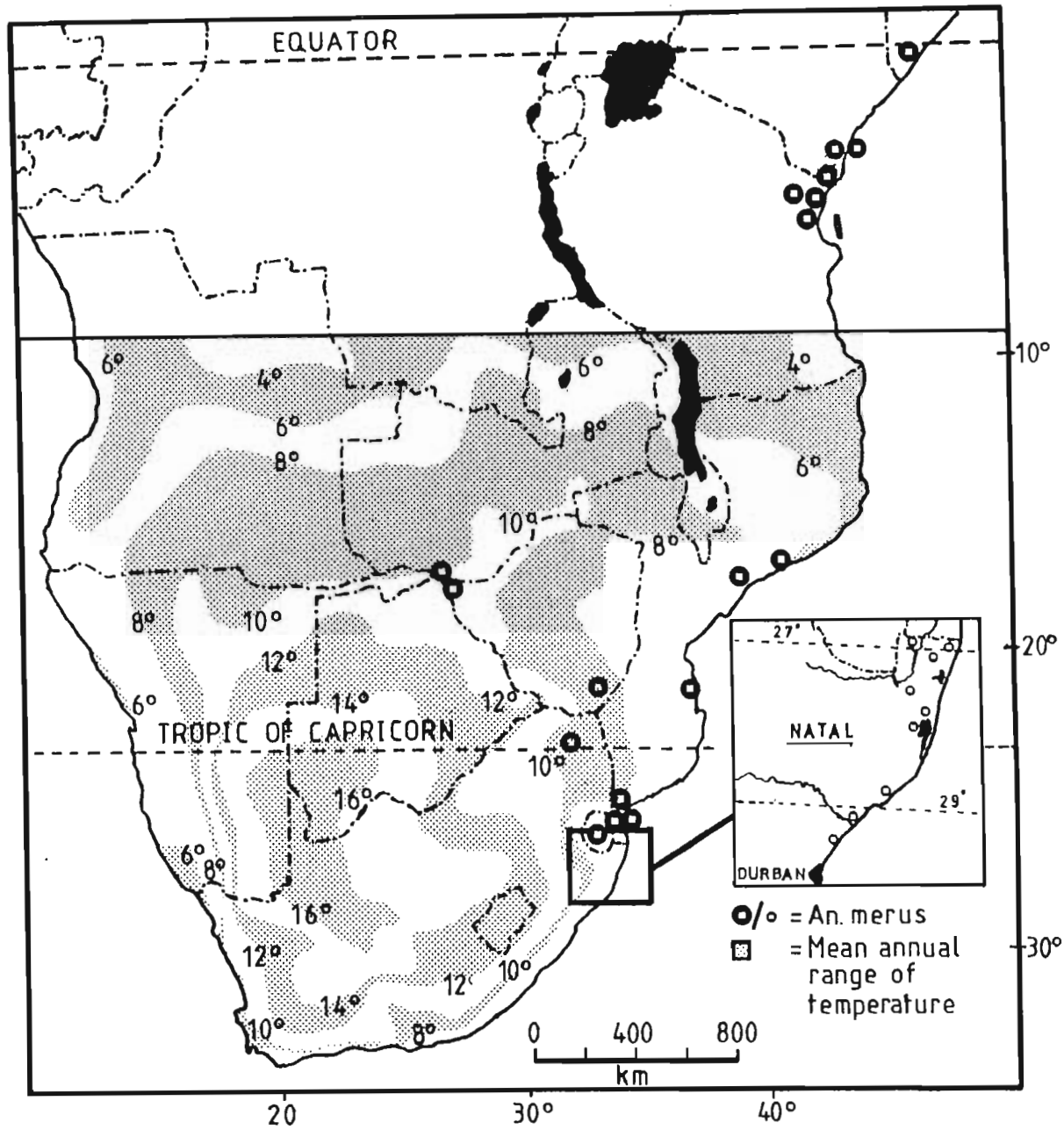


Figure 15. Mean annual range in temperature for southern Africa (Knoch & Schulze, 1956) and the distribution of *An. merus* (Gillies & De meillon, 1968; Gillies & Coetzee, 1987: le Sueur & Sharp, 1988).

If absolute size characters are to be used for entomological identification purposes, then the annual temperature range of the study locality should be taken into account. If the mean range is 4°C or more, then both winter and summer samples should be evaluated. For *An. merus*, this temperature range resulted in a decrease in wing length of approximately 5%.

In the identification key to adult Afrotropical anophelines by Gillies & Coetzee (1987), wing length is used as a diagnostic criterion for 15 species (Table 9). Considering the separation of *An. ruarinus* and *An. rhodesiensis rupicolus/An. caroni* (in part), the use of wing length can probably be considered reasonable as the laid down limits are separated by 1 mm . The maximum annual range in temperature for *An. ruarinus* throughout its distribution is 8 to 10°C and temperature-related changes in wing length would probably not result in significant overlap. In contrast the separation of *An. brucei* (in part) from *An. freetownensis/An. demeilloni* which is delimited by only 0.1 mm, is unreliable when their distributions are considered in relation to temperature range and the size variation this would engender (Figure 15). Similarly, the use of wing lenghth for identification of all the other species quoted in Table 9 on the basis of wing length appears questionable .

If mosquito taxonomic keys are to include absolute size criteria , or any other phenotypic characters which are influenced by abiotic factors, then morphological variation should be assessed seasonally throughout the species

Table 9. Summary of the use of wing length as a diagnostic criterion in the key for identification of Afrotropical anophelines, with the limits of distribution of these species (Gillies & De Meillon, 1968; Gillies & Coetzee, 1987).

SPECIFIC NAME	WING LENGTH	DISTRIBUTION	
	(millimeters)	Latitude	Longitude
		(degrees)	
An. ruarinus	4.5 or more	11S to 27S	13E to 33E
vs			
An. rhodesiensis rupicolus	3.5 or less	30N to 10N	18E to 47E
An. caroni (in part)		5S	14E
An. demeilloni (berg river form)	4 or less	33S	19E
vs			
An. carteri (in part)	4.4 or more	17S to 29S	28E to 31E
An. brucei (in part)	2.8 or less	7S	7E
vs			
An. freetownensis	2.9 or more	13N to 7S	9W to 15E
An. demeilloni		18N to 34S	18E to 46E
An. keniensis (in part)	3.2 or more	2N to 8S	31E to 40E
vs			
An. moucheti	3.0 or less	8N to 12S	4E to 34E
An. bervoetsi		6S	18E
An. rivulorum (in part)	2.5 - 3.3	15N to 28S	13W to 39E
vs			
An. demeilloni (in part)	2.9 - 4.2	16N to 34S	10E to 45E
An. demeilloni (in part)	3.3 or more	16N to 34S	10E to 45E
vs			
An. parensis (in part)	3.3 or less	26S to 28S	32E
An. funestus group		16N to 32S	17W to 45E
An. demeilloni (in part, mainly highlands)		16N to 34S	10E to 45E
An. cameroni (extreme southern Africa only)		34S	19E
An. sergenti machmahoni		17N to 1N	13W to 51E

distribution. The findings of this study also reinforce the necessity for genetically based identification in the assess-

ment of phenotypic variation, bearing in mind that intraspecific variation may be due either to genetic polymorphism or to environmentally or physiologically induced variation, as in this case study.

3.2.2 HINDLEG BANDING PATTERN IN SPECIES SEPARATION.

The following publication deals with species separation in the *An. gambiae* complex, using the width of the pale band between tarsomere 3 and 4, of the hindlegs. Coetzee *et al.* (1982) and Coetzee (1986) demonstrated that *An. gambiae/An. arabiensis* could be distinguished from *An. merus/An. quadriannulatus* using the above criteria. In order to assess the accuracy of this technique in the field, it was deçided to compare the width of this band in newly erupted and free-flying females. The object was to gauge whether with age, the size of this band would be affected by scale loss, thus affecting the accuracy of the technique. This was found not to be the case. The study did however elucidate the fact that there was a correlated increase in the average band size, for *An. arabiensis* in regions where the intra-domiciliary application of DDT was carried out. This increase in mean band size reduces the accuracy of the technique. Sharp *et al.*, (1990) point out that this difference may reflect: intraspecific, genetically determined variation, with DDT acting as the selective pressure;or an interspecific difference within *An. arabiensis* which is highlighted in the presence of DDT. In the publication all the data relating to larval eruptions were collected and processed by myself.

Mosquito Systematics · Vol. 21(2) 1989

The value of hindleg banding patterns in the identification of species of the *Anopheles gambiae* Giles complex (Diptera: Culicidae) in Natal, South Africa

by

B. L. Sharp, D. leSueur and Frances Ridl[1]

ABSTRACT. The validity of using hindleg banding patterns for the identification of *Anopheles merus*, *An. quadriannulatus* and *An. arabiensis* was investigated in Natal. A high percentage of *An. merus* and *An. quadriannulatus* were correctly identified. Of two geographically separated *An. arabiensis* populations only one closely conformed to the identification criteria.

INTRODUCTION

Species identification of members of the *Anopheles gambiae* complex is vital to the efficient management of malaria vector control programs in Africa. Due to lack of suitable morphological characters, species identification within this complex of mosquitoes has mainly depended on cytological (Coluzzi 1968) and biochemical (Miles 1978, 1979) techniques. Coetzee et al. (1982) and Coetzee (1986) demonstrated that *An. gambiae/An. arabiensis* could be distinguished from *An. merus/An. quadriannulatus* by the width of the pale band at the apex of hind tarsomeres 3 and 4. Coetzee (1986) cautioned that the reported measurements might only apply to the localities sampled, and not to other areas in Africa. The reasoning behind this statement was not outlined by the author. Due to the practical implications of this technique for the field entomologist, an investigation was launched to evaluate the effectiveness of this method of identification of *An. gambiae* sensu. lato. species in Natal.

MATERIALS AND METHODS

The legs of wild caught females, their adult female progeny and adult females raised from wild caught larvae were mounted according to the technique of Hunt and Coetzee (1986). No more than 5 F1 females per family or 5 females from larvae collected in any one pool were used in scoring leg banding.

1 Research Institute for Diseases in a Tropical Environment of the South African Medical Research Council, P. O. Box 17120, Congella 4013, Rep. South Africa.

Measurements were taken of the pale band at the junction of hindtarsomeres 3 and 4 using a compound microscope (magnification x100) fitted with an eyepiece micrometer. The maximum and minimum length of pale scaling were measured and the mean calculated. For each species, specimens were treated as two groups; (a) wild caught adults (WCA) and, (b) rearings from wild caught larvae or adult progeny of wild caught females (LE).

All material was collected in the Natal province of the Republic of South Africa at the following grid references: *An. arabiensis* Dondota (31°58'E, 28°34'S), Mamfene (32°15'E, 27°22'S); *An. merus*, Ophansi (32°16'E, 27°32'S), Chubu (32°51'E, 28°2'S), *An. quadriannulatus*, Numaneni (32°16'E, 26°58'S), Sandspit road (30°57'E, 30°48'S), Ophansi (32°16'E, 27°32'S), Mamfene (32°15'E, 27°22'S), Lumbongwenya stream (32°12'E, 27°51'S), Numaneni (32°15'E, 26°58'S), Buwensi (32°11'E, 27°12'S), Makhanis Drift (32°17'E, 27°1'S), Sihangwane (32°33'E, 27°5'S).

Species were identified by isoenzyme electrophoresis (Miles 1978, 1979), polytene chromosome analysis (Coluzzi 1968; Hunt 1973) and the physiological salt tolerance method (Muirhead-Thomson 1951).

RESULTS

A total of 440 females viz. 206 *An. arabiensis* 118 *An. merus* and 116 *An. quadriannulatus* were examined. Measurements were subjected to the Coetzee (1986) method in order to determine percentage correct identification. Where the pale band at the junction of hind tarsomeres 3 and 4 measures less than 0,099 mm in *An. gambiae/An. arabiensis*, and more than 0,1 mm in *An. merus/An. quadriannulatus*.

The percentage of *An. merus* correctly identified was >92% for both reared and wild caught females and >79% for *An. quadriannulatus* (Table 1). The Dondota *An. arabiensis*, population (A), revealed correct identification in excess of 86%, whereas only 19.6% were correctly identified in the case of the Mamfene *An. arabiensis*, population (B).

The distribution of leg banding measurements for *An. arabiensis* populations (A) and (B) are presented in Figure 1. In population (A) peak frequency was at 0,07 mm and 0,1 mm in population (B). 68% of the leg-band measurements in population (B) were wrongly identified using the Coetzee (1986) method in contrast with the 7.6% in population (A). This difference was statistically significant (Chi-square = 136.842, $P < 0.001$).

The percentage WCA and LE legs incorrectly identified according to the Coetzee (1986) method is shown in Table 2. Within species, the number of specimens in the WCA and LE groups wrongly identified by the method were not significantly different (Table 2).

Mosquito Systematics Vol. 21(2) 1989

DISCUSSION

A high percentage of measurements conformed to the Coetzee (1986) criteria in *An. merus*, *An. quadriannulatus* and *An. arabiensis* population (A). In all cases this was lower than the 94,0% correct grouping found by Coetzee (1986). This may be due to the use of greater magnification, the method of measurement or the use of a calculated mean measure in this study. Since the scaling of the leg-band may become rubbed with age there is the possibility that this might affect identification. To investigate this possibility all samples within species were separately treated as either freshly emerged or wild caught adults prior to pooling the data. None of the three species showed statistical differences between these groups with respect to specimens that were wrongly identified by the Coetzee (1986) method.

Two populations of *An. arabiensis*, 120 km apart were investigated during this study: population (A) from an area that had never been subjected to the intra-domicillary application of D.D.T. and population (B), from an area which is sprayed annually. A much higher percentage of measurements from population (A) fitted the Coetzee criteria than from population (B). The peak in distribution of the leg-banding measurements in population (B) was 0,1 mm, coincident with the measurement used by Coetzee (1986) for *An. merus/An. quadriannulatus*.

Coetzee (1986) states that of the four species examined, *An. arabiensis* showed the greatest variability. This finding is highlighted by the results of the present study. We speculate that the major difference between these two collection areas is the application of D.D.T.. The majority of the areas from which *An. arabiensis* were collected by Coetzee (1986) were not subject to a malaria vector control program. These data compare well with those collected in the present study from the unsprayed area.

The pooling of the *An. arabiensis* data from this study resulted in only 56% correct identification. This result seriously detracts from the use of leg-banding for the separation of the *An. gambiae* complex member species in the Natal region and highlights the necessity of confirming morphological identifications by biochemical and/or cytological means.

ACKNOWLEDGEMENTS

The authors are indebted to Dr. C.H.J. Schutte (R.I.D.T.E.), Dr. Maureen Coetzee (S.A.I.M.R.) and Mr. K. Newberry (N.I.T.D.) for the critical reading of, and comments on the manuscript; the S.A. Medical Research Council for permission to publish.

LITERATURE CITED

Coetzee, M. 1986. Practical use of hind leg banding patterns for identifying members of the *Anopheles gambiae* group of mosquitoes. Mosq. Syst. 18:134-138.

Coetzee, M., K. Newberry, and D. Durand. 1982. A preliminary report on a morphological character distinguishing important malaria vectors in the *Anopheles gambiae* Giles complex in Southern Africa. Mosq. Syst. 14:88-93.

Coluzzi, M. 1968. Cromosomi politenici delle cellule nutrici ovariche nel complesso *gambiae* genere *Anopheles*. Parassitologia 10:179-183.

Hunt, R. H. 1973. A cytological technique for the study of *Anopheles gambiae* complex. Parassitologia 15:137-139.

Hunt, R. H., and M. Coetzee. 1986. Field sampling of *Anopheles* mosquitoes for correlated cytogenetic, electrophoretic and morphological studies. Bull. of W.H.O. 64:897-900.

Miles, S. J. 1978. Enzyme variation in the *Anopheles gambiae* Giles group of species (Diptera: Culicidae). Bull. ent. Res. 68:85-96.

Miles, S. J. 1979. A biochemical key to adult members of the *Anopheles gambiae* group of species (Diptera: Culicidae). J. Med. Ent. 15:297-299.

Muirhead-Thomson, R. C. 1951. Studies on salt-water and fresh-water *Anopheles gambiae* on the East African coast. Bull. ent. Res. 41:487-502.

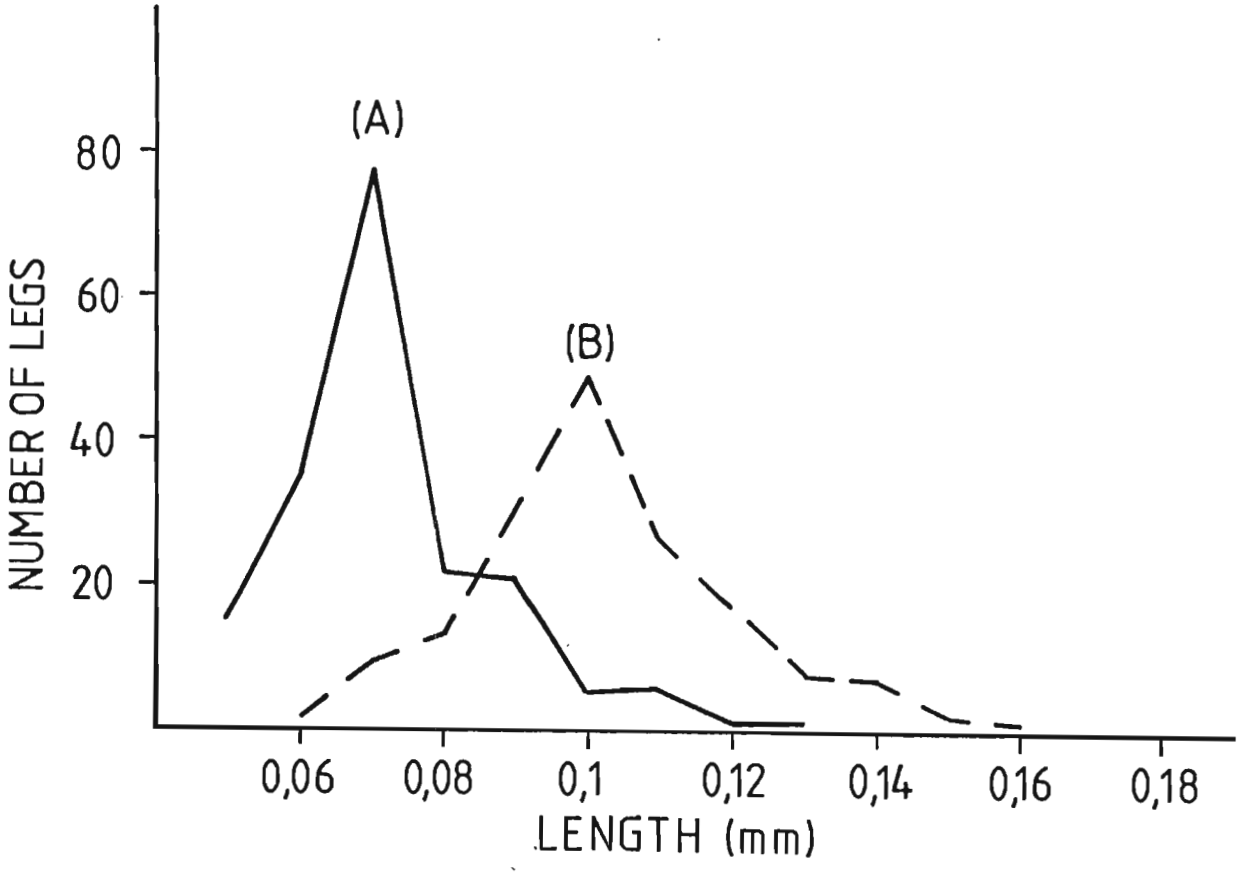
Table 1. Percentage correct identification of *An. gambiae* complex species using the Coetzee (1986) method.

Species	Status of Adult	Number of Adults	% Correct Identification
An. merus	WCA	52	92,3
	LE	66	93,9
	Total	118	93,2
An. quadriannulatus	WCA	48	81,3
	LE	68	79,4
	Total	116	80,2
An. arabiensis (A)	WCA	33	93,9
	LE	76	86,8
	Total	109	89,0
An. arabiensis (B)	WCA	97	19,6

Table 2. Comparisons of WCA and LE leg measurements within species wrongly identified by the Coetzee (1986) method.

	Percentage of leg measurements wrongly identified.			
Species	WCA	LE	Chi sq.	P=
An. merus	4.2	3.2	0.009	N.S.
An. quadriannulatus	6.1	13.6	2.599	N.S.
An. arabiensis (A)	4.6	7.8	0.142	N.S.

Figure 1. Distribution of the leg-banding measurements of *Anopheles arabiensis* populations (A) and (B).



3.2.3 THE EFFECT OF SEASON ON MORPHOLOGY

The occurrence of a melanic form within *Pyretophorus costalis* was first reported and described as a distinct variety by Theobald in 1903. This specimen originated from the Gambia and could thus not have been *An. merus*, for which data is presented below. Both decreases and increases in white scaling have been reported in mosquitoes. In *Aedes pseudoscutellaris* the amount of white scaling increases with temperature (Marks, 1954). Ghelelovitch (1950) reported that low temperatures result in a decrease in the dark patches on the abdominal sternites of *Culex pipiens* (Linnaeus).

It is thus obvious that it is important to assess how "fixed" taxonomic criteria used for an individual species are. During the investigation on the effect of temperature on head capsule width and wing length (Section 3.2.1), it was noted that concurrent with the increase in wing size, at low temperatures, was a tendency for the mosquito to be more melanic in appearance. It was thus decided to investigate how this affected pale markings on the wings and legs, which constitute important taxonomic criteria within the *An. gambiae* complex. A further aim was to assess whether the variation reported (for *An. arabiensis*) in section 3.2.2 could not have been an artifact of temperature.

MATERIALS AND METHODS

The origin and collection of the material were the same as that given in sections 3.2.1. and 3.2.2 for *An. merus* and *An.*

arabiensis respectively.

The wing nomenclature is the same as that used by Gillies and Coetzee (1987). The pale and dark markings on the costa between and including the areas **A** and **D** were measured. Measurements were carried out with a Wild M7A Stereomicroscope using a 10X eyepiece and 31X objective and an eyepiece micrometer with 120 divisions, each equal to 31.0 um on the focal plane.

The measurements of the pale bands at the junction of tarsomeres 3 and 4 (Evans, 1938) were carried out as described in section 3.2.2. Tarsal length was measured at the same magnification.

RESULTS AND DISCUSSION

Figure 16 shows a comparison of the size of the dark and pale areas on the Costa of the wings of *An. merus* specimens in both winter and summer. It is interesting to note that all of the 19,6% increase in wing size reported in section 3.2.1 occurs in the dark areas. Thus the size of the dark areas **A,B,C & D** increases significantly (t= 4,6; 10,0; 7,4 & 6,4 respectively , $P<0.001$ in all four) between winter and summer. In contrast the size of the pale areas **b,c & d** remain unchanged (t = -0,41; 1,6 & -0,13 respectively, $P>0,05$ in all three).

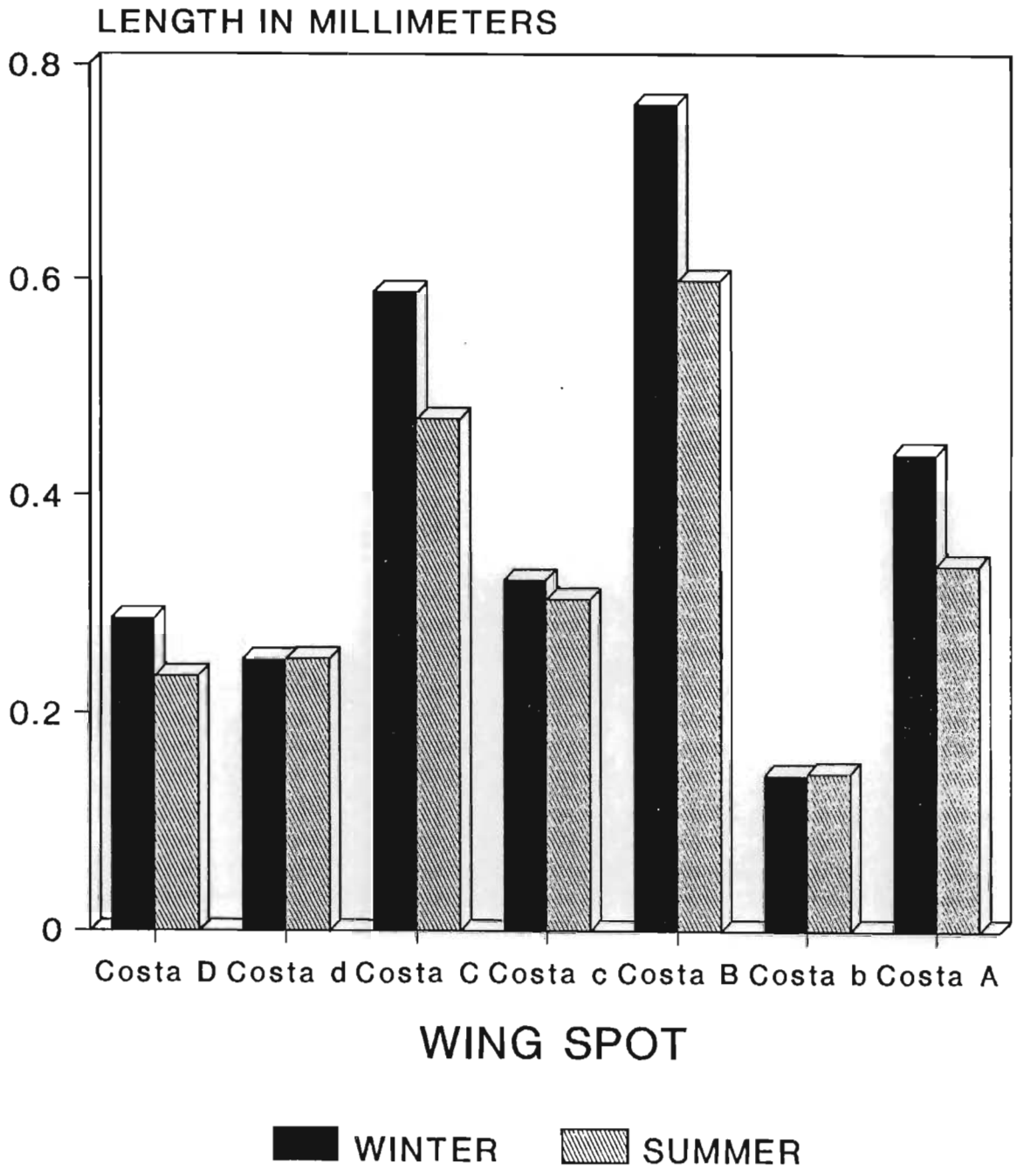


Figure 16. Comparison of the size of the pale and dark Costa areas, of the wings of *An. merus* and *An. arabiensis*, during winter and summer

These results suggest that the pale wing markings are unaffected by environmental factors such as temperature and are thus suitable as taxonomic characteristics for species determination. Table 11 shows a comparison of the size between these pale markings for *An. merus* and *An. arabiensis*.

Table 11. Comparison of the size of three pale, Costa, wing markings for *An. arabiensis* and *An. merus*.

PALE MARK	SPECIES	MEAN (mm)	S.D.	n	t-TEST (P)
d	*An. merus*	0,25	0,048	79	P<0,001
	An. arabiensis	0,28	0,043	72	
c	*An. merus*	0,30	0,049	79	P>0,05
	An. arabiensis	0,29	0,048	72	
b	*An. merus*	0,14	0,032	79	P>0,05
	An. arabiensis	0,15	0,036	72	

The pale markings **b** and **c** show no significant difference in size for the two species. Pale mark **d** was however significantly larger in *An. arabiensis*. Only 11,6% of the two samples failed to overlap, indicating that this criterion is of little taxonomic use. These mean values are similar to those reported by Coetzee (1986b). The large standard deviations in both studies indicate that they would not be significantly different. Coetzee (1986b) also found that the mean size for pale mark **d** of *An. arabiensis* was greater than that for *An. merus*. The difference was however not significant.

In section 3.2.2 it was shown that there was no significant abrasion of the pale band at the junction of tarsomeres 3 and

4 (of the hindlegs). Table 10 shows a similar result for the pale areas on the Costa of the wings.

Table 10. Comparison of the pale Costa wing markings, for larval erupt (le) and free-flying (ff) adult *An. arabiensis*

PALE MARK	SOURCE	MEAN in mm	n	t-TEST (P)
b	le	0,15	72	P>0,05
	ff	0,15	57	
c	le	0,30	72	P>0,05
	ff	0,29	57	
d	le	0,28	72	P>0,05
	ff	0,29	57	

The relationship between wing size and the size of tarsi 3 and 4 for *An. arabiensis* was investigated. This showed a linear relationship between these criteria, with an increase in wing size resulting in an increase in tarsi size;

1. Wing length and length of tarsus 3, **y = 4,16x + 32,6.** Correlation coefficient = 0,79.
2. Wing length and length of tarsus 4, **y = 2,99x + 26,5.** Correlation coefficient = 0,82.

In contrast there was no relationship between tarsus size and the size of the pale band at the junction of tarsomeres 3 and 4 (correlation coefficient = 0,31). This indicates that as in the case of the pale markings on the Costa, the above hindleg band is not affected by environmental factors bringing about changes in body size. Similarly it indicates that all the increase in size occurs entirely within the melanised region of the tarsi.

The initial aim of this portion of the study was to establish a suitable technique for the separation of larval instars and determine its accuracy. This technique was then to be used in population dynamics studies (Chapters 4 and 5). However as indicated earlier a number of environmentally -induced changes (Section 3.2.1 & 3.2.2) were found which were of taxonomic importance as are those reported in this section. They indicate that the pale areas of the wing costa and the hindlegs (tarsomere3/4) are good taxonomic criteria when considered in relation to seasonality. However the results have implications for previous work conducted on the *An. gambiae* complex. Coluzzi (1964) suggested that "the ratio of the length of the white ring to the length of tarsus usually gives discriminatory results." Coetzee (1986b) considered the results from the pale band to be adequate. As indicated by this study the use of tarsal length would in fact reduce the accuracy as this character is a product of mosquito size (and seasonality/temperature) whereas the pale band is not.

Zahar *et al.*, (1970) reported on the use of wing spot ratios. In view of the fact that dark wing spots were shown in this study to increase in size during winter, while the pale bands remain constant, then the validity of the use of this ratio in the field is questionable. The use of the palpal ratio (segments IV + IV/III), (Bryan, 1980; Bushrod, 1981; Coluzzi, 1964) for species separation in the *An. gambiae* complex is however unlikely to be affected as any seasonally induced changes in the length of the segments should occur propor-

tionately.

The results reported in this section demonstrate a limitation of using material which is raised under constant conditions (i.e. insectary) in the investigation of taxonomic characteristics suitable for species separation. The need to ascertain how "fixed" a particular characteristic is, under varying environmental conditions is highlighted.

References

Abdel-Malek, A. & Goulding, R.L. (1948). A study of the rate of growth of two sclerotised regions within larvae of four species of mosquitoes.-*Ohio J. Sci.* 48, 119-128.

