THE BIONOMICS OF THE MALARIA MOSQUITO *ANOPHELES GAMBIAE SENSU LATO* IN SOUTHEAST TANZANIA:

Adult size variation and its effect on female fecundity, survival and malaria transmission

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Edith Onesmus Kirenga Lyimo

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PROPOSITIONS

1. Knowledge of variability in mosquito vector parameters is important in understanding malaria transmission. However, knowing the variability alone is not enough, but the relationship between parameters and how they vary and/or co-vary in relation to each other is essential.
   This thesis.

2. The *Anopheles gambiae s.l.* population in the Kilombero area maintains a body size range which ensures the survival of the species in this area.
   This thesis.

3. The development rate of the aquatic stages of mosquitoes is so dependent on abiotic and biotic characteristics of the environment that the larvae occupy, that it is impossible to generalise, even within a confined geographic area.
   This thesis.

4. The high pre-gravid rate found in *Anopheles gambiae s.l.* makes it difficult to estimate the survival rate of the females of this species with precision because the pre-gravid females stay longer in the nulliparous state than the females that do not undergo a pre-gravid phase.
   This thesis.

5. *Anopheles gambiae s.l.* appears to select oviposition sites randomly, but recent findings in several *Culex* species suggest that chemical cues may direct the gravid *Anopheles* female to specific sites.
   This thesis.
   Millar, J.G., Chaney, J.D. & Mulla, M.S. (1992) J. Am. Mosq. Control Assoc. **8**: 11-17.
   Beehler, J.W., Millar, J.G. & Mulla, M.S. (1993) J. Chem. Ecol. **19**: 635-644.

6. Arbitrary measures of heterogeneity are tempting and very popular, but their ability to reflect the relevant properties of the system of interest is unclear and questionable.
   Kolasa, J. & C.D. Rollo (1991). The Heterogeneity of Heterogeneity: A Glossary. *in* Ecological Heterogeneity (J. Kolasa and S.T.A. Pickett, eds.), Ecological Studies **86**, Springer-Verlag New York Inc., New York.

7. To say that a disease depends on certain factors is not to say much, until we can also form an estimate as to how largely each factor influences the whole result.
   Ross, R. (1911). The prevention of malaria. Murray, London. Pg. 651.

8. Overall development programmes can have an impact on transmission of malaria. A strong cross-sectoral approach is therefore required in order to lessen the potential burden of disease on the very people this development seeks to help.
   Gwadz, R.W. (1991). Malaria and development in Africa. A cross-sectoral approach. AAAS, Washington DC.

9. External support to health projects in developing countries often leads to problems once such support comes to an end, as most of these projects are not sustainable.

10. There is nothing like 'a finger in the dyke' for malaria control in the sub-Saharan Africa. What is needed are 'fingers'.

11. It is too bad the mosquito is such a pain - (not to mention the itch) - its life cycle is quite fascinating.

Barnard, B. (1991). Agricultural Research, USDA-ARS, Beltsville, MD.

Propositions with the thesis "The bionomics of the malaria mosquito *Anopheles gambiae sensu lato* in Southeast Tanzania - adult size variation and its effect on female fecundity, survival and malaria transmission" by Edith O.K. Lyimo.

Wageningen, December 1, 1993.

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This is to Benjamin, for his patience

Foreword

With the initiation of collaborative research between the Wageningen Agricultural University and the University of Nijmegen in The Netherlands, the Swiss Tropical Insitute, Basel and WHO-IRTC Diagoms Laboratory in Geneva, Switzerland and The National Institute for Medical Research and Ifakara Centre in Tanzania, a project on the epidemiology of malaria in the Kilombero district came into effect. The Kilombero Malaria Project (KMP) as it came to be known, was designed not only to look at the scientific aspects of malaria transmission but also to train Tanzanian cadre from the research and laboratory level. As such, I was able to join the project to assist in mosquito entomology as well as pursue a PhD programme within the set-up of the KMP.

The work presented here was accomplished as a result of contributions from many people individually or as a team, as well as from National and International organisations and I would like to extend my sincere acknowledgment to them all. Nevertheless, some people deserve my special and heartfelt gratitudes, Prof. W. L. Kilama, the Director General of the National Institute for Medical Research, for his inititives, support and encouragement in securing the PhD studentship. Prof. Joop van Lenteren, for accepting to be my promotor and supervisor despite his already heavy schedule. His assistance is highly prized. Dr. ir. Willem Takken, apart from being co-promotor and immediate supervisor, made sure my stay in Wageningen was always pleasant. Your field visits to Tanzania though always short and very tightly programmed were of great help. To my field supervisor Dr. Derek Charlwood, I would like to say thank you very much for throwing me in at the deep end. Prof. Marcel Tanner, head Department of Epidemiology, at the Swiss Tropical Institute in Basel was a great help when I visited Basel. Your interest in my work, and the many discussions and critical comments did much to improve my work. Dr. C. F. Curtis, London School of Hygiene and Tropical Medicine and Dr. P. Billingsley, Imperial College London advised and made critical reviews of most of the chapters. Their comments were of great help in improving this work.

At this point I would like to thank the administrative staff of the Entomology Department at Binnenhaven especially Mrs. Ineke Buunk and Ans Klunder for their practical help. Ineke, thank you so much for all the letters you typed for me and for being ready to listen when I came up with my sometimes trivial requests and questions. Your cheerfulness, competence and willingness to help will always be remembered. Gerard Pesch, Mrs. T de Vries, Irene van Nes-Keereweer and Otteline Crommelin, thank you for your help with correspondences, bits and pieces. The Binnenhaven librarians Mrs. J.H.D. Brouwer, Ina Otter-Beenen, Marian Roseboom de Vries, H.T. de Lange, J. Wolsing and Mr. J. Soolsma went a long way searching and providing my literature requests. I would like to thank all those people who helped with mosquito rearing in Wageningen, especially Leo Koopman and Frans van Aggelen for being so tolerant and understanding particularly

when I messed up and caused havok with the mosquito colonies.

I am very much indebted to the Ifakara Centre management, the former director Dr. C. MacPherson and most of all his successor Dr. Thomas Teuscher, and the rest of his administration gave me the opportunity to work at their Centre and made my stay and working in Ifakara very pleasant indeed. Financial help provided by the Institute is highly acknowledged. The field team of the entomology section at the Ifakara Centre, without you I would not have managed. George Mwambeta thank you for helping me with the many trips to the villages, organising the many mosquito collections especially with the night bait catches even when lions were rampaging in the village. Stephen Ngatunga, Kebby Kembamba, Balbina Mariwa Seydina Bakari and Simon Sama thank you all for your support and the many dissections you did for me and the drivers who drove us up and down to Michenga and Namawala. I would like to acknowledge the villagers who invited me into their houses without even a question. Without your cooperation this work could never have being accomplished. The technicians Miraj Hussein and Honorary Urassa were of much help with the sporozoite ELISA technique.

Special thanks are due to my colleague and friend Dr. Jacob Koella. You were a great help to me all the time, right from the very beginning especially when things were so difficult in the field and I was about to give up. You showed so much interest in my work all the time, advising, discussing and helping all along and thank you for allowing me to employ the malaria transmission model you developed. My many colleagues at Ifakara as well as at the Binnenhaven helped and encouraged me to push on. Bart Knols, you were always there to listen to my worries and to talk to. Thank you for teaching me the many graphical programmes and producing some of the figures presented in this thesis. Other colleagues helped in one way or other and are acknowledged, Theo Jetten and Ruurd de Jong in Wageningen, Peter Smith at STI in Basel, Peter Odi and Nicole Hurt at Ifakara.

Françoise, Willem, Nicolas and Daniel made me feel welcome at your home. The moments we shared together (which were many) were a great boost to my sometimes dwindling spirit. Your concern for my well being is highly acknowledged. The UVV group in Wageningen advised me on how to live in Wageningen, where to shop and what to buy. Your concern and friendship is highly acknowledged. The Pastors for international students, Ben Verbene and Hinne Wagenaar and members of the Cross Roads group brought me into contact with other students and our discussion evenings provided an atmosphere of ease and friendship. Last but not least, I would like to thank my family, my parents, Martha and Onesmus and all my sisters, nieces and nephews for supporting me morally and spiritually during the whole period of my study.

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Publications resulting from this dissertation

Chapters of this dissertation will be published as the following journal articles:

Chapter 2
Lyimo, E.O., Takken, W. & Koella, J.C. (1992). Effects of rearing temperature and larval density on larval survival, age at pupation and adult size of *Anopheles gambiae*. *Entomologia Experimentalis et Applicata* **63**: 265-271.

Chapter 3
Lyimo, E.O. & Takken, W. Temperature effects on the development rate of larvae of *Anopheles gambiae s.l.*. To be submitted to: *Entomologia experimentalis & applicata.*

Chapter 4
Lyimo, E.O. & Takken, W. The ecology of the aquatic stages of *Anopheles gambiae* Giles *s.l.* (Diptera: Culicidae) in southeastern Tanzania. To be submitted to: *Bull.ent.Res.*

Chapter 5
Lyimo, E.O., Takken, W. & Charlwood, J.D. Adult bionomics of *Anopheles gambiae s.l.* in relation to malaria transmission in southeastern Tanzania. To be submitted to: *American Journal of Tropical Medicine and Hygiene.*

Chapter 7
Lyimo, E.O. & Takken, W. (1993) Effects of adult body size on fecundity and the pre-gravid rate of *Anopheles gambiae s.l.* females in Tanzania. *Medical and Veterinary Entomology* (in press).

Chapter 8
Lyimo, E.O., Charlwood, J.D., Billingsley, P.F. & Takken, W. The relationship between adult body size and survival as measured by parity in field populations of *Anopheles gambiae sensu lato.* To be submitted to: *Acta Tropica.*

Chapter 9
Lyimo. E.O. & Koella J.C. (1992). Relationship between body size of adult Anopheles gambiae s.l. and infection with the malaria parasite *Plasmodium falciparum. Parasitology* **104**: 233-237.

Other publications

The author has also contributed to the following publications in the field of Medical Entomology:

Lines, J.D., Lyimo, E.O. & Curtis, C.F. (1986) Mixing of indoor- and outdoor-resting adults of *Anopheles gambiae* Giles s.l. and *A. funestus* Giles (Diptera: Culicidae) in coastal Tanzania. *Bulletin of Entomological Research* **76**: 171-178.

Lyimo, E.O. & Irving-Bell, R.J. (1988) Circadian flight activity of mosquitoes entering and leaving septic tanks in central Nigeria. *Insect Science and its Application* **9**: 493-498.

Lines, J.D., Wilkes, T.J. & Lyimo, E.O. (1991) Human malaria infectiousness measured by age-specific sporozoite rates in *Anopheles gambiae* in Tanzania. *Parasitology* **102**: 167-177.

Lyimo, E.O., Msuya, F.H.M., Rwegoshora, R.T., Nicholson, E.A., Mnzava, A.E.P., Lines, J.D. & Curtis, C.F. (1991) Trial of pyrethroid impregnated bednets in an area of Tanzania holoendemic for malaria Part 3. Effects on the prevalence of malaria parasitaemia and fever. *Acta Tropica* **49**: 157-163.

Smith, T., Charlwood, J.D., Kihonda, J., Mwankusye, S., Billingsley, P., Meuwissen, J., Lyimo, E., Takken, W., Teuscher, T. & Tanner, M. (1993) Absence of seasonal variation in malaria parasitaemia in an area of intense seasonal transmission. *Acta Tropica* **54**: 55-72.

Alonso, P.L., Tanner, M., Smith, T., Hayes, R.J., Armstrong Schellenberg, J., Lopez, M.C., Bastos de Azevedo, I., Menendez, C., Lyimo, E., Weisss, N., Kilama W.L., Teuscher, T. (1993) A trial of the synthetic malaria vaccine SPf66 in Tanzania: rationale and design. *Vaccine*
(in press).

Samenvatting

De grootte van volwassen muggen kan zowel de populatie dynamiek van hen als de overdracht van ziekteverwekkers beïnvloeden. De resultaten van studies aan dit onderwerp verschillen per soort. Bij enkele muggesoorten is gevonden dat de grotere individuen gekenmerkt worden door een hoge vruchtbaarheid en langere levensduur (Steinwascher, 1982; Nasci, 1986a; 1986b; 1987) maar bij andere soorten leidt een grotere vorm niet tot een langere levensduur (Walker *et al.*, 1987; Landry *et al.*, 1988; Pumpuni & Walker, 1989). Vergelijkbare gegevens zijn verkregen aangaande de transmissie van ziekteverwekkers. Enkele studies toonden aan dat kleinere muggen virussen zoals Japanse Encephalitis, West Nile en La Cross, efficiënter overbrengen dan grotere individuen (Takahashi, 1976; Baqar *et al.*, 1980; Grimstad & Haramis, 1984), terwijl andere studies geen verschil in de efficiency van virustransmissie konden aantonen tussen kleine en grote muggen (Kay *et al.* 1989). Bij *Plasmodium* parasieten vond Ichimori (1989) geen verband tussen de grootte van de mug *Anopheles stephensi* Liston en het aantal oöcysten van *P. yoelii nigeriensis* dat tot ontwikkeling kwam, terwijl Kitthawee *et al.* (1990) aantoonden dat de grotere individuen van *An. dirus* Peyton and Harrison méér *P. falciparum* oöcysten ontwikkelden dan de kleinere muggen.