Bryan, J.H. (1980). Use of palpal ratio and the number of pale bands on the palps in separating *Anopheles gambiae* Giles *s.s.* and *Anopheles melas* Theobald (Diptera:Culicidae).-*Mosq. Syst.* 12, 155-163.

Bushrod, F.M. (1981). The *Anopheles gambiae* Giles complex and Bancroftian filariasis transmission in a Tanzanian coastal village.-*Ann. Trop. Med. Parasit.* 75, 93-100.

Christie, M. (1954). A method for the study of larval populations of *Anopheles gambiae* and other pool-breeding mosquitoes.-*Ann. trop. Med. Parasit.* 48, 271-276.

Clarke, J.L. (1971). Potential use of the spermatheca in the separation of species A and B females of the *Anopheles gambiae* complex in northern Nigeria.-*Bull. Wld. Hlth. Org.* 45, 260-263.

Clements, A.N. (1963). *The physiology of mosquitoes*.-393 pp. *International series of monographs on pure and applied biology*. vol. 17. Pergamon Press, Oxford.

Coetzee, M. (1986a). Practical use of hind leg banding patterns for identifying members of the *Anopheles gambiae* group of mosquitoes.-*Mosq. Syst.* 18, 134-138.

Coetzee, M. (1986b). A morphometric study of four members of the *Anopheles* (*Cellia*) *gambiae* complex (Diptera: Culicidae).-132 pp. Phd. Thesis, University of the Witwatersrand, Johannesburg.

Coetzee, M., Newberry, K. & Durand, D. (1982). A preliminary report on a morphological character distinguishing important malaria vectors in the *Anopheles gambiae* Giles complex in southern Africa.-*Mosq. Syst.* 14, 88-93.

Coluzzi, M. (1964). Morphological divergences in the *Anopheles gambiae* complex.-*Riv. Malar.* 43, 197-232.

Coluzzi, M. (1968). Cromosomi politinici delle cellule nutrici ovariche nel complesso *gambiae* del genere *Anopheles*. -*Parassitologia*. 10, 179-183.

Coluzzi, M. & Sabatini, A (1969). Cytological observations on the saltwater species *Anopheles merus* and *Anopheles melas* of the *gambiae* complex.-*Parassitologia* 11, 177-187.

Deane, M.P. & Causey, O.R. (1943). Viability of *Anopheles gambiae* eggs and morphology of unusual types found in Brazil.-*Amer. J. trop. Med.* 23, 95-101.

Evans, A.M. (1931). Observations made by Dr. M. A. Barber on a melanic coastal race of *Anopheles costalis*, Giles (gambiae) in southern Nigeria.-*Ann. trop. Med. Parasit.* 25, 443-453.

Evans, A.M. (1938). Mosquitoes of the Ethiopian Region. II. *British Museum (natural History)*. London.

Ghelelovitch, S. (1950). Etuede genetique de deux characteres de pigmentation chez *Culex autogenicus* Robaud. *Bulletin Biologique de la France et de la Belgique*, 84, 217-224.

Gillies, M.T. & Coetzee, M. (1987). A supplement to the Anophelinae of Africa south of the Sahara.-*Publ. S. Afr. Inst. Med. Res.* no. 55, 143pp.

Gillies, M.T. & De Meillon, B. (1968). The Anophelinae of Africa south of the Sahara.-*Publ. S. Afr. Inst. Med. Res.* no. 54, 343pp.

Gillies, M.T. & Shute, G.T. (1954). Environmental influences and the maxillary index of *Anopheles gambiae*.-*Nature*. 173, 409-410.

Green, C.A. (1971). The practical problem of identifying

members of the *Anopheles gambiae* complex in autecological studies.-*Parassitologia*. 13, 421-426.

Green, C.A. (1972) .Cytological maps for the practical identification of females of the three freshwater species of the *Anopheles gambiae* complex.-*Ann. trop. Med. Parasit*. 66, 143-147.

Green, C.A. (1981). Malaria epidemiology and anopheline cytogenetics.-pp. 21-29. in Pal, R., Kitzmiller, J.B. and Kanda, T. (Eds.). *Cytogenetics and the genetics of vectors*.-265 pp. Kodansha Ltd., and Elsevier Biomedical. Tokyo and New York.

Green, C.A. & Hunt, R.H. 1980. Interpretation of variation in ovarian polytene chromosome of *Anopheles funestus* Giles, *A. parensis* Gillies and *A. aruni*?.-*Genetica* 51, 187-195.

Harbach, R.E. & Knight, K.L. (1980). *Taxonomists glossary of mosquito anatomy*. Plexus Publ. Inc., New York.-415pp.

Heuval, M.J. van den (1963). The effect of rearing temperature on the wing length, thorax length, leg length and ovariole number of the adult mosquito, *Aedes aegypti* (L.).-*Trans. R. ent. Soc. Lond.* 115, 197-216.

Holstein, M.H. (1954). Biology of *Anopheles gambiae*. Monograph series of the World Health Organisation, Geneva.-172pp.

Hunt, R.H. (1973) A cytological technique for the study of the *Anopheles gambiae* complex.-*Parassitologia* 15, 137-139.

Hunt, R.H. (1987). Location of genes on chromosome arms in the *Anopheles gambiae* group of species and their correlation to linkage data for other anopheline mosquitoes.-*Med. Vet. Entomol.* 1, 81-88.

Hunt, R.H. & Coetzee, M. (1986). Field sampling of *Anopheles*

mosquitoes for correlated cytogenetic, electrophoretic and morphological studies.-*Bull. Wld Hlth Org.* 64, 897-900.

Jones, J.C. (1953). Some biometrical constants for *Anopheles quadrimaculatus* Say larvae in relation to age within stadia.-*Mosq. News* 13, 243-247.

Knoch, K. & Schulze, A. (1956). Niederschlag, temperatur und schwule in Afrika, *World Atlas of Epidemic Diseases*. vol 2. Heidelberger & Akademie der wissenschaften, Heidelberg.

Le Sueur, D. & Sharp, B.L. (1988). The breeding requirements of three members of the *Anopheles gambiae* Giles complex (Diptera:Culicidae) in the endemic malaria area of Natal, South Africa.-*Bull. ent. Res.* 78, 549-560.

Le Sueur, D. and Sharp, B. L. (1991). Temperature-dependent variation in the head capsule width and wing length of *Anopheles merus* Donitz: Implications for anopheline taxonomy.-*Med. Vet. Entomol.*, 5, 8pp.

Mahon, R.J., Green, C.A. & Hunt, R.H. (1976). Diagnostic allozymes for routine identification of adults of the *Anopheles gambiae* complex (Diptera: Culicidae).-*Bull. ent. Res.* 66, 25-31.

Mahon, R.J., Green, C.A. & Hunt, R.H. (1976). Diagnostic allozymes for routine identification of adults of the *Anopheles gambiae* complex (Diptera: Culicidae).-*Bull. ent. Res.* 66, 25-31.

Marks, E.N. (1954). A review of the *Aedes scutellaris* subgroup with a study of variation in *Aedes pseudoscutellaris* Theobald (Diptera: Culicidae). *Bulletin British Museum (Natural History) Entomology*, 3, 349-414.

Miles, S.J. (1978). Enzyme variation in the *Anopheles gambiae*

Giles group of species (Diptera:Culicidae). *Bull. ent. Res.* **68**, 85-96.

Miles, S.J. (1979). A biochemical key to the adult members of the *Anopheles gambiae* group of species (Diptera : Culicidae).-*J. med. Ent.* **15**, 297-299.

Mitrofanova, J. (1929). On the growth of the head in the larvae of *Anopheles maculipennis* Meig.-*Bull. ent. Res.* **19**, 361-366.

Muirhead-Thomson, R.C. (1948). Studies on *Anopheles gambiae* and *Anopheles melas* in and around Lagos.-*Bull. ent. Res.* **38**, 527-558.

Muirhead-Thomson, R.C. (1951). Studies on salt-water and freshwater *Anopheles gambiae* on the East African coast.-*Bull. ent. Res.* **41**, 487-502.

Rattanarithikul, R. & Green, C.A. (1986). Formal recognition of the species of the *Anopheles maculatus* group (Diptera: Culicidae) occurring in Thailand, including the descriptions of two new species and a preliminary key to females.-*Mosq. Syst.* **18**, 246-278.

Ray, C. (1960). The application of Bergmann's and Allen's rules to the poikilotherms.-*J. Morphology* **106**, 85-108.

Ribbands, C.R. (1944). Differences between *Anopheles melas* (*Anopheles gambiae* var. *melas*) and *Anopheles gambiae*. I.-The larval pecten.-*Ann. trop. Med. Parasit.* **38**, 85-99.

Service, M.W. (1970). Ecological notes on species A and B of the *Anopheles gambiae* complex in the Kisumu area of Kenya.-*Bull. ent. Res.* **60**, 169-180.

Service, M.W. (1973). Mortalities of the larvae of the *Anopheles gambiae* Giles complex and detection of predators by the precipitin test. *Bull.ent.Res.* **62**, 350-369.

species B of the *Anopheles gambiae* complex in Kenya: comparison between rice fields and temporary pools, identification of predators, and effects of insecticidal spraying.-*J. med. Ent.* 13, 535-545.

Sharp, B.L. (1983). *Anopheles merus* Donitz its biology in relation to malaria transmission in Natal. Master of Science thesis. 162pp. University of the Witwatersrand, Johannesburg.

Sharp, B.L., le Sueur, D. & Ridl, F.C. (1989). The value of hind leg banding patterns in the identification of species of the *Anopheles gambiae* Giles complex (Diptera: Culicidae) in Natal, South Africa.-*Mosq. Syst.* 21 (1), 21-26.

Shinkarenko, L., Hulsman, K., Mottram, P., Dale, P. & Kay, B.H. (1986). Reliability of using head capsule width and body length to identify larval instars of *Aedes vigilax* (Diptera: Culicidae).-*J. Austral. Ent. Soc.* 25, 37-40.

White, G.B. & Muniss, J.N. (1972). Taxonomic value of spermatheca size for distinguishing four members of the *Anopheles gambiae* complex in East Africa.-*Bull. Wld Hlth Org.* 46, 793-799.

Soper, F.L. & Wilson, D.B. (1943). *Anopheles gambiae* in Brazil, 1930 to 1940. 262pp. New York: Rockefeller Foundation.

World Health Organisation. (1975). *Manual of Practical entomology*. II. Techniques. 191pp. W.H.O. Geneva.

Zahar, A.R., Hills, M. & Davidson, G. (1970). An attempt to group freshwater species of the *Anopheles gambiae* complex by some morphological larval and adult characters.-*Parassitologia* 12, 31-46.

CHAPTER 4

LARVAL GROWTH AND PRE-IMAGINAL DURATION

INTRODUCTION

Knowledge of larval growth and metamorphosis is an essential prerequisite to the investigation of the dynamics of larval populations and of malaria transmission. The importance of the larval stages is highlighted by the fact that it is to a large extent their growth rates and survivorship which are responsible for the observed fluctuations in adult populations. In addition larval nutrition and fat deposition play a important role in the fitness and ultimate survival of the ecloded adult. Population size and individual longevity are ultimately the factors which determine the transmission efficiency of the vector.

In this chapter the investigation of larval growth patterns and rates is reported. The utilization of these larval growth rates in the determination of survivorship is reported in chapter five.

Dyar (1890) measured the head capsule width of caterpillars and concluded that the ratio of increase was constant and thus best described by a geometric progression. A number of authors have subsequently investigated the growth of the head capsule in mosquito larvae and was shown to conform to Dyar's rule in the following species; *An. sergentii* (Kettle, 1948), *Culex quinquefasciatus* (Deslongchamps & Tourneur, 1980),

Culex territans (De Olivera & Durand, 1978) and *Culex pipiens fatigans* (Sen & Das Gupta, 1958). The following species did however not conform to Dyar's rule; *Aedes vigilax* (Shinkarenko *et al.*, 1986), *An. quadrimaculatus* (Jones, 1953), *Aedes aegypti* and *Aedes trivitatus* (Abdel-Malek & Goulding, 1948).

Jones (1953) and Deslongchamps & Tourneur (1980) working respectively on *An. quadrimaculatus* and *Culex quinquefasciatus*, noted that larvae from the same egg batch, under uniform conditions showed considerable variation in growth rates. Thus their appears to exist individual variation, which is not a result of factors such as nutrition and temperature. Retardation of larval growth rates due to insufficient food has been documented (Marcovitch, 1960; Nielsen & Haeger, 1954; Wiggelsworth, 1929). Marcovitch (1960) found that on a diet of cornstarch, *Aedes aegypti* larvae reached the third instar and remained alive for 120 days. Ouda *et al.*, (1986) reported the occurrence of a slow growth larval phenotype (sgl) for *Culex quinquefasciatus*, which could only be maintained as a heterozygous line.

Considerable investigation into the effect of temperature on larval/pupal growth rates and instar duration have been carried out. These have included studies of the optimal temperatures for development and of the effect of constant versus fluctuating temperatures (Clements, 1963; Huffaker, 1944; Milby & Meyer, 1986; Mottram *et al.*, 1986). From these studies the following conclusions regarding temperature may be made;

1) That for each species there is an optimal temperature at which development is rapid and the duration of the larval/pupal (immatures) stages is short.

2) That at this development rate the amount of available food or the rate at which it can be gathered may become limiting and result in small adults.

The duration of the immature stages of the *An. gambiae s.l.* have been recorded by a number of authors prior to the elucidation of the complex. Holstein (1954) recorded a pre-imaginal duration of 8,2 days at temperatures above 26°C and 13,2 days when the temperature fell below 25°C. Jepson et al. (1947), working in Mauritius estimated that the optimal temperature for development was 25-26°C and hypothetically calculated that at temperatures below 18°C, the duration of the immature cycle would be in excess of one month. These estimates were based on measured larval to pupal/adult duration for saline and freshwater breeding sites. They would thus have included the three members of the *An. gambiae* complex which occurred on Mauritius viz; *An. gambiae s.s.*, *An. arabiensis* and *An. merus*. Other field records for the *An. gambiae s.l.* range between 6 and 13 days (Ethes, 1939; Mathis, 1935). Service (1973) estimated the duration of the immature stages to be 11,8 days in Kisumu, Kenya during November/December. The *An. gambiae* population he was studying was estimated to consist predominantly (70-80%) of *An. arabiensis*, the remainder being *An. gambiae s.s.*

MATERIALS AND METHODS

4.2.1 MORPHOLOGICAL MEASUREMENTS

The methodology relating to morphological measurements reported in this chapter are given in Chapter 3.

4.2.2 LARVAL GROWTH TRIALS

4.2.2.1 LABORATORY

Laboratory larval trials were initially carried out under constant temperature regimes, using a temperature controlled waterbath. The food utilised in the initial trials was different to that of the latter trials in that it contained more activated yeast. Subsequent trials were carried out using a Conviron programmable growth cabinet. Seasonal temperature and light profiles based on field recordings were used. Larvae were placed individually into the wells of a microtitre plate. and approximately 250 were used in each trial. In later trials larvae were not separated and approximately 1000 larvae were raised simultaneously in a plastic bowl. The larval food utilised in all fluctuating temperature trials originated from the same manufactured batch and was of the same constitution as that utilised in the insectary. Larval food was always provided *ad libitum* and the water changed daily to prevent fouling.

A subsample of thirty larvae was removed daily and the following measurements recorded for each larva; head capsule width, abdomen length, thorax width, head capsule length and collar width (for explanation, see section 3.1, materials and

methods). Larvae were measured in a drop of water on a glass slide and then returned to their wells. Instar duration was monitored at six hour intervals and change in instar noted by the presence or absence of larval skins. Morphological measurements were not taken during the aggregated larval trials.

All larval growth trials were carried out with *An. merus*; one with colony material and three with field material. The field material was from gravid adult females collected at cattle kraals (at Ophansi, 27°36'S 32°16'E) and placed in breeding tubes to oviposit. Eggs were held over on damp filter paper for 48 hours and then placed in water. Larvae were identified using the salinity tolerance test and electrophoresis of the parent female. Two initial trials (field material) were conducted in distilled water and subsequent ones (two field and one colony) using water containing 7,82g NaCl per liter.

4.2.2.2 FIELD

A single growth trial was carried out in the field during winter (July 1987). This portion of the study was carried out at Nceswana pan in the Ophansi district (Chapter 2, Appendix 2, Plate 18). Six pools of approximately the same dimensions (23 x 17 x 3 cm) were selected, drained and flushed. The water was then filtered to remove all larvae, eggs and predators. The pools were then reconstituted using the filtered water and the salinity noted. Larvae were collected and sorted into instars, using head capsule width, as described in Chapter 3. Larvae in each particular instar were then introduced to the pools in pre-determined densities, approxi-

mating those recorded from the July 1984 collections. All individuals in one pool were in the same instar. Two pools were set up for fourth instar larvae one based on the average winter density and the other at 25% of this density. The pools were then emptied daily and changes in instar noted. First instar duration was measured using eggs obtained from gravid females collected at a nearby pit shelter. The individuals in each pool were then followed through two changes in development (ie. instar or eclosion), and these were plotted. The difference between the means of the two mortality corrected distributions gave the instar or pupal duration. Pools were covered with gauze covers at night to prevent oviposition by free-flying females and the entry of potential predators.

4.2.3 PROCESSING FOR SCANNING ELECTRON MICROSCOPY

Larval specimens were fixed in 3% glutareldahyde for 24 h and then washed twice (2 X 30mins.) in 0.05M cacodylate buffer. The specimens were then fixed in 2% Osmium tetroxide for two hours. The two 30 minute washes in cacodylate buffer were then repeated. Dehydration was carried out in the following series of ethanol;

10% for 10 minutes

20% for 10 minutes

50% for 10 minutes

70% for 10 minutes

90% for 10 minutes

100% for 2 X 15 minutes

The specimens were then transferred to baskets for critical

point drying (CPD), but kept immersed in ethanol all the time. Critical point drying was carried out in liquid CO_2 using a Hitachi HCP II critical point dryer. Viewing of the specimens was carried out at 8KV using a Hitachi S5-70 scanning electron microscope (SEM).

4.2.4 RATIONALISATION OF MULTIPLICATIVE AND EXPONENTIAL REGRESSION MODELS

This study used the exponential model ($y = e^{a+bx}$) of regression analysis in the investigation of Dyar's rule. The majority of authors have however utilised the multiplicative model ($y = ab^x$). In the latter, assuming conformation to Dyar's rule, 'b' is the factor by which head capsule width increases between successive instars. In order that the factorial increases in this study and those of other studies could be compared, the following transformation was carried out;

$$y = e^{a+bx}$$
$$= e^{a} * e^{bx}$$
$$= e^{a} * (e^{b})^{x}$$

Thus in the two models , the following are equivalent;

	Multiplicative	**Exponential**
	a	$=e^{a}$
and	b	$=e^{b}$

4.3 RESULTS

4.3.1 DYAR'S RULE

The equations for the geometric regressions of head capsule

width against instar are given below. It is evident from the values for R-squared that the data closely follow a geometric progression and that variation in instar can be explained in terms of head capsule width. The increase in head capsule width is constant between successive instars and the data thus conforms to Dyar's rule.

Table 12. Geometric regression equations of head capsule width against instar for three members of the *An. gambiae* complex. The factorial increase between instars and the R-squared values for the equations are also given.

Species and origion	Geometric equation	Factorial Increase	R-squared
An. arabiensis colony	$y = e^{-2,31 + 0,46x}$	1,58	99,08%
An. arabiensis wild	$y = e^{-2,25 + 0,45x}$	1,57	99,07%
An. merus colony	$y = e^{-2,27 + 0,50x}$	1,65	98,78%
An. merus wild	$y = e^{-2,21 + 0,48x}$	1,62	98,56%
An. quadriannulatus colony	$y = e^{-2,29 + 0,46x}$	1,58	99,30%

4.3.2 LARVAL GROWTH

Figure 17 shows the relationship between abdomen length and thorax width with time, under simulated winter temperature conditions. The equations for the lines are as follows;

		R-squared
Abdomen length:	$y = e^{-0.48 + 0.061x}$	86.4%
Thorax width :	$y = e^{-1.86 + 0.069x}$	82.5%

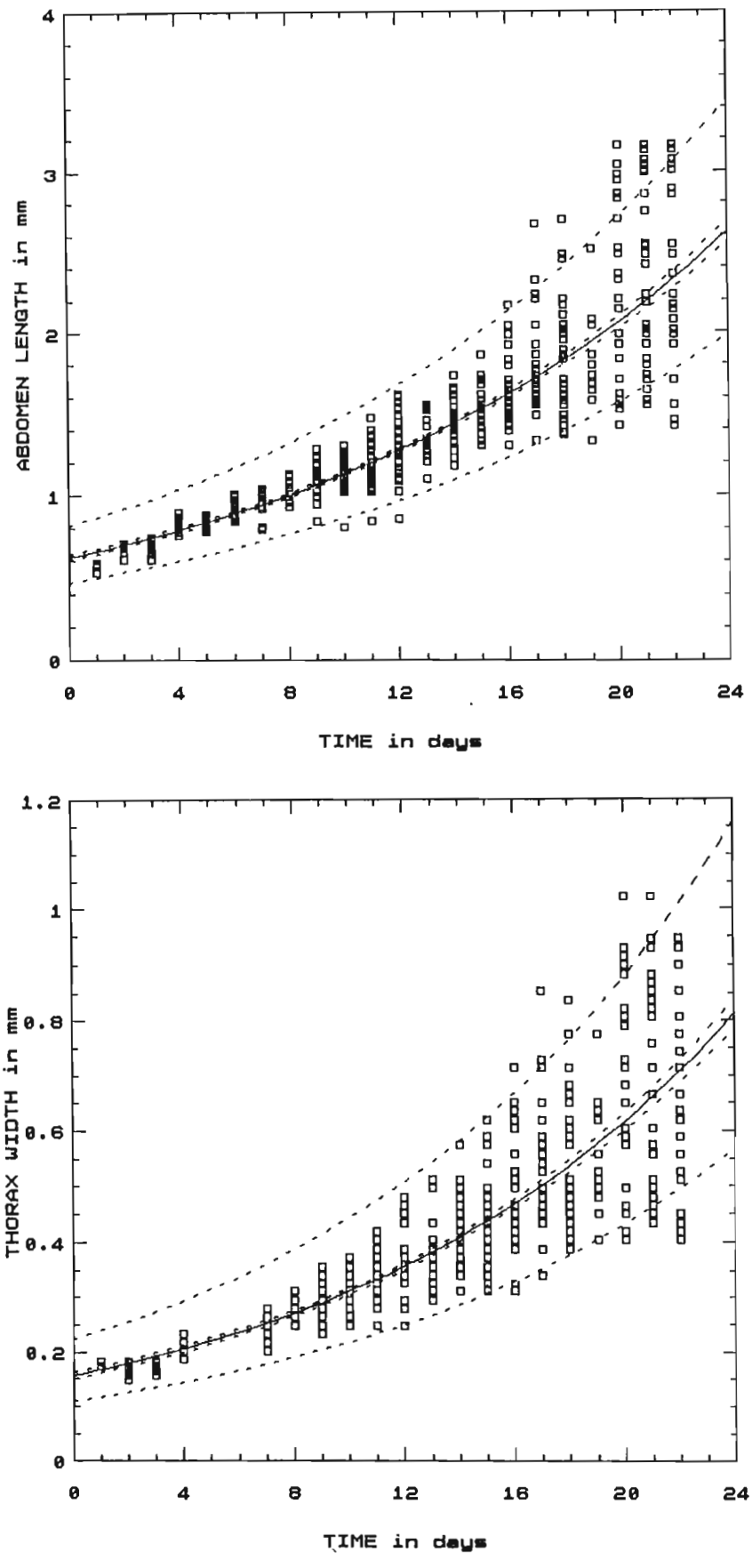


Figure 17. The increase in abdomen length (**top**) and thorax width (**bottom**) with time for all instars combined, under simulated winter conditions. The inner dotted lines represent the prediction limits and the outer lines the 95% confidence limits.

The R-squared values indicate that between 86 and 83% of the respective variation in abdomen length and thorax width can be explained in terms of time (age). It is however evident that the correlation decreases with time. These figures represent all instars and it was thus decided that a clearer picture would be obtained by investigating growth within each instar. The increase in abdomen length with time for instars I to III is shown in Figure 18. It is immediately evident that differential growth rates of individuals are responsible for the increase in the observed range of values for abdomen length with time. This is mirrored by the decrease in the measured values of R-squared;

Instar		R-squared
I	=	81,5%
II	=	40,7%
III	=	10,3%

Note should also be taken of the individuals in the graph for first instar larvae, which lie outside the 95% confidence limits.

In this growth trial the larvae were raised individually in the wells of a microtitre plate. It was thus possible to follow the development of selected individuals and growth profiles of six larvae are shown in Figure 19. There appear to be two distinct growth patterns. The former is best described by the exponential model and the latter by the linear model. The equation for the lines are shown in Table 13.

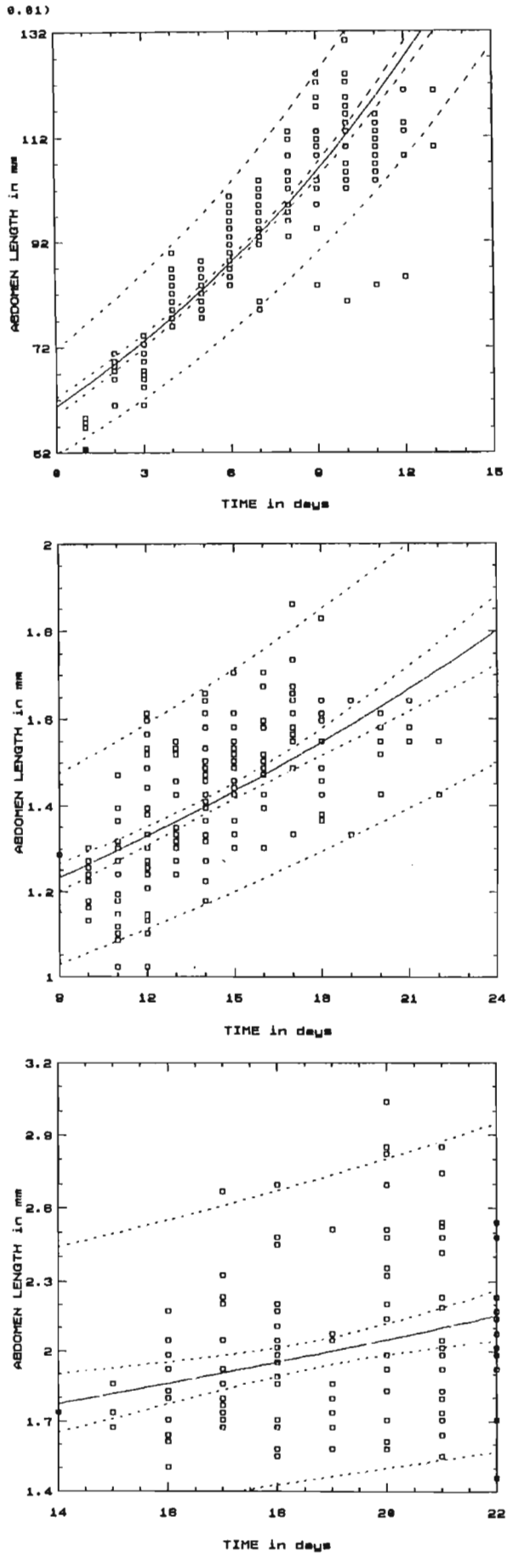


Figure 18. Regressions of abdomen length against time for instars one to three. Simulated winter conditions.

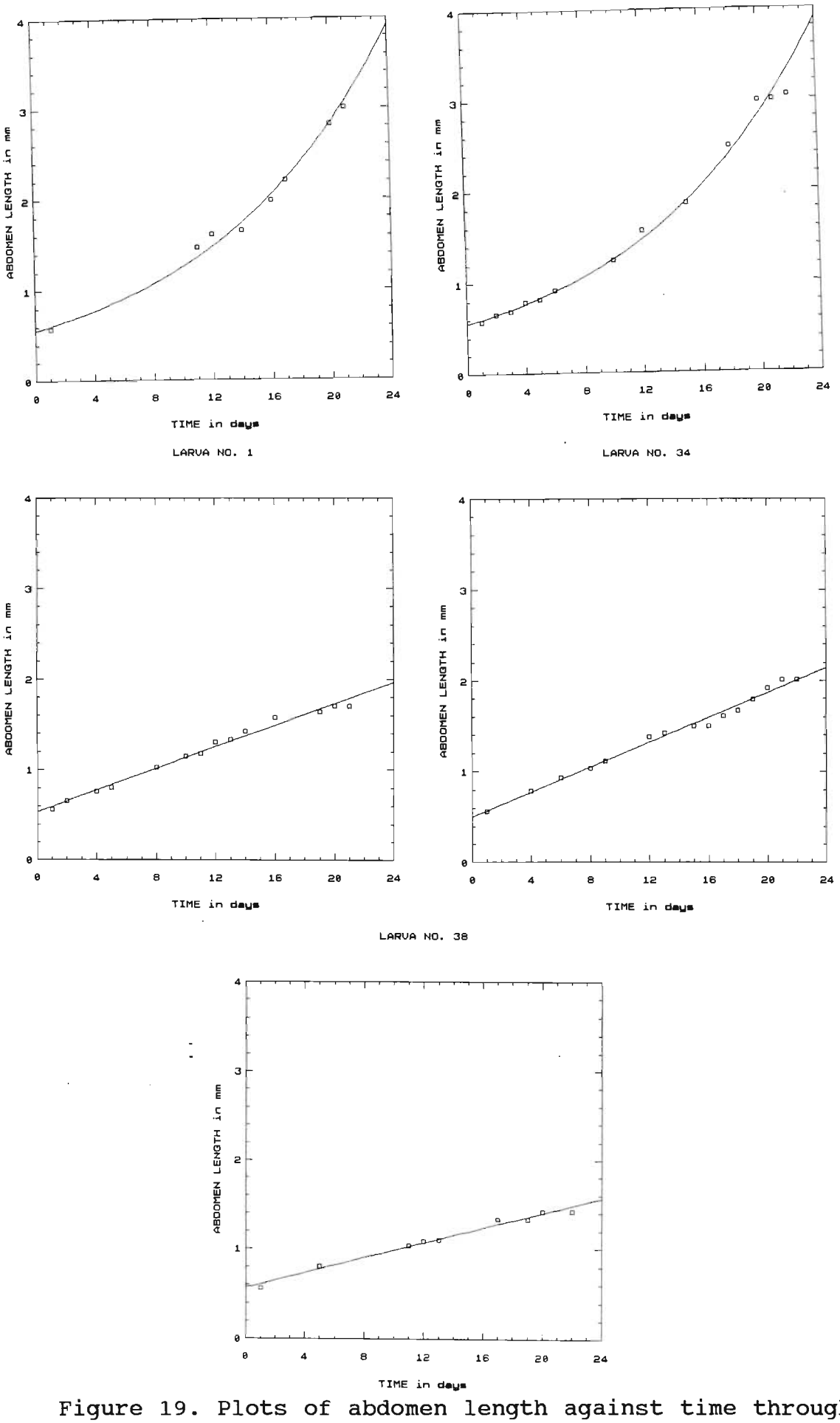


Figure 19. Plots of abdomen length against time through all larval stages for six individual larva. Larvae were maintained under simulated winter conditions. Arrows indicate points were change in instar could be accurately determined.

Table 13. Mathematical equations and correlation coefficients describing the growth of six individual larvae under simulated winter conditions

Larva No.	Equation	Correlation Coefficient
	Linear	
38	$y = 0.57 + 0.042x$	0.992
61	$y = 0.54 + 0.051x$	0.998
34	$y = 0.50 + 0.068x$	0.995
1	$y = 0.54 + 0.059x$	0.994
	Exponential	R-squared
131	$y = e^{-0.59 + 0.081x}$	99.6%
104	$y = e^{-0.58 + 0.081x}$	99.0%

Figure 20 shows a plot of the number of larvae in each stage against time. The important points to note here are;

1) the fact that the first adults erupted on day 28, at which stage some larvae were still in the first instar.

2) that some larvae were still in the fourth instar by day 44.

3) the tailing effect which the slow growth larvae cause and which is especially noticeable in the third and fourth instars.

4.3.3 LABORATORY TRIALS ON THE EFFECT OF TEMPERATURE ON LARVAL AND PUPAL GROWTH RATES

A comparison of larval growth rates under constant and fluctuating temperature regimes is shown in Table 14 below. The fluctuating temperature of 15^{o}C represents the mean over 24 hours. The actual winter temperature profile used is shown in Appendix 3a. The important factors to note are that growth in the first and second instars appear to be less temperature

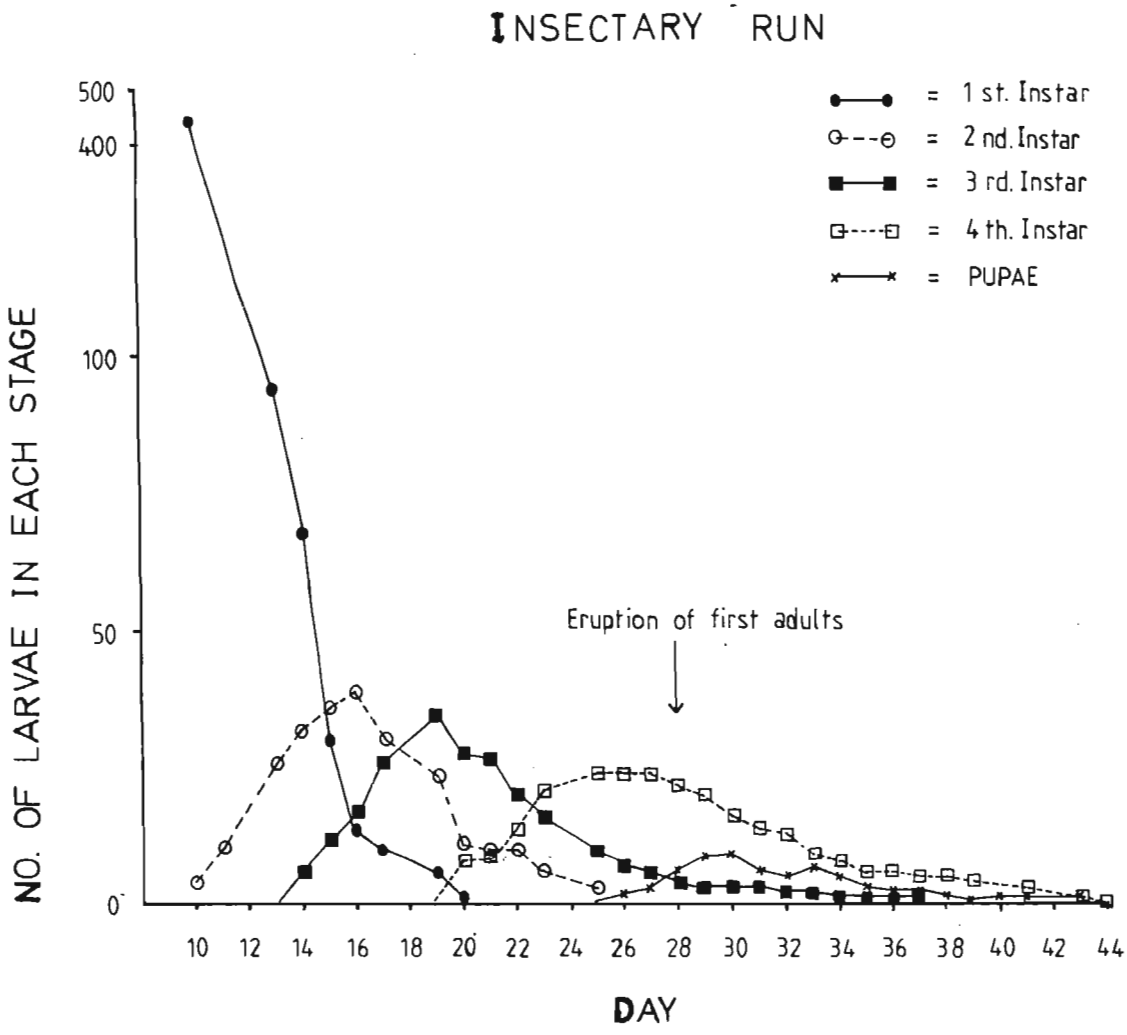


Figure 20. Plot of the number of larvae in each stage against time. Insectary *An. merus* were used for the trial and were maintained under simulated winter conditions.

Table 14. Comparison of larval growth rates under fluctuating and constant water temperatures (units = days)

Temperature	Larval instar				Total instar duration
	I	II	III	V	
Constant 18°C	2,8	5,0	4,7	9,6	22,1
Constant 25°C	2,8	5,1	2,1	5,1	15,1
Fluctuating 15°C mean	9,7	4,6	4,2	7,5	26,8 (21,6-24,0)

dependent. The high value recorded for first instars during the fluctuating temperature trial is a product of the food utilised. Evidence for this conclusion will be given later. The values quoted in brackets represent the corrected duration using the field recorded value of 5.2 days and the value of 2.8 recorded at 18°C.

Table 15 gives the summarised results of all laboratory trials on the effect of fluctuating temperatures on the duration of the immature stages. Initial trials were conducted using distilled water, but were unsuccessful due to high mortalities. These were subsequently found to be a result of a parasitic fungus. Figure 21 shows a comparison between the combined mortalities recorded for the two trials conducted in freshwater and those recorded for the trial using field material (trial 3, Table 15) and saline water. The effect of the parasitic fungus is evident and no larvae attained fourth instar. The egg to adult survival for the trial conducted in saline water was 28.6%.

Table 15. The effect of temperature on the duration of larval and pupal stages, for *An. merus* (units=days).

		Larval instar				Pupae	Pre-imaginal cycle		
		I	II	III	IV		Mean	Range	
This study Winter	Insectary material	13,7	8,2	7,8	6,0	2,8	38,5	30,0-48,0	Saline water 7,82g NaCl/l or 10 p.p.t.
	Field material (3)	9,7	4,6	4,2	7,5	3,8	29,8	25,0-39,0	
This study Summer	Field material	2,1	2,0	1,4	2,4	1.2	9,1	6,5-11,5	
Service Summer 1978	Field recorded	1,4	2,9	1,9	3,8	1,8	11,8		
This study Winter	Field material (1)	9,7	6,6	3,2	Fungal contamination				Distilled water (0 p.p.t.)
	Field material (2)	10,0	4,5	6,1					

The extent of parasitism is shown in Plate 22 a, b, c, d, e and f. Plate 22a is of the valve mechanism of the respiratory siphon and the area surrounding it. The fungus forms a dense mat over the body surface. Plate 22b shows the extensive parasitism of a single larval setal branch. Plate 22c is of the anal papillae, the outline of which can be barely made out due to the density of the fungal infection. From initial observations it appeared as if the hyphae were penetrating through the body surface, as in the case of *Coelomyces* fungi (Plate 22d). However upon closer investigation it became apparent that the fungus was in fact attached to the surface (Plate 22e). Plate 22f is of the head capsule of a parasitised larvae. This plate shows the unusual linear growth patterns of the fungus, as well as apparent penetration of

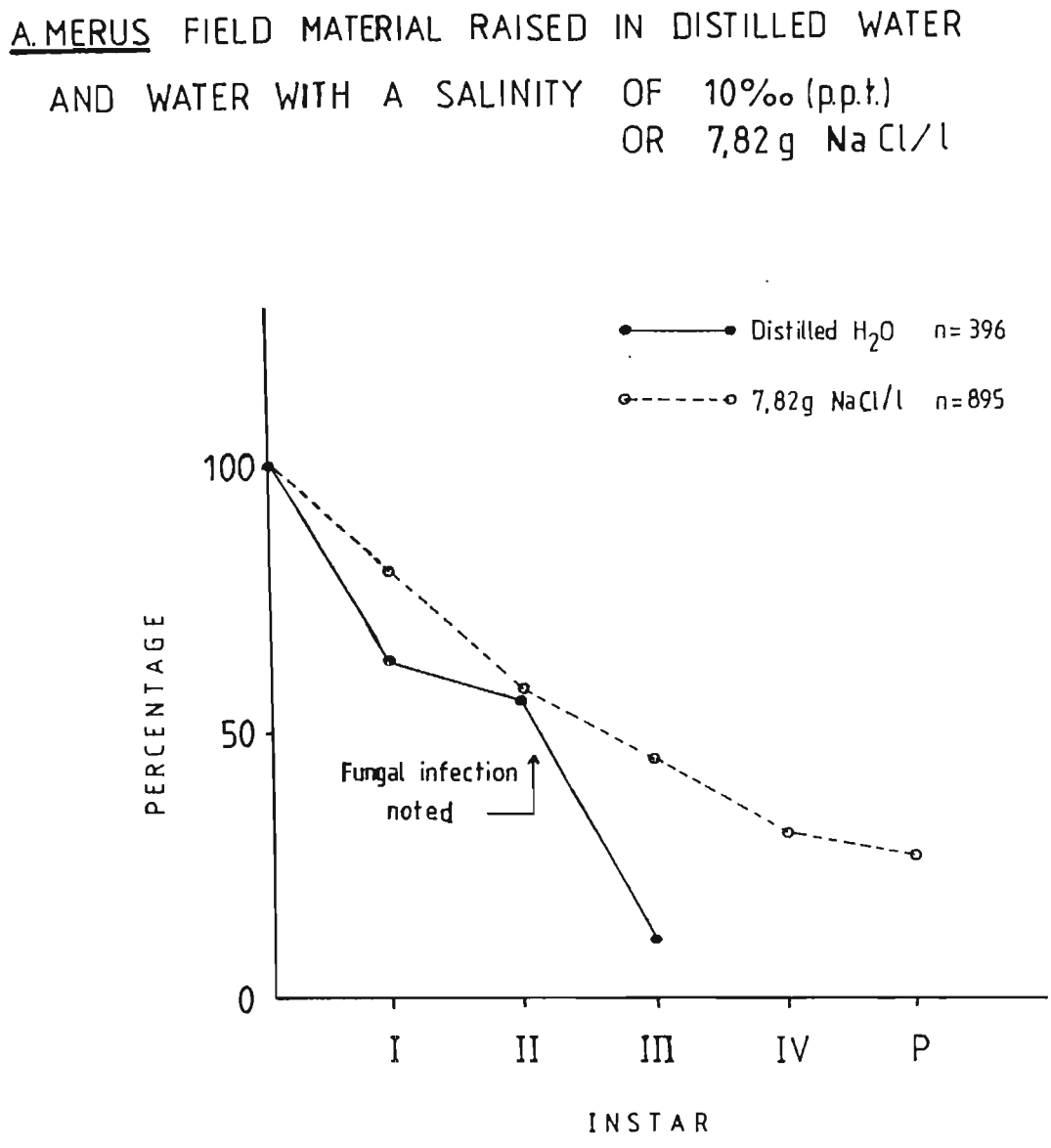


Figure 21. Comparative survival rates of *An. merus* larvae raised in saline and distilled water.

Plate 22

A : Scanning electronmicrograph showing the valve mechanism of the respiratory siphon and the parasitisation of the surrounding body surface.

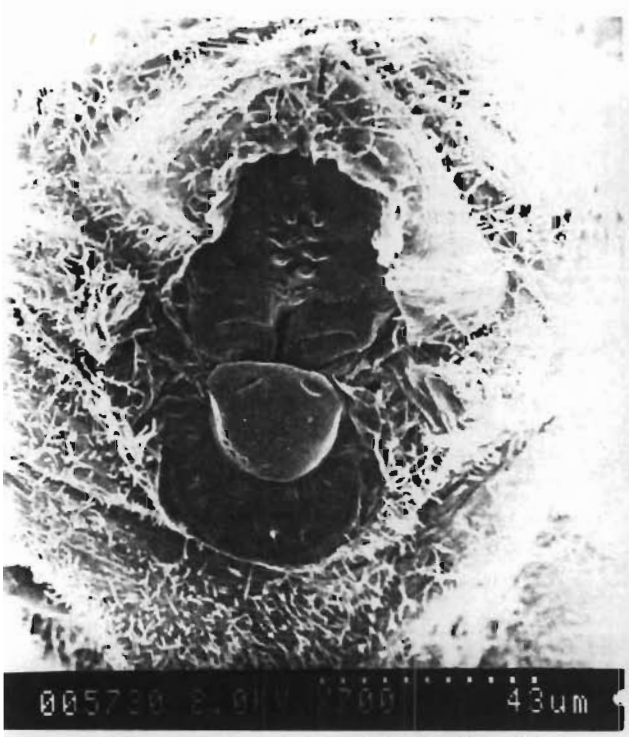
B : Parasitisation of a single larval setal branch.

C : Scanning electron-micrograph of anal papillae, the outline of which can be barely recognised due to the density of the fungal infection.

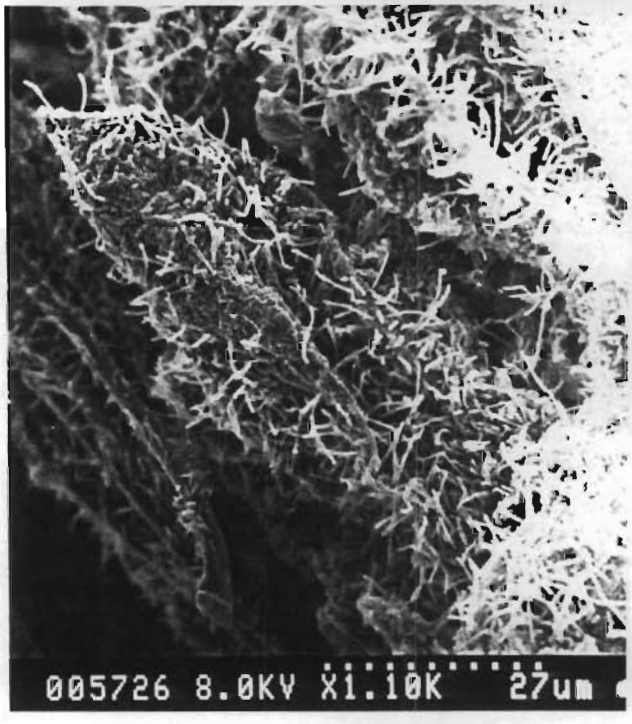
D : Apparent penetration of body surface by fungus.

E : Close-up showing that fungus is in fact attached to the exterior and that holdfast is responsible for giving the appearance of penetration seen above.

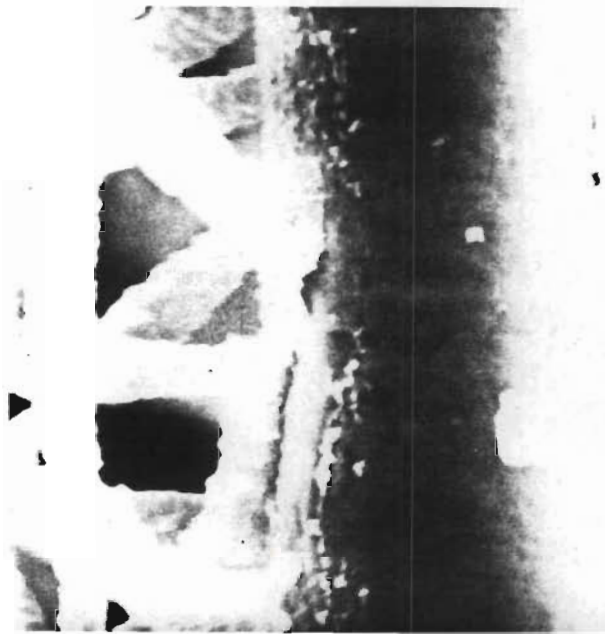
F : Scannining electron-micrograph of fungus showing unusual curvi-linear patterns and apparent penetration.


43um


005725 8.0KV X2.49K 12.0um


005726 8.0KV X1.10K 27um


005728 8.0KV X10.0K 2.31um



the head capsule. Attempts to have the fungus identified by South African mycologists proved unsuccessful. There are however two records of external parasitic fungi for *An. maculipennis*, one belonging to the family Saprolegniaces and the other described as a *aspergillus* type of fungus (Walker, 1938).

More recently a lot of attention has focussed on a fungal pathogen *Culicinomyces clavisporus*, which was first reported from laboratory colonies of *Anopheles hilli* and *Anopheles quadrimaculatus* in 1972 (Sweeney, 1985). This a Deuteromycetes fungus which colonises the body cavity of living larvae and produce a layer of asexual spores. The fungus appears to closely resemble that described, however the question of penetration raised earlier needs to be clarified. The apparent penetration of the head capsule described earlier has not been reported for this species. Electron micrographs have been sent to Dr. A.W. Sweeney for positive identification. One of the advantages of this fungal pathogen is that it produces true conidia in submerged culture, thus facilitating mass production (Sweeney et al., 1983).

Due to the fact that the field collection site is situated more than 400km from the laboratory, it was decided to use insectary material in the next growth trial. Extremely high first instar mortalities (97%) however occurred. The survivorship curve is shown in Figure 22. Fifty five larvae attained second instar and the mortalities of these through the remainder of the cycle were slightly higher than those recorded for field material. The low numbers available for the

latter stages of this trial precluded the accurate determination of the larval and pupal durations.

The following points (Table 15) summarise the important result from all the trials;

1) The high values for the preimaginal cycle under winter conditions (29.8) compared to the values for summer (9.1).
2) The high value recorded for first instar during the summer trial, relative to the values for the other instars and to those recorded by Service (1973).
3) The consistently high values recorded for the first instar under winter conditions ie.13,7; 9,7; 9,7; 10,0.

The temperature profile used in the summer trials is shown in shown in Appendix 3b. The temperatures used in this profile are based on the highest pool temperatures recorded from the field (Chapter 2, Appendix 1).

4.3.4 WINTER, FIELD DURATIONS OF THE LARVAL AND PUPAL STAGES.

Figure 23 shows the duration of first and second instars recorded in the field, as well as the method of calculation. The calculated values for first and second instars were 5,2 and 5,5 days respectively. Temperature was recorded at hourly intervals throughout the trial and the mean for each time (eg, 10h00), over the trial period was plotted (Appendix 3c). A sample of the hourly temperatures recorded over a 5 day period is shown in Appendix 1 (Chapter 2). The duration for

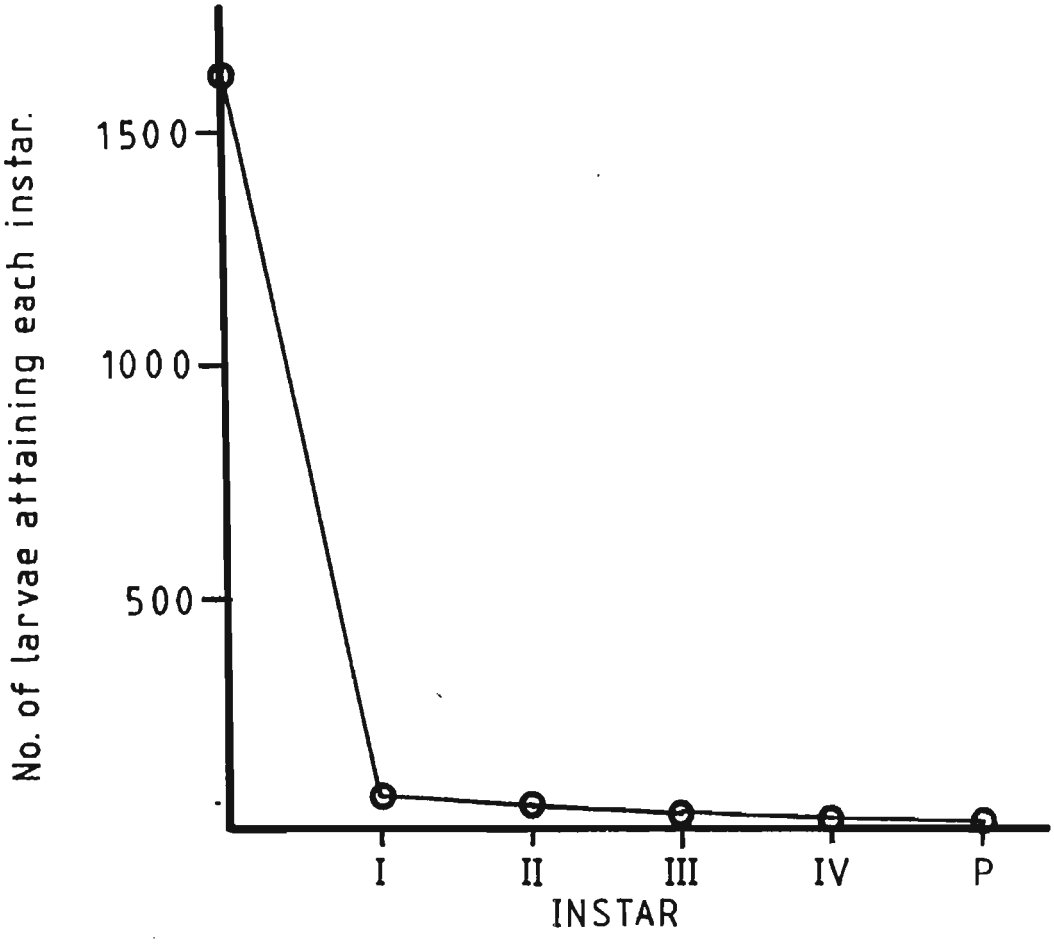


Figure 22. Survival of *An. merus* colony material (usually maintained at 24°C) when raised under simulated winter conditions.

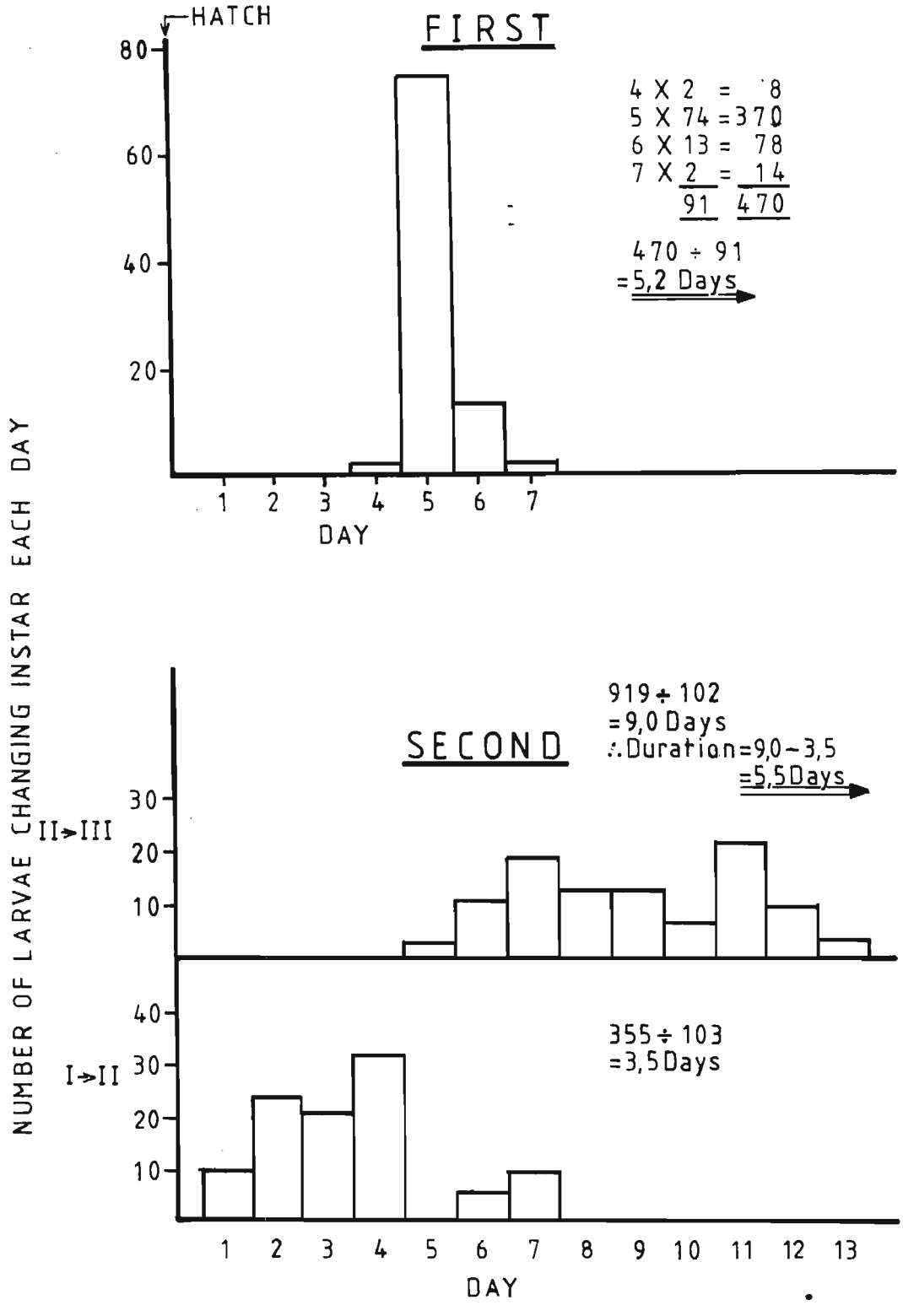


Figure 23. Mortality corrected plots of the number of larvae in a particular instar, against time, as a measure of mean instar duration. Winter field calculated instar durations for first and second stage larvae are shown. Only a single plot is shown for first instar as the starting point is the time of hatch. The difference in growth rates (lack of synchrony) necessitates the use af a mean starting and end point in later instars (i.e. two to pupae)

third instars was measured as 8,6 days and is shown in Figure 24. For the investigation of the duration of the fourth instar, pools with a low and high larval density were created and the duration measured (Figure 25). The fourth instar duration for the low density pool was measured as 11,4 days. That of the high density pool could not be measured as no individuals had reached the pupal stage. The only conclusion which can thus be drawn for the high density pool is that the duration is definitely in excess of 21 days. The pupal duration was calculated as 3,6 days (Figure 26). This calculation had to be based on the first three days of both distributions due to high mortalities incurred on days 10 and 11. The cause of mortality is unknown, but may have been due to the presence of a spider (*Thalassius* spp.) which occurs at this site and is often noted on the water surface of pools.

4.4 DISCUSSION

4.4.1 DYAR'S LAW

Dyar's law suggests that the size of the head capsule in each instar is determined by ;

1) The size of the head capsule in the first instar
2) The factorial increase between instars
3) The number of moults which have occurred

Figures 9 and 11 show that the head capsule width of the three species are remarkably similar in the first instar, the difference of the means between first instar freshwater members and *An. merus* being 4%. The same comparison between individuals in the fourth instar yields a 12% difference in

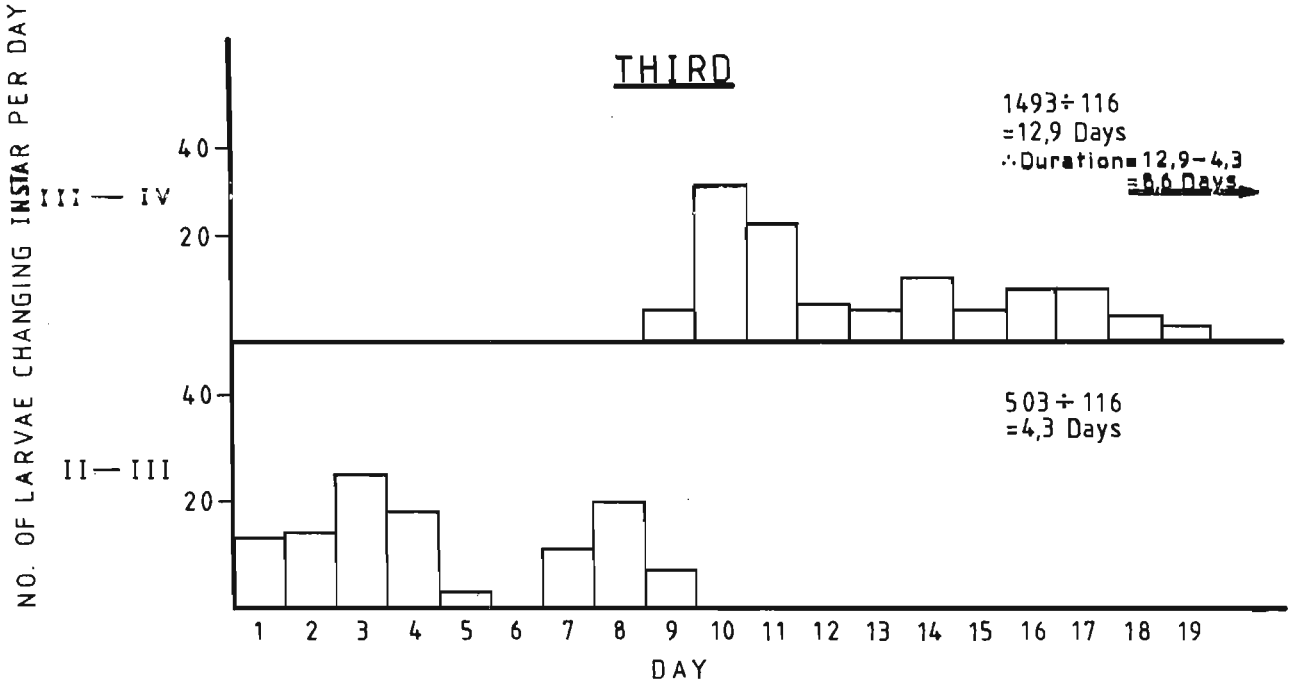


Figure 24. Instar duration of third stage larvae under winter conditions in the field.