De variatie in de grootte van muggen is geassocieerd met het type broedplaatsen dat een soort bezet. Muggen die tijdelijke broedplaatsen bezetten vertonen méér variatie in grootte dan muggen welke broeden in permanent aanwezige habitats (Haramis, 1983; 1985; Fish, 1985; Nasci, 1987). *An. gambiae* Giles, het onderwerp van deze studie, broedt bij voorkeur in habitats gekenmerkt door hun tijdelijk karakter en vertoont onder de anophelinen van sub-Sahara Afrika de meeste variatie in grootte. Tot nu toe is de invloed van de grootte van deze mug op haar bionomie niet bestudeerd. Nadere kennis hierover kan helpen om de biologie van deze belangrijke muggesoort beter te begrijpen.

Het onderzoek beschreven in dit proefschrift is uitgevoerd om de volgende algemene vragen te beantwoorden: (1) door welke factoren wordt de variatie in grootte van *An. gambiae* veroorzaakt en (2) op welke wijze beïnvloedt de grootte van de mug belangrijke eigenschappen van het vrouwtje zoals reproduktie en levensduur alsmede de overdracht van de malariaparasiet. Specifieke doelstellingen waren:

- te bestuderen of temperatuur en larvale dichtheid een effect hebben op de ontwikkeling en overleving van onvolwassen stadia van *An. gambiae* en op de grootte van de adulten.
- te bestuderen of de grootte van de volwassen mug van invloed is op de voedselopname (bloedzuigen) en vruchtbaarheid.
- te bestuderen of de grootte van het volwassen muggevrouwtje van invloed is op haar levensduur.
- te onderzoeken of een verband bestaat tussen de grootte van de mug en haar

infecties met malariaparasieten.

Tijdens het onderzoek zijn met verschillende vangmethoden 50.321 vrouwelijke *An. gambiae* gevangen en werden 11.097 vleugels gemeten. Vleugellengte is een algemeen aanvaarde maat voor de grootte (Christophers, 1960; Haramis, 1983).

Factoren die de larvale ontwikkeling en de grootte van de volwassen mug bepalen zijn zowel in het laboratorium als in het veld bestudeerd. In het laboratorium zijn muggen gekweekt onder verschillende constante temperaturen en dichtheden (hoofdstuk 2). In het veld zijn muggen gekweekt onder constante dichtheden bij natuurlijke fluctuaties van de temperatuur (hoofdstuk 3). De ontwikkelingsduur en overleving van de onvolwassen stadia (larven en pop) werden gemeten alsmede de vleugellengten van de pas uitgekomen vrouwtjes. De interactie tussen dichtheid en temperatuur was bepalend voor de larvale ontwikkelingsduur en overleving alsmede de grootte van de volwassen mug.

Ter bepaling van relatieve dichtheden en overleving van onvolwassen stadia werden natuurlijke broedplaatsen bestudeerd. De sterfte onder de onvolwassen stadia was hoog (gemiddeld 95%) en werd in hoofdzaak veroorzaakt door pathogenen en predatoren maar ook door weersomstandigheden. Grote, semi-permanente broedplaatsen produceerden grotere vrouwtjes dan kleine plasjes van een tijdelijk karakter (hoofdstuk 4). Verschillen in grootte van muggen gevangen op diverse lokaties en op verschillende tijdstippen van het jaar, werden óók bestudeerd in veldpopulaties van *An. gambiae s.l.*. Tussen populaties van verschillende lokaties werd een significant verschil in grootte gevonden, en ook tussen muggen gevangen op dezelfde lokatie maar op diverse tijdstippen over een periode van twee jaar, waarbij de grotere vrouwtjes gevonden werden tijdens de koelere maanden van het jaar (hoofdstuk 5). De dichtheid van vrouwtjes van *An. gambiae* in woningen bereikte een maximum tegen het einde van de regentijd in mei, wanneer ook het aantal infectieve beten per mens (Entomological Inoculation Rate) het hoogst was. Hieruit volgt dat de intensiteit van malariatransmissie het grootst was aan het einde van de regentijd (hoofdstuk 6).

De vraag of de grootte van de mug van invloed is op het zgn. "pre-zwangerschap" stadium en op de vruchtbaarheid is onderzocht bij binnenshuis gevangen volgezogen muggen en bij pas uitgekomen muggen welke in het laboratorium een bloedmaaltijd kregen. Vrouwtjes die eieren ontwikkelden na één bloedmaaltijd waren groter en produceerden méér eieren per legsel dan die welke meer dan één bloedmaaltijd nodig hadden voor een eilegsel (hoofdstuk 7). Uit een vergelijking van de grootte van pas uitgekomen vrouwtjes afkomstig van in het veld verzamelde poppen met de grootte van gastheerzoekende vrouwtjes vóór en na de eerste eileg, kon de overlevingsduur van volwassen muggen bestudeerd worden (hoofdstuk 8). Pas uitgekomen vrouwtjes waren, gemiddeld, significant kleiner dan de gastheerzoekende vrouwtjes. De gastheerzoekende vrouwtjes vóór en na de eerste eileg verschilden daarentegen niet in grootte. Kleine vrouwtjes werden in dezelfde mate geïnfecteerd tijdens het bloedzuigen als haar grotere

soortgenoten, maar de grotere vrouwtjes produceerden meer oöcysten.
Het percentage muggen met sporozoieten was echter het hoogste bij vrouwtjes van gemiddelde grootte (hoofdstuk 9). In een afrondende studie is het effect van de muggengrootte op de malaria transmissie in haar geheel bestudeerd met behulp van een malaria-transmissiemodel zoals beschreven door Koella (1991) (hoofdstuk 10). Het model voorspelt dat muggengrootte een gering effect heeft op de transmissie van malaria. De mogelijke redenen hiervoor worden besproken.

De conclusies van het onderzoek zijn:

(1) Milieufactoren, met name de watertemperatuur, en de dichtheid van larven hebben een direct effect op de hoeveelheid voedsel beschikbaar voor de larven en beïnvloeden de ontwikkelingssnelheid en overlevingskans van onvolwassen stadia zowel als de grootte van volwassen *An. gambiae s.l.*.

(2) De grootte van de volwassen mug is bepalend voor het tijdstip van het eerste eilegsel en de vruchtbaarheid van *An. gambiae s.l.* en derhalve ook van invloed op de "fitness" van ieder vrouwtje afzonderlijk.

(3) Kleine muggen sterven reeds vroeg in het volwassen stadium en dragen niet veel bij aan de volgende generatie.

(4) De relatief grote muggen produceren veel oöcysten. Deze muggen overleven echter niet voldoende lang om de malariaparasiet over te brengen, vermoedelijk vanwege hun zware infectie met oöcysten.

(5) De grootte van de volwassen mug heeft een gering effect op malariatransmissie in haar geheel vanwege het effect van co-variatie bij de transmissieparameters.

Summary

Size of adult mosquitoes is known to affect both population dynamics as well as disease transmission. Studies devoted to this topic have given different results for different species. For example in some mosquito species, large size was found to be associated with high fecundity and longer survival (Steinwascher, 1982; Nasci, 1986a; 1986b; 1987) but in others large size did not result in longer survival (Walker *et al.*, 1987; Landry *et al.*, 1988; Pumpuni & Walker, 1989). Similar data were found for disease transmission. Some results indicated that smaller mosquitoes transmit Japanese Encephalitis, West Nile and La Cross viruses more efficiently than larger mosquitoes (Takahashi, 1976; Baqar *et al.*, 1980; Grimstad & Haramis, 1984), while other results did not show any difference between small and larger mosquitoes in their ability to transmit viral diseases (Kay *et al.*, 1989). With *Plasmodium* parasites, Ichimori (1989) did not find any relationship between *Anopheles stephensi* Liston female size and the number of *P. yoelii nigeriensis* oocysts developed, whereas Kitthawee *et. al.* (1990) showed that large *An. dirus* Peyton and Harrison developed more *P. falciparum* oocysts than small ones.

Variation in mosquito adult size is associated with the type of breeding sites used by a species. Several studies have shown that temporary habitat breeders are more variable in size than permanent habitat breeders (Haramis, 1983; 1985; Fish, 1985; Nasci, 1987). *An. gambiae* Giles, the subject of this study, breeds preferably in temporary water bodies and is one of the most size variable anophelines in the sub-Saharan region. No work has previously been undertaken to study the effect of adult size on the bionomics of this mosquito, information which could elucidate our understanding of the biology of this important mosquito.

The present research study was initiated in order to answer the following general questions: (1) what causes adult size variation in *An. gambiae* and (2) how does adult size affect important female characteristics such as reproduction, survival duration and malaria transmission. The specific aims of the study were:

- to investigate the effects of temperature and larval density on development and survival of immature *An. gambiae* and on the size of adults.
- to investigate the effects of adult size on blood feeding and on fecundity.
- to investigate the effects of adult size on survival.
- and to find out the relationship between adult size and malaria parasite infections.

In the course of this research, a total of 50,321 female *An. gambiae s. l.* were caught using various sampling methods, and 11,097 wings were measured, wing length being an accepted measurement of body size (Christophers, 1960; Haramis, 1983).

Factors affecting larval development and adult size were studied in the laboratory as well as in the field. Mosquitoes were reared in the laboratory under various constant

temperatures and densities (chapter 2). In the field, larvae were reared at constant densities under natural fluctuating temperatures (chapter 3). Developmental times and survival rates of immatures under different conditions were monitored and the wing length of emerged females compared. Rate of larval development and immature survival as well as size of adults were determined by the interaction between density and temperature.

Natural breeding sites were monitored to determine relative densities and survival of immature stages, and the size of emerging adults. Mortality of immatures was very high (on average 95%), caused mainly by pathogens and by predators, as well as weather conditions. Large, semi-permanent breeding sites produced larger sized females than the small temporary puddles (chapter 4). Spatial and temporal differences in adult size were investigated in field populations of *An. gambiae s. l.* There was a significant variation in adult size of populations from different localities, and also a seasonal variation in size of mosquitoes collected from the same locality over a two year period, with larger females being caught during the cooler months of the year (chapter 5). Density of female *An. gambiae* inside houses peaked towards the end of the rainy season in May, which was accompanied by an increase in entomological inoculation rates (the number of infective bites per person per night). Thus, the intensity of malaria transmission was higher towards the end of the rainy season (chapter 6).

Effect of adult size on pre-gravidity and on fecundity was examined for blood fed indoor resting mosquitoes and for newly emerged wild females fed in the laboratory. Females which developed eggs after a single blood meal were larger than those which required more than one meal to produce one batch of eggs, and produced more eggs per batch (chapter 7). Survival of adults was investigated by comparing the size of newly emerged females from field collected pupae with that of nulliparous and parous host seeking females (chapter 8). Newly emerged females were significantly smaller than the host seeking females. There was no difference in mean size between nulliparous and parous host seeking mosquitoes. Small-sized females were equally likely to be infected during blood feeding as were large-sized females, but large females produced more oocysts. The proportion of mosquitoes with sporozoites, however, was highest in intermediate sized females (chapter 9). Finally, the effect of adult mosquito size on the overall malaria transmission was examined using a malaria transmission model described by Koella (1991), (chapter 10). The model predicts that mosquito size has little effect on malaria transmission. Possible reasons for this are discussed.

The conclusions from these studies are:

(1) Environmental factors, notably the temperature of breeding water, and the density of larvae directly affect the amount of food available to larvae and influence the development and survival of immatures and the size of adult *An. gambiae s. l.*

(2) Adult size affects time of first reproduction and fecundity of *An. gambiae s. l.*, hence fitness of individual females.

(3) Small sized mosquitoes die early in adult life and do not contribute much to the bionomics of the species.

(4) Large-sized mosquitoes produced many oocysts, but they did not survive well enough to transmit the parasites (probably due to their heavy oocyst infections).

(5) The effect of adult size on the overall malaria transmission is negligible due to the effects of co-variation in the transmission parameters.

Chapter 1.

Introduction

This thesis deals with the bionomics of the malaria vector, *Anopheles gambiae sensu lato* in southeast Tanzania. The main question concerns the effect of adult size on female reproduction, survival and disease transmission. Before discussing the details of this specific project, I will summarize malaria as a disease, give a brief biology of the *An. gambiae* group, the potential ways to control malaria and finally I will specify the research questions and aims of my study.

An overview of malaria as a disease

Malaria is an infectious parasitic disease of man, transmitted from infected to non-infected individuals by anopheline mosquitoes. The disease occurs in most tropical areas of the world, notably in Africa, Asia and Latin America (Bruce-Chwatt, 1987). The World Health Organization estimates that over 40% of the world's population in more than 100 countries is at risk from the disease (Figure 1). At least 110 million cases occur annually worldwide of which 90 million are in tropical Africa. Deaths due to malaria are estimated at about 1 million a year worldwide (WHO, 1990). Despite the fact that in the 1950's and 1960's malaria was eradicated from over 30 countries and significantly reduced by control measures in much of Asia and South America, malaria is presently on the increase (WHO, 1992). This is due to the increasing drug resistance of the parasite and insecticide resistance of the vector, and is exacerbated by a failure to develop adequate alternative intervention methods. As a result malaria remains a major public health problem and an obstacle to socioeconomic development.

Malaria is caused by protozoan parasites of the *Plasmodium* group. There are four *Plasmodium* species which cause malaria in humans; *P. falciparum* Welch, 1897, *P. malariae* Laveran, 1881, *P. vivax* Grassi and Feletti, 1890, and *P. ovale* Stephens,1922. In tropical Africa *P. falciparum* is the most prevalent parasite, it is also the most malignant form of the disease. In some parts of East Africa *P. falciparum* accounts for more than 90% of all infections. In Tanzania it accounts for 95-98% of all malaria cases (Clyde, 1967; Kilimali & Mkufya, 1985; Amani Medical Research Centre annual report, 1980).

The life cycle of the parasite is summarized in Figure 2. Parasites in the form of sporozoites are injected into the human body by the bite of the female *Anopheles* mosquito when feeding. Injected sporozoites pass to the liver where, after about 12 days, they develop into merozoites.

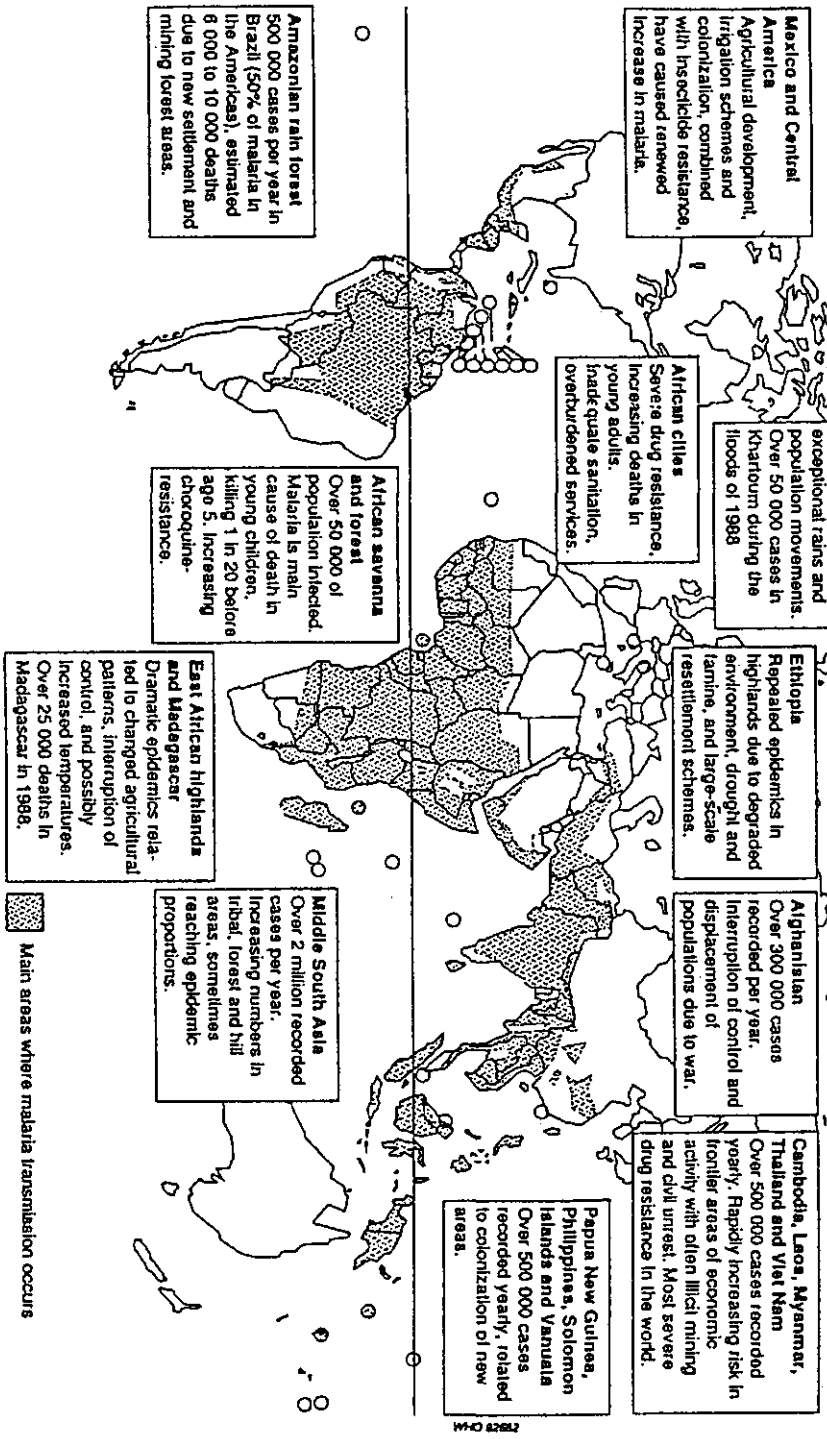

Fig. 1. Worldwide malaria distribution and problem areas. (from WHO doc. CTD/MAL/EXP/ 93.2)

The merozoites are released back into the blood stream and invade the red blood cells where they multiply to give rise to more merozoites. Invaded red cells rupture to release the merozoites which in turn invade more red blood cells. The intermittent rupture of the parasitised cells give rise to the periodic fevers and the symptoms characteristic of malaria episodes. Some merozoites however differentiate into sexual stages, the female (macro-) and male (micro-) gametocytes. A mosquito ingesting blood containing male and female gametocytes can become infected. The male gametocytes exflagellate in the mosquito and fertilize the female gametocyte to form a zygote. This transforms into an ookinete which migrates through the midgut wall and rests on the outer wall where it develops into an oocyst. After 8 to 10 days depending on temperature, mature oocysts burst to release sporozoites which migrate to the salivary glands. Sporozoites are injected into a new host blood stream when the mosquito feeds, thus completing and continuing the cycle.

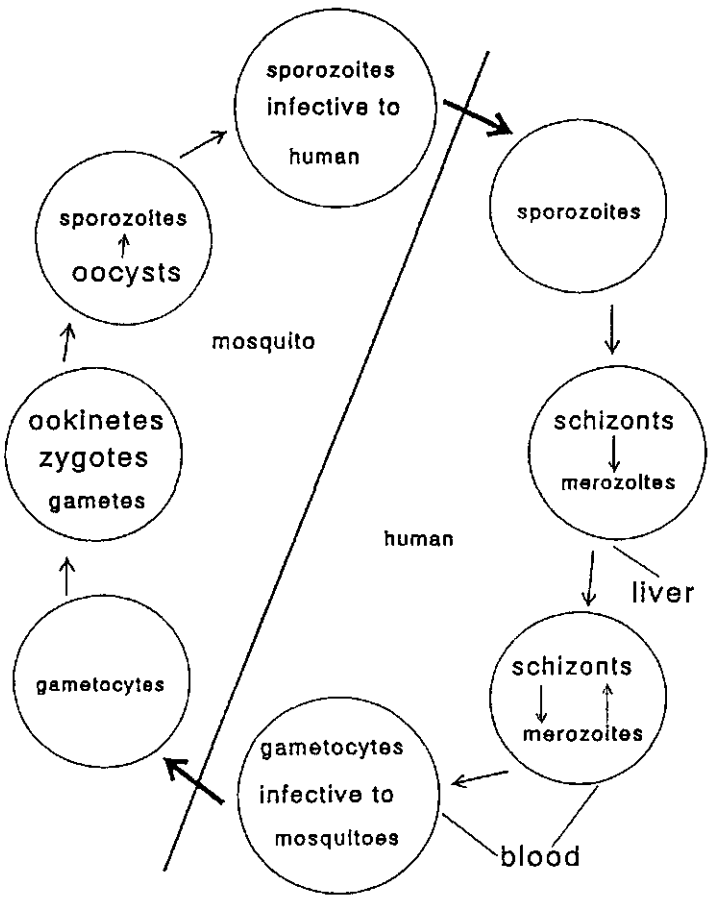

Fig. 2. Life cycle and transmission of the malaria parasite *Plasmodium falciparum*.

In many areas of sub-Saharan Africa malaria is one of the most important causes of morbidity and mortality, especially among children and pregnant women. It has been estimated that about 5% of children die directly or indirectly due to malaria before the age of five years. In pregnant women malaria causes anaemia, miscarriage, low birth weight and other complications (Janssens & Wery, 1987; Warren *et al.*, 1990; Paluku, 1991).

The pattern of malaria transmission in sub-Saharan Africa is very variable, from unstable malaria with cyclical epidemics in some areas to a stable condition with varying degrees of endemicity in others. In some parts of the region, transmission is intensive and people receive up to 300 infective bites per person per year (Molyneaux & Gramiccia, 1980; Beier *et al.*, 1990; Paluku, 1991). The transmission pattern depends principally on eco-geographic features, which differ from the forested areas in the south to the Sahara in the north.

In Tanzania the problem of malaria remains one of the most challenging of health problems (Kilama, 1985; UNICEF, 1985), threatening 70% of the population of about 23 million people. The Tanzanian ministry of health reports estimate that about 4.5 million cases are reported annually and of these 3500 die from the disease, mostly young children below the age of five years. This is most likely an underestimation as not all hospitals file their reports with the ministry and most of these reports are incomplete. Clyde (1967) described the endemicity of malaria in Tanzania, dividing the country from meso-endemic, hyper-endemic to holoendemic areas, depending on the duration of the transmission season. However, the transmission patterns may have changed since his studies. For example, transmission has intensified and stabilized in areas previously classified as having low transmission. Additionally, the disease has spread to areas which were previously free from malaria, such as highland areas and the islands. These changes have been brought about by environmental changes which favor breeding and survival of the vectors, such as cutting down of forests to give way to farm lands. Changes in agricultural practice have stimulated vector development as well, for example the growing of irrigated rice in areas where rice growing was only seasonal (Khatibu, 1991). Furthermore, the parasite has been spread by the movement of infected people from malarious areas to non-malarious areas introducing the parasites into these previously malaria-free areas. The movement of non-immune people from non-endemic areas into areas where malaria is endemic, due to population and economic pressures (such as in the Moshi area), is also increasing the incidence of the disease (Rita Njau, personnal communication). Resistance of the parasites to drugs, and of the vectors to insecticides have also contributed to this intensification of malaria transmission.

The anopheline mosquito vectors in Africa

The main vectors of malaria in tropical Africa are members of the two sibling groups

Anopheles gambiae Giles, 1902 and *An. funestus* Giles, 1900. Minor vectors include *An. nili* Theobald, 1904, *An. pharoensis* Theobald, 1901 and *An. squamosus* Theobald, 1901. Gillies and De Meillon (1968) listed about a dozen incidental vectors whose status remains unclear. The *An. gambiae* group is a complex of three fresh water species: *An. gambiae s.s, An. arabiensis* Patton, 1905 and *An. quadriannulatus* Theobald, 1911; one mineral water species, *An. bwambae* White, 1985; and two salt-water species, *An. merus* Dönitz 1902 and *An. melas* Theobald, 1903 (White, 1985). The species are genetically different but morphologically indistinguishable. As such, the best way of identifying them is by species-specific chromosomal inversions (Coluzzi, 1984) or more recently by DNA probes (Gale & Crampton, 1987; Hill, *et al.* 1991).