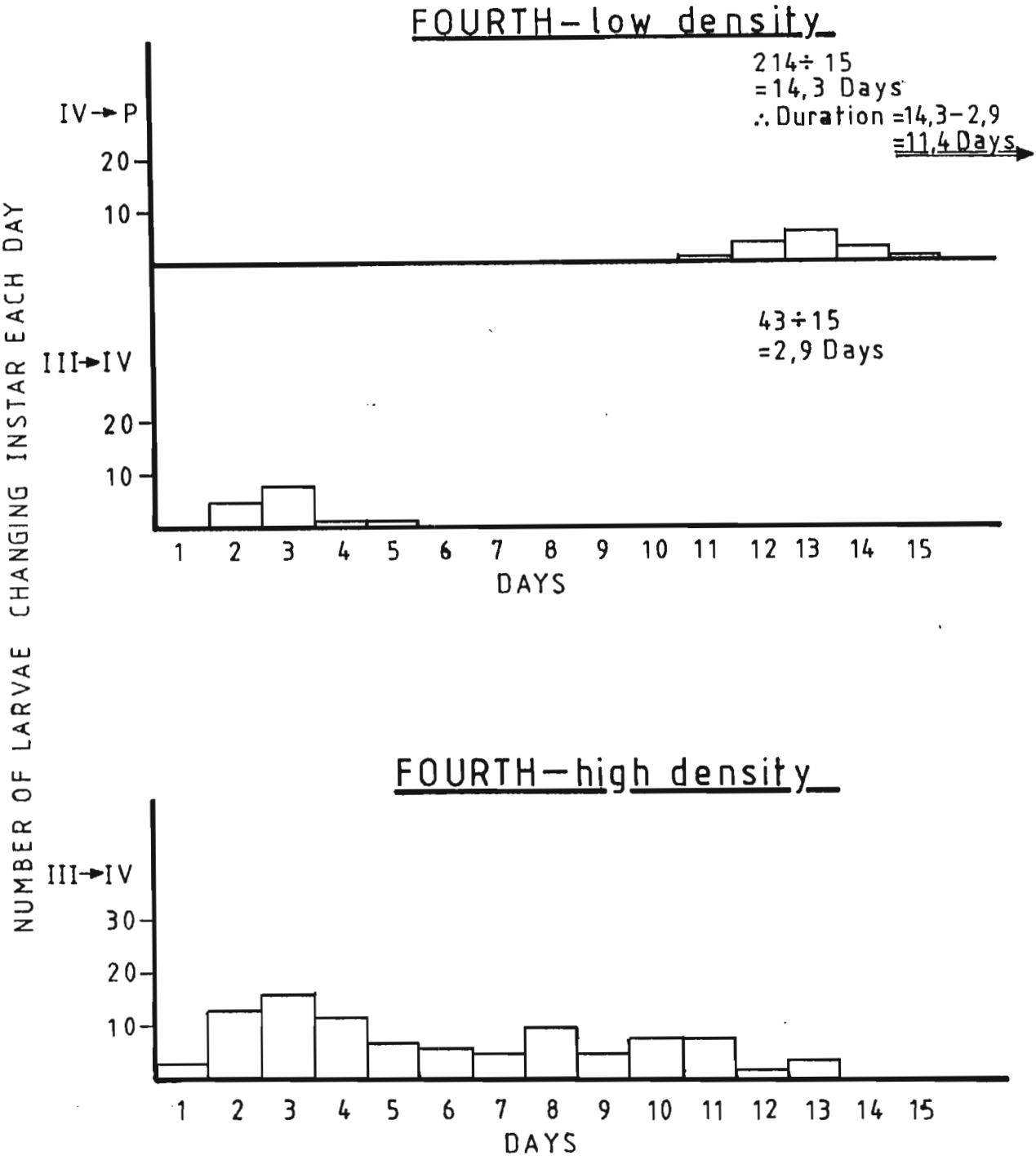


Figure 25. Winter, field measured, instar duration for fourth instar larvae under high and low pool densities.

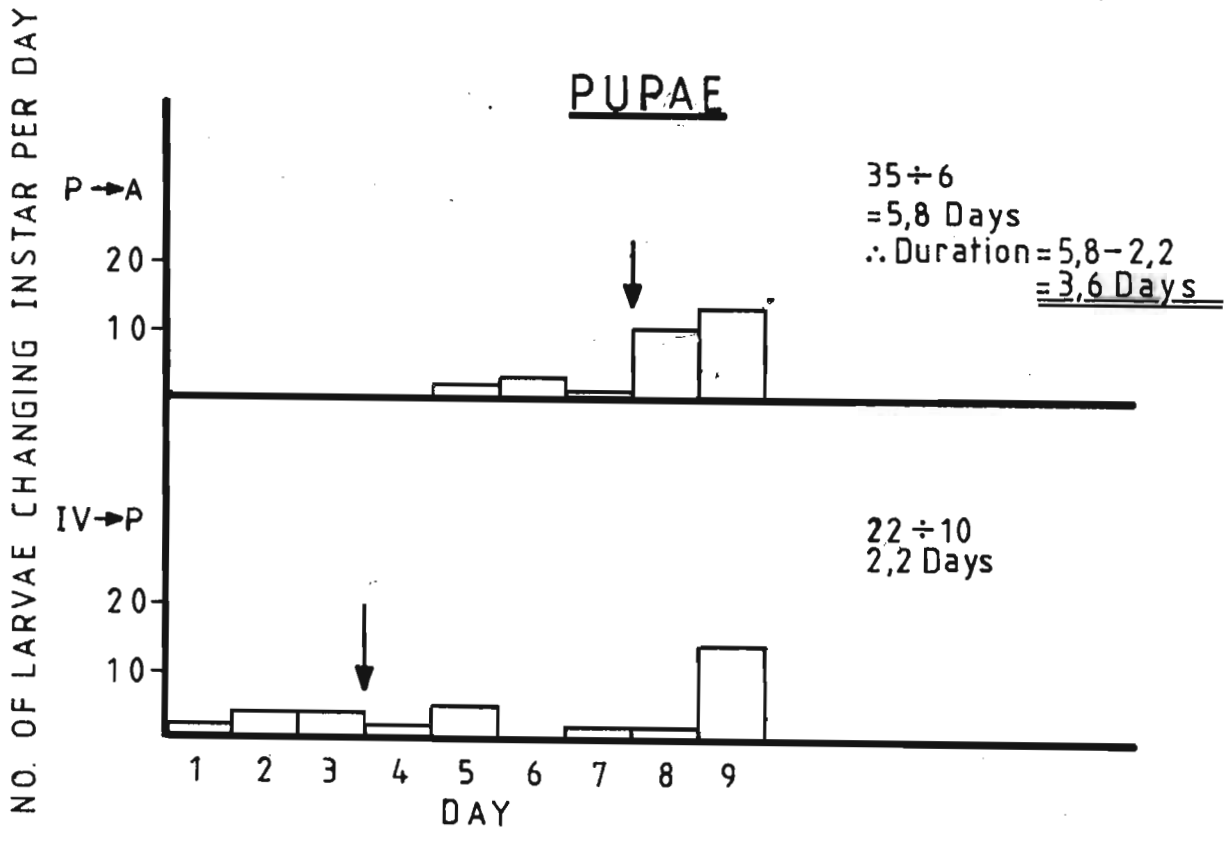


Figure 26. Winter, field measured pupal duration. The arrows indicate that the preceeding portion of the distribution curves were used to calculate the pupal duration, due to the high mortalities recorded during the latter stage of the trial.

the means. This difference is a result of the compounding effect of the factorial increase. Thus the greater factorial increase accounts for the differences in head width between this species and the two freshwater members. However as explained in Chapter three, this difference was a product of temperature. It is thus evident that irrespective of environmental conditions, growth of the head capsule conforms to Dyar's rule in all three species investigated in this study.

This is important as Dyar's rule can be applied in the separation of larval instars. Accurate separation will thus be possible irrespective of environmentally induced morphometric changes in head capsule width.

4.4.2 LARVAL GROWTH

There are obvious survival advantages to floodwater mosquitoes such as *Aedes aegypti* in having eggs which hatch over a period of time Gillet (1955a). Hotchkin (1985) further demonstrated that the time of hatch affected the subsequent growth rates of the larvae, with second-hatch larvae developing significantly faster than first-hatch larvae. Gillet (1955b) demonstrated that it was a genetically acquired trait. Eggs of the *An. gambiae s.l.* are not very resistant to desiccation unless maintained on a damp substrate (Holstein, 1954) although Deane and Causey (1943) identified so-called resistant eggs which were morphologicaly different to normal eggs, while working on *An. gambiae* material from Brazil. It is however likely that this was merely a reflection of the differences in egg morphology existing between members of the *An. gambiae* complex. Support for this conclusion is given by

the fact that Soper and Wilson (1943) reported the capture of *An. gambiae* var. *melas* from a plane arriving in Natal, Brazil from Africa.

In the author's own experience, eggs will hatch spontaneously if held over on damp filter for more than three days at approximately 24^{o}C. Differential hatch rates have also been observed for all three members studied. However the first hatch is highly synchronised, and includes the vast majority of the eggs. The hatching of a few eggs appears to be delayed for approximately 24 h. In this study larvae from the first hatch were used in all growth trials. The possibility of time of hatch accounting for the different larval growth rates observed in this study can thus be discounted.

From the decreasing values of R-squared reported in section 4.3.2 , it can be concluded that in the first instar 81,5% of variation in size (abdomen length) can be explained in terms of age (time). However, the decreasing values of R-squared indicate that by the third instar, as little as 10% of the variation can be explained in terms of age. From Figure 22, it can be concluded that .this is a direct result of the individual variation in larvae. The respective values for the correlation coefficient (r) and the coefficient of determination (R) indicate that at the individual level, variation in size can be explained in terms of age and differential growth patterns.

For the purposes of this discussion the differential larval

growth rates will be divided into three categories and notated as follows;

1) Fast Growth (FGL): Those accounting for the exponential growth curves in Figure 18.

2) Average Growth (AGL): Those accounting for the linear growth curves in Figure 18.

3) Slow growth (SGL): Those falling outside the 95% confidence limits in Figure 19.

It is interesting to note that the ratio of the AGL larvae to the FGL was approximately 2:1. The proportion of SGL larvae to AGL or SGl was not noted. It may be speculated that these differential growth rates reflect individuals adapted to specific conditions. In other words the SGL individuals are best adapted to warm (summer) conditions and the FGL to cooler (winter) conditions. If this were the case, then there would be a number of selective advantages:

1) The differential growth rates result in a spread in eruption dates (Figure 22) which would be advantageous from a survival point of view, when considering the variability and potential adversity of climatic conditions.

2) It is well documented, that at high temperatures, at which growth occurs at maximum velocity, smaller and often weak individuals are produced (Clements, 1963; Ray, 1960). These individuals may be considered "products of an ectothermic, metabolic burnout". In this situation, if the slow growth larvae result in larger more robust individuals, it would once again be of selective advantage.

The genetics of these differential growth rates within a single egg batch requires further investigation by means of line selection and crossing experiments.

4.4.3 LABORATORY TRIALS ON THE EFFECT OF TEMPERATURE ON LARVAL AND PUPAL GROWTH RATES.

4.4.3.1 COMPARISON OF FLUCTUATING VERSUS CONSTANT TEMPERATURES.

Milby and Meyer (1986) concluded that there was no difference in the larval duration of *Culex tarsalis* under constant or fluctuating temperatures, if the mean of the fluctuating temperature was equivalent to that of the constant temperatures. This conclusion although true under the temperature regimes they utilized, would not necessarily apply if these regimes included temperatures exceeding the optimal development temperature.

This is illustrated in Figure 27 where there are two theoretical, fluctuating temperature profiles. Both have the same mean temperature, however if one considers the reciprocal catenary curve for *An. quadrimaculatus* from Huffaker (1944), it is immediately evident that these two profiles would result in different pre-imaginal durations. The important point here is the inclusion of temperatures above the optimal temperature, as at these temperatures the assumption hat the growth rate increases with temperature, no longer holds.

There is however a tendency for growth rates to be accelerated under fluctuating temperature regimes (Clements, 1963).

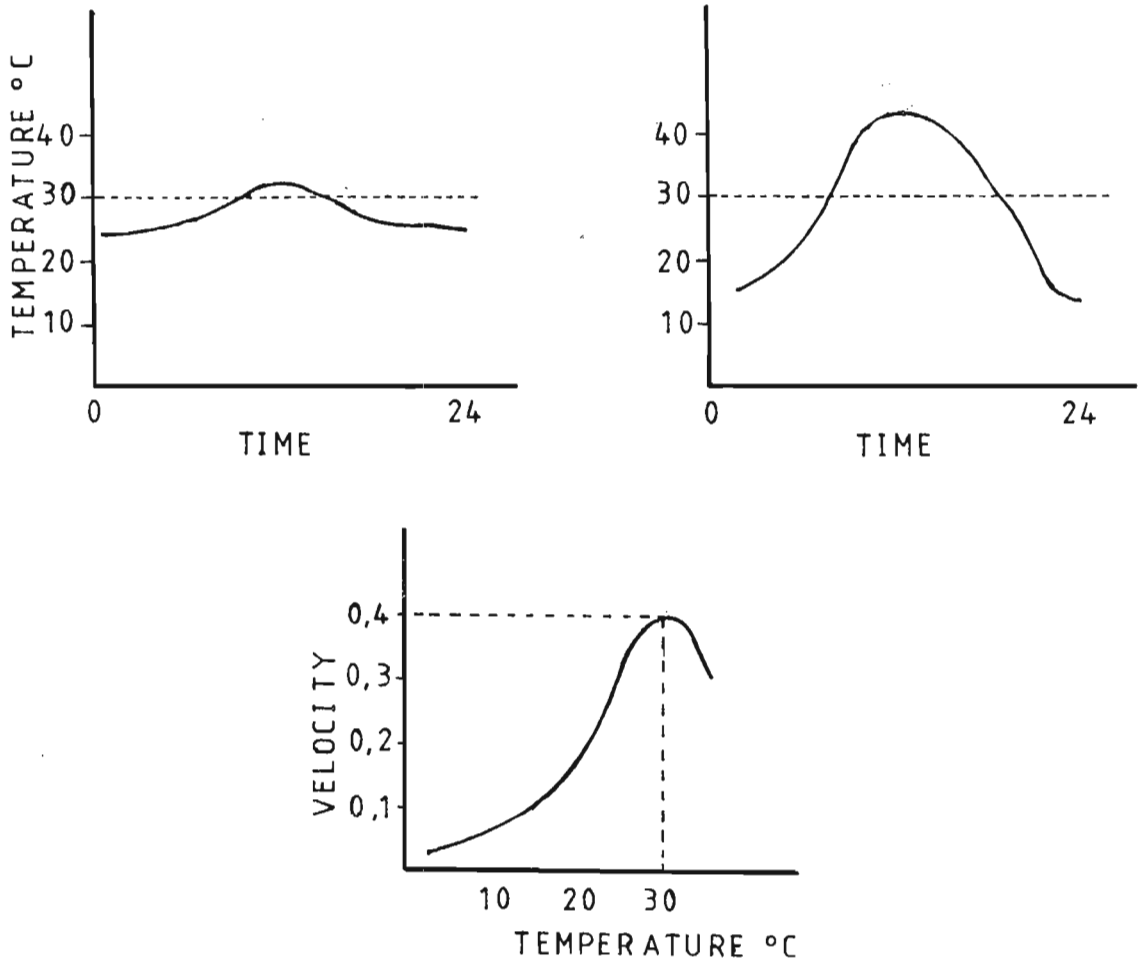


Figure 27. Two theoretical fluctuating temperature profiles, with the same mean temperature. The reciprocal catenary curve is for *An. quadrimaculatus* and is reproduced from Huffaker (1944).

Huffaker (1944) explained this in terms of 'Blackman's concept' , that the maximal initial rate of metabolism falls off with time. Thus it is suggested that fluctuating temperatures allow for a recovery period and then the resumption of metabolism, at the initial maximal rate at high temperatures. Huffaker (1944) however also failed to consider the effect of temperature profiles, which incorporated a significant portion above the optimal range.

The extremely long first instar duration (Table 15) in this study during the fluctuating temperature trials is thought to be due to the particle size of the food available resulting in it being a limiting factor. This is supported by the fact that the second instar duration is greater than expected, when compared to the values obtained for other instars and those obtained by Service (1973). The food source used in the constant temperature trials was different and contained a larger amount of activated yeast. The duration derived from the field study was thus substituted and yielded an overall duration of 21,6 days. The expected value if development occurred at the same rate under constant and fluctuating temperatures would be 25,8 days. Fluctuating temperatures thus appear to have an accelerating affect on the growth of *An. merus*. However before this can be concluded, growth rates with fluctuating temperature profiles of different cyclical magnitude and mean temperatures would have to be investigated.

4.4.3.2 FLUCTUATING TEMPERATURE TRIALS

An. merus only occurs in saline waterbodies in Natal (Chap-

ter 2). It is however usually maintained in distilled water in insectaries (Coetzee and le Sueur, 1988). The initial trials were thus conducted in distilled water, but extremely high mortalities were experienced (Figure 21). These mortalities were subsequently found to be due to a parasitic fungus, which could not be identified locally. It was found that problems were not experienced with this fungus if larvae were raised in water containing 7,8g NaCl per litre (10 p.p.t.). The third trial was conducted in saline water and an egg to adult survival of 31% attained (Figure 21.). It may be speculated that the susceptibility of *An. merus* to this fungus may preclude it from freshwater habitats in the wild. In view of this it is suggested that colonies of *An. merus* should be maintained in saline water. Extremely high larval mortalities were noted in the *An. merus* colony used in this study, on a number of occasions. The cause of these mortalities was not established at the time, however recurrence has not been noted since NaCl has been added to the water.

After the two unsuccessful trials in distilled water a trial was conducted with saline water using material from the *An. merus* colony, due to the logistical problem of obtaining gravid females from the field. This trial was however unsuccessful due to extremely high mortalities incurred in the first instar (94,3% - Figure 22). Mortalities in this trial and those for remaining instars were higher than those measured for field material under the same conditions. There are two possible reasons for the poor survival of insectary material in comparison to field material.

1. The colony had been maintained under insectary conditions for approximately 84 generations and had undergone a series of bottlenecks, due to population crashes. It is possible that under these conditions, individuals best adapted to the warm constant environment of the insectary, were selected for.

2. That this was not a result of genetic selection, but due to acclimation to insectary conditions and subsequent thermal shock, when exposed to the simulated winter conditions.

The latter is however unlikely as the field material for this trial was collected during summer. It is thus likely that the high mortalities are a result of the colonisation process. The fact that colonies are also often started from a few individuals which do not necessarily represent the genetic pool of the species, should be borne in mind. Before any conclusions concerning acclimation could be drawn, a similar trial using material from the same region , collected during winter would have to be carried out and the relative mortality rates and instar durations compared.

4.4.3.3 MEASURED PRE-IMAGINAL DURATIONS AND INTERPRETATION.

The mean temperature for all profiles used, or measured in the study was calculated (from the figures, a,b and c in Appendix 3). This allowed comparisons to be made between the measured durations of this study and the predicted values according to the catenary model derived by Jepson *et al.*, (1947) and are quoted below;

Table 16. Comparison of the pre-imaginal durations measured at specific temperatures in this study and those predicted by the Catenary model (Jepson *et al.*, 1947) for the same temperatures.

		MEASURED MEAN TEMPERATURE	ACTUAL DURATION	PREDICTED DURATION
FLUCTUATING:	SUMMER (LAB)	30,8	(8,1) 9,1	9,0
	WINTER (LAB)	15,1	(24,6) 29,8	INFINITE
	WINTER (FIELD)	16,3	31,2	INFINITE
CONSTANT:	LABORATORY	25,0	15,1	16,5
	LABORATORY	18,0	22,1	INFINITE

The values quoted above in brackets include the corrected values for first instar and the adjacent values the uncorrected. The catenary curve which Jepson *et al.*, (1947) derived from field measured, pre-imaginal durations is reproduced in Figure 28. It is immediately evident that at high temperatures, there is a close correlation between the actual values and those predicted by Jepson *et al.*,'s model. However the model predicts that at temperatures below 18^{o}C the growth velocity is so low that the pre-imaginal duration cannot be defined. The actual values measured do not concur with this. It is however obvious from Figure 28 that there is a lack of data below 25^{o}C, which probably accounts for the inaccuracy of the model at low temperatures. The data from this study was thus added to Jepson *et al.*,'s and a reciprocal regression plotted (Figure 29). This regression improves the accuracy of the model, due to the inclusion of values at lower temperatures. The preimaginal duration was indefinable at 16,5^{o}C according to Jepson *et al.*, (Figure 28) and at 12,2 in

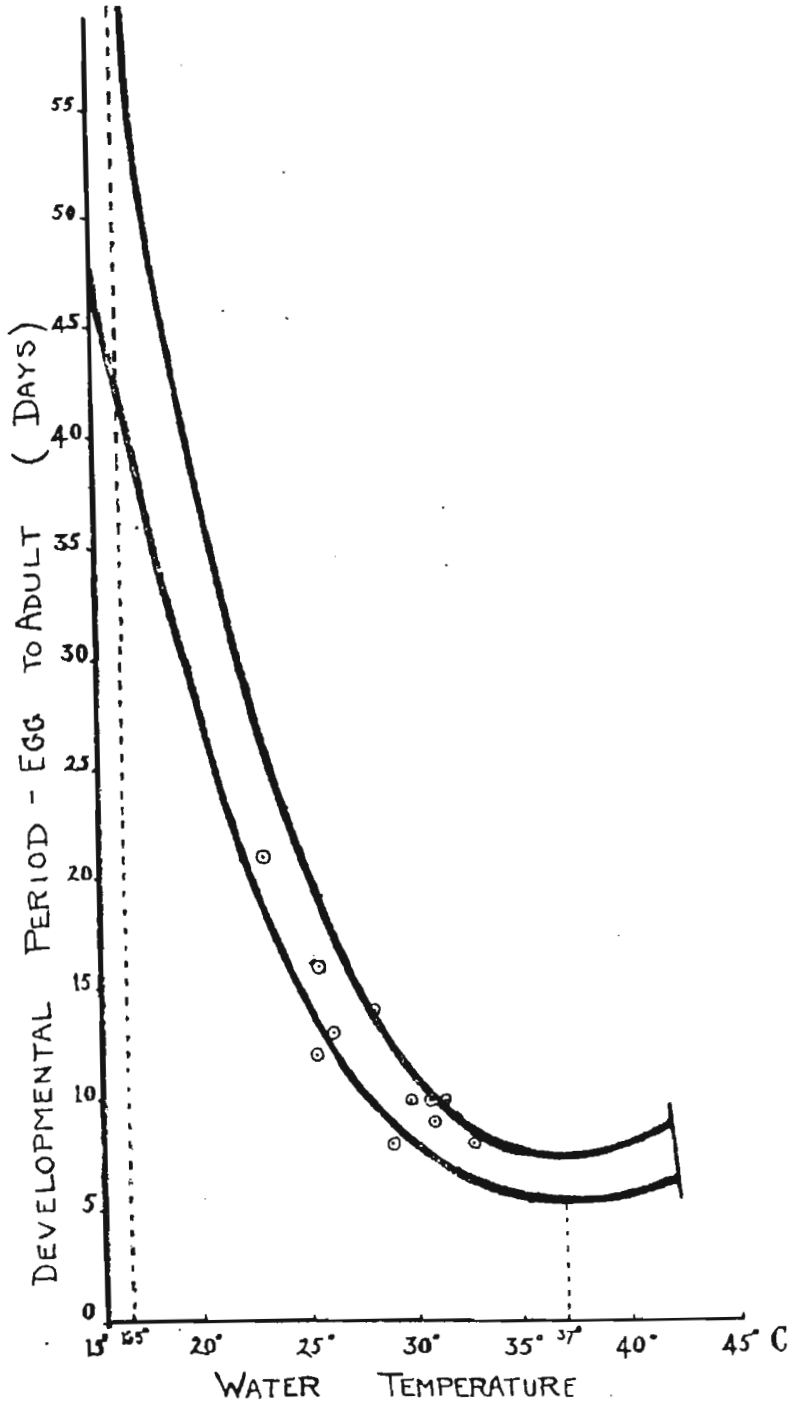


Fig. 3.

Figure 28. The catenary curve produced by Jepson *et al.*, (1947) for the *An. gambiae s.l.* on Mauritius.

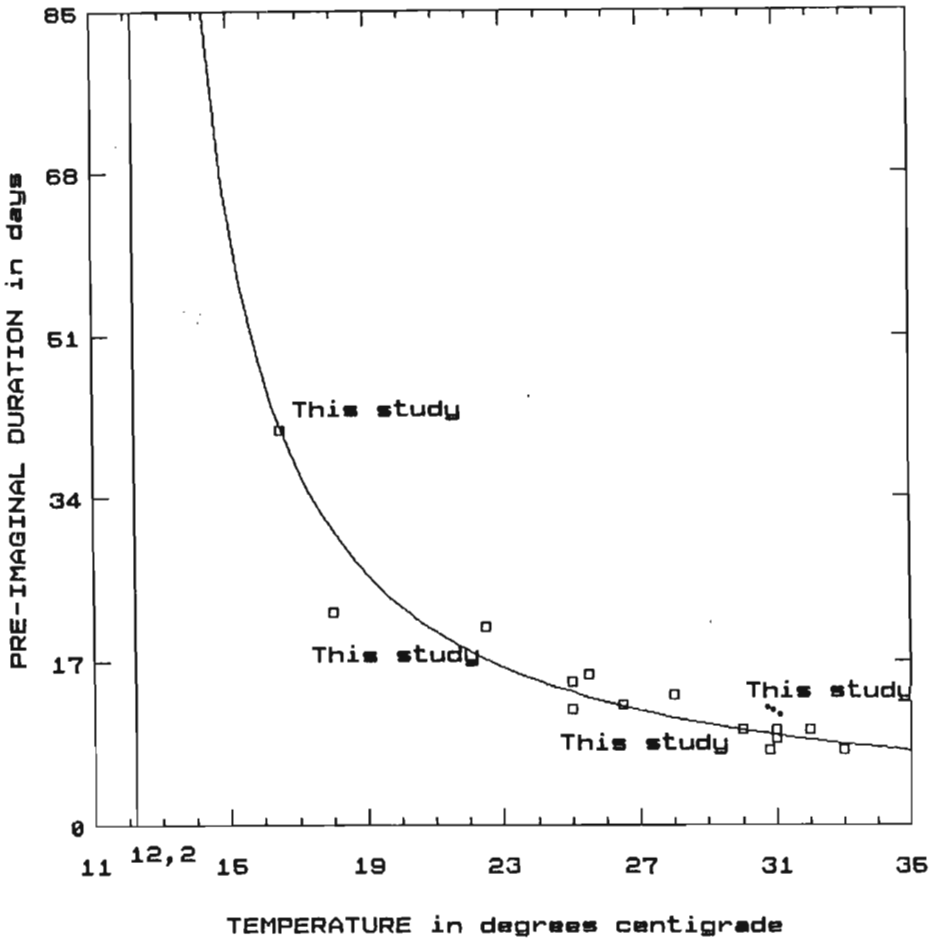


Figure 29. The reciprocal regression curve including data from this study and from Jepson *et al.*, (1947). The marked points are from this study. The solid vertical line adjacent to the y axis indicates the estimated temperature at which growth ceases.

the corrected model (Figure 29, R-squared = 81,2%).

The corrected curve shown in this study is a more accurate representation of the field situation. However in section 4.4.4 it will be shown that density, through nutrition, plays a role in pre-imaginal durations. In addition it will be shown in Chapter 5 that low temperatures in winter result in high larval densities per pool and the consequent extension of larval life, due to food being limiting. It is thus felt that this curve could be fine-tuned with the addition of more field data from winter months. The curve is however useful as it may be used to predict the interval at which larviciding should be carried out, when correlated to climatic data at different times of the year. This is extremely important in the implementation of an efficient and cost effective larviciding programme.

Service (1973) concluded from laboratory and field observations that the durations of instars I to IV and of pupae were 1,4; 2,9; 1,9; 3,8 and 1,8 days respectively, in November/December in Kisumu, Kenya. In a later study conducted in July in the same region Service (1977) concluded that the field instar durations were the same. This is in distinct contrast to the summer/winter situation in this study. Due to the fact that Kisumu, Kenya is situated just north of the equator, one would expect little seasonal variation. An explanation for there being no change in the duration measured for two periods situated in opposite seasons may be summarised as follows;.lm 0.50"

1) that the seasonal variation in temperature is minimal,

2) that the annual daily temperature is always high and falls within the optimal range for development (ie. approximately 30^{o}C).

If this is the case, then the mean temperature and range would fall within the region of the curve (Figure 29, 22 to 33^{o}C) where it would make little difference to the preimaginal duration, due to its reduced metabolic effect. The situation is however vastly different in areas in which the mean winter temperature is seven degrees or more below the mean summer value. This is the situation in northern Natal and even more so in adjacent Swaziland (Figure 4), due to the decreased buffering effect of the warm Mozambique sea current on air temperature.

Kelly & Cory (1984) used the instar durations determined by Service as "a rough guide" in their estimation of larval survivorship in Swaziland. Extrapolation such as this from one region to another may lead to false conclusions if differences in temperature are not taken into account. In this instance howeverKelly & Cory's (1984) survey was carried out in February, a time, when temperatures in Swaziland are probably at their most comparable to those in Kisumu Kenya.

This study indicates that in more temperate regions the accuracy of survivorship estimates is dependent on concurrent measurement of larval/pupal durations. This is especially so in the winter months, where small changes in temperature

result in large increases in preimaginal duration (Figure 29).

4.4.4 WINTER FIELD TRIAL

The technique used in the estimation of larval instar durations requires daily correction for mortalities if they are not to be underestimated. This was possible in the present study since the pools used were small and could be emptied daily but the problem of how this could be done in larger natural sites remains. Mark release/recapture methods could be used, but would be difficult as changes in instar ratios could be interpreted as being due to mortalities or metamorphosis. Service (1973) does not describe the methods he used to obtain his instar durations from large water bodies. However once again, if temperatures approximate those for optimal development, then smaller artificial pools adjacent to the larger waterbodies could probably be used, ignoring the possible effects of density dependent factors. The situation under cooler, more temperate conditions, would however be more complex.

The difference in the preimaginal cycle under winter conditions in the field and laboratory is a direct result of nutrition. In winter the larval durations increase, due to reduced metabolic rates. This results in an accumulation of larvae in pools. In Chapter 5 an almost 300% increase in the number of larvae per pool is reported. This increased density results in food becoming limiting and the larval cycle is further extended (Figure 25). All other instars were run at high pool densities and only the third instar showed signifi-

cant increases relative to the laboratory results. The third and fourth instars thus appear to be nutritionaly limited in winter. This is however not surprising as their nutritional requirements would be greater than that of the earlier instars, due to their larger size. The value for the pupal stage in the field is similar to that recorded in the laboratory which is to be expected since the pupal stage does not feed and its duration is thus essentially temperature dependent. Nielsen & Evans (1960) concluded that temperature was the only factor affecting the the pupal duration of *Aedes taeniorhynchus*.