Four members of the complex have been described in Tanzania: *An. gambiae s.s, An. arabiensis, An. merus* and *An. quadriannulatus* (White, 1974; Bushrod, 1978). *An. gambiae s.s* and *An. arabiensis* occur sympatrically as is the case in many other parts of continental Africa. The proportion of the two species varies according to ecological features. Thus, the ratio of *gambiae:arabiensis* decreases from the humid coastal and humid lacustrine areas towards the more semi-arid and dry areas. *An. merus* is found along the coast where it co-exists with *An. gambiae s.s* (Marchand & Mnzava, 1985; Mnzava & Kilama, 1986) and *An. quadriannulatus* has only been reported from Zanzibar (Odetoyinbo & Davidson, 1968). In the Kilombero area, Mnzava and Kilama (1986) and Biro (1987) reported a distribution of 98% *An. gambiae* and 2% *An. arabiensis* during the rainy season. However, recent information based on species specific DNA-probes reveals that this ratio may be close to 70:30 (Dr. S. Hill, personal communication.).

Biology of the An. gambiae complex

Immature stages

Studies conducted on the breeding behaviour of the *An. gambiae* group seem to show that the fresh water species prefer to colonise transient rain pools, and there is no major difference between the sibling species in selection of these habitats (Chinery, 1970; Surtees *et al., 1970;* Service, 1970b, 1977; White *et al.,* 1972). The female mosquitoes lay their eggs in a variety of habitats, but mostly in shallow open sunlit pools resulting from rain and seepage water. Such types include borrow-pits, drains, brick-pits, car-tracks, foot and hoof-prints around ponds and water-holes, and rainwater collecting in natural depressions.

Eggs are laid singly on the water surface or on wet mud at the edges very close to the water. The eggs cannot survive desiccation and die quickly if dry. Eggs hatch within one to two days into first instar larvae. There are four immature stages through which larvae feed and grow before pupation. Larvae spend most of their time at the surface where they feed and breath like most Culicidae. Larvae are filter feeders, feeding on organic material

suspended in the water. Pupae are very active but do not feed.

Most breeding sites are temporary or semi-permanent in nature, and in some areas breeding is highly seasonal, following the rainfall pattern of that particular area. Since *An. gambiae* exploit these freshly formed rain pools well ahead of the other aquatic insects, they tend to escape predation and possible inter-specific competition. As a result, breeding is prolific during the peak rainy season. In the dry season breeding shifts to more permanent habitats such as wells, edges of permanent swamps or drying river beds (Gillies & Coetzee, 1987). In West Africa, *An. gambiae* has been reported breeding in domestic clay pots (Bruce-Chwatt, 1957; Chinery, 1984) and in Madagascar, Subra *et al.* (1975) reported breeding of *An. arabiensis* in open water storage tanks. Irrigated rice fields are also favourable habitats of the fresh water breeding *An. gambiae s.l.* The salt water breeding sibling species of the complex prefer brackish ponds along the coast where salinity is high. Breeding of these species inland occurs in association with salt treatment pans (Cross & Theron, 1983 quoted by Gillies & Coetzee, 1987).

Immature development can be rapid, and is temperature dependent. The egg to adult cycle takes 6-10 days (Jepson *et al.*, 1947; Gillies & Shute, 1954; Service, 1977). The mortality of immature stages of *An. gambiae s.l.* in nature is very high. Service (1971, 1973, 1977) estimated about 93% mortality from egg-hatch to adult emergence. Parasitism and predation are the main causes of mortality. Other factors such as lack of food and unsuitable breeding conditions, e.g. drying of sites and pollution, may also contribute to mortality of immatures.

Adult stages

Adult emergence takes place in the early evening. Males and females rest for a short period of time and, depending on the time of emergence, mating may take place the same night. Males form swarms in the twilight and females fly into these swarms and are mated (Charlwood *et al.*, 1980). Males feed entirely on plant sugars and most females take a plant sugar meal before a blood meal. Dispersal from breeding sites to feeding areas depends on the local topography and availability of host. In open and sparsely populated areas flight range is greater than in well vegetated and densely populated areas. Gillies (1961) estimated a range of between 1 to 1.5 kilometers in coastal Tanzania, while records of flight range of 3 to 6.5 kilometers have been observed in Zambia (Gillies & De Meillon, 1968).

Female members of the *An. gambiae* complex feed and rest readily both indoors (endophagic and endophilic) or outdoors (exophagic and exophilic). None of the species of the complex are exclusively exophagic, endophagic, exophilic or endophilic. All species exhibit a mixture of these extremes of behaviour. (For example, *An. arabiensis* often prefer to feed indoors but rest outdoors, whereas *An. gambiae* favour indoor feeding and resting (Molineaux & Gramiccia, 1980; Service *et al.*, 1978; Clarke *et al.*, 1980).

Similarly, host choice is not obligate and some members tend to feed on both humans and non-humans. However the degree of anthropophagism and zoophagism varies according to species. For example, *An. arabiensis* commonly feeds on cattle as well as on man, whereas *An. gambiae* feeds principally on man (Gillies & Coetzee, 1987). These behaviours are very important in the epidemiology of malaria since an anthropophilic member of the complex is likely to be a better vector of malaria than is a zoophilic one.

The biting cycle of the members of the *An. gambiae* complex has been studied in many parts of sub-Saharan Africa (Gillies & Coetzee, 1987). Principally there is very low activity up to 21.00-22.00 hours, followed with an increase in feeding activity to a peak between midnight and 04.00 hours, and decreasing again to shortly before dawn (Gillies, 1957). Exceptions may be found in densely forested areas where females feed readily during the day (Haddow, *et al.*, 1946). Various factors, including environmental, physiological and genetic act together to determine the biting cycle, and this may vary from place to place.

Mosquito densities inside houses differ extensively depending on the season and location. The abundance of *An. gambiae s.l.* adults tends to follow the seasonal pattern of rainfall. In areas with two wet seasons mosquito populations have two annual peaks, whereas only one peak occurs in areas with a single wet season (Gillies, 1954a; Service, 1963). However, local and seasonal factors also affect breeding and modify these patterns, thus it is difficult to generalize. In areas with a prolonged dry season most breeding sites disappear during the dry period. At these times *An. gambiae s. l.* breeds in less suitable habitats such as large ponds and marshes, where mortality is high and adult numbers drop (Christie, 1959; White & Rosen, 1973). In areas with an extreme dry seasons, individuals survive dry periods as adults, resting in shaded areas. Omer and Cloudsley-Thompson (1968; 1970) found that in the Sudan *An. gambiae s. l.* (probably *An. arabiensis*) survived the 9 month dry season as adults, taking small blood meals and not developing eggs, thus undergoing a period of quiescence.

Longevity of the *An. gambiae* complex has been studied mainly in terms of the proportion parous, which by itself does not give accurate estimates of longevity. The limited data available suggest a longevity of 1 to 5 weeks (Gillies & Wilkes, 1965). Daily survival rates of between 0.75 to 0.94 have been reported in many parts of Africa (Krafsur, 1970; White, 1972; Molineaux & Gramiccia, 1980). Re-analysis of published data on survivorship of several vectors by Clements and Paterson (1981) showed that mortality increased with age of the females. Using cross-correlation analysis, Mutero and Birley (1987) demonstrated that in the coastal area of East Africa, *An. gambiae s.s.* and *An. merus* survived longer than *An. arabiensis*.

Control of malaria parasites and vectors

Malaria control aims at interrupting transmission, either by attacking the parasite in the human host, or by attacking the mosquito vector at its various stages. Usually a combination of various methods, integrated to suit local conditions, needs, and available resources is the most effective, but also the most difficult to apply. In some areas of the world, such as the United States of America, and parts of Europe, e.g Italy, Greece and The Netherlands, malaria has been eliminated. This has been accomplished either by changes in socioeconomy or by eradication programmes. In these areas extensive residual spraying and larviciding with DDT, coupled with land drainage and better land management to eliminate breeding sites, substantially reduced, or completely eliminated the mosquito vectors (Russell, 1955; Bruce-Chwatt & Zulueta, 1980; Bruce-Chwatt, 1987). Coupled with vector control, active case detection and extensive chemotherapy eliminated the parasite in the human host.

In sub-Saharan Africa and other tropical countries, however, the problem has intensified after an initial reduction in the 1950's and 1960's. This is due to many factors including socioeconomic conditions as well as the complex relationship between the parasites, the vectors and the human hosts. The parasites are now extensively resistant to the cheap and easy to use antimalarial drugs (Peters, 1990). In Tanzania for example, chloroquine which is the first line drug is no longer effective in more than 40% of cases (Mutabingwa *et.al*, 1985). Evidence of resistance to fansidar (a combination of sulpadoxine and pyrimethamine) is also available (Kilimali & Mkufya 1985). The problem of drug resistance and the absence of a malaria vaccine available for use in the tropics in the near future, calls for increased emphasis on vector control strategies in the control of malaria (WHO, 1992).

Vector control

Vector control methods aim at reducing the vector population and are directed towards either the larval or adult stages. Larval control may be achieved by applying chemical larvicides, introducing biological agents in breeding habitats or implementing environmental management operations (Service, 1985b). Adult control operations aim at reducing mosquito longevity so that they die before they can transmit the disease. However, adult control methods which depend on the behaviour of the females are more selective against that proportion of the mosquito population which enters houses, feeds and rests indoors. Available vector control methods can be classified as either chemical, mechanical, biological or environmental.

Chemical and Mechanical methods

Chemical control with insecticides gained momentum with the advent of DDT and other organochlorines in the 1940s, prompting the worldwide malaria eradication programme of the fifties. Chemical insecticides can be applied as:-

-*Larval spraying*: this method is directed against the larval stages. Earlier chemicals used in larviciding included crude oils and Paris green. After the second world war, new and more effective larvicides with low mammalian and fish toxicity replaced the old chemicals. Organophosphorus compounds such as temephos, chlorpyriphos and fenthion are among the chemicals used for larviciding. Larviciding has limited application when used against *An. gambiae,* due to the fact that they breed in temporary habitats most of which are difficult to locate and treat. Also, during the rainy season when mosquitoes are at their highest densities there is extensive breeding. Control operations become logistically difficult during this period.

-*Residual spraying*: this method is still the most effective and feasible for chemical control of mosquito vectors. The technique consists of spraying insecticides with a persistent effect on all surfaces where mosquitoes are likely to rest. Directed against endophilic mosquito vectors, the technique relies on the mosquitoes resting on the sprayed surfaces and picking up enough chemical to be killed. The residual effect of the insecticide depends on the type of the compound, its formulation, dosage applied and the surfaces sprayed, and varies from a few weeks to several months. Chemicals available for residual spraying are organochlorines (DDT, dieldrin and HCH), organophosphates (malathion, fenitrothion), and carbamate compounds (propoxur). These compounds were used with success in many malaria control programmes including the WHO malaria eradication campaigns of the 1950s. Resistance of mosquito vectors to these compounds however has reduced their effectiveness. Also, there is an increased concern about the environmental and human effects of these highly toxic substances. A new pyrethroid insecticide, lambdacyhalothrin (Icon), a synthetic compound which resembles natural pyrethrins gave promising results in trials in Tanzania (Matola *et al.*, 1990, unpublished report).

-*Space spraying*: atomized insecticide droplets are applied indoors against adult mosquitoes. The mist produced contains droplets that remain airborne for periods of up to six hours and kill flying mosquitoes. Pyrethrum, synthetic pyrethroids and organophosphorus compounds are widely used in aerosols. Techniques for open space spraying have been developed such as the ULV technique. However, they require sophisticated equipment and trained manpower. Their high operational costs preclude the routine use of these techniques. This method has not been used extensively in Africa

because in most places the house structure is unsuitable for its use. Either houses do not have enough openings to allow the insecticide to penetrate indoors or they are too open to prevent the insecticide from being blown off by winds.

-*Personal protection*: although they are used mainly to avoid the nuisance of biting insects, personal protection methods, when effective, also reduce vector borne diseases. Methods include putting wire mesh on windows and doors or around eaves, and sleeping under bednets. Recently, pyrethroid impregnated fabrics such as bed nets and window or bed curtains have been shown to have a major impact on malaria transmission. In many parts of Africa and Asia trials with treated nets and curtains have been very encouraging (Lindsay *et al.*, 1988; Lyimo *et al.*, 1991; Magesa *et al.*, 1991). Personal protection methods are becoming increasingly popular due to their effectiveness and easy application. Community use of treated bednets extensively reduces vector population size and longevity (Curtis, 1992). Treated nets can also be used outside without losing their effectiveness. In addition, treating nets uses relatively less insecticide than spraying walls.