It has become evident from this study that there are too many factors affecting larval growth for accurate simulation to be carried out in the laboratory. For this reason estimates of preimaginal durations for use in the investigation into larval/pupal dynamics should always be derived from field data. The accuracy and value of the estimation of longevity of *An. culicifacies* and *An. stephensii* in the laboratory by Reisen and Mahmood (1980) and its interpretation in terms of malaria transmission is therefore questionable. Similarly the comparison of development rates in the laboratory between their study and other authors, is questionable when the effect of different food sources alone is considered. The variability of larval growth rates with different food types is illustrated in this study and is also well documented in the literature (Clements, 1963). Studies of vector population dynamics for use in the interpretation of disease transmission should thus be field based. Laboratory based,seasonal

comparisons for a single species may however still be of value.

4.4.5 SUGGESTED FURTHER STUDY RELATING TO MALARIA TRANSMISSION IN NATAL.

Figure 30 is reproduced from Sharp *et al.*, (1988) and shows the pattern of late season transmission which characteristically occurs in Natal. Peak transmission thus occurs in Autumn, whereas one would expect it to occur in mid-summer, when larval growth velocities are at their maximum. Transmission efficiency by the vector is however obviously a combination of population size and longevity. It is thus possible 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 at high temperatures may result in the peak in the adult population occurring in late summer. Data from Shelley (1972) for *An. arabiensis* in the Zambezi Valley, Zambia supports this conclusion, as an index of the adult population peaked in March and April. The increased size/nutritional fitness of individuals produced during winter has been demonstrated in this study. It could thus be theorised that Autumn transmission is a result of an optimal balance between population size and longevity, at cooler temperatures. The effect of temperature on parasite development rates within the mosquito should also be borne in mind. This hypothesis

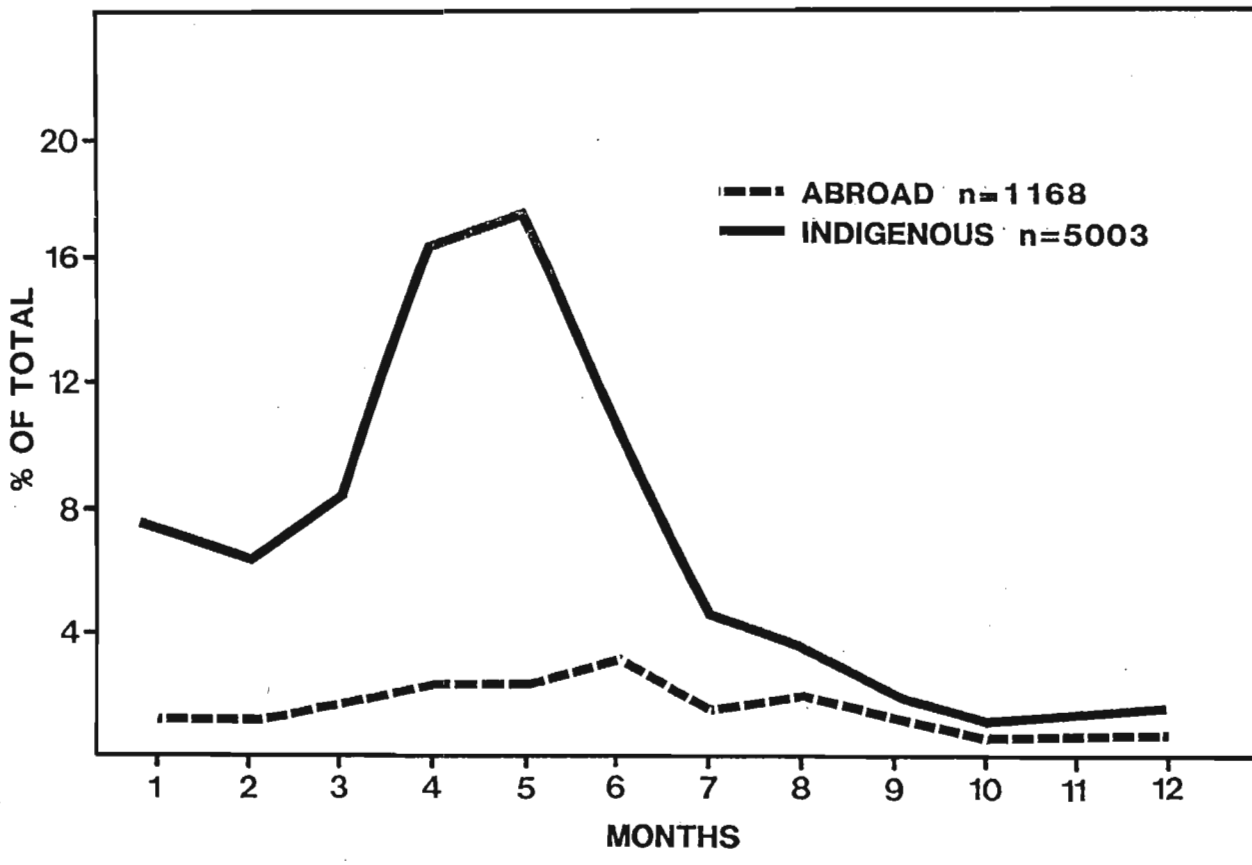


Figure 30. The pattern of late season transmission characteristically occurring in Natal. Reproduced from Sharp *et al.*, (1988).

would however have to be tested by the field investigation of seasonal changes in population size, longevity, fecundity and parasite development.

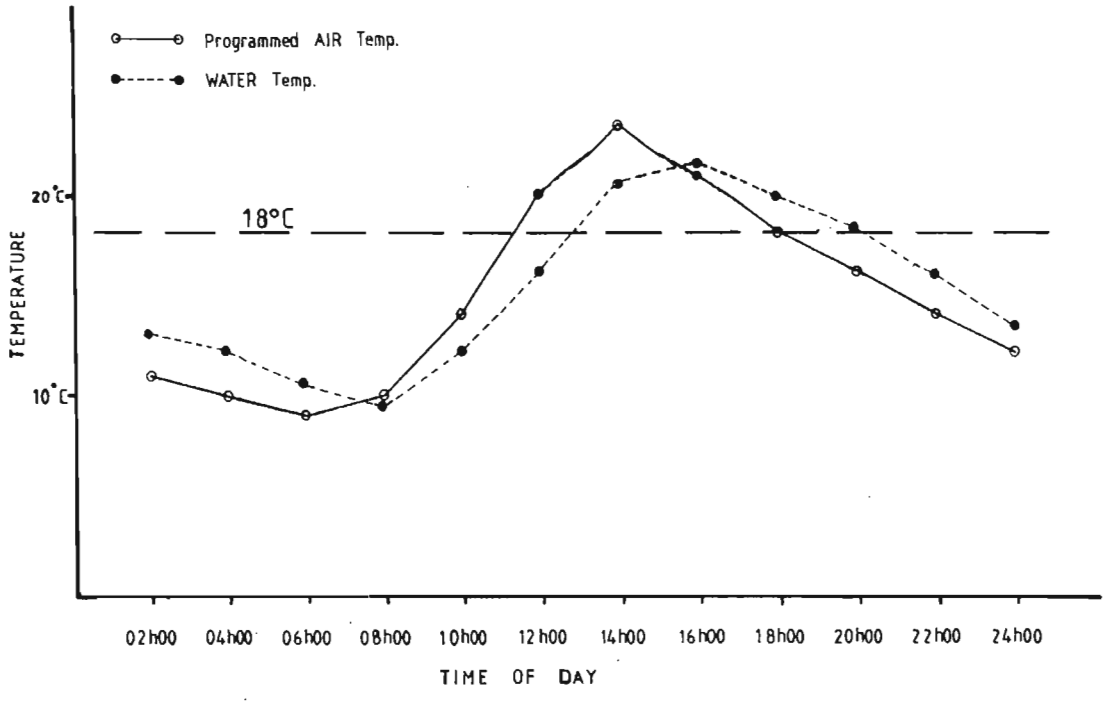
When estimating survivorship by means of age structure, during winter, careful attention has to be paid to temperature since, as shown in Figure 29, at low temperatures, small variations can result in large changes in instar duration. Thus when collections for survivorship estimates are made, it is the temperatures in the period preceding the collection which need to be considered. This is problematic and seriously detracts from the usefulness of this technique under winter temperature conditions such as those of Natal. The technique is however still useful as long as this limitation is borne in mind .

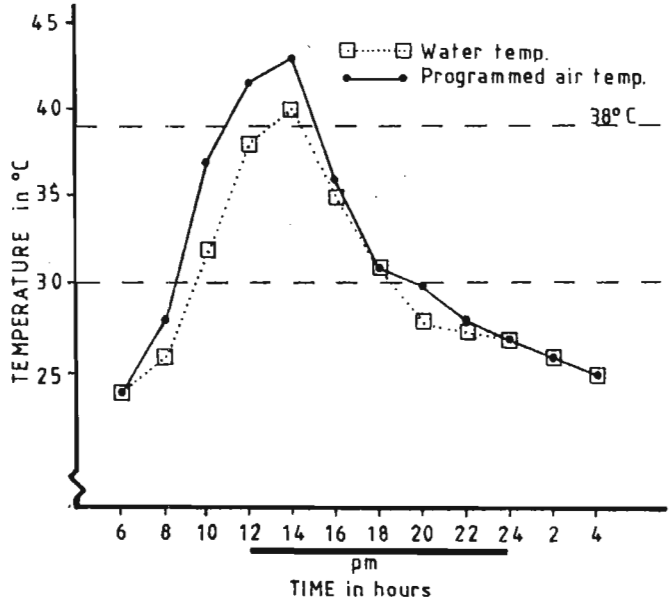
APPENDIX 3

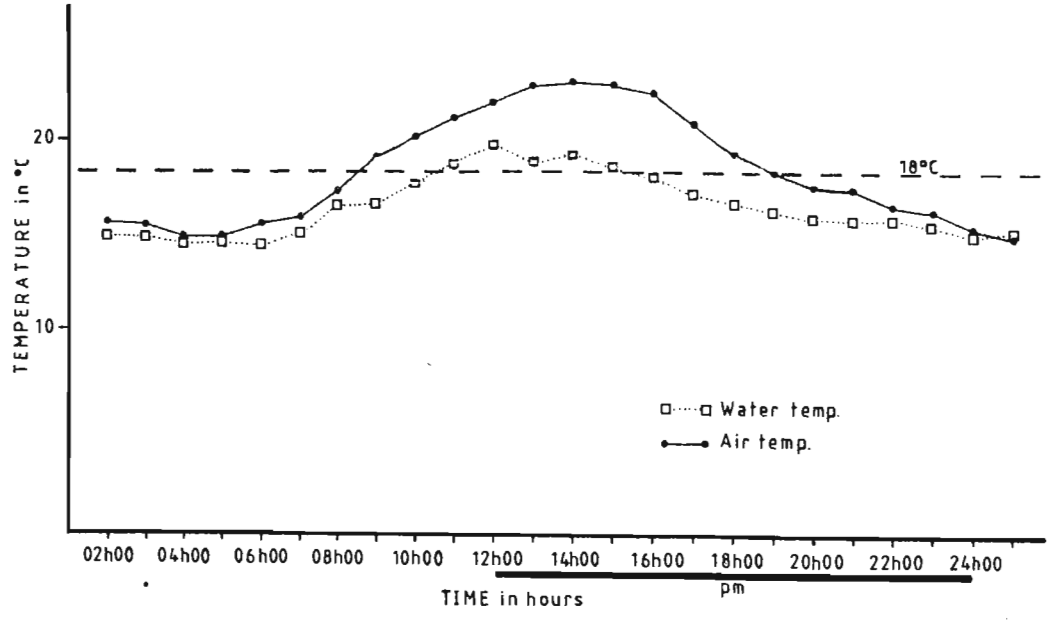
a. Winter temperature profile programmed into the conviron growth cabinet and the resultant water temperatures at which larvae were raised. The 18^{O}C line is included to allow comparoson with c., below).

b. Summer temperature profile used to programme the Conviron growth cabinet and the resultant water temperature, at which *An. merus* larvae were raised. The dotted lines indicate the area which was considered to fall within the optimal temperature range for *An. gambiae s.l.* (Figure 29).

c. Profile of measured, hourly mean temperatures for the duration of the winter field trial. The dotted 18^{O}C line represents the mean of the summed hourly temperatures.


Programmed AIR Temp.
WATER Temp.
20°C
18°C
10°C
TEMPERATURE
02h00
04h00
06h00
08h00
10h00
12h00
14h00
16h00
18h00
20h00
22h00
24h00
TIME OF DAY


45
40
35
30
25
Water temp.
Programmed air temp.
38°C
TEMPERATURE in °C
6
8
10
12
14
16
18
20
22
24
2
4
pm
TIME in hours


20
10
TEMPERATURE in °C
18°C
Water temp.
Air temp.
02h00
04h00
06h00
08h00
10h00
12h00
14h00
16h00
18h00
20h00
22h00
24h00
pm
TIME in hours

REFERENCES

Abdel-Malek, A. and Goulding, R.L. (1948). A study of the rate of growth of two sclerotised regions within larvae of four species of mosquitoes.-*Ohio J. Sci.* **48**, 119-128.

Bates, M. (1941). Field studies of the *Anopheline* mosquitoes of Albania.-*Proc. Ent. Soc. Wash.*, **43** (3), 37-58.

Clements, A. N. (1963). *The physiology of moquitoes.International Series of Monographs on Pure and Applied Biology.* **vol. 17**.Pergamon Press.-393pp.

Coetzee, M and le Sueur, D. (1988). Effects of salinity on the larvae of some afrotropical *Anopheline* mosquitoes.-*J. Med. Vet. Ent.* **2**, 385-390.

Deane, M.P. and Causey, O.R. (1943). Viability of *Anopheles gambiae* eggs and morphology of unusual types found in Brazil.-*Amer. J. Trop. Med.* **23**, 95-102.

De Oliveira, D. and Durand M. (1978). Head capsule growth in *Culex territans* Walker.-*Mosq. News* **38(2)**, 230-233.

Deslongchamps, P and Tourneur, J.C. (1980). Head capsule growth and early sexual dimorphism in *Culex quinquefasciatus* Say (Diptera: Culicidae).-*Mosq. News* **40(3)**, 351-355.

Dyar, H.G. (1890). The number of molts of Lepidopterous larvae.-*Psyche.* **5**, 420-422.

Ethes, Y. (1939). in Biology of *Anopheles gambiae*. Research in French West Africa. Holstein, M.H. (1954). W.H.O. page 68.

Gillet, J.D. (1955a). Variation in the hatching response of *Aedes* eggs (Diptera: Culicidae).-*Bull. ent. Res.* **46**,

241-254.

Gillet, J.D. (1955b). The inherited basis of variation in the hatching-response of *Aedes* eggs (Diptera: Culicidae).-*Bull. ent. Res.* **46**, 255-265.

Holstein, M.H. (1954). The Biology of the *Anopheles gambiae*.-World Health Organisation. 173pp.

Hotchkin, P.G. (1985). The duration of larval life of *Aedes aegypti* as affected by the time of hatch.-*J. Am. Mosq. Contr. Assoc.* **1(4)**,489-492.

Huffaker, C.B. (1944). The temperature relations of the immature stages of the malarial mosquito, *Anopheles quadrimaculatus* Say, with a comparison of the developmental power of constant and variable temperatures in insect metabolism.-*Ent. Soc. Am.* **37(1)**, 1-27.

Jepson, W.F., Moutia, A. and Courtois,C (1947). The malaria problem in Mauritius: the bionomics of Mauritian *Anopheline*s.-*Bull. ent. Res.* **38**, 177-208.

Jones, J.C. (1953). Some biometrical constants for *Anopheles quadrimaculatus* Say larvae in relation to age within stadia.-*Mosq. News* **13(4)**, 243-247.

Kelly, P.M. and Cory, J.S. (1984). Report of the Oxford malarial mosquito expedition to Swaziland 1984. Oxford university. 54pp.

Kettle, D.S. (1948). the growth of *Anopheles sergentii* Theobald (Diptera: Culicidae), with special reference to the growth of the anal papillae in varying salinities.-*Ann. trop. Med. Parasit.* **42**, 5-29.

Marcovitch, S. (1960). Experiments on prolongation of the life of mosquito larvae by underfeeding.-*J. Econ. Ent.* **53**, 169.

Mathis, M. (1935). Sur la nutrition sanguine et la fecondite de Stegomyia:*Aedes aegyptii*.-*Bull. Soc. Pat. Exot.* **28**, 231-234.

Milby, M.M. and Meyer, R.P. (1986). The influence of constant versus fluctuating water temperatures on the preimaginal development of *Culex tarsalis*.-*J. Am. Mosq. Contr. Assoc.* **2(1)**, 7-10.

Miura, T., Takahashi, R.M. & Wilder, W.H. (1984). Impact of the mosquitofish (*Gambusia affinis*) on a rice field ecosystem when used as a mosquito control agent.-*Mosq. News* **44**, 510-517.

Molineaux, L. and Gramiccia, G. (1980). *The Garki Project* W.H.O., Geneva.

Mottram, P., Kay, B.H. and Kettle, D.S. (1986). The effect of temperature on eggs and immature stages of *Culex annulirostris* Skuse (Diptera: Culicidae).-*J. Aust. ent. Soc.* **25**, 131-135.

Nielsen, E.T. and Evans, D.G. (1960). Duration of the pupal stage of *Aedes taeniorhynchus* with a discussion of the velocity of development as a function of temperature..-*Oikos*. **11**, 200-222.

Nielsen, E.T. and Haeger, J.S. (1954). Pupation and emergence of *Aedes taeniorhynchus* (Wied.).-*Bull. ent. Res.* **45**, 71-95.

Ouda, N.A., Al- Chalabi, B.M. and Dikran, B.B. (1986). Observations on slow growth larvae: A new character in the mosquito *Culex quinquefasciatus*. Say (Diptera: Culicidae).-*J. Biol. Sci. Res.* **17(1)**, 211-218.

Ray, C. (1960). The application of Bergmann's and Allen's rules to the poikilotherms.-*J. Morphology* **106**, 85-108.

Reisen, M.K. and Mahmood, F. (1980). Horizontal life table characteristics of the malaria vectors *Anopheles culicifacies* and *Anopheles stephensi* (Diptera: Culicidae).-*J. Med. Entomol.* **17(3)**, 211-217.

Sen, P. and Das Gupta, S.K. (1958). "Dyar's Law" in the determination of larval instars in mosquitoes.-*Bull. Calcutta. Sch. Trop. Med.* **6**, 69-70.

Service, M.W. (1973). Mortalities of the larvae of the *Anopheles gambiae* Giles complex and detection of predators by the precipitin test.-*Bull. ent. Res.* **62**, 359-369.

Service, M.W. (1977). Mortalities of the immature stages of species B of the *Anopheles gambiae* complex in Kenya: comparison between rice fields and temporary pools, identification of predators, and effects of insecticidal spraying.-*J. Med. Ent.* **13** (4-5), 535-545.

Sharp, B.L., Ngxongo, S., Botha, M.J., Ridl, F.C. & le Sueur, D. (1988). An analysis of 10 years of retrospective malaria data from the KwaZulu areas of Natal.-*S. Afr. J. Sci.* **84**, 102-106.

Shelley, A.J. (1973). Obsevations on the behaviour of *Anopheles gambiae* species B in Kambole village in the Zambezi valley, Zambia.-*Ann. trop. Med. Parasit.* **67** (2), 237-247.

Shinkarenko, L., Hulsman, K., Mottram, P., Dale, P. and Kay, B.H. (1986). Reliability of using head capsule width and body length to identify larval instars of *Aedes vigilax* (Diptera: Culicidae).-*J. Aust. ent. Soc.* **25**, 37-40.

Smith, C.A. (1914). The development of *Anopheles punctipennis* Say.-*Psyche.* **37(2)**, 1-19.

Soper, F.L. and Wilson, B.D. (1943). *Anopheles gambiae* in Brazil 1930 to 1940. The Rockerfeller Foundation. 262pp.

Sweeney, A.W. (1985).The potential of the fungus *Culicinomyces clavisporus* as a biocontrol agent for medically important Diptera. in *Integrated mosquito control methodologies*. (eds. M. Laird and J.W. Miles).-444 pp. Volume 2. Chapter 13. Biocontrol and other innovative components and future directions. 269-284.

Sweeney, A.W., Cooper, R., Medcraft, B.E., Russel, R.C., O'Donnel, Panter, C. (1983). Field tests of the mosquito fungus *Culicinomyces clavisporus* against the Australian encephalitis vector *Culex annulirostris*.-*Mosq. News* **43**, 290-297.

Walker, A.J. (1938). Fungal infections of mosquitoes, especially of *Anopheles costalis*.-*Ann. trop. Med. Parasit.*, **32**, 231-244.

Wiggelsworth, V.B. (1929). Delayed metamorphosis in a predaceous mosquito larva and a possible practical application.-*Nature*. **123**, 17.

CHAPTER 5

LARVAL DYNAMICS, OVERWINTERING AND SURVIVORSHIP

INTRODUCTION

The overwintering of mosquitoes is of interest for a number of reasons. In some instances there is the possibility that certain mosquito species may serve as winter reservoirs for viruses of medical importance, such as those responsible for encephalitis (Reisen *et al.*, 1986). In the case of *Anopheles atroparvus* it was shown that the cases of so called "family malaria", were the result of transmission between family members by an overwintering female (Ramsdale and Wilkes, 1985). These studies and others were conducted in regions experiencing fairly harsh winters, where overwintering is carried out largely by the non-autogenous female (Mitchell & Briegel, 1989) and the eggs (Stewart, 1973). However in more temperate regions, reduced numbers of gonotrophically active females may be present and larvae still occur (Reisen *et al.*, 1986). The versatility of certain mosquito species is indicated by the study of Sichinava (1974) on *Culex pipiens molestus* in Georgia, USSR. During winter no breeding occurs in open waterbodies, but is localised to the dark, flooded basements of centrally heated buildings and both autogenous and non-autogenous females are present.

In this study the aim was to ascertain the role of the immature stages, if any, in the overwintering of *An. gambiae s.l.* populations in the endemic malaria region of Natal. This

study was carried out on *An. merus* due to the availability of a suitable population and study site.

Fundamental to the investigation of any population (adults or immatures) is the ability to accurately and representatively sample it and thus obtain accurate population estimates. Such estimates are important in:

1. The structuring and scheduling of population control measures (Miura, 1984);

2. The construction of life tables for both larval and adult populations for use in the planning of integrated control measures (W.H.O., 1975);

3. The assessment of the biting and resting behaviour of the vector population and consequently, the efficiency of control measures, as well as the assessment of models such as that of uniform and non-uniform exposure to applied insecticide (Molineaux and Gramiccia, 1980).

Sampling must take into account the spatial and temporal distribution of the population being studied and also necessitates an understanding of the life cycle and behaviour of the organism. However much of this information can only be obtained by sampling the population (Service, 1971). Thus preliminary population sampling in the elucidation of such data should be carried out prior to attempts at the absolute estimation of populations being made. The importance of

absolute figures in the assessment of mortality factors was stressed by Southwood (1976).

Estimates of the larval population are obviously important in vector surveillance, in that they precede the adult population and may be used as indicators of the presence or absence and abundance levels of a vector species. Larval assessment has the advantage that it may be carried out during daylight hours and is not subject to factors such as differences in biting behaviour and the effects of rain, wind and temperature. Although the latter factors obviously do ultimately effect the larval population, they do not have effects such as reduced catches of adults on windy nights (Sharp, 1983). Adult population catches are also affected by trap type and bait preference (Sharp, 1984; Leemingsawat, 1989). However larval sampling is made difficult by the diversity of breeding sites as well as the non-random distribution of individuals within such sites (Service, 1971). One such limitation is illustrated in this study (Chapter 2), where under conditions of low population density, larval sampling for *An. arabiensis* proved ineffective, while specific adult population sampling techniques were. A similar conclusion was reached by Knight (1964) and Soper and Wilson (1943). The efficiency and sensitivity of sampling techniques for surveillance are also greatly affected by inadequate staffing and supervision (W.H.O., 1975).

Rogers *et al.*, working on *Glossina palpalis* pointed out that studies of the natural dynamics of an organism may be used in the prediction of the effectiveness of control measures.

Rogers *et al.*, (1984) demonstrated that the application of insecticide affected natural population regulation, due to its action on non-target organisms. Similarly, Service (1977) demonstrated that the application of insecticide to rice paddies in Kisumu, Kenya, resulted in greater pre-adult survivorship of *An. arabiensis* . This was thought to be due to the fact that recolonisation of the paddies by *An. gambiae* complex immatures was more rapid than that of naturally occurring predators. There have been relatively few investigations of instar mortalities in mosquitoes. Service (1971, 1973 and 1977) investigated survivorship of the immatures of the *An. gambiae* complex. Lakhani and Service (1974) and Southwood *et al.* (1972) respectively investigated the instar mortalities of *Aedes cantans* and *Aedes aegypti*.

Lakhani and Service (1974) stated that density dependent factors, such as competition for food or space, or the accumulation of toxic substances (possibly excretory in origin), may be more important in regulating larval population numbers, than is generally realised. Studies relating the number of larvae per dip and the number of larvae per area sampled have been carried out (Andis *et al.*, 1983; Russell *et al.*, 1945). However few studies of mosquitoes which attempt to assess the relationship between larval numbers and pool size and the effect of density dependent factors such as those suggested by Lakhani and Service, appear to have been conducted.

Since *An. merus* is a salt water breeder it was considered

important to investigate salinity in relation to larval dynamics and possible physiological limitations. The most comprehensive field study on larval salinity tolerance and its effect on the population dynamics of *An. merus* is that of Mosha and Mutero (1982). They suggested that the salinity tolerance of *An. merus* may vary with season as the tolerance of first instar larvae varied at different times of the year. They do however not consider that salinity tolerance of larvae may undergo physiological acclimation, when the population is subject to conditions of increasing salinity. Sharp (1983) demonstrated that the salinity tolerance of second and fourth instar *An. merus* larvae was greater, when they were raised in salt water, as opposed to distilled water. He did however not investigate this possible increase in salinity tolerance in first instar *An. merus*.

5.2 MATERIALS AND METHODS

5.2.1 FIELD COLLECTIONS AND PROCESSING

The field study on survivorship and larval population dynamics was conducted at Nceswana lake in the Ophansi area (site 47, Figure 3). A picture of the breeding site used in the study is shown in plate 18, Appendix 2, Chapter 2. The site was situated at the edge of the pan and water levels in the pools were maintained by seepage. The small pools were formed by cattle hoof prints and the entire site extended for approximately 200 m along the edge of the pan, and was four to six metres wide.

Monthly (one week per month) collections were made at the

site over the period May 1984 to January 1985. The site was inundated in February 1985 with the advent of heavy cyclonicly induced rainfall. At each collection, the entire contents of approximately 15 pools were emptied into individual, marked buckets. Measurements such as pool size, salinity, temperature and dissolved oxygen levels were also recorded. Three transects were conducted across the width of the site at each survey, due to the presence of a salinity gradient between the water's edge and the top of the site. The five pools selected in each sample were approximately five metres apart. The origin point, ie. at which each transect was started, was selected randomly.

The buckets were then taken back to the field station and the contents passed through a series of filters. The larvae/pupae thus obtained were placed in sample bottles containing a 4% formalin solution for subsequent analysis. The filtered water was then added back to the sediment which remained in the bucket. These buckets were then observed successively for two hours in case any larvae had remained in the sediment left in the bucket during filtration. During one survey the contents of five pools of approximately the same size were emptied with a 200ml soup ladle and each successive dip was emptied into a separate, marked honey jar. The subsequent processing of the contents of these jars was the same as the buckets. The larvae obtained in these collections were separated into instars on the basis of head capsule width (Chapter 3). Identification was the same as reported in Section 3.2.1, Identification.

5.2.2 STANDARDISATION OF FIELD COLLECTIONS

To ensure that all larvae were collected from the pools in the study site when they were emptied, the following procedure was carried out:

1. Approximately 300 larvae in each instar were collected and divided into five groups (60 larvae), consisting of only one instar (eg. first instar).

2. Five pools which had dried out were flushed (3x) with tap water from the field station. The pools were then filled and each group of larvae added to a single pool.

3. The pools were then emptied into buckets and processed as described earlier. More water was then added to the pools which were again emptied and the contents placed into separate buckets.

4. The filtered samples were then processed and the recovery rates for each instar, for the initial emptying and the flush were then calculated.

The data for adults is based on collections from a pit shelter situated approximately 200 m from the site (Thomson,-Muirhead, 1948).

5.3 RESULTS

5.3.1 STANDARDISATION OF LARVAL FIELD COLLECTIONS

The percentage recovery of larvae from pools into which known

numbers of larvae were placed is shown in Figure 31. The recoveries for instars I to IV and pupae were 86,9; 90,0; 89,1; 94,0 and 90 percent respectively. These percentages were used in the standardisation of the collection technique.

5.3.2 SEQUENTIAL SAMPLING

Figure 32 shows the recovery of larvae and pupae during the sequential sampling trial. The overall trend of increasing numbers of larvae with successive dips should be noted (Figure 32, all larval stages). It is interesting that the maximum larvae per dip in the first instar was obtained with the last dip. In contrast the other instars (II-IV) showed their peak return at the eighth or ninth dip, with a decrease thereafter. The initially low return obtained in the first six dips, for first and fourth instar is evident. The recovery of pupae is apparently random, unlike that of larvae and does not follow any distinct trend.