Biological methods

Biological control of mosquitoes consists of the utilization of natural enemies and biological toxins. These are directed against the aquatic stages. Natural enemies include larvivorous fish (for example *Gambusia affinis)* and other mosquito fishes. *Gambusia affinis* has been tested in paddy fields in the USA and showed mass reduction of mosquito larvae (Hoy & Reed, 1971; Hoy *et al.*, 1971). A very successful operation involving the use of mosquitofish in mosquito control was done in Somalia, where *Tilapia spilurus spilurus* was used to stock underground water tanks, the only available breeding habitats for *An. gambiae* during the dry season. There was a sharp reduction of larvae as well as adults in treated villages (Alio *et al.*, 1985). Other species of mosquitofish have been used in China, Korea and Russia (Curtis, 1991).

Invertebrate predators such as *Toxorhynchites* and other insects, nematodes, protozoa and fungi are also used in control of mosquitoes. The spore-forming bacteria *Bacillus thuringiensis israelensis* and *B. sphaericus* are highly toxic to mosquito larvae and are the most used biological larvicides for mosquitoes as well as for blackflies. However, these bacteria are more effective in *Culex* than in *Anopheles* larvae control because they normally settle within minutes of application and are not available in the feeding zones of larvae which feed at the air-water interface (Kramer, 1984). When incorporated in surface-bound particles the bacteria have shown to be effective in controlling *Culex* mosquitoes in India and in the USA (Curtis, 1991).

For *An. gambiae* application of biological agents is impossible in most cases because breeding habits of the species are so diverse and it is not possible to achieve the necessary

wide coverage of breeding sites. Also it is often difficult to predict performance of biological control agents, as this may be affected by the presence of alternative prey other than the mosquito larvae and by weather conditions. Further, logistic problems such as mass rearing, maintenance, storage and transportation, limit the value of manipulative types of biological control.

Environmental management

Environmental management for mosquito control covers a wide range of operations which can be classified under:

- *Environmental modification*: permanent physical transformation of land, water and vegetation to eliminate vector breeding habitats, for example drainage, filling, land levelling and transformation of impoundment margins (Service, 1989).
- *Environmental manipulation*: activities aimed at producing temporary conditions unfavourable for the breeding of vectors. These include stream flushing and other physical changes of the habitats.
- *Modification of human habitats and/or behaviour*: this form of environmental management aims at reducing man-vector contact. Examples of this kind of approach are mosquito proofing of houses, siting of settlements away from breeding sites and personal protection.

Practical problems with vector control

In Tanzania, as in most tropical areas where malaria is a problem, the control methods are faced with several set-backs. These include resistance of vectors to most of the common insecticides, lack of managerial and logistic support, and high cost. Most of the newly developed insecticides are expensive. Behavioural changes of vectors as a result of control activities such as avoidance of treated surfaces or shifting of biting time, are also posing problems. Human behaviour and beliefs, and low socioeconomic status result in poorly constructed dwellings which cannot keep out the mosquitoes. Inadequate research support towards malaria control also contributes to the problem. No single control method can be successfully applied and integrated control, which combines the available methods against both the vectors as well as the parasites, is the advocated approach. Several methods need to be employed which are proving more demanding in terms of resources and knowledge. In order to develop an integrated control programme, more information is needed on the biology, ecology and behaviour of the vectors.

The problem and aims of the study

An. gambiae s. l. is the most important mosquito species transmitting malaria in Africa. Because of its significance it is also the most studied. Nevertheless, aspects concerning the ecology of this vector remain unanswered. It is often speculated that adult mosquito female size has a strong effect on both population dynamics and the probability of transmission of malaria. As earlier published reports were inconsistent or conflicting, I have concentrated on the effect of female size on the population dynamics and on the transmission of malaria.

A number of recent studies indicate that the nature of variation in the size of mosquitoes may reflect larval conditions and that large individuals survive longer than their smaller (and therefore probably less well nourished) siblings (Reisen *et al.*, 1984; Fish, 1985; Haramis, 1985; Nasci, 1986b). The nature and extent of the variation apparently depend on the type of larval habitat that the mosquitoes occupy. In general, those which breed in temporary habitats are subject to greater stress resulting from crowding, insufficient food and relatively high temperatures, and thus show a larger amount of variation in adult size than insects occupying more permanent habitats (Haramis, 1983; 1985; Fish, 1985; Nasci, 1987). *An. gambiae* is the temporary pool breeder *par excellence*. The reported range in wing size (itself a measure of overall size) of 2.8-4.4mm by Gillies and De Meillon (1968) for this species is one of the largest among the sub-Saharan anopheline. Nothing, however, is known about the effect that size has on the survival and consequent vectorial capacity of *An. gambiae*. Could it be that larger individuals live longer and therefore are more likely to transmit malaria parasites than their small siblings?

Previous studies on other mosquito species have indicated that large individuals survive longer (Steinwascher, 1982; Reisen *et al.*, 1984; Hawley, 1985b; Nasci, 1986a; 1986b; Nasci, 1987; Packer & Corbet, 1989), were more successful in obtaining a blood meal (Nasci, 1987; Packer & Corbet, 1989), and produced more offspring, (Steinwascher, 1982, Nasci, 1986a, Packer & Corbet, 1989) than small ones. Nevertheless, Landry *et al.* (1988) correlated the body size of adult *Ae. triseriatus* collected from host baits and from oviposition traps with age, and found no strong evidence that size was advantageous to survival. In other studies with the same mosquito (Walker *et al.*, 1987; Pumpuni & Walker, 1989), mark-recapture methods could not detect any differences in survival between large and small mosquitoes.

Studies on the effects of female size on the transmission of mosquito-borne diseases have given variable results. Takahashi (1976) and Baqar *et al.* (1980) showed that smaller *Culex tritaeniorynchus* were more efficient in transmitting Japanese Encephalitis and West Nile viruses. Grimstad and Haramis (1984) obtained the same results for La Cross virus in *Ae. triseriatus*. On the other hand, Kay *et al.* (1989) found no difference in the ability of

large and small individuals of *Culex annulirostris* to acquire and transmit Murray Valley Encephalitis virus. As for protozoa, Kitthawee *et al.* (1990) showed in laboratory reared *An. dirus* infected with *Plasmodium falciparum*, that large females developed more oocysts. Ichimori (1989) found no relationship between *An. stephensi* female size and the number of oocysts of the parasite *P. (yoelii) nigeriensis*.

These studies demonstrate that mosquito female size may affect population as well as disease transmission parameters. The probability of a mosquito picking up and transmitting the pathogen depends on its feeding behaviour as well as its survival. A proportion of *An. gambiae* females require two blood meals for the completion of their first gonotrophic cycle, perhaps as a result of deficient larval nutrition. The proportion of so called pre-gravid females in a population varies between season and locality according to the availability of breeding sites (Gillies & Wilkes, 1965). Despite the increased chance of pre-gravid females acquiring the malaria parasite because of their double feeding, nothing is known about their subsequent chances of transmitting it, which depends on the insect's survival. This is because a suitable marker, identifying previously pre-gravid females once they have become parous, has hitherto not been available. Adult size may be a suitable marker for this. Since this was a poorly studied area, it seemed appropriate to investigate the biological and ecological factors that determine mosquito size, as well as the role of adult size in survival and malaria transmission. Such studies could give more information on the ecology of *An. gambiae*, and if found useful such information could be used in the planning of control strategies, such as manipulation of breeding sites, or the timing of pesticide application so as to achieve maximum impact.

In this thesis the variation in size of females of the *Anopheles gambiae* complex causes of this variation and its effect on female characteristics were investigated. The specific aims of the study were:
(1) to investigate the effects of temperature, larval density and nutrition on the development rate and survival of immature stages, and on the size of adult mosquitoes in the laboratory and in the field.
(2) to study the effect of adult size on blood feeding and fecundity.
(3) to study the effect of adult size on survival of adult females.
(4) to investigate the relationship between adult size and malaria parasite acquisition and transmission.

The laboratory studies were undertaken at the Wageningen Agricultural University, Department of Entomology and the field studies were conducted in Michenga village near Ifakara, southeastern Tanzania and the Ifakara Centre laboratories.

Part I. FACTORS AFFECTING ADULT SIZE IN *ANOPHELES GAMBIAE S. L.*

Chapter 2.

Effect of rearing temperature and larval density on larval survival, age at pupation and adult size of *Anopheles gambiae*

Abstract

The effects of temperature and larval density on survival of larvae, growth rate, age at pupation, and adult size (measured as wing length and dry weight) of laboratory-reared *Anopheles gambiae* (Diptera: Culicidae) were studied. Larvae were reared at three temperatures (24, 27 and 30°C) and three densities (0.5, 1, and 2 larvae/cm^2). The effects of density and temperature strongly interacted to determine the mosquitoes' life-history parameters. Survival was highest at the intermediate temperature of 27°C. The differences between the temperatures increased with increasing density. At 30°C survival decreased as density increased, but at 27°C increasing density led to higher survival. Age at pupation increased as temperature decreased from 30°C to 24°C and as density decreased from 2 to 0.5 larvae/cm^2. Adult size also increased as temperature decreased, but showed a negative correlation with density only at 27°C. In contrast, at 24°C and 30°C a decrease in density led to a decrease in adult size. Growth rate showed a similar pattern. At 27°C growth rate decreased as density increased, but at other temperatures the opposite trend was observed.

Introduction

Anopheles gambiae may, with some justification, be called the most important mosquito species for humans in Africa because of its role in the transmission of malaria, killing about one million children every year (WHO, 1990). In spite of the significance of *An. gambiae* as a vector, many aspects of its population dynamics are not yet understood. However, an understanding of the dynamics and of the processes that govern the dynamics may be helpful in controlling the mosquito population densities and thus malaria transmission.

Any explanation of mosquito population dynamics must include the mechanisms that form the life-history traits (Stearns, 1976; Istock, 1985), which directly affect the rate of population growth. For many mosquito species, two of the traits that have the greatest influence on population growth are age at pupation and adult body size. Age at pupation is strongly correlated with age at maturity, which in turn is the one trait that has the largest effect on any organism's population growth (Charlesworth, 1980). Adult size influences population growth because, in general, large mosquitoes produce more offspring

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(Steinwascher, 1982; Nasci, 1986; Packer & Corbet, 1989) and survive longer (Steinwascher, 1982; Reisen *et al.*, 1984; Hawley, 1985a; Packer & Corbet, 1989) than small individuals. A positive correlation between body size and fecundity has also been observed for various *Anopheles* species (Briegel, 1990), and seems to be particularly marked after the first blood meal.

A number of recent studies have shown that age at pupation and adult size of various mosquito species may reflect the environmental conditions during growth of the larval stages (Reisen *et al.*, 1984; Fish, 1985; Haramis, 1985). In general, species that breed in temporary habitats are subject to greater stress, such as crowding, insufficient food or relatively high temperatures, and show greater variation in adult size than species that breed in permanent habitats (Haramis 1985; Hawley 1985b; Fish, 1985; Nasci, 1987). Laboratory studies have shown that larvae reared at high temperatures and under food stress develop into small adults and experience high mortality (Siddiqui *et al.*, 1976; Reisen *et al.*, 1984; Nayar, 1969). As temperature increases, growth rate generally increases and age at pupation decreases (Brust, 1967; Hagstrum & Workman, 1971).

Such studies are largely lacking for *An. gambiae*. Although considerable variability in adult size has been observed (Gillies & De Meillon, 1968), it is not known what controls this size variation. Variation of other life-history traits or the effect of the environment on life-history traits have yet to be studied.

This study considers how larval survival, age at pupation and adult size of *An. gambiae* are affected in the laboratory by the temperature and the density at which larvae are raised.

Materials and methods

Experiments were performed at Wageningen Agricultural University, where the 'Galisua' strain of *An. gambiae sensu strictu* from Liberia (courtesy Prof. Colluzzi, Rome) had been reared for 19 generations. Mosquito larvae were reared at three temperatures (24±0.5°C, 27±0.3°C and 30±0.5°C) and at three densities (100, 200, and 400 larvae per liter), which resulted in nine treatments. Each treatment was replicated four times.

First instar larvae were added to one liter of demineralized water in 20x10.5x8 cm plastic pans, yielding approximately 0.5, 1 and 2 larvae per cm^2 of surface area. 0.2mg of fish food (Tetramin) per surviving larva was added daily until all larvae had pupated. Water was added as necessary to compensate for evaporation. Pans were checked daily, and dead larvae or pupae were removed. Age at pupation was recorded to the nearest 24 hours, and pupae were put into separate cages. Twenty four hours after emergence, adults were killed, put individually into gelatin capsules, and dried for 48 hours at 40°C. Then they were weighed with a Cahn electrobalance to the nearest 0.001 mg. One wing of each weighed mosquito was glued onto a slide, and its length was measured with an ocular

micrometer from the distal end of the alula to the tip, excluding the fringe scales, to the nearest 0.03mm.

Growth rate of individual mosquitoes was estimated as weight at emergence divided by the age at pupation, and thus indicated the average increase of weight per day throughout the larval period. Survival rate, S, was calculated as the proportion of larvae that survived for ten days by the equation $S = p^{10/t}$, where p is the proportion of larvae that survive to pupation, and t is the mean age at pupation within the treatment. The effects of temperature and of density on wing length, weight, growth rate, and age at pupation were evaluated with an analysis of variance (Sokal & Rohlf, 1981), taking account of replicates and of interactions between temperature and density. For the evaluation of survival the mosquitoes within each treatment were pooled over all replicates. The differences between survival rates were then compared to the confidence interval for the difference of two proportions (Fleiss, 1981).

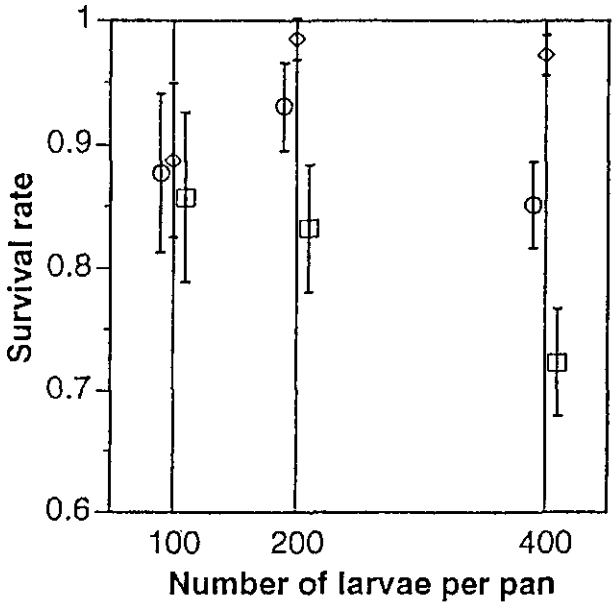

Fig. 1. Mean survival rates of larvae reared at different densities and temperatures. The vertical bars denote 95% confidence intervals. Circles represent larvae reared at 24°C, diamonds represent 27°C, and squares represent 30°C.

Results

For all treatments combined survival was 83.4%. Age at pupation ranged from 6 to 17 days with an average of 9.79 days. Adult dry weight at emergence was between 0.12 and 0.5mg with a mean of 0.25mg, and wing length was between 2.17 and 4.14mm with a mean of 2.83mm. Growth rate was between 0.011 and 0.055mg/day with a mean 0.026mg/day.

Survival was higher at 27°C than at the lower and higher temperatures (Fig. 1), though the differences were significant only at the two higher densities. The effect of density on survival depended on the temperature. Whereas at 30°C survival decreased as density increased, no significant differences could be detected at 24°C, and at 27°C survival peaked at the intermediate density.

Age at pupation decreased as temperature or density increased (Fig. 2).

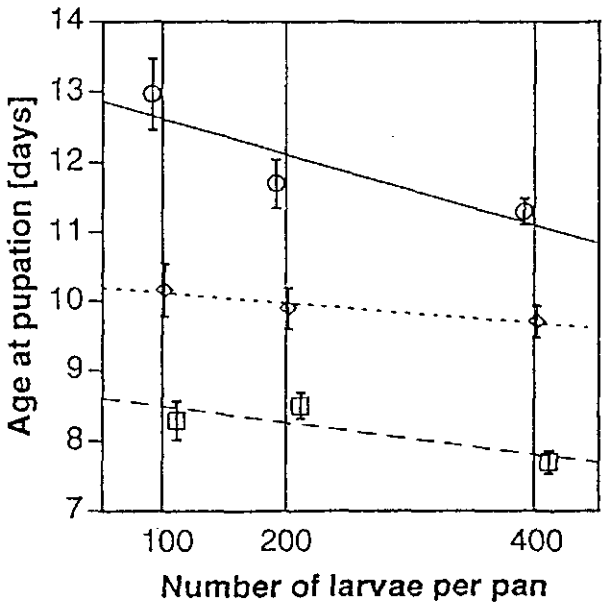

Fig. 2. Mean age at pupation of mosquitoes reared at different densities and temperatures. The vertical bars denote 95% confidence intervals of the means. Regression lines of age at pupation on density of larvae are shown for each temperature. Circles represent larvae reared at 24°C (regression: solid line), diamonds represent 27°C (regression: dotted line), and squares represent 30°C (regression: dashed line).

Table 1. Analysis of variance table for age at pupation

Source	DF	Sum of squares	F ratio	P
Replicate	3	18.716	2.557	0.0538
Density	2	141.652	29.027	<0.0001
Temperature	2	811.281	166.244	<0.0001
Density x temperature	4	70.992	7.274	<0.0001
Error	1687	4116.150		

Table 2. Analysis of variance tables for adult size

(a) Wing length

Source	DF	Sum of squares	F ratio	P
Replicate	3	0.6922	15.194	<0.0001
Density	2	1.4355	47.264	<0.0001
Temperature	2	2.7661	91.077	<0.0001
Density x temperature	4	1.5118	24.888	<0.0001
Error	1687	25.6188		

(b) Dry weight

Source	DF	Sum of squares	F ratio	P
Replicate	3	0.4418	51.713	<0.0001
Density	2	0.0036	0.621	0.5377
Temperature	2	0.3361	59.081	<0.0001
Density x temperature	4	0.1807	15.865	<0.0001
Error	1687	4.8037		

Table 3. Analysis of variance table for growth rate

Source	DF	Sum of squares	F ratio	P
Replicate	3	0.00870	93.393	<0.0001
Density	2	0.00038	6.124	0.0023
Temperature	2	0.00075	12.050	<0.0001
Density x temperature	4	0.00384	30.918	<0.0001
Error	1687	0.05238		

Mean age at pupation decreased from 13 days at 24° and a density of 100 mosquitoes to 7.7 days at 30°C and 400 mosquitoes. The effect of density was strongest at 24°C and weakest at 27°C, and the effect of temperature was strongest at 100 larvae and weakest at 200 larvae. The effects of density and temperature and their interaction were statistically significant (Table 1).

Temperature and density interacted strongly to determine adult size (Table 2). At 24°C and 30°C wing length (Fig. 3a) and weight (Fig. 3b) increased as larval density increased, but at 27°C higher larval densities tended to lead to smaller adults. At all densities mosquitoes were smallest at 30°C, but at the lowest density differences between 24°C and 27°C were not significant.

A similar pattern was observed for growth rate (Fig. 4, Table 3). At 24°C and 30°C growth rate increased as larval density increased, but at 27°C higher larval densities led to slower growth. At a density of 400 larvae growth rate increased as temperature increased, but at lower densities growth rate was highest at 27°C.

Female wing length was positively correlated with dry weight ($r^2 = 0.71$, df_{254}, F=634.49). Mosquito dry weight could be predicated from the regression equation: weight = 0.352 x wing length - 0.73.

Discussion

Rearing conditions had a clear effect on several life-history traits of *Anopheles gambiae*. Survival was highest at 27°C, which suggests that in the laboratory 27°C was close to the optimal rearing temperature for larval growth. As temperature increased from 24°C to 30°C, mean age at pupation decreased by about three days (30%), adult dry weight decreased by about 0.055mg (20%), and wing length decreased by about 2.5mm (10%).

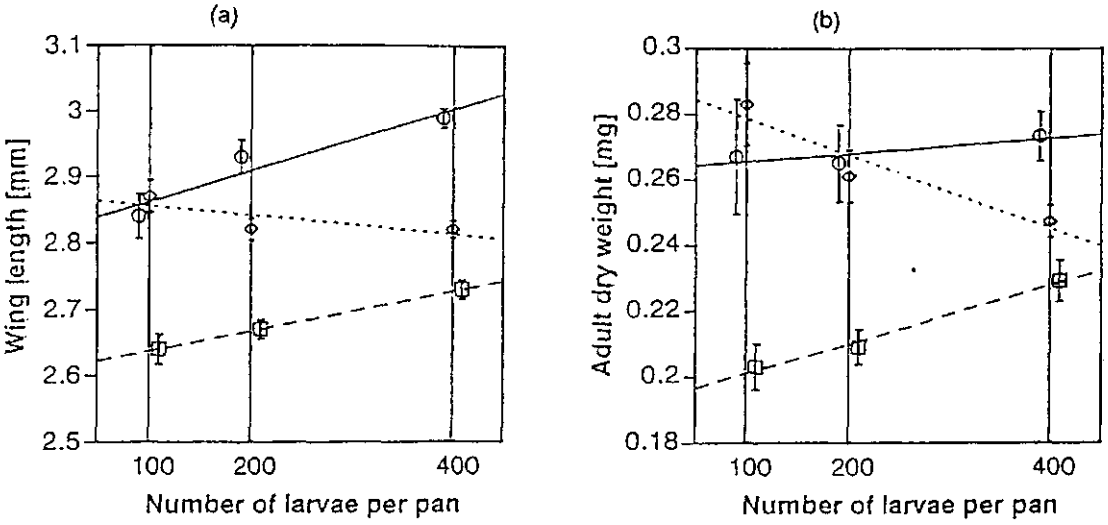

Fig. 3. Mean wing length (a) and dry weight (b) of adults reared at different densities and temperatures. The vertical bars denote 95% confidence intervals of the means. Regression lines of size on density of larvae are shown for each temperature. Circles represent larvae reared at 24°C (regression: solid line), diamonds represent 27°C (regression: dotted line), and squares represent 30°C (regression: dashed line).

These changes were accompanied by an increase in larval growth rate, in particular at high densities. Such a pattern is generally observed in the development of mosquitoes, e.g. *Aedes vexans* and *Ae. nigromaculis* (Brust, 1967), *Culex tritaeniorhynchus* (Baqar *et al.*, 1980), *Cx. tarsalis* (Hagstrum & Workman, 1971; Bock & Milby, 1981; Reisen *et al.*, 1984), and *Cx. nigripalpus* (Day *et al.*, 1990).

As density increased from 100 to 400 larvae per liter, age at pupation decreased by 1.7 days (13%) at 24°C, by 0.5 days (5%) at 27°C, and by 0.6 days (7%) at 30°C. Such a decrease in developmental time has been reported for other mosquito species,

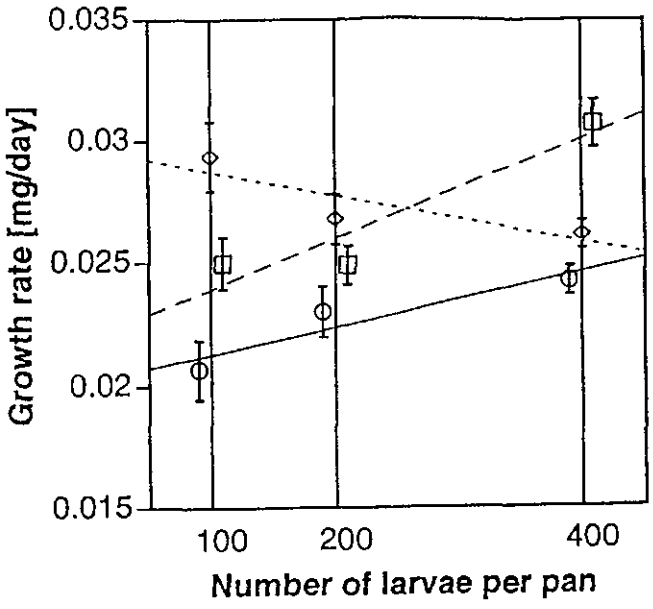

Fig. 4. Mean growth rates of mosquitoes reared at different densities and temperatures. The vertical bars denote 95% confidence intervals of the means. Regression lines of growth rate on density of larvae are shown for each temperature. Circles represent larvae reared at 24°C (regression: solid line), diamonds represent 27°C (regression: dotted line), and squares represent 30°C (regression: dashed line).

e.g. *Cx. tritaeniorhynchus* (Siddiqui *et al.*, 1976), and may reflect an accumulation of various nutrients from food or larval metabolites, as suggested by Reisen (1975). Because the amount of food each larva received was kept constant in this study, the observed effects of density were apparently due to larval rearing space, in contrast to Suleman's (1982) study on *Cx. quinquefasciatus*, in which development was most influenced by amount of available food per larva.Perhaps the most intriguing aspect of the present study was the strong interaction between temperature and density on larval development. At 27°C wing length, dry weight, and larval growth rate decreased as density increased. This

pattern is expected if high density leads to increased competition. In contrast, at 24°C and 30°C as density increased wing length, weight and growth rate increased. An explanation for this opposing pattern is not apparent, but might be based on the assumption that large, rapidly growing larvae have a competitive advantage over small, slow ones only when conditions are limiting. Therefore, at the extreme temperatures and high densities only the large larvae survive, so that surviving adults are on average large. In contrast at lower densities a mixture of large and small larvae survive, so that surviving adults are on average smaller. The observed relationship between temperature, density and survival lends weight to this possibility, though we have no data on size-specific survival within treatments.