Figure 33 shows the relationship between the number of larvae recovered in the **n**th dip of a pool and the total number of larvae which remain in the pool at the **n-1**th dip. Note should be taken of the numbering of the points. The numbers refer to the dip number. The equation for the line (Figure 33) and the correlation coefficient are given below;

$$Y = 104,9 - 0,176X \qquad \text{Corr. Coef.} = -0,912$$

The relationship for the individual larval stages was investigated. The equation for the lines and the correlation coefficients are given below;

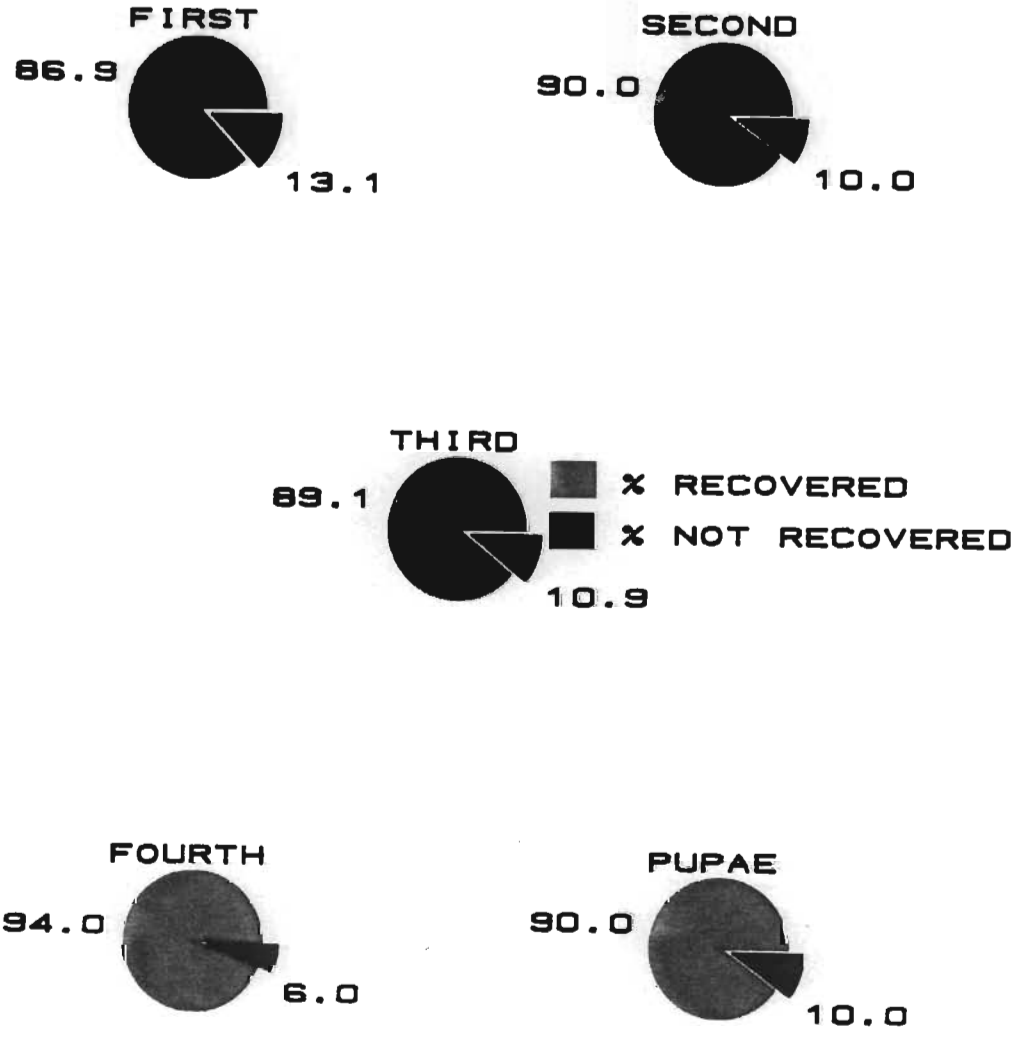


Figure 31. Percentage recovery for *An. merus* larvae and pupae at Ophansi, when pools to which known numbers had been added, were emptied.

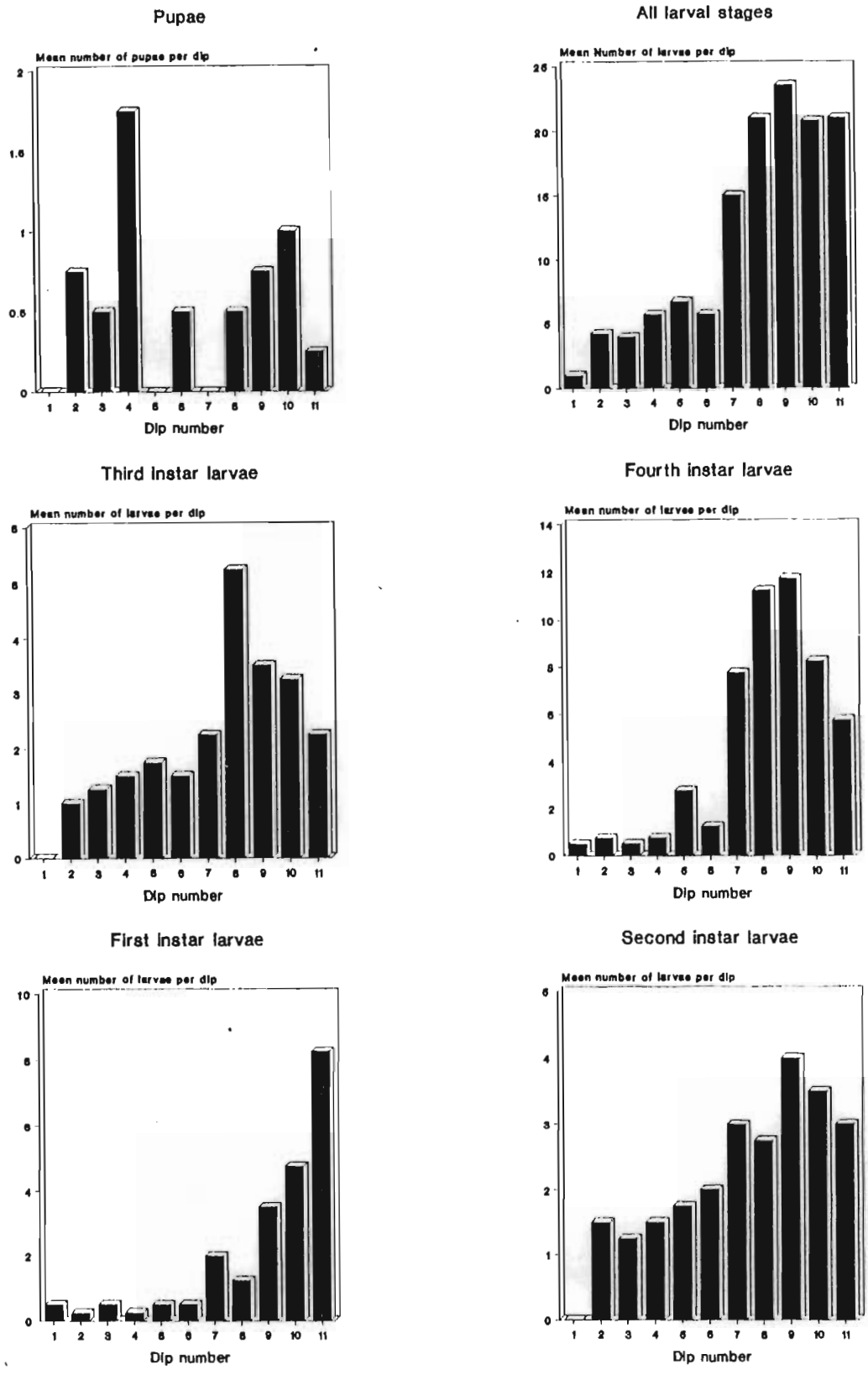


Figure 32. The number of larvae (total and individual stages) obtained in each of eleven successive dips, which emptied the pools.

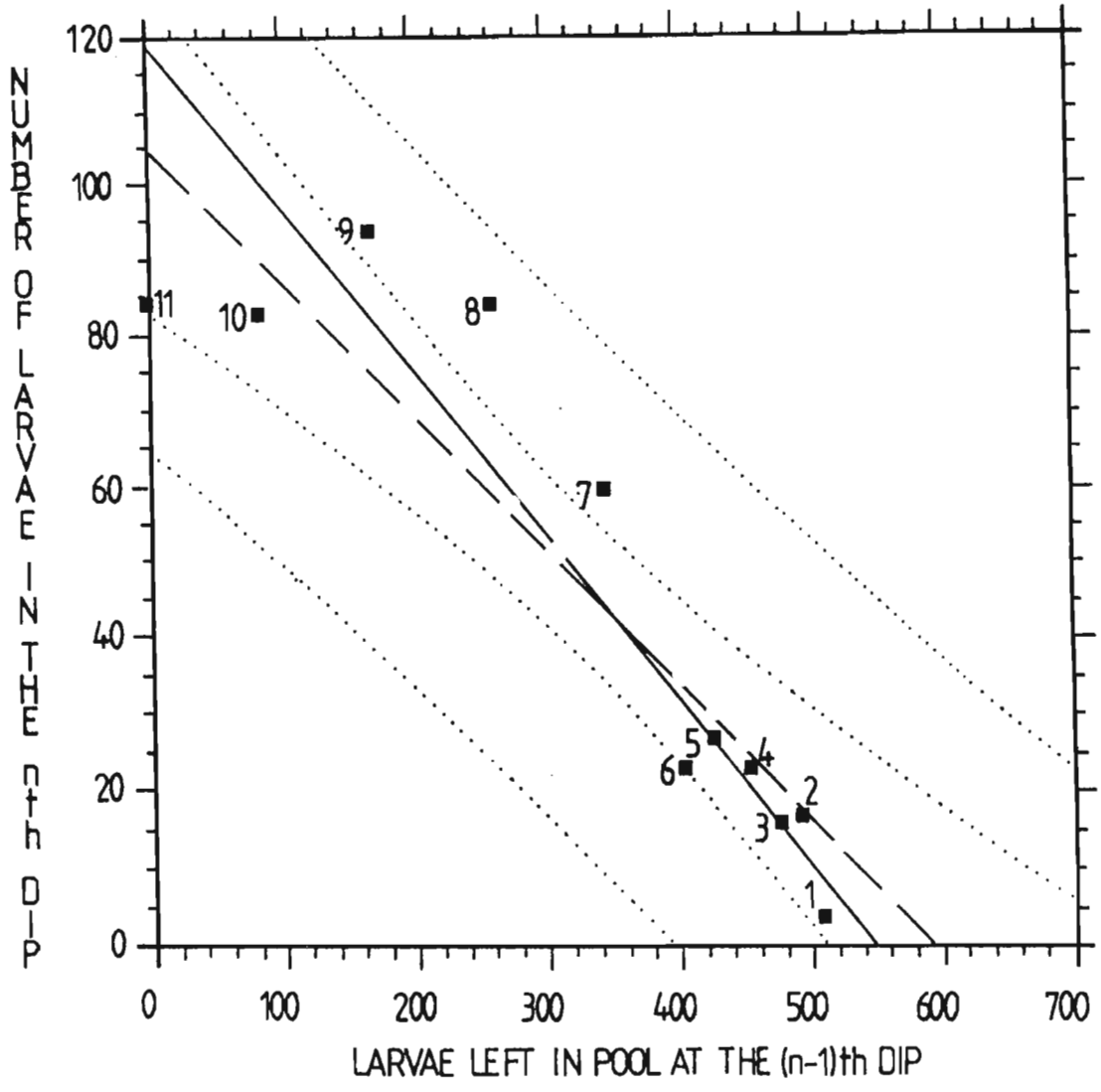


Figure 33. The relationship between the number of larvae per sequential unit sample and those remaining in the pool. All larval stages. The solid line is fitted by eye and ignores the data points (10 & 11) collected after the return per unit sample had peaked (ie. 9).

		Corr. Coef.
FIRST	Y = 32,2 - 0,367X	-0,990
SECOND	Y = 15,8 - 0,123X	-0,875
THIRD	Y = 15,3 - 0,121X	-0,710
FOURTH	Y = 42,1 - 0,174X	-0,749
PUPAE	Y = 2,66 - 0,043X	-0,162

Note should be taken of the high correlation coefficient for firsts and the fact that this value tends to decrease in subsequent instars.

5.3.3 MONTHLY LARVAL RECOVERIES

The number of larvae recovered in each instar over the ten months of May to December was corrected using the rates shown in Figure 31. This data was then corrected for any differences in the number of pools sampled each month. The resultant data for the mean, monthly number of larvae per pool and in each instar, is shown in Figures 34 and 35. The important points to note in these figures are;

1. The mean number of larvae per pool. The recorded values are high during the months of April (265) and May (194) and then decline in July (99). The mean density of larvae then increases in August (343) and then begins to decline again, reaching its lowest level in December. This overall trend is easily visualised in Figure 37 (top). Special note should be taken of the low values obtained for the months of July and December.

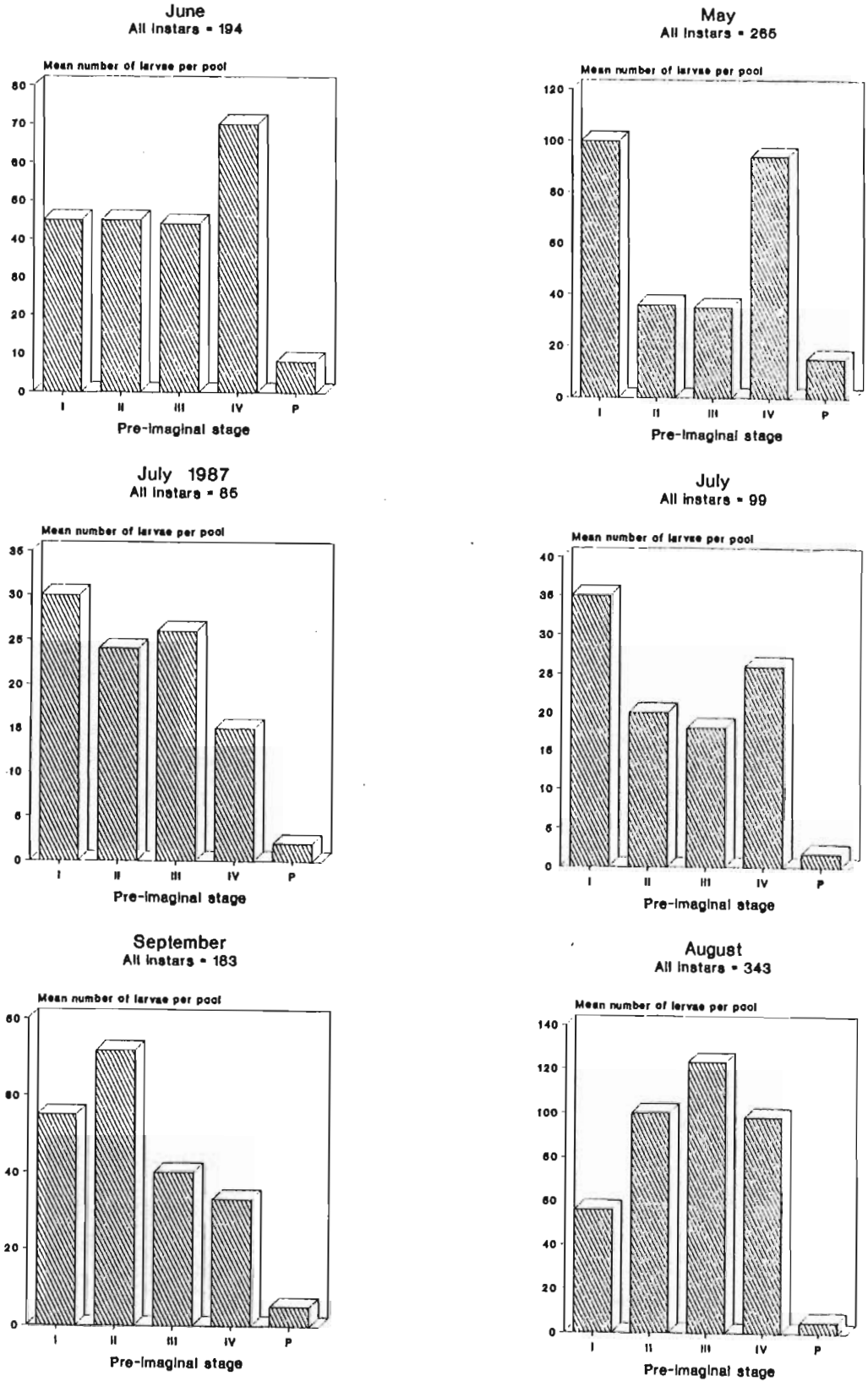


Figure 34. Mean number of larvae of each instar per pool, for the months of May to September 1984 and July 1987 (corrected values)

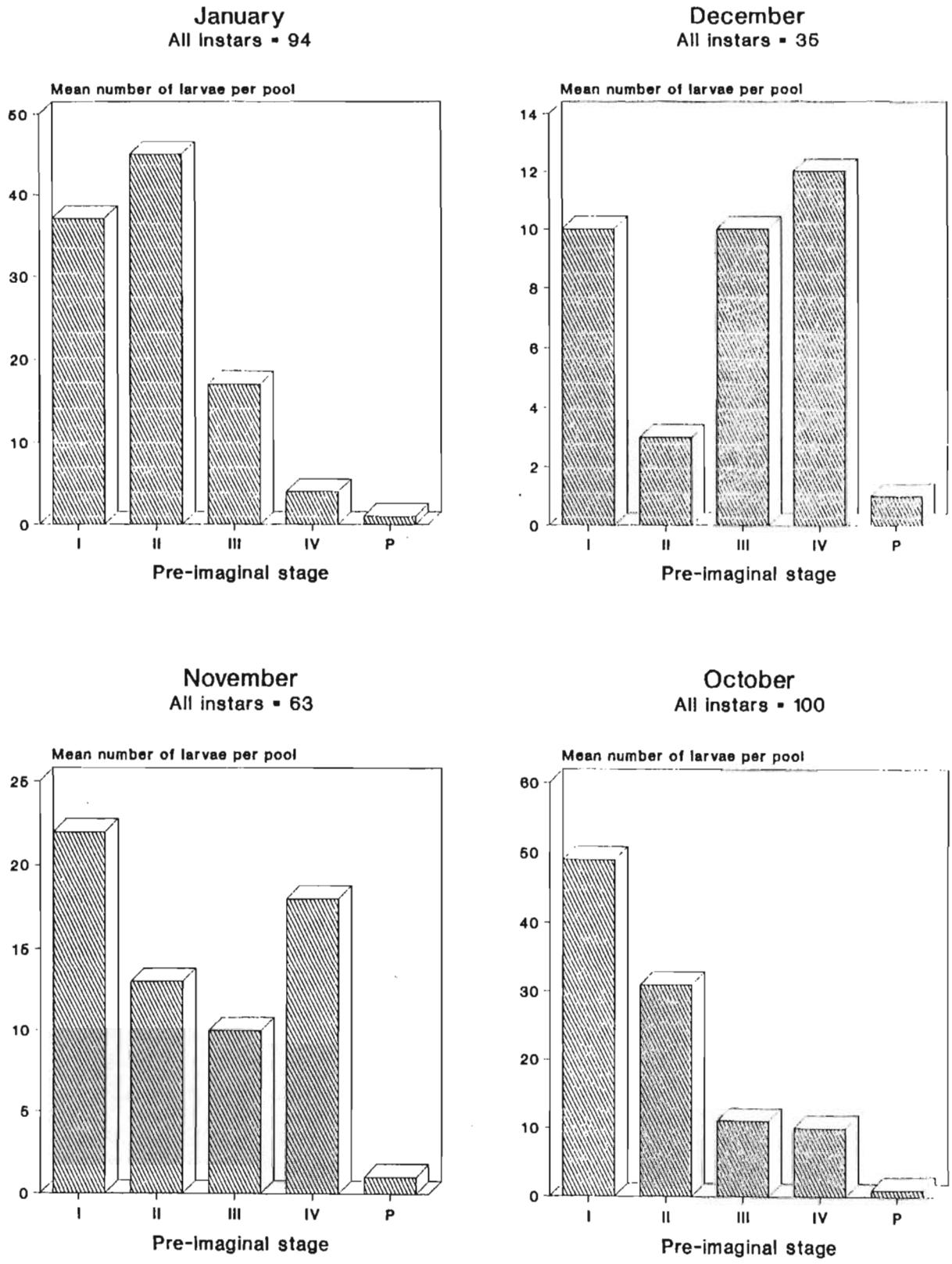


Figure 35. Mean number of larvae of each instar per pool, for the months of October to January 1984 (corrected values).

2. The relative proportion of larvae in each instar. The accumulation of larvae in the fourth instar during the winter months of May, June and July. The deficit of first instar larvae in the month of August and subsequent increase in the proceeding months. Note should also be taken of the unexpected instar proportions obtained for the month of December.

The mean monthly temperature for the region over the period 1968 to 1983 is shown in Figure 36. This data will be used in the explanation and discussion of the results shown in Figures 34 and 35. Figure 37 (top) shows the mean, monthly number of larvae per pool together with an index of the adult population. The adult index is based on the mean, daily number of adults collected from a pit shelter, with the pit shelter being cleared daily. The important point to note here is the disparity between the adult index and the larval index in May. During the next two months, the two indices follow each other closely. With the advent of the summer months the larval population declines, while the adult population increases. The dip in the adult and larval population indices during the month of December should be noted.

Rainfall data recorded over the study period, together with the fifteen-year mean for the region is shown in Figure 37 (bottom). During the study, the rainfall for the winter months was unusually high when compared to the figures for the fifteen-year mean. The rainfall recorded for the month of November during the study is similar to that for the fifteen-year mean but is low relative to that recorded for

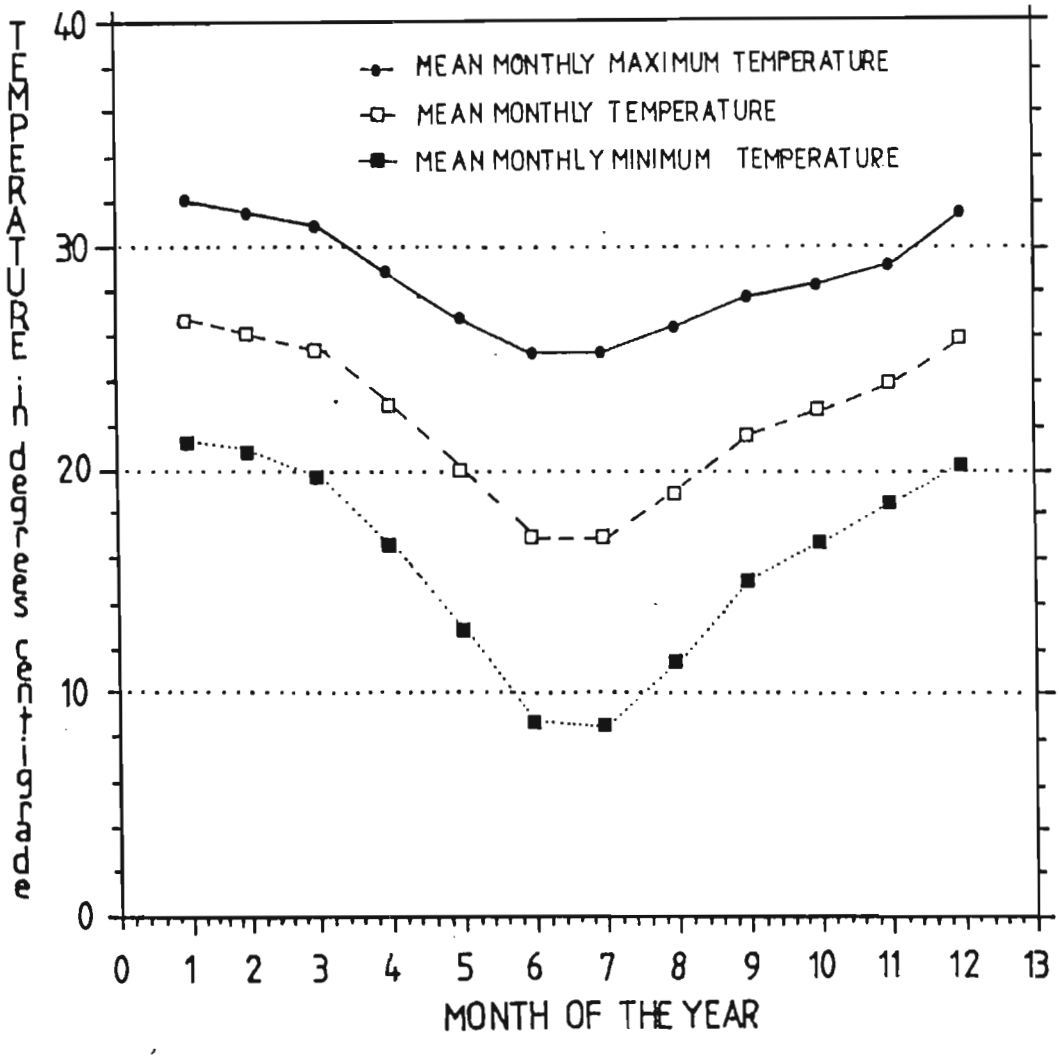
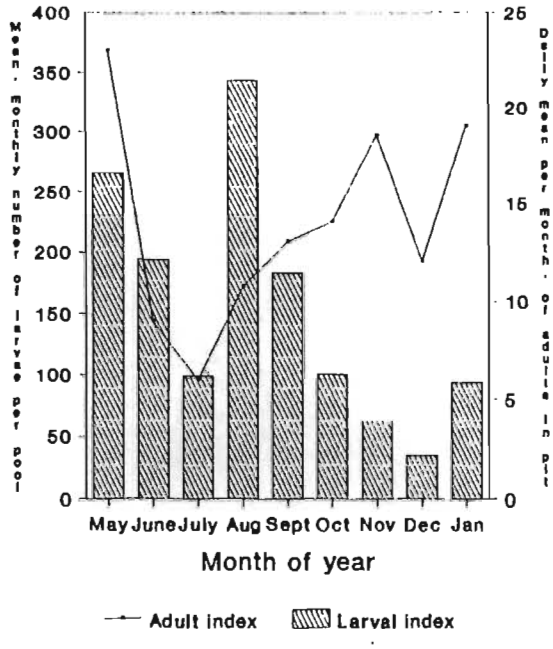


Figure 36. The monthly, mean, minimum and maximum temperature recorded for the study area over the period 1968 to 1983.



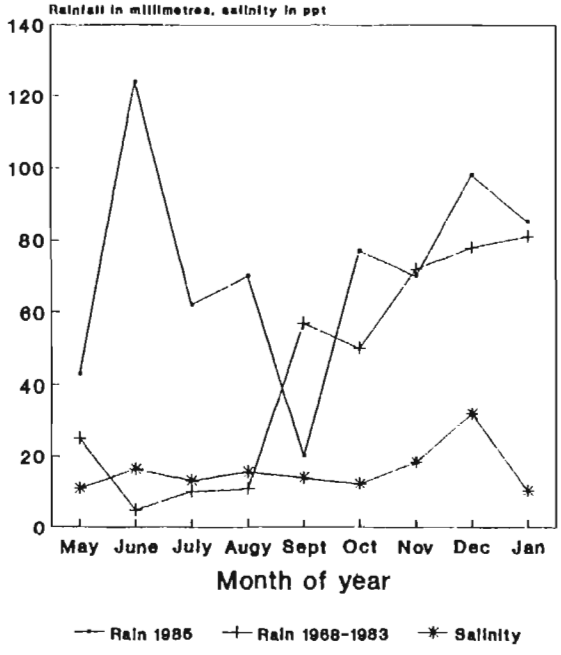


Figure 37. **Top** = The mean monthly number of larvae per pool together with an index of the adult population. **Bottom** = The mean monthly, rainfall and pool salinity recorded over the duration of the study. The mean monthly rainfall for the period 1968 to 1983.

the month of October 1985.

The mean monthly salinity for the pools sampled during the study is included on the same figure in parts per thousand (ppt). The mean recorded salinity was fairly consistent throughout the study period, excluding the month of December. The frequency of occurrence of salinities recorded from the study site over the nine month period is shown in Figure 38 (top) and the comparative data for the month of December in Figure 38 (bottom). The displacement of the fitted distribution curve to the right is due to the high frequency of salinities above 30ppt. The approximate means of the two distributions, all months, and December only, are 15 and 30 parts per thousand respectively.

The apparently high number of larvae per pool during the winter months (Figure 37 top) was tested for significance. The mean number of larvae per pool was 221 for winter and 80 for summer and was significantly different ($n = 20\ 375$, $P < 0.001$).

5.3.4 LARVAL SURVIVORSHIP

Survivorship curves were constructed using the larval numbers sampled in January and December and the summer instar durations determined in this study, and those measured by Service (1973) in Kisumu, Kenya (Figure 39). Smooth curves could not be fitted to the month of December, irrespective of the two instar duration profiles utilised. The instar duration profiles used to estimate survivorship in this study are shown

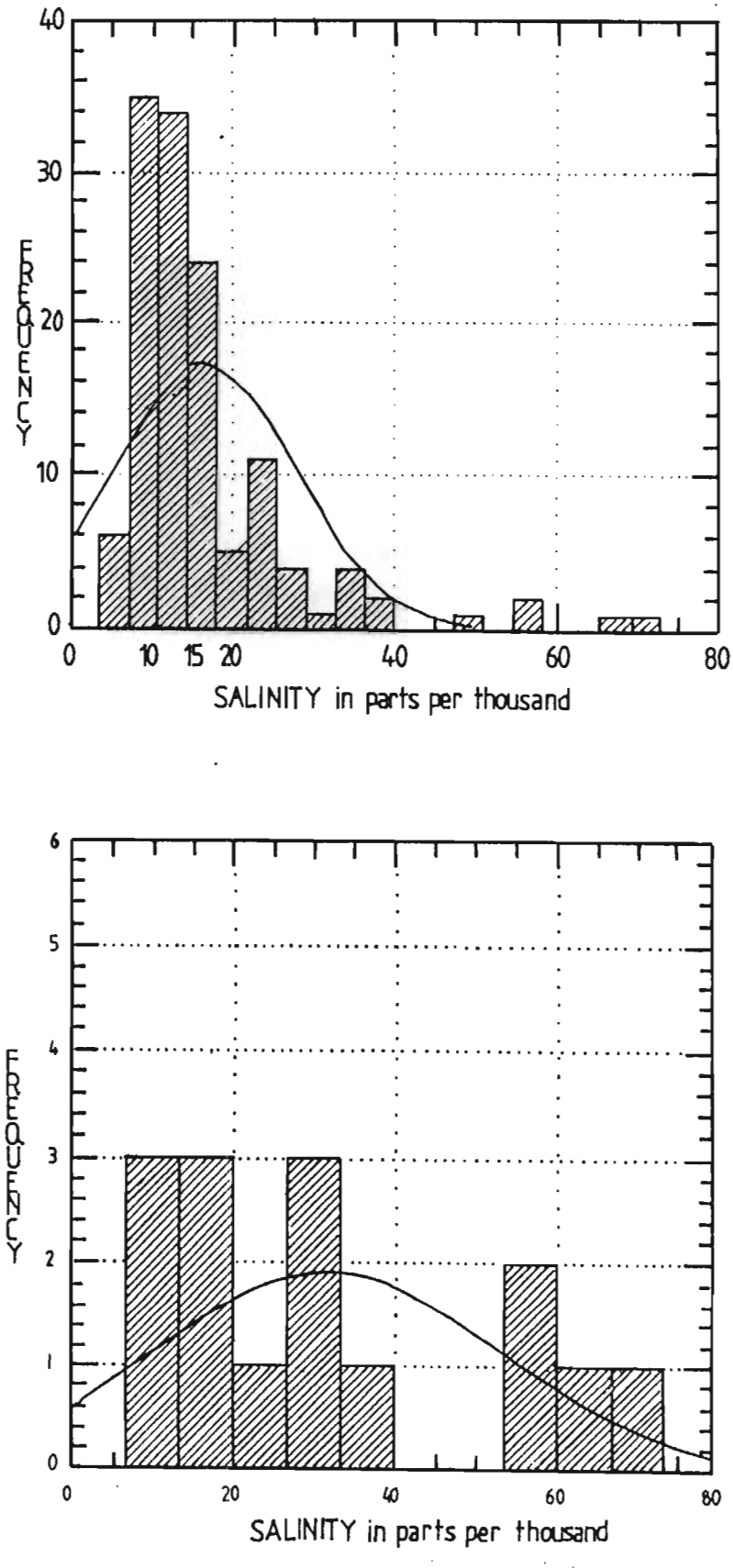


Figure 38. **Top:** The frequency with which specific salinities were recorded from the study site over the ten monthly sampling period, with a fitted normal distribution curve. **Bottom:** Frequency histogram of the salinities of pools sampled during the month of December and a fitted normal distribution curve.

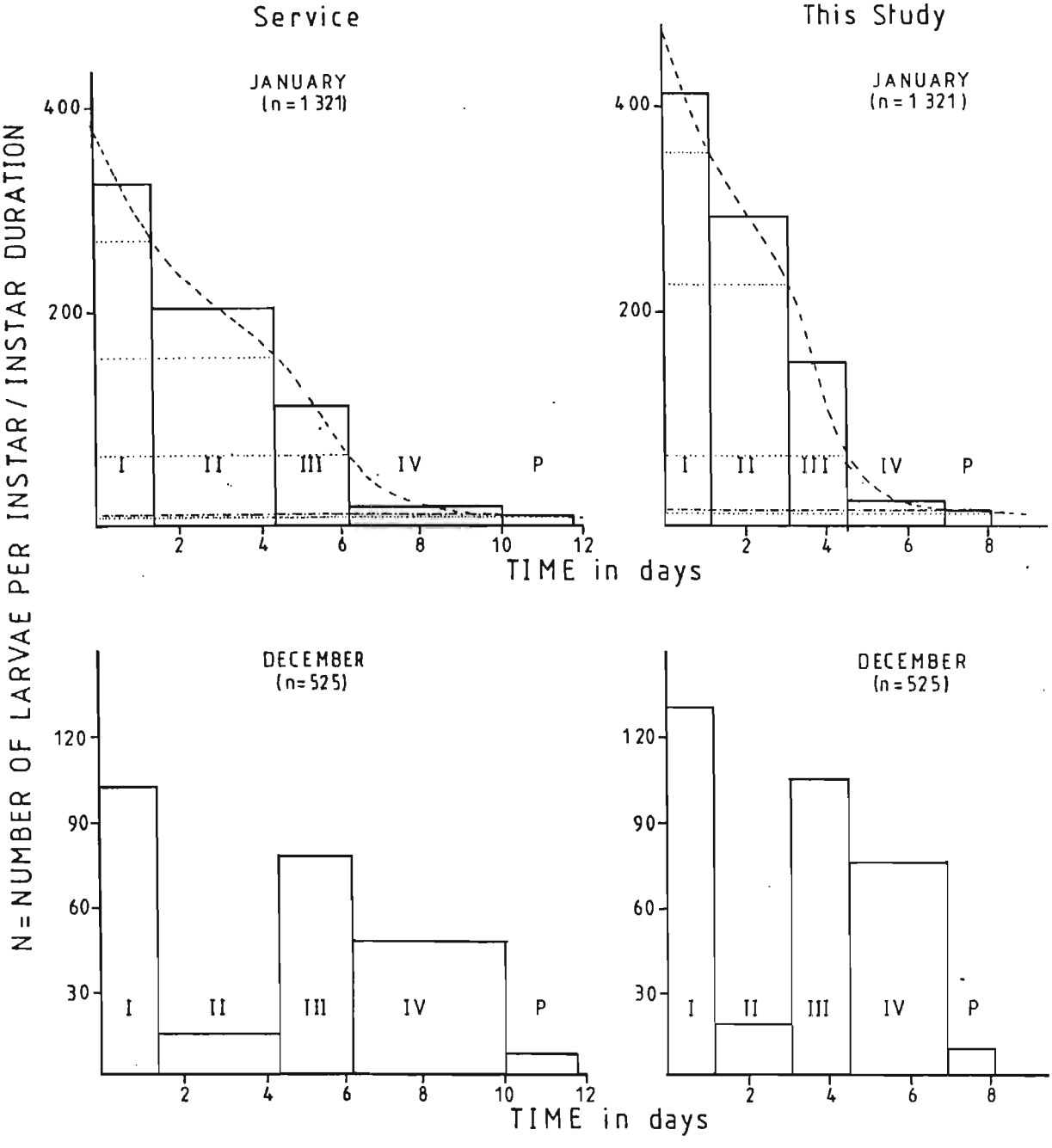


Figure 39. Survivorship curves for the data collected from Ophansi, for the months of December and January, using instar durations derived in this study and those of Service, 1973.

in Figure 40.

Survivorship profiles for the months of June, July 1985 and July 1987 were constructed using the field determined larval instar durations. The profiles derived using the durations for fourth instar under low and high density (number/pool) conditions are shown in Figures 41 and 42 respectively. That the instar durations estimated in July 1987 do not apply to the data collected in July 1985 is indicated by the high values for the fourth instar (Figure 41 middle & bottom). The difference in mean pool density between July 1984 and July 1987 should be noted. The application of the instar duration measured for fourth instar under high pool densities results in the survivorship curves for June and July following the expected profile of a decline in numbers in successive instars, due to mortalities incurred in each. Inherent in this statement is the fact that the population is stable and that the apparent build up of fourth stage larvae was not a product of a change in either output or input to or from the larval population. The merits of this assumption will be dealt with in the discussion.

The estimated instar mortalities together with the relative proportions dying daily in each instar derived from these curves are shown in Table 17. The estimated first instar to adult mortalities for January, using the instar durations derived by Service (1973) and those from this study are 97,3 and 97,9% respectively. The estimated larval instar mortalities show a similar trend with an increase in the percentage mortality being recorded in successive instars.

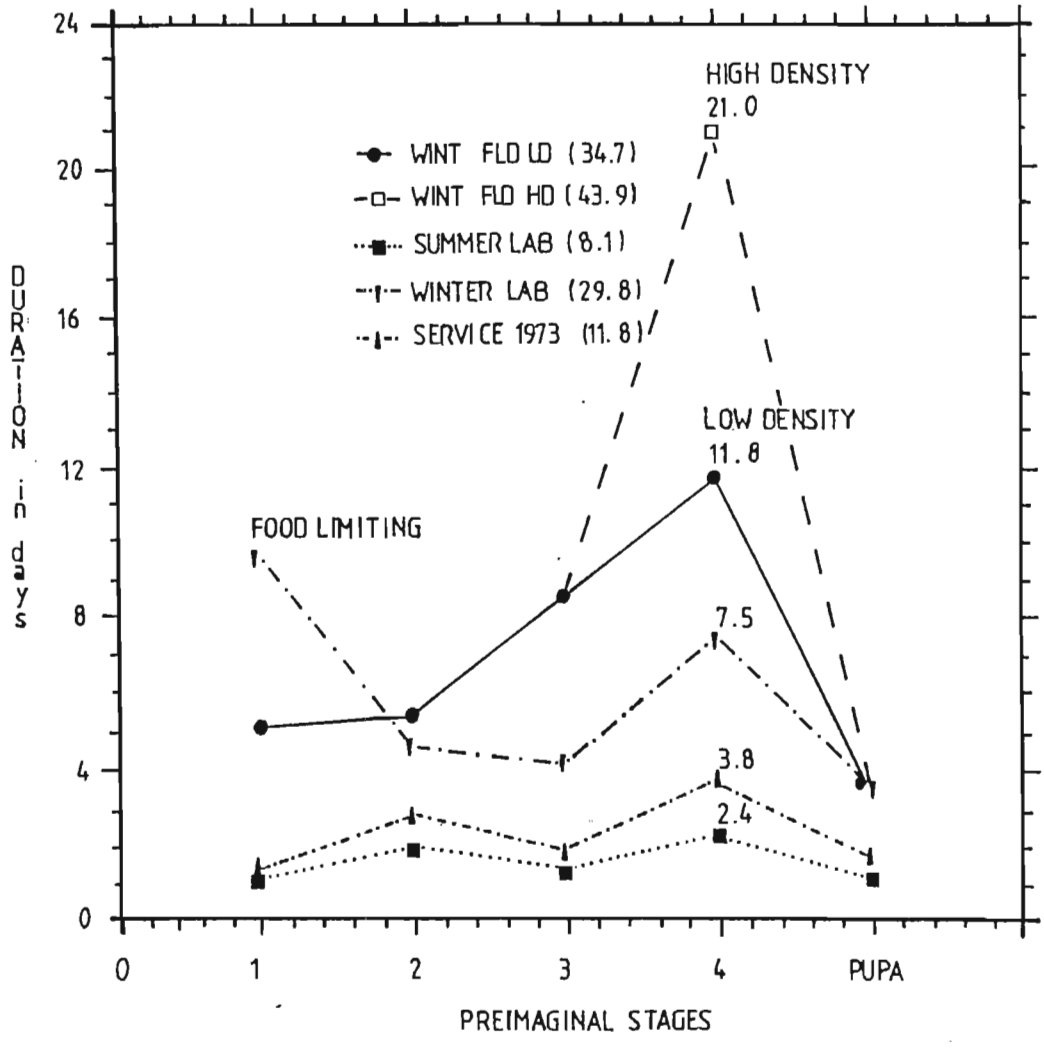


Figure 40. Summary of the pre-imaginal durations measured under various temperature condition.

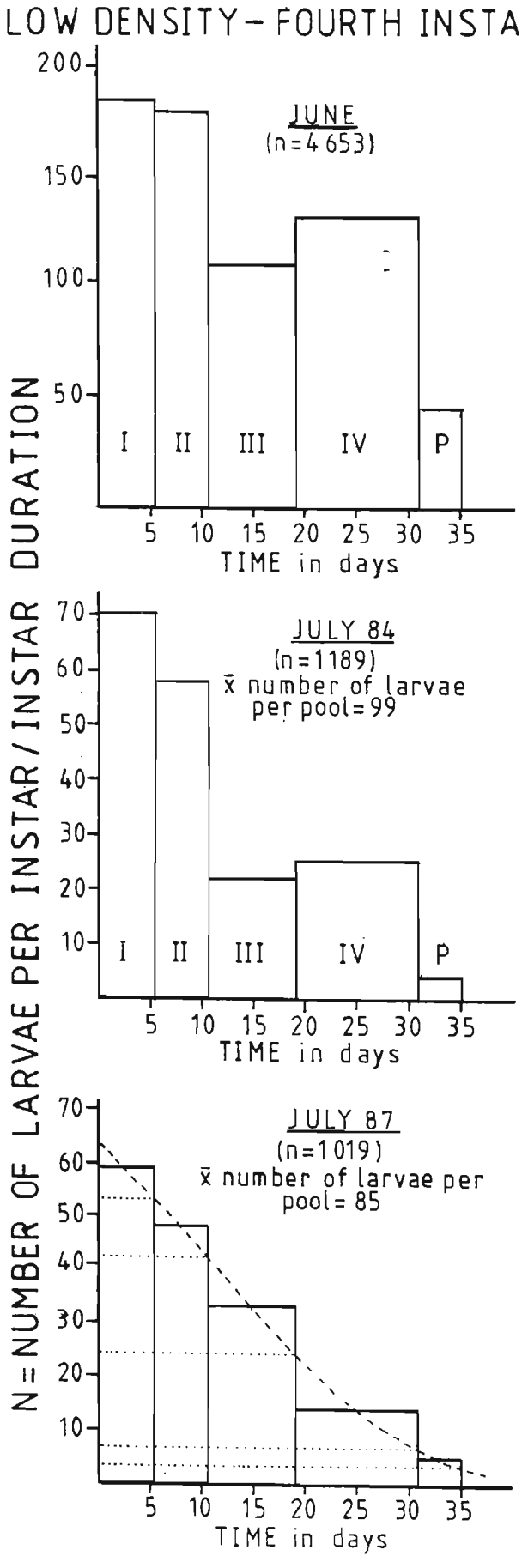


Figure 41. Survivorship curves for the months of June and July 1984 and July 1987, using the fourth instar durations measured at low density (larvae/pool).

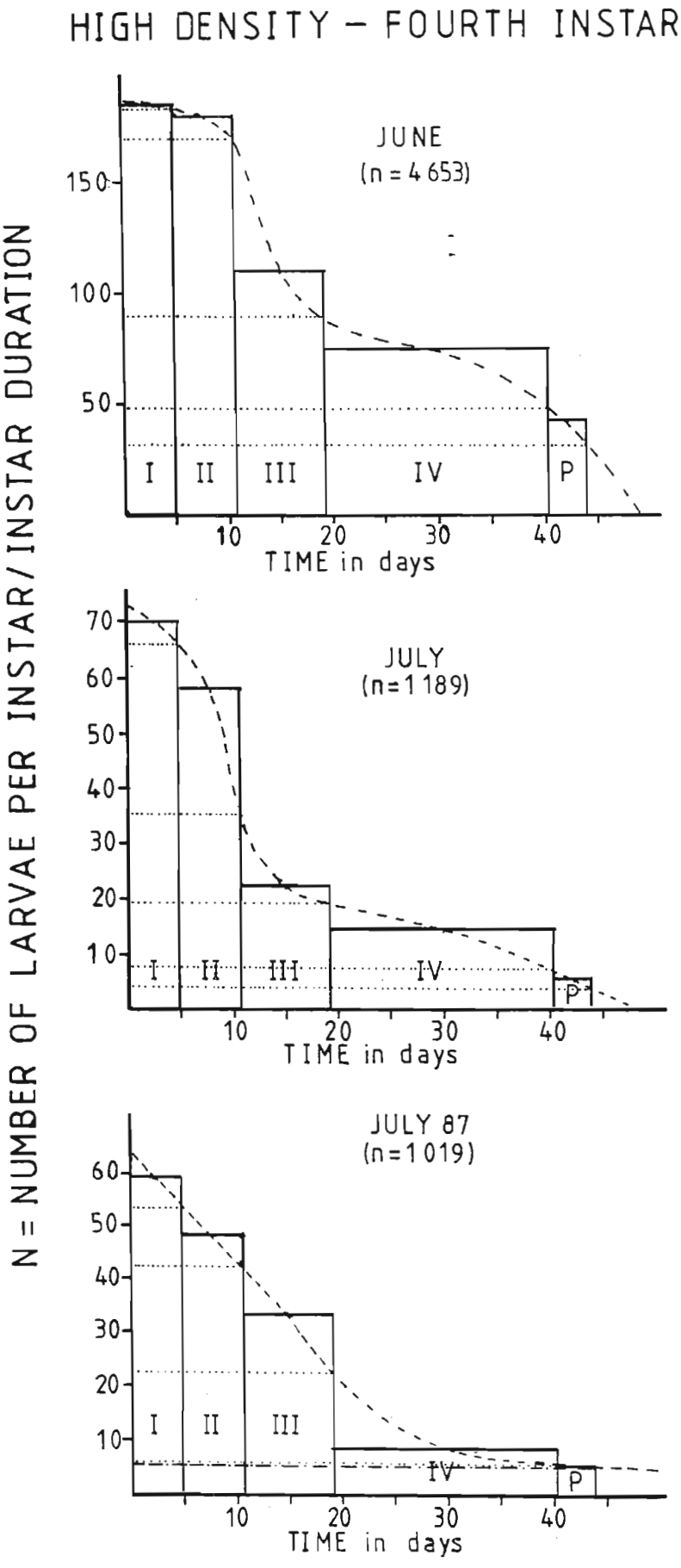


Figure 42. Survivorship curves for the months of June and July 1984 and July 1987, using the fourth instar durations measured at high density (larvae/pool).

Table 17. Estimated mid-summer and mid-winter mortalities, together with the relative proportion dying daily in each instar. (High and low IV instar density, winter conditions)

Instar (i)	Age in days at beginning instar (t_{i-1})	No. entering instar (St_{i-1})	Death in Instar (Di)	Relative proportion dying in ($\frac{Di}{St_{i-1}}$)	Proportion dying daily in instar $1-(\frac{St_i}{St_{i-1}})^{1/d}$
Instar durations measured in this study					
January					
I	0	476	120	0,252	0,232
II	1,1	356	120	0,252	0,200
III	3,1	228	165	0,724	0,601
IV	4,5	63	45	0,714	0,407
P	6,9	18	5	0,278	0,238
Adult					
Instar durations measured by Service (1973)					
January					
I	0	380	106	0,279	0,208
II	1,4	274	116	0,423	0,173
III	4,3	158	95	0,601	0,384
IV	6,2	63	53	0,841	0,384
P	10,0	10	2	0,200	0,117
Adult	11,8	8			
High density fourth instar					
June	19,3	90	42	0,467	0,030
P	40,3	48	17	0,354	0,114
Adult	43,9	31			
July					
I	0	73	7	0,020	0,019
II	5,2	66	31	0,470	0,115
III	10,7	35	16	0,457	0,069
IV	19,3	19	11	0,579	0,020
P	40,3	8	4	0,500	0,175
Adult	43,9	4			
July 1987					
I	0	64	11	0,172	0,036
II	5,2	53	11	0,208	0,044
III	10,7	42	20	0,476	0,072
IV	19,3	22	16	0,727	0,060
P	40,3	6	1	0,167	0,049
Adult	43,9	5			
Low density fourth stage					
July 1987					
I	0	64	10	0,156	0,032
II	5,2	54	12	0,222	0,045
III	10,7	42	17	0,236	0,059
IV	19,3	25	18	0,720	0,106
P	30,7	7	3	0,429	0,144
Adults	34,3	4			

This pattern of low first instar mortality and an increase in the successive instars was also present in the estimates for the winter months. The estimates of pupal mortality for the months of June and July 1985 were high (35 and 50% respec-

tively), when compared to those for January (20 to 28%) and July 1987 (17%). The effect of the low and high fourth instar density on the estimation of mortalities is also shown.

The increased duration of the fourth instar under high density conditions resulted in higher estimated mortalities in the third instar (48 vs 24%) and a decrease in the estimated pupal mortalities (17 vs 49%). The first instar to adult mortalities for the winter months June and July 1984 and July 1987 with high fourth instar density were 83,6, 94,5 and 92,2 percent respectively. The first instar to adult for July 1987 with low density fourth instar conditions was 93,8%.

5.3.5 POOL SIZE AND LARVAL DENSITY

The relationship between total larval numbers and pool size is shown in Figure 43. The low degree of correlation between these two parameters is indicated by the correlation coefficient of 0,346.

5.3.6 SALINITY

The mean number of larvae per pool for all the juvenile stages is shown as a function of pool salinity in Figure 44. The horizontal axis increases in increments of three. Thus the first division represents salinities between 0 and 3 ppt, the second between 4 and six, etc.

The following factors should be noted in the graph for first instar larvae;

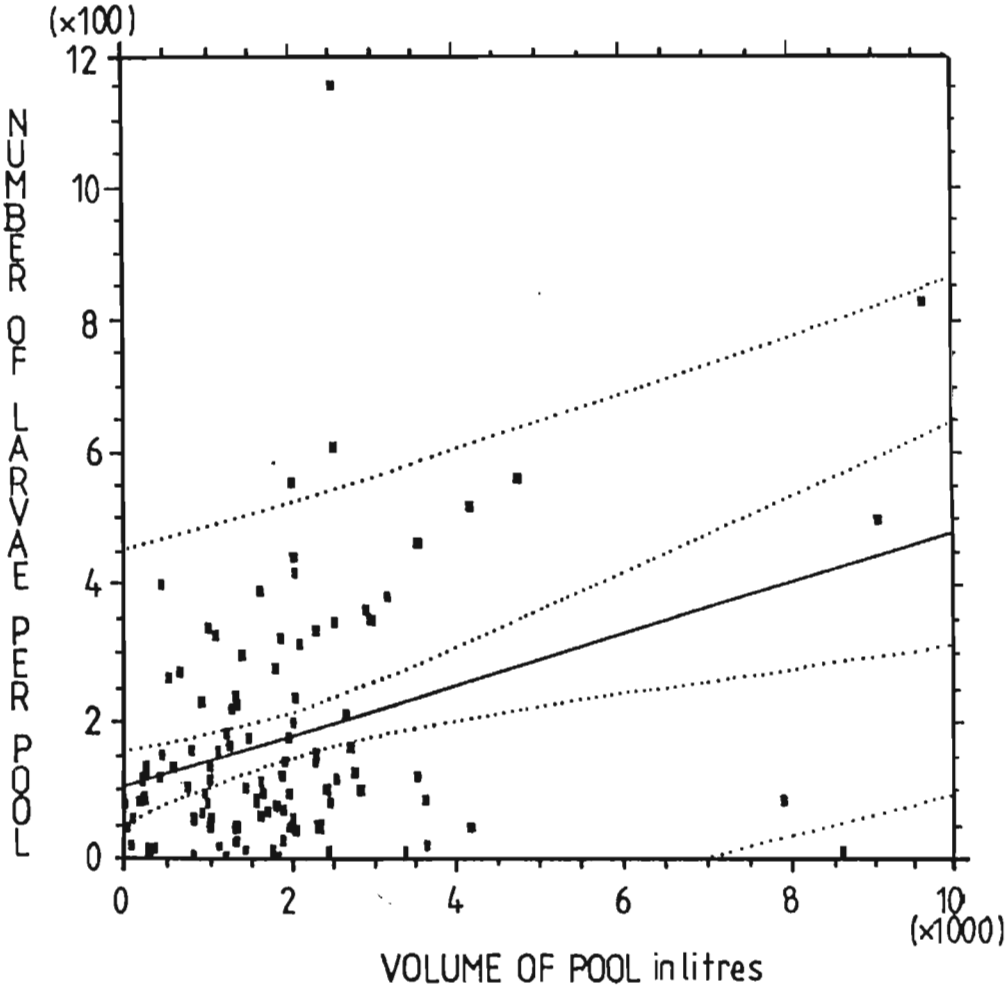


Figure 43. The relationship between pool size and the number of larvae per pool.

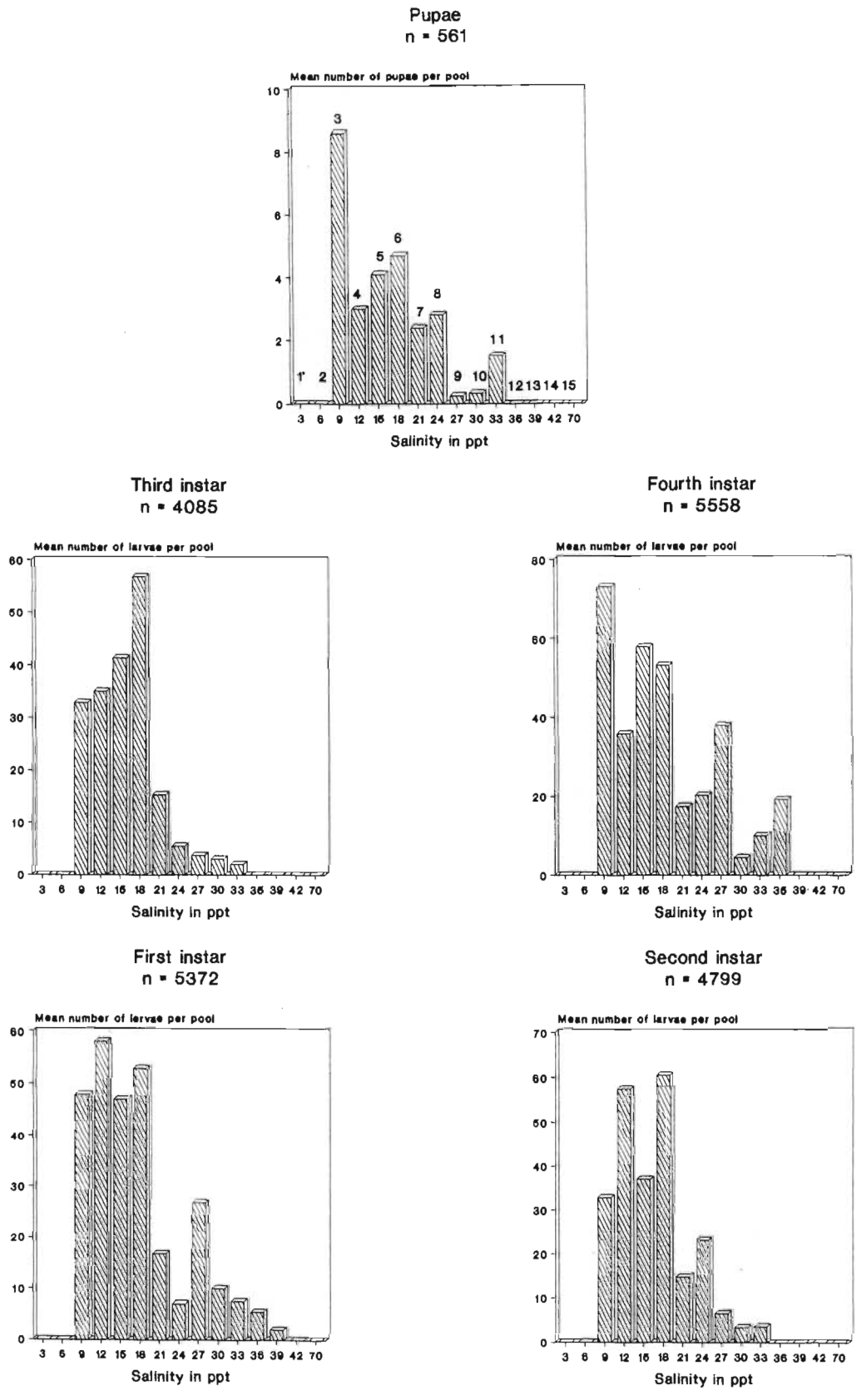


Figure 44. The mean number of larvae per pool for a specific salinity range. The salinity increase in units of three parts per thousand. The bars for the pupae chart are numbered and the same notation applies to other instars. The numbers of the bars correlate to the t-Tests which were conducted.

1. That the maximum, mean number of larvae per pool was between nine and eighteen parts per thousand.

2. That no larvae were recorded at salinities less than eight parts per thousand.

3. That the high numbers recorded for vertical bar number nine were due to a single pool and that only four pools of this salinity were sampled. The mean number of first instar larvae for the four pools was as follows; 9, 1, 87 and 10.

4. That the maximum value at which first instar larvae were recorded was 39 parts per thousand.

5. Student's t-tests were conducted between the mean number of larvae recorded at various salinities and the results are shown below. The numbering of the bars is as for pupae. Thus a student's t-test between bars 2 and 3 represents a test for significance in the mean number of larvae occurring at 4-6 ppt. and that at 7-9 ppt.

Students t-test between

2 and 3	t = 3,6 ;	P < 0,001
3 and 4	Not significant (NS)	
3 and 7	NS	
3 and 8	t = 2,78;	P < 0,001
4 and 8	t = -2,81;	P < 0,01
5 and 8	t = 3,4 ;	P < 0,01
6 and 8	t = 2,2 ;	P < 0,05
7 and 8	NS	
4 and 7	NS	
4 and 9	NS	
4 and 10	NS	
4 and 11/12/13	NS	

The results obtained for second instar are similar to those for first instar, with the mean maximum number of larvae per pool being recorded between a salinity of 7 and 18 ppt. The high value for vertical bar 8 was due to the high number of

larvae recorded from a single pool in this salinity range. The maximum salinity from which second instar larvae were collected was 33 ppt. The following students t-tests were conducted;

Students t-test between

2 and 3	t = -3,1; P < 0,01
3 and 7/8/9/10	NS
4 and 8/9/10	NS
6 and 9/10	NS

The mean maximum number of third instar larvae per pool was from salinities ranging between 7 and 18 ppt. The apparent increase in numbers with salinity within this range was not significant (t-Test between bars three and six). The maximum salinity from which second instar larvae were collected was 36 ppt.

The decline in the mean number of second and third instar larvae at salinities above 18 ppt. should be noted. This decline also occurred in the first instar, but was less marked at higher salinities. Fourth instar larvae and pupae do not show a sudden drop as in case of the earlier instars, but an overall trend of a progressive decline in numbers with increasing salinity is apparent. The apparent increase in the mean number of fourth instar larvae and pupae with salinities between 28 and 36 ppt. is not conclusive due to small sample size (n = 3, 2 and 1 for columns 10, 11 and 12 respectively, for fourth stage larvae and pupae). The following t-Test were conducted and none of the means tested were significant, except that between columns 2 and 3 (fourth instar t = 3,2; P < 0,01: pupae t = -1,82 P < 0,077 therefore

NS for a 95% confidence interval for the difference in means).

Fourth instar, t-Test between	Pupae, t-Test between
3 and 10	3 and 7/8/9/10/11
5 and 10	7 and 4/5/6

5.4 DISCUSSION

5.4.1 STANDARDISATION OF LARVAL AND SEQUENTIAL SAMPLING IN THE FIELD.

The lowest recovery rate for any instar was that for firsts (87%) and the highest that for fourths (94%), with the recovery of the remaining two instars and pupae being in the region of 90% (Figure 31). The recovery rate of successive instars thus appears to increase with size. This does however not take into account possible differences in behavioural avoidance by the individual larval stages. These will be covered later.

Christie (1954) attempted to assess the recovery rate of instars two to four. The technique he used was similar to that in this study in that the whole pool was emptied, but his pools were considerably larger. He was unable to assess the recovery of first instar larvae due to the size of the pool used, and the possibility that eggs may have been stranded on the side and did not look at recovery rates for pupae. He did not separate his instars so that metamorphosis of individuals from one instar to the next during the four-hour sampling interval precluded an assessment of the recovery rates for individual instars. His overall larval recovery

rate was 95,5%.

The placing of groups containing only one instar into each pool allowed the accurate assessment of recovery for each instar. This was considered important as it allowed a correction factor to be applied for each instar during the study. The use of such a factor is of obvious importance to the accurate assessment of larval instar mortalities. In this instance failure to apply the correction factor would have resulted in an under estimation of first instar mortality and an over estimation of that of fourths. It may be argued that the differences in this instance are relatively small, however when sampling a population, the aim is to obtain as accurate a measure as possible. In this study it was possible to accurately sample the larval population because of the nature of the breeding sites utilised. Similarly the technique could have been applied to the majority of the sites utilised by the other members of the *An. gambiae* complex within the study area, due to their small size (Chapter 2). This would however not have been the case with the winter sites utilised by the freshwater members and the saline marshes used by *An. merus*.

With the sequential sampling trials, the trend was for the number of larvae recovered, to increase with each successive dip (Figures 32). This was the case with all instars except pupae which appeared to be randomly recovered. The initial recovery of first instars was lower than that of the other instars and also peaked with the last dip (no. 11). All the other instars peaked 3 or 4 dips before the last dip ie. dip 8 or 9. Thus all the immature stages exhibit avoidance beha-

viour to prevent capture. In the case of pupae however this was relatively ineffective and probably related to the fact that they tended to 'bob up and down', only remaining submerged for a short periods. First instar larvae on the other hand were the most efficient at avoiding capture and this was obviously related to their ability to remain submerged for a longer period of time. This may have been related to their small size and consequent large surface area to volume ratio enhancing their ability to absorb oxygen from the water. The high dissolved oxygen levels of the pools investigated in this study and the ability of larvae to remain submerged was discussed in Chapter 2. It should however be borne in mind that the pools in this study were small (2-3 litres in volume) and that the observed trend is thus a result of avoidance by the larvae and a concentrating effect with the decrease in the volume of the pool with each dip.

Nielsen and Nielsen (1953) observed that first instar larvae of *Aedes taeniorhynchus* spent most of their time on the bottom of pools and also concluded that this may have been size related. They also reported similar avoidance behaviour with stimuli such as light/shadow, sound and vibration. The importance of this behavioural trait is that there is a tendency for untrained or inadequately supervised surveillance staff to dip a pool once and then walk on to the next one. This will obviously lead to the conclusion that there is a small or no larval population within the region. Secondly the numbers collected may be few and little may remain for species specific identification purposes by the time the

sample reaches the laboratory due to mortalities incurred (drowning, etc.).