The range of wing lengths reported in the present study was comparable to the range collected in a natural population in Tanzania (chapter 4 in this thesis), where wing lengths between 2.3 and 3.5mm, with a mean of close to 3mm, were measured. In the same environment, temperatures of breeding sites fluctuated between about 25°C in the cool dry season and 35°C in the hot rainy season (Lyimo, unpublished data). Therefore seasonal variation in body sizes, as shown for other species such as *Cx. nigripalpus* (Day *et al.*, 1990) and *Anopheles merus* (Le Sueur & Sharp, 1991), and in age at pupation are expected. Because of the correlations of body size with fecundity and of age at pupation with generation time, this variation could in turn lead to considerable differences in rate of population growth.

The presented study shows that the environment can have a considerable effect on various life-history traits of *An. gambiae*. Perhaps more importantly, it shows that environmental parameters interact to influence larval development, so that we cannot understand the life-history by considering the effect of one environmental parameter in isolation. The way in which the environment modifies the life-history traits is not yet clear. Much work is still required if we want to untangle the complex relationships between the environment and the life-history traits and to understand the population dynamics of *An. gambiae*.

Acknowledgments

Thanks are due to Jacqueline Schouten, Leo Koopman and Frans van Aggelen for their help in rearing the mosquitoes and data collection, and to Derek Charlwood for helpful comments on earlier versions of the manuscript. The first author gratefully acknowledges the financial support from the Directorate General for International Cooperation of the Government of the Netherlands.

Chapter 3.

The effects of constant versus fluctuating temperature and of predators on the larval development of *Anopheles gambiae sensu lato*

Abstract

Larval development and survival of *An. gambiae s.l.* reared in water with constant or fluctuating temperatures and at different densities were studied in the laboratory and in the field with or without predators. Larvae were reared at four different densities (0.03, 0.06, 0.25 and 0.5 larvae/cm^2). Mean age at pupation increased with increasing density of larvae. At constant temperature (27 ± 0.5 °C) development was faster at the lower densities, whereas at diurnal fluctuating temperatures (range 19.5-40.5 °C), development was faster at the higher density tested. Survival of larvae was higher in the laboratory than in the field, and in both field and laboratory treatments larval survival decreased with increasing density. In the field predators caused larval mortality but non-predatory factors seemed to have higher effects on survival than predation.

Introduction

A number of studies have examined the influence of the environment and other factors on development of the aquatic stages of several mosquitoes species. Water temperature and density of larvae were found to affect development and survival of immature stages as well as the size of the resultant adult mosquitoes (Brust, 1967; Hagstrum & Workman, 1971; Reisen *et al.*, 1984; Haramis, 1985; Hawley, 1985). Laboratory studies with *An. gambiae s.s.* showed that larval density and temperature interacted strongly in their influence on the development of immatures. At high temperature and high density development was faster than at low temperature or low density (Lyimo *et al.*, 1992). *An. gambiae s.l.* breeds in a variety of habitats which may differ significantly in their ecology. Service (1971, 1973, 1977) studied *An. gambiae* immature development and survival in some of these habitats and observed different survival patterns for different habitats. His studies however, investigated the role of biotic factors e.g. predators and parasites only. So far, no studies have attempted to look at the effects of the physical environment on the development of immature *An.gambiae*.

The aims of this study were to investigate the effect of predators on survival of immatures in the field and to examine the effects of larval density and constant or fluctuating breeding water temperatures on immature development, survival and on adult size.

Materials and Methods

Field experiments were conducted in Michenga village, Kilombero district, southeastern Tanzania. The area is described in detail in chapter 4 of this thesis. The laboratory experiments were done at the Department of Entomology, Wageningen Agricultural University. Six pairs of small rectangular pits measuring 40x40x40 cm were dug near the edge of a large swamp and allowed to fill with seepage water to about 15-20 cm deep. The pits were seeded with 6-12 hours old first stage larvae hatched from eggs of wild caught females of *An. gambiae s. l.* Fifty, 100, 400 or 800 larvae were added to the pits, resulting in densities of 0.03, 0.06, 0.25, and 0.5 larvae per square centimeter respectively. One pit in each pair was covered by a plastic mesh screen to prevent oviposition by other wild mosquito or access by predators. The other pit in each pair was left uncovered. Three replicates were performed for each treatment. Predators, previously identified as natural enemies of mosquito larvae (Jenkins, 1964; Service, 1973), or by direct observation were identified daily. Immature development was monitored daily until pupation, and all pupae were collected in small test tubes and allowed to emerge in the laboratory at the Ifakara Centre. Wing lengths of emerging females were measured as described by Lyimo *et al.* (1992).

For the second experiment, the same pits and same mosquito densities as in the first experiment were used, but all pits were covered to exclude predation. Daily early morning (7.00-8.00 hours) and mid afternoon (14.00-15.00 hours) temperatures of the breeding water were recorded with a normal mercury thermometer at a depth of 5 cm. Larval development was monitored daily until pupation. Pupae were collected and allowed to emerge at the Ifakara laboratory and female wing lengths were measured as in the first experiment. The laboratory cohort were reared in plastic pans measuring 26x12x8 cm. Larvae were added at a density of 10, 19, 78 and 156 larvae to give the same densities per square centimeter of 0.03, 0.06, 0.25 and 0.5 larvae. Three replicates were done for each density. Cultures were kept in the insectary at a constant temperature of 27° C, and 0.1 mg fish food (Tetramin) was added to the trays daily until pupation started after which food was added every other day. Water temperatures were recorded each afternoon. Larval development was monitored up to pupation. Pupae were allowed to emerge and female wing lengths were measured as mentioned above.

Differences in survival rates, developmental time and wing lengths between the different treatments were compared by analysis of variance and by paired t-tests.

Results

A number of predatory insects were present during the experiment, notably *Culex tigripes* larvae, a number of water beetles and adults of Dolichopodidae flies. Two to three days

after the start of the experiment, first stage larvae of *Culex tigripes* were observed in all uncovered pits. The number of these predatory larvae varied from 14 to 83 per pit at the early stages but decreased as the experiment progressed, and in most cases only one or two larvae pupated. In addition, hundreds of other *Culex spp.* larvae and pupae were present. The mean survival rate of immature *An. gambiae s.l.* was 0.20 in the absence of predators and 0.01 in the presence of predators. For the predator free treatment, the mean wing length of females decreased as density increased ($F_{(df3,33)} = 57.91$; $p < 0.001$, ANOVA test). Too few females emerged from the pits which were open to predation to allow for density group comparison of wing sizes. However, the mean wing length of females subjected to predation was significantly shorter than those from covered, predator free pits ($F_{(df1,46)} = 202.286$; $p < 0.001$, t-test) (Table 1).

Table 1. Mean comparison of developmental time and survival rates of immature *An. gambiae s. l.* and of female wing lengths in the presence or absence of predation.

Parameter	Covered pits	Uncovered pits
Developmental time (days)	8.6	8.9
Survival rate	0.193	0.007
Mean wing length (mm)	3.24 ± 0.01 (n=37)	2.44 ± 0.03 (n=13)
Predators	absent	present -*Cx. tigripes* -Dolichopodidae adults -water beetles

In the field experiment, measured diurnal water temperatures fluctuated from 19.5 °C to 40.5 °C, with an average of 28.7 °C. Temperature in the laboratory was 27.5 ± 0.5 °C. Age at pupation ranged from 6 to 14 days with an average of 8.9 days for mosquitoes reared in the field, and from 6 to 19 days with an average of 9.9 days for the laboratory group. For both groups, age at pupation increased with increasing larval densities (Fig. 1). Mean age at pupation increased from 6.5 days at 0.03 larvae per cm^2 to 11.5 at 0.5 larvae per cm^2 in the laboratory. The increase in the field group was from 6.8 days at the lower density to 10.4 at the higher density tested. At comparable densities, mean age at pupation was significantly lower in the laboratory group at the densities of 0.03 and 0.25 larvae per cm^2

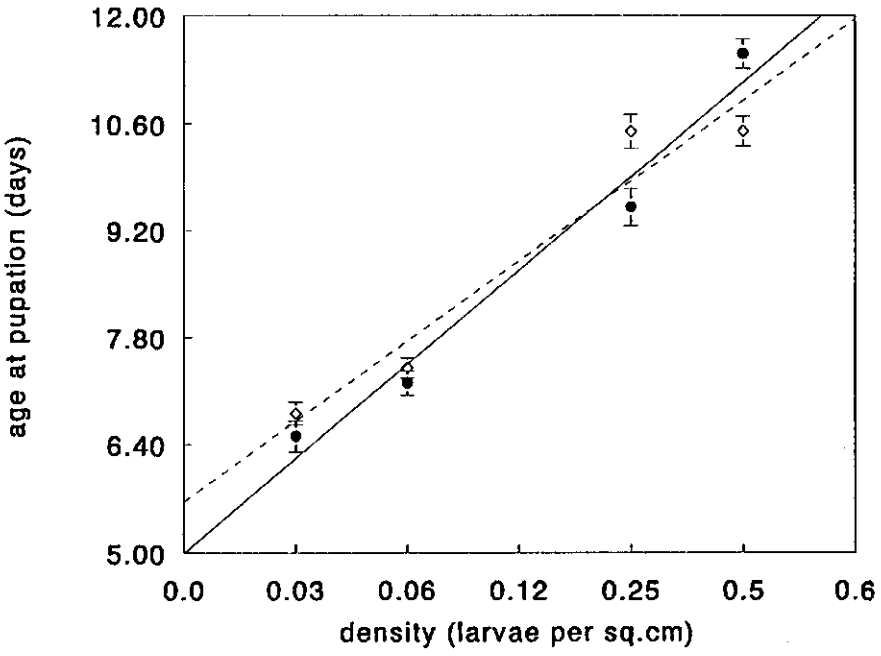

Fig. 1. Mean age at pupation of larvae reared at different densities and either at constant temperature in the laboratory (closed circles,regression solid line) or at fluctuating temperature in the field (diamonds, regression dotted line). The vertical bars denote 95% confidence intervals.

(F-value = 12.36, $df_{(1,78)}$; $p< 0.05$), but at the higher density of 0.5 larvae per cm^2 development was faster in the field reared mosquitoes (F-value = 39.26, $df_{(1,488)}$; $p< 0.001$, ANOVA test). There was no significant difference in mean age at pupation at the 0.06 larvae per cm^2. Analysis of covariance was then performed to compare the mean age at pupation between the laboratory and the field reared mosquitoes, with mean temperatures as covariable. There was a significant decrease in age at pupation in the field reared mosquitoes and a significant increase in age at pupation at high densities. The interactions between the treatments and mean temperature, and treatment and density were also

significant (Table 2).

Survival of the immatures, calculated as $S=p^{10/t}$, where p is the proportion of larvae surviving to pupation, and t is the mean age at pupation within the treatment (Lyimo, *et al.*, 1992), decreased with increasing density (Fig. 2). Survival was significantly higher in the laboratory than in the field. Mean wing lengths of females decreased with increasing density (Fig. 3). In the laboratory, the mean wing lengths of females from the two lower densities were not significantly different, but the two higher densities were significantly different from each other.