The estimation of the number of mosquito larvae in a confined natural breeding site using removal methods has been estimated by various authors (Miura, 1980; Wada, 1962a and b). The basic assumption in this technique is that there is a relationship between the number of larvae collected from a pool in a particular unit sample and the remaining larval population. The above authors investigated this method in confined breeding sites containing *Culex quinquefasciatus* and *Aedes togoi*. In both these species the number of larvae decreased with each successive dip or unit collection. This is theoretically what one would expect, as at dip one the population is at its maximum and then decreases with each subsequent dip. In addition the following conditions should hold true:

1. The removal technique should not decrease the probability of an individual being caught;
2. The population should be stable during the sampling period;
3. The sampling instrument should not be limited in the number of individuals it may hold.
4. The probability of being caught should be the same for all individuals.

The results of this study are however in direct contrast to those of Miura (1980) and Wada (1962a & b). From Figure 32 it is quite obvious that the number of larvae per dip is at a

minimum at dip one and then increases, with each subsequent dip (this excludes pupae and ignores for the moment the decline which occurs from dips 8 to 9 for instars 2-4). The data from this study were plotted in the same fashion (Figure 33) as that of the above authors, except that the dip number increased from right to left, whereas in their studies it decreased (ie. they had dip or sample unit one closest to the y axis).

Inherent in the manner in which the data have been plotted is the assumption that the sampling technique does reduce the probability of an individual being caught (see 1 above) due to avoidance behaviour. This factor together with the decrease in pool volume results in a concentrating effect and thus increases this probability with each subsequent dip. Figure 33 also makes the assumption that the probability of individuals of each instar being caught is the same (see 4 above). From Figure 32 it is obvious that this assumption does not hold as first instar larvae for example, are more efficient at avoidance than the others.

Thus the goodness of fit of the various regression lines (pg. 197) is a reflection of the ability of each instar to avoid capture. The high correlation coefficient for firsts (0,990) indicates that they were able to remain submerged for almost the entire sampling period. Thus in this case the increase in the number of firsts purely reflects the increased probability of being caught, due to the decreasing volume of the pool with each dip. In the case of instars 2 to 4, they were

unable to remain submerged over the sampling period and this resulted in the peak at dip 7 or 8 and subsequent decline. Thus in their case the probability of being caught was not purely a function of decreased volume, but was increased by their inability to remain submerged over the sampling period.

Southwood (1966) points out that the major problem in this removal technique lies in the assumption that there is an equal probability of all stages being caught (see 4 above). From the present study it is clear that this assumption does not hold for *An. merus*. Neither Wada (1962a & b) nor Miura (1980) consider this possibility. Miura (1980) states that this is an assumption of the technique in his introduction, but does not investigate whether or not it holds for *Culex quinquefasciatus*. These author's data also indicate a lack of behavioural avoidance to capture for these two species. Observations on the *Culex quinquefasciatus* colony in our insectary appear to contradict this as they certainly do exhibit diving behaviour in response to the stimuli mentioned earlier.

A possible explanation for this apparent difference would be that the above authors only commenced sampling once the majority of individuals had been forced to return to the surface and that their was no concentrating effect due to reduced pool volume.

Southwood (1966) discussed the fitting of a line to the data by eye or using regression methods. He pointed out that it is not strictly correct mathematically to use the latter as the

data being plotted on the x axis are not independent of that on the y axis and are in fact derived from it. He also pointed out that fitting by eye is only acceptable if the data very closely approximate a straight line. It is however felt that fitting a line mathematically is preferable to visual estimation and serves the intended purpose. A visual line was therefore fitted to Figure 33 (which ignored the data points at which the recovery of larvae began to decline). This resulted in a better approximation of the larval population. The solid lines represent those fitted by eye.

5.4.2 MONTHLY LARVAL RECOVERIES

The importance of applying the collection correction factor (Figure 31) for different instars to the larval collection data was covered in section 5.4.1. The same data corrected for differences in the number of pools sampled are shown in Figures 34 and 35. It is interesting to note the decrease in the number of first instar larvae in proportion to other instars in June. This is easily explained by the fact that in the months of April and May the air temperature declines rapidly and drops below 20^{o}. This results in a rapid decline in the circulating adult population during the latter weeks of May (Figure 37, top), which in turn results in a reduced input of eggs to the larval breeding sites.

Over this period (May & June) the drop in temperature also results in an increase in the duration of the larval lifespan (Chapter 4). The effect of this increased larval duration is

reduced adult output and thus an accumulation in the number of individuals in the larval stages. This is reflected in the high mean numbers of larvae per pool (May = 265 and June = 194). By July however the mean number of larvae per pool has dropped considerably as many of the larvae present in June have now either erupted or died, but the input is still low due to the small circulating adult population. Initially it was thought that the unusually high rainfall during July 1985 (Figure 37, bottom) had played a role in reducing the larval numbers by flushing the sites. However a repeat survey in July 1987 yielded a similar result of 85 larvae per pool compared with the 99 in July 1985 (Figure 34).

In August ambient temperatures begin to increase (Figure 36) and result in increased larval growth velocity. This factor together with the still low egg-input from the adult population (Figure 37, top) results in the characteristic shape of the barchart for August, in which the increased temperature has resulted in most of the larvae progressing to later instars and increased eclosion. This is confirmed by the increase in the adult population. As the adult population increases so does the egg-input to the breeding site, with a consequent increase in the ratio of first instar larvae to subsequent instars. This is illustrated by the data for September and October (Figure 34 and 35). Thus by November we see the expected picture of a decline in larval numbers for each successive instars. The high number of fourths is due to the fact that the data are uncorrected for differences in larval instar duration. The longer duration of fourth instar (Chapter 4) results in the apparent accumulation of larvae in

this stage. It was not possible to correct these data for differences in larval instar duration as ideally should be done. The reasons for this are obvious when the effect of temperature on instar duration (Figure 40) and the large seasonal changes in environmental temperature are considered (Figure 36).

From the data on rainfall over the period 1968 - 1983 (Figure 37, bottom) it is obvious that there is a trend for the mean monthly rainfall to increase progressively from October to January. However during October 1985 the rainfall was higher than expected from the 15 year summary. In November it approximated expected levels but was less than in October. This resulted in the upper sites which normally would only have been filled later in the rainy season, receiving insufficient seepage water to balance losses by evaporation. This caused increase in the salinity of the breeding sites in December. This is clearly shown in Figure 38 where the fitted distribution curve peaks at slightly more than 30 ppt. (Figure 38 bottom), as opposed to 15 ppt.(top), for the rest of the year. This increased salinity accounts for the unexpected larval data for the month of December (Figure 35) and also results in a sharp decrease in the adult population (Figure 37, top). From this it may be concluded that the salinity was limiting and had resulted in a decrease in larval population numbers. It was the upper salinities in the region of 60 ppt. (Figure 38, bottom) as these pools contained no larvae. The decrease in salinity levels (Figure 37, bottom) in January resulted in the larval data

collected approximating the expected pattern (Figure 35) and an increase in the adult population (Figure 37, top).

A t-Test was conducted between the mean number of larvae per pool in winter and in summer and showed that there were significantly more larvae per pool in winter. This is important as during mid-winter (June and July) the adult population is low and the bulk of the population is in larval stages. In addition larvae are localised to the winter breeding sites (Chapter 2). These three factors support the theory that winter larviciding in problem areas may assist vector control. From the data on larval instar duration (Chapter 4), the most cost effective interval at which larviciding should be carried out can be calculated. From Figure 37 (top) it is clear that the best time to do this would be in late July, prior to the August increase in the adult populations, due to increased eclosion. Quite by chance, larviciding was carried out in Mamfene (site 38, Figure 3) by the resident malaria control personnel during the winter months of June, July and August. During the 1986/87 malaria season this area had the highest number of malaria cases for any region and a large *An. arabiensis* population was present. During the 1987/88 season, following the winter application of larvacides, the adult population did not attain the previous years levels (B.L. Sharp pers. comm.). Thus winter larviciding may offer an important supplementary control measure for specific problem areas. Winter larviciding has now been introduced as a routine measure and has played an important role in reducing the annual case rate for the area from 600-700 cases per annum to less than 70.

The high number of larvae per pool in winter has interesting implications for the use of larval sampling indices in surveillance. From Figure 37 (top) it is obvious that if dipping was used as an index of the vector population for surveillance purposes, without considering the effects of temperature on larval growth rates, then the following erroneous conclusions would be drawn:

1. That there is a fairly large vector population present during winter.
2. That there is a relatively small vector population present in summer.

The important point here is larval turnover. In mid-summer the larval instar duration is approximately 8 days and in mid-winter approximately 42 days. Thus in mid-summer the relatively small larval population turns over rapidly and generates a large adult population and vice versa in winter (Figure 37 top).

A review of literature relating larval and adult indices elicited the following short-comings:

1. Some authors do not appreciate the relationship between larval growth rates, temperature and larval population size and do not present any temperature data (Vanhara, 1985).

2. In other studies larval and adult population indices include between 6 and 15 species (Bates and Zulueta, 1949; Begum *et al.*, 1986; Stewart, 1973). This lumping of species would result in a masking of differences in physiological response to the effects of temperature.
3. Some studies were conducted in regions in which there was little variation in annual temperature and the mean minimum temperature was in excess of 20°C (Bates, 1941).

In all the above studies the larval and adult indices followed each other, with the adult population showing the expected lag. The important point here is that we are working with a species at the southern most limit of its distribution, which is normally afro-tropical. Thus if one is to compare the relationship between larval and adult indices with season, then it preferably should be with other species with a similar distribution. Comparison with species adapted to colder climates (Stewart, 1973) is not valid as they are physiologically adapted to low temperatures and will thus respond differently. In addition the site being investigated was a permanent (winter and summer) breeding site.

5.4.3 ESTIMATION OF FIELD MORTALITIES

When attempting to estimate instar mortalities using the relative proportions of each instar and their durations, the most important assumption is that the population is at equilibrium. Thus the difference between input and output from the sites is purely a function of incurred larval mortalities. If this is not the case then an increase in the propor-

tion of third instar larvae may for example not reflect decreased mortality, but an increase in input that occurred at an earlier time interval, equivalent to the combined durations of the first and second instars at that temperature regime.

From Chapter 4 it was clear that at temperatures approximating the developmental optimum, the effect on instar durations was small. However at increasingly sub-optimal temperatures, the effect of a single ^{o}C change on instar temperature may be large. The importance of this is that in tropical regions where ambient temperatures approximate the optimum (Figure 29) the effect of small changes in temperature will not significantly increase eclosion and thus not alter population equilibrium. This applies to the summer situation in this study, but not the winter one in which small changes in temperature will lead to fluctuations in output. It should however be noted that these fluctuations may occur within an overall population trend of decrease or increase (Figure 37b). It should also be borne in mind that when population turnover is rapid (ie. short larval duration), then equilibrium of the population may be attained within a short time period. However when it is long, equilibrium is not attained rapidly and environmental conditions may alter before it as reached.

These factors detract from the use of the technique outlined by Service (1971) for the estimation of larval instar mortalities, when temperatures do no approximate the optimum range. From Figure 29 this range could be considered to be a mean

monthly temperature of 23 to 32 ^{0}C for *An. merus*. From Figure 36 it is obvious that the winter temperatures from the study area fall outside this range. In addition the adult index indicates that the population is not at equilibrium but is either increasing or decreasing, except during July (Figure 37, top). Thus the application of this technique to data collected from the study area in winter is questionable. Should it be applied however, then the most applicable month would be July. As outlined above, it is felt that it is not as necessary to take successive samples during summer due to the reduced effect of temperature (Figure 29). In addition if one is trying to obtain a smoothing effect then one should not necessarily sample on successive days (Service, 1971, 1973 and 1977), but at intervals which take into account the larval instar durations.

Lakhani and Service (1974) used a sampling interval of seven days when working on *Aedes cantans*. During this study they estimated the approximate instar durations in the laboratory under constant temperature regimes. They point out that these are only approximations because field temperatures fluctuate, but do not mention the possible effects of nutrition. The effect of nutrition and density on instar duration have been demonstrated in Chapter 4. In this study laboratory estimation of instar durations under simulated winter temperature conditions poorly approximated the field situation, with the error being in the region of 30%. It is thus felt that a prerequisite to this technique should be the estimation of larval instar durations in the field. This seriously limits

the technique as although it may be possible to estimate these in small confined sites it is extremely difficult to do so in larger sites. The assumption by Service (1973 and 1977) that the instar durations for different larval sites (small and large) are the same may be acceptable in respect of temperature in equatorial regions, but requires further investigation relating to nutritional and density-dependent status.

The conclusion was made above that the technique is best applied in the summer months in this region, when the effects of temperature fluctuations on larval growth are minimised (Figure 29). However the effect of other factors which might upset population equilibrium were not considered. The influence of rainfall and salinification are two such factor (Figure 37). These points illustrate that an oversimplified approach to the assumption of population equilibrium can be made and that an additional population index such as one of adults, should be used simultaneously. Ideally this index should also be corrected for longevity (which may vary seasonally), before an accurate conclusion concerning adult population equilibrium can be made. The adult indices based on the indoor catches of Joshi *et al.*, (1975) at Kisumu did not correct for longevity but suggest that the adult *An. arabiensis* and *An. gambiae s.s.* populations fluctuated greatly. The *An. arabiensis* adult index was however similar during the months of January to May. The assumption that the trapping technique is equally effective throughout the year is also being made here.

From Figures 35 and 37, it can be seen that the high salinities in November did effect the population and that it cannot be considered to be at equilibrium in December. This is supported by the data shown in Figure 39. The data for January suggest that the salinity effect had ceased, but the ratio of first instar to second was still showing the effects and was lower than expected (Figure 35). Thus in Figure 39, the first instar mortalities would be under estimated. The estimated mortalities (Table 17), show a similar trend of increasing mortality with instar, to that demonstrated by Service (1971 and 1973). The curves are negatively skewed and most similar to Type I of Slobodkin (1961) in which mortalities are concentrated in the older animals. The overall first instar to adult mortalities of 97,3% and 97,6% respectively, using the instar durations recorded in this study and those of Service (1973), compare well with the data recorded for *An. arabiensis* and *An. gambiae s.s.* by Service;

		First instar to adult mortality
1971	Marsh	95,6%
	Borrow pit	97,4%
1973	Pond	98,0%
	Pond	97,2%
	Ditch	100,0%
1977	Rice paddies	97,6%
	Pools and ponds	98,0%
	Rice (sprayed)	86,9%

The high mortalities are extremely interesting from a population dynamics point of view. Also of interest is the higher survivorship in the paddies when sprayed, which Service (1977) explained in terms of more rapid recolonisation by

An. gambiae s.l. than by its predators.

The pupal mortalities he recorded and those of this study were lower than that of the later instars. It is difficult to speculate on why this should occur, but there are a number of possiblities.

1. Predation levels might increase with instar size.
2. The ability to remain submerged may play a role.
3. The probability of predation may increase with instar duration.

The data for pupae in relation to size, ability to remain submerged (Figure 32) and measured mortalities (Table 17) contradict 1 and 2 above. Similarly the reason in 3 is contradicted by the data for third instar larvae (Table 17 and Figure 39). It is however possible that it is a combination of the above. The most commonly encountered predator at the study site was a spider *(Thalassius sp.)* found on the surface of the water. The reduced occurrence of potential predators in the pools relative to freshwater sites was probably related to salinity.

Bearing in mind the limitations concerning the application of the technique to data collected in winter, it was decided to investigate the effect of low and high-density larval instar durations on the estimation of survivorship. The effect of under-estimating the duration of fourth instar larvae is demonstrated by the graphs for June and July (1984) in Fig-

ures 40 and 41. Since the larval instar durations under conditions of high density most closely approximate the real situation, then the incorrect low density durations result in an under estimation of third instar mortalities and an over estimation of those of pupae.

With respect to winter survivorship, only the curve for July 1987 will be discussed as these data are the closest to fulfilling the requirements of the technique. The population was at its seasonal minimum (Figure 37, top) in July and thus most closely approximated being at equilibrium. The temperatures over the months of June and July (Figure 36) were relatively stable. The estimations of instar duration were carried out in the field and in the same month. The factors related to minor temperature fluctuations and sampling mentioned earlier should however also be borne in mind. The overall trend is similar to that for summer in that the mortalities of the early instars are low (Table 17). The mortalities recorded for third stage larvae were however lower than those recorded in mid-summer (January) while those for pupae were higher. The reason for this is not known. The only possible explanation is that the small Coleoptera present during summer but not in winter may have played a predatory role. If they did they would have most probably preyed on the early instars as they were relatively small (approximately 4 mm in length). The spider noted at the study site was present during winter. The first instar to adult mortality of 92,2% was lower than that for summer.

5.4.4 POOL SIZE AND LARVAL DENSITY

There appears to be no relationship between larval density and pool size. These data however are based mainly on winter collections. In endothermic organisms, density dependent factors such as nutrition have a potentially greater role in population regulation due the requirement of maintaining a constant body temperature and associated metabolic levels. In ectotherms this limitation does not exist, except under conditions of high temperature. Thus under conditions in which an endotherm would starve and be unable to maintain its body processes, the ectotherm merely grows more slowly or ceases to grow. Such an ability has been demonstrated in this study and is documented in the literature but does not apply at high temperatures (see Chapter 4). I would thus alter the suggestion by Lakhani and Service (1974) that "competition for food or space may be more important in regulating population size than is generally realised" to include a reference to temperature (ie. at high environmental temperatures).

The suggestion that toxic products of excretory origin play a role may be important (at optimal, metabolic temperatures), however it should be noted that at low temperatures the quantities of waste product would parallel the low metabolic rate of ectotherms.

Lack of pool volume data for the summer months precluded a seasonal (temperature) comparison of density and metabolic dependent factors on population numbers.

5.4.5 SALINITY

The data on salinity suggest that *An. merus* is able to distinguish between sites of low and high salinity when ovipositing. The lack of larvae from sites with a salinity of six parts per thousand or less was statistically significant for all instars. This suggests that the adult females avoid the sites of low salinity as mortalities could not have accounted for this total absence. Mosha and Mutero (1982), and Rogo *et al.* (1985) working at Jimbo, in Kenya recorded that low salinities resulted in a decrease in the *An. merus* population. The latter authors also failed to find larvae when the salinity dropped below 13% (4,6ppt.). Mosha and Mutero (1982) also reported the only documented occurrence of *An. merus* occurring in freshwater in the field. The ability of gravid females of certain species to distinguish between sites of different tone, water type and the presence of conspecific immatures is well documented (McCrae, 1984; Reisen,1978; Roberts, 1965 and Chapter 2).

The aim of the student's t-tests done here was to assess whether or not there was a significant reduction in numbers of any instar at high or low salinities. The results indicate that at the higher salinities investigated , only first instar appeared to be limited at values in the region of 24 ppt. or 69% sea water. The high number recorded for first instar larvae at 25-27 ppt., was due to a single pool and was also influenced by the small sample size (only 4 pools). No statistical conclusions could be expected for data in the 28 to 39 ppt. range as the total sample size was six pools. Five

pools with salinities ranging between 44 and 70 ppt., were sampled and no larvae were present. For all instars, the highest pool population means were recorded at salinities of between 8 and 18 ppt., (23 to 51% sea water). This compares well with conclusions drawn by Mosha and Mutero (1982) in which they stated that as the salinity increases, so does the *An. merus* population until an optimum of about 45% sea water and thereafter it declines.

Muirhead Thomson (1951) reported that continuous breeding of *An. merus* was no longer possible at salinities of 83% sea water. Sharp (1983) found that first instar *An. merus* raised in distilled water showed increased mortality from a salinity of 50% sea water and no survival at a salinity of above 75%. This study suggests that salinity in the region of 70% is limiting. The variation in the data presented above and the apparent seasonal variation in salinity tolerance is most likely to be related to physiological acclimation. Sharp (1982) demonstrated that the salinity tolerance of second and fourth instar *An. merus* raised in 40 % sea water was greater than if raised in distilled water. The probable mechanism for this increased salinity tolerance is the decrease in size of the anal papillae of *An. merus* when raised in saline water as opposed to those raised in distilled water (Coetzee and le Sueur, 1988). It should however be borne in mind that the ability to acclimate will also be affected by the rate at which salinity increases and this is likely to be related to abiotic factors such as temperature, rainfall and wind.

Muirhead Thomson (1951) suggested the use of salinification as a control method for *An. merus*. However in view of present knowledge of its limited importance as a vector (Bushrod, 1981; Mosha and Petrarca, 1983) and its inherent salinity tolerance, this is not considered feasible. This possibility is nevertheless worth considering for the freshwater members. Sharp (1983) investigated the salinity tolerance of *An. arabiensis* and *An. gambiae s.s.* larvae using colony material. The results for the two species were the same and as follows:

First instar	30% sea water
Second instar	35%
Third instar	35%
Fourth instar	40%

Considering the relatively small sized pools generally utilised by freshwater members in the endemic malaria region of Natal (Chapter 2) and the above data for salinity tolerance, it is felt that larval control by salinification may thus be feasible. To attain larvicidal activity approximately 12 grams of sodium chloride would have to be added per litre of pool volume. The present cost of coarse salt is R8-19c (approx. $3 U.S.) per 50 kilogram bag and the method is thus likely to be cost-effective. This method would only be used in areas where the presence of an *An. arabiensis* population had been established and intensive breeding was occurring, such as Mamfene in this study (Chapter 2). The technique thus appears to be feasible from an economic and practical point of view, but would require field assessment. The possibility that such sites may be subsequently invaded by *An. merus* cannot be ruled out but this is considered preferable to

utilisation by *An. arabiensis*, the vector efficiency of which is well established (White, 1974). Salinification of the soil is not considered a problem at the levels recommended and dilution/dispersion would occurr result with the onset of heavy rains. The application of salt as opposed to many of the accumulative insecticides /larvicides may thus be preferable but requires evaluation.

REFERENCES

Andis, M.D., Meek, F.L., Wright, V.L. (1983). Bionomics of Louisiana riceland mosquito larvae I. A comparison of sampling techniques.-*Mosquito News* **43** (2), 195-203.

Bates, M. (1941). Field studies of the anopheline mosquitoes of Albania.-*Proc. Ent. Soc. Wash.*, **43** (3), 37-58.

Bates, M. and Zulueta, de- J. (1949). The seasonal cycle of anopheline mosquitoes in a pond in Eastern Colombia.-*Am. J. Trop. Med.* **29**, 129-150.

Begum, A., Biswas, B.R. and Elias, M. (1986). The ecology and seasonal fluctuations of mosquito larvae in a lake in Dhaka city.-*Bangladesh J. Zool.* **14** (1), 41-48.

Bushrod, F.M. (1981). The *Anopheles gambiae* Giles complex and Bancroftian Filariasis transmission in a Tanzanian coastal village.-*Ann. Trop. Med. and Parasit.* 75 (1), 93-100.

Christie, M. (1954). A method for the numerical study of larval populations of *Anopheles gambiae* and other pool-breeding mosquitoes.-*Ann. Trop. Med. and Parasit.* **48**, 271-275.

Coetzee, M and le Sueur, D. (1988) Effect of salinity on the larvae of some Afrotroical anopheline mosquitoes.-*Med. Vet. Entomol.* **2**, 385-390

Joshi, G.P., Service, M.W. and Pradhan, G.D. (1975). A survey of species A and B of the *Anopheles gambiae* Giles complex in the Kisumu area of Kenya prior to insecticidal spraying with OMS-43 (fenitrothion).-*Ann. Trop. Med. Parasit.* **69** (1), 91-104.

Knight, K.L. (1964). Quantitative methods for mosquito larval

surveys.-*J. Med. Ent.* 1 (1), 109-115.

Lakhani, K.H. and Service, M.W. (1974). Estimated mortalities of the immature stages of *Aedes cantans* (Mg.) (Diptera,Culicidae) in a natural habitat.-*Bull. ent. Res.* **64**, 265-276.

Leemingsawat, S. (1989). Field trials of different traps for malaria vectors and epidemiological investigations at a foot hill basin in Kanchanaburi, Thailand.-*Jpn. J. Sanit. Zool.* **40** (3), 171-179.

McCrae, A.W.R. (1984). Oviposition by African malaria vector mosquitoes. II. Effects of site tone, water type and conspecific immatures on target selection by freshwater *Anopheles gambiae* Giles, *sensu lato*.-*Ann. Trop. Med. Parasit.* **78**, 307-318.

Mitchell, C.J. and Briegel, H. (1989). Inability of diapausing *Culex pipiens* Diptera Culicidae to use blood for producing lipid reserves for overwinter survival.-*J. Med. Entomol.* **26** (4), 318-326.

Miura, T. (1980). Estimation of mosquito population size in confined natural breeding sites.-*Proc. Pap. Ann. Conf. Calif. Mosq. Vector Control Assoc. Inc.* **48**, 108-112.

Molineaux, L., Shidrawi, G.R., Clarke, J.L., Boulzaguet, J.R. and Ashkar, T.S. (1979). Assessment of insecticidal impact on the malaria mosquito's vectorial capacity, from data on the man-biting rate and age-composition.-*Bull. Wld. Hlth. Org.* **57** (2), 265-274.

Mosha, F.W. and Mutero, C.M. (1982). The influence of salinity on larval development and population dynamics of *Anopheles merus* Donitz (Diptera:Culicidae).-*Bull. ent.*

Res. **72**, 119-128.

Mosha, F.W. and Petrarca, V. (1983). Ecological studies on *Anopheles gambiae* complex sibling species on the Kenya coast.-*Trans. R. Soc. trop. Med. Hyg.* **77**. 344-345.

Muirhead-Thomson, R.C. (1948). Studies on *Anopheles gambiae* and *Anopheles melas* in and around Lagos.-*Bull. ent. Res.* **38**, 527-558.

Muirhead-Thomson, R.C. (1951). Studies on salt-water and freshwater *Anopheles gambiae* on the east African coast.-*Bull. ent. Res.* **41** 448-502.

Nielsen, E.T. and Nielsen, A.T. (1953). Field observations on the habits of *Aedes taeniorhynchus*.-*Ecology*. **34**, 141-156.

Ramsdale, C.D. and Wilkes, T.J. (1985). Some aspects of overwintering in southern England of the mosquitoes *Anopheles atroparvus* and *Culiseta annulata* (Diptera:Culicidae).-*Ecological Entomology*. **10**, 449-454.

Reisen, W.K. and Siddiqui, T.F. (1978). The influence of conspecific immatures on the oviposition preference of the mosquitoes *Anopheles stephensi* and *Culex tritaeniorhynchus*.-*Pakistan J. Zool.*, **10** (1), 31-41.

Reisen, W.K., Meyer, R.P. and Milby, M.M. (1986). Overwintering studies on *Culex tarsalis* (Diptera:Culicidae) in Kern county, California:Survival and the experimental induction and termination of diapause.-*Ann. Ent. Soc. Am.* **79** (4), 664-685.

Rogers, D.J. and Randolph, S.E. and Kuzoe, F.A.S. (1984). Local variation in the population dynamics of *Glossina palpalis palpalis* (Robineau-Desvoidy) (Diptera: Glossinidae). I. Natural population regulation.-*Bull. ent.*

Res. 74, 403-423.

Rogo, L.M.N., White, G.B. and Odhiambo, R.C. (1985). Salinity relationships of mosquitoes breeding in a brackish pond on the Kenya coast.-*Insect Sci. Applic.* 6 (1), 91-95.

Russell, P.F., Ramachandra Rao, T. and Persis Putnam. (1945).

An evaluation of various measures of *Anopheles* larva density.-*Amer. J. Hyg.* 42, 274-298.

Service, M.W. (1971). Studies on sampling larval populations of the *Anopheles gambiae* complex.-*Bull. Wld. Hlth. Org.* 45, 169-180.

Service, M.W. (1973). Mortalities of the larvae of the *Anopheles gambiae* Giles complex and detection of predators by the precipitin test.-*Bull. ent. Res.* 62, 359-369.

Service, M.W. (1977). Mortalities of the immature stages of species B of the *Anopheles gambiae* complex in Kenya: comparison between rice fields and temporary pools, identification of predators, and effects of insecticidal spraying.-*J. Med. Ent.* 13 (4-5), 535-545.

Sharp, B.L. (1983a). *Anopheles merus* (Donitz) its biting cycle in relation to environmental parameters.-*J. ent. Soc. sth. Afr.* 46 (2), 367-374.

Sharp, B.L. (1983b). *Anopheles merus* Donitz its biology in relation to malaria transmission in Natal.-Master of Science Thesis. University of the Witwatersrand. 162pp.

Sharp, B.L., Quicke, F.C. and Jansen, E.J. (1984). Aspects of the behaviour of five anopheline species in the endemic malaria area of Natal.-*J. Ent. Soc. Sth. Afr.* 47 (2), 251-258.

Sichinava, S.H.G. (1974). Results of winter observations on the biology and ecology of *Culex pipiens* in Sukhumi Georgian-SSR USSR.-*Meditsinskaia parazitologia i parazitarnye bolezni*. **43** (5), 557-562.

Slobodkin, L.B. (1961). Growth and regulation of animal populations. Holt, Rhinehart and Wilson. New York. 184pp.

Soper, F.L. and Wilson, B.D. (1943). *Anopheles gambiae* in Brazil 1930 to 1940.-The Rockerfeller Foundation. 262pp.

Southwood, T.R.E. (1976). Ecological Methods with particular reference to the study of insect populations. Chapman and Hall. London. 391pp.

Southwood, T.R.E., Murdie, G., Yasuno, M., Tonn, R.J. and Reader, P.M. (1972). Studies on the life budget of *Aedes aegypti* in Wat Samphaya, Bangkok, Thailand.-*Bull. Wld. Hlth. Org.* **46**, 211-226.

Stewart, W.W.A. (1973). Seasonal occurrence and species composition of mosquito adults and larvae in a fresh water breeding site near Saskatoon, Sakatchewan.-*Mosquito News* **33** (4), 545-553.

Vanhara, J. (1985). Influence of the waterworks constructed near Nove Mlyny (southern Moravia) on the mosquito community (Culicidae,Diptera).-*Ekologia (CSSR)* **4** (3), 251-265.

Wada, Y. (1962a). Studies on the population estimation for insects of medical importance. I. A method of estimating the population size of mosquito larvae in a fertiliser pit.-*Endem. Dis. Bull. Nagasaki* **4**, 22-30.

Wada, Y. (1962b). Studies on the population estimation for insects of medical importance. I. A method of estimating

the population of larvae of *Aedes togoi* in the tide-water rock pool.-*Endem. Dis. Bull. Nagasaki* 2, 141-156.

White, G.B. (1974). *Anopheles gambiae* complex and disease transmission.-*Trans. Roy. Soc. Trop. Med. Hyg.* **68** (4), 278-301.

World Health Organisation. (1975). Manual on practical entomology in malaria. I. Vector Bionomics and Organisation of anti-ma