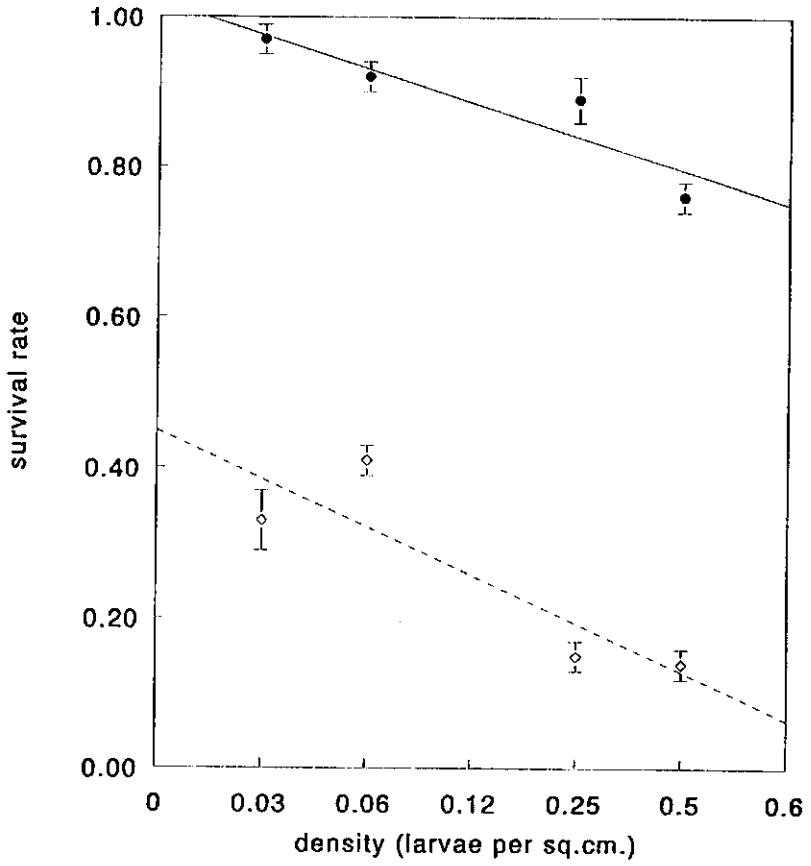

Figure 2. Mean survival rates of larvae reared in the laboratory under constant temperature (closed circles, regression solid line) or in the field at fluctuating temperature (diamonds, regression dotted line). The vertical bars denotes 95% confidence intervals.

The field cohort however, showed an opposing pattern with mean wing length of the two lower densities significantly different while at the higher densities they were not significantly different. Mean wing lengths of females from the field group were significantly larger than those from the laboratory group ($F_{(df1,1184)} = 9.07$; $P = 0.01$, ANOVA-test).

Table 2. Summary results of the analysis of covariance for developmental time of laboratory and field reared cohorts of *An. gambiae*.

Source of variation	sum of squares	degree of freedom	F-ratio	significance
Treatment	19.71	1	8.50	0.0036
Density	496.77	1	213.13	<0.0001
Treat x density	47.13	1	20.19	<0.0001
mean temp.	31.00	1	13.31	0.0003
Treat x mean temp	22.90	1	9.82	0.0018
error	2748.10	1184		

Discussion

Small collections of water such as the pits used in the present studies are generally considered to be predator free (Christie, 1958). However, as Service (1973) noted and from the present study, this is not so. The pits used in this study were freshly dug, and free of any predators, but colonization by *Culex tigripes* was very rapid. Larvae of this predator were found in pits two days after the pits were dug. These larvae preyed voraciously on all stages of *An. gambiae* larvae. Dolichopodidae adults were also observed taking larvae from pits. However, no distinction was made between aquatic and surface predators on their contribution to larval mortality. Pits open to predation produced fewer adults and smaller females. With predation, one would expect these pits to produce larger females because of reduced density of competing larvae. However, open pits attracted other mosquitoes, mostly *Culex spp*.

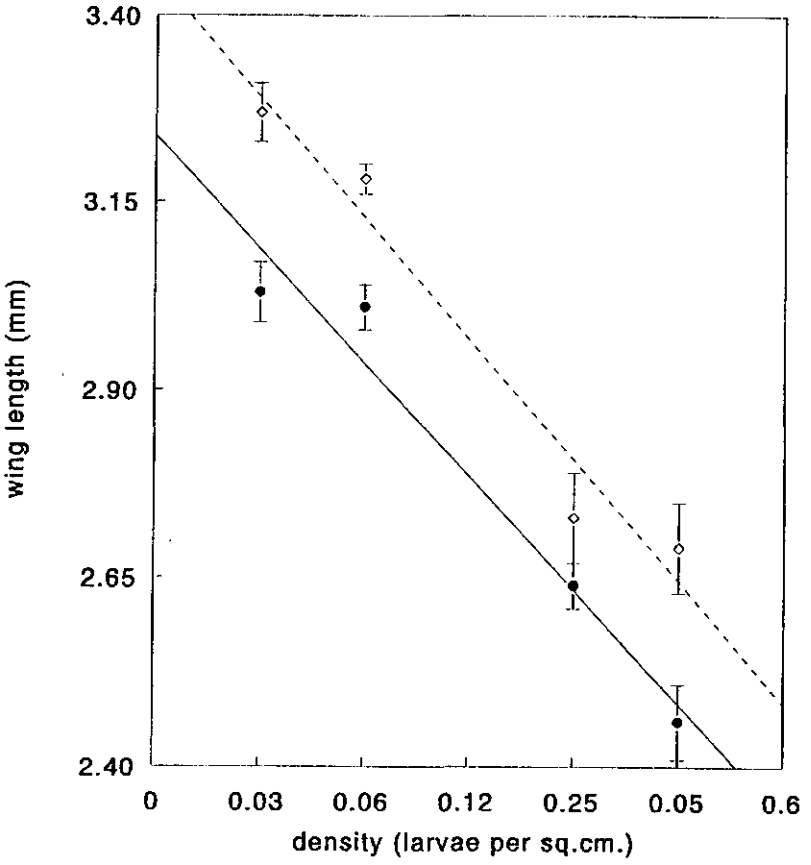

Fig. 3. Mean wing length of females reared in the laboratory under constant temperature (closed circles, regression solid line) or in the field at fluctuating temperature (diamonds, regression dotted line). The vertical bars denotes 95% confidence intervals.

The presence of these other mosquito larvae may have affected the *An. gambiae* larvae, either by direct competition for nutrients, or by other interference mechanisms such as growth retardant factors (Suleman, 1982). Another possibility is that predators might have selected for the larger, faster growing larvae and only the smaller ones survived to pupation.

Although predators contributed to mortality of immature *An. gambiae*, other mortality factors were also responsible, and these other factors seemed to be more important. In the present study for example, 80% mortality was recorded in predator-free treatments. Assuming that the same was true for the pits which were open to predation then overall mortality due to predators was less than 20%, meaning that predation was not the main cause of mortality in immature *An. gambiae*. The actual causes of mortality in the predator-free pits were not investigated but several groups of fungi such as *Coelomomyces spp*, microsporidia and other entomopathogenic fungi are known to cause mortality in *An. gambiae* (Service, 1973; Jenkins, 1964; Fox & Weiser, 1959). Larvae collected in a ditch near our experimental pits were found infected with *Coelomomyces*. These pathogens may have been responsible for the observed mortality of immatures in our experimental pits.

Initial density of larvae affected survival of immatures. In the second experiment, survival decreased with an increase in density both in the laboratory and in the field. Previous studies of a laboratory colony of *An. gambiae s.s.* by Lyimo *et al.* (1992) showed similar results. The laboratory cohort showed much higher survival rates than the field cohort chiefly because no mortality factors other than competition for food and space were acting upon them. The field cohort might have been affected by pathogens, as was observed in the first experiment. No attempts were made to quantify total nutrients in the field pits but as all were within half a meter of each other, it was assumed that nutrients did not vary much between the pits. Although the laboratory cohort was offered 0.1 mg of food daily, there was less mortality of larvae which meant less food per larvae, as compared to the field cohort which had higher mortalities and may have had less competition. This may be the reason for the observed large adults emerged from the field mosquitoes.

Age at pupation increased with increasing density both at constant and fluctuating temperatures. However, the developmental pattern was not consistent. At the higher densities pupation was faster at fluctuating temperatures while at the lower densities pupation was faster at constant temperature. These differences were probably caused by the interaction between high density of larvae and temperature. The field temperature fluctuations resulted in higher mean temperatures which on interacting with the density acted as a stress factor, thus modifying the developmental process. Such a phenomenon was observed by Lyimo, *et al.* (1992) in the laboratory for *An. gambiae s.s.* and also by Reisen (1975) in *An. stephensi* who found that density and temperature interacted strongly in determining larval development.

The results of this study support previous laboratory studies (Lyimo *et al.*, 1992) by showing that the interaction between rearing water temperatures and density of larvae is important in modifying immature development as well as size of *An. gambiae s. l.* The mechanism of the interaction is still not clear, but as suggested by Hagstrum and Workman (1971), this interaction may alter feeding rates of the larvae, thus affecting their

development. Non-predatory factors, such as entomopathogenic fungi are probably more important as causes of mortality in *An. gambiae* immatures in small breeding sites than predators.

Acknowledgements

Many thanks are due to my field assistant Seydina Bakari and to the entomology field workers of the Ifakara Centre. Dr. J. C. Koella for his critical comments and statistical advice, and Dr. W. Takken for his comments. Financial support was generously provided by the Directorate General for International Cooperation of the Government of the Netherlands.

Part II. ECOLOGICAL ASPECTS OF *ANOPHELES GAMBIAE S. L.* IN SOUTHEAST TANZANIA

Chapter 4.

Life history aspects of *Anopheles gambiae s. l.* immature stages: Breeding activity, density and survival.

Abstract

Breeding of *Anopheles gambiae s.l.* in Michenga village in South East Tanzania was monitored during the main rainy season of 1991, by daily surveillance of breeding sites. Several categories of breeding sites were identified which included rice fields, water holes, rain pools, ditches, borrow pits, puddles and footprints. All breeding sites had prolific larvae but were not equally productive of pupae. Life tables were constructed from age distribution histograms and instar mortalities estimated. Overall mortality of immatures was estimated to be at least 94.6% and was mainly due to predation and parasitism. Drying up of breeding sites also contributed to mortality of immatures. Mean wing lengths of females collected from water holes and rain pools were significantly longer than of those females collected from puddles and footprints. Temperature of the breeding water and crowding of larvae could be the explanation for this observation.

Introduction

Understanding the ecology of vectors is important in explaining population dynamics and disease transmission. Different populations of the same species may have subtle ecological differences, which could prove important in the interaction of that species with its environment. The affluence of *Anopheles gambiae s. l.* for example, may be due partly to the genetic complexity of the species, and partly to its ability to use a variety of breeding habitats. The population dynamics of *An. gambiae* is associated with the availability of breeding sites and adult densities fluctuate seasonally depending on the pattern of rainfall (Gillies & De Meillon, 1968; Krafsur, 1977; White *et al.*, 1972). Rainfall creates temporary breeding sites such as shallow pools and foot prints, and replenishes permanent sites such as swamps. Because of its breeding habits *An. gambiae* quickly colonises these newly formed temporary rain fed water bodies ahead of other aquatic fauna (Christie, 1959). In the Kilombero area of Tanzania, Freyvogel & Kihaule (1968) and Biro (1987) showed that *An. gambiae* densities peaked during the rainy season and dropped during the dry season.

In this study, immature of *An. gambiae* were monitored until emergence, as part of a study of the population dynamics of this mosquito in a Michenga village. We examined breeding site characteristics, the development and survival of immature stages of *An. gambiae*, and the size of adult *An. gambiae* females emerging from different sites.

Materials and Methods

Study area

These studies were conducted in Michenga village, Kilombero district, in southeastern Tanzania (Fig. 1). The Kilombero district lies in the Kilombero valley and covers an area of 250 kms southwest-northeast and 60 kms east-west. The altitude averages 270 m and is approximately 240 at Michenga. To the north the district is bordered by the foothills of the Udekwa mountains with savanna type of *Brachystegia* vegetation. Michenga village is a rural community about 5 kilometers west of Ifakara town. The Lumemo river, a tributary of the Kilombero river, originates from the Udekwa mountains and runs along the western border of the village. The village is very flat with several semi-permanent ponds scattered within the village. Open water holes are the main source of domestic water in addition to a few hand pumped shallow wells. Some of these water holes have water throughout the year. During the rainy season, the village is flooded and many temporary water bodies are present.

The seasons vary from a hot and humid period in December through May, a cool and dry period in June through August and a hot dry period in September through November. The rainy season starts in November/December and extends through May, with a peak in April. However, monthly precipitation varies considerably from year to year. The annual rainfall varies from 1200 mm in the plain to over 1800 mm on the mountain range. The mean temperature is 26 °C, with a minimum of 16 °C and a maximum of 37 °C. Relative humidity fluctuates from 50% to over 95% (Fig. 2).

The Kilombero valley consists of grassland in the plains and *Brachystegia* woodland (Miombo woodland) on the terraces and the foothills. People engage in subsistence agriculture and grow mostly rice and maize. Cassava and bananas are the semi-permanent crops. Rice is grown on ridges or flat plots and no irrigation is practiced in this area. The Michenga village has a total of 2485 inhabitants in 442 households. The average number of persons per household is 4.2. The age distribution forms a 14:33:53 ratio of under fives:schoolchildren:adults (1989 census, Kilombero Malaria Project report). Houses are mostly made of mud bricks with palm leaf thatch. A few houses are of burnt bricks with corrugated iron roof. The most common animals in the village are dogs. Other large animals include some cows and goats. Almost every household keeps chickens or ducks for eggs and meat.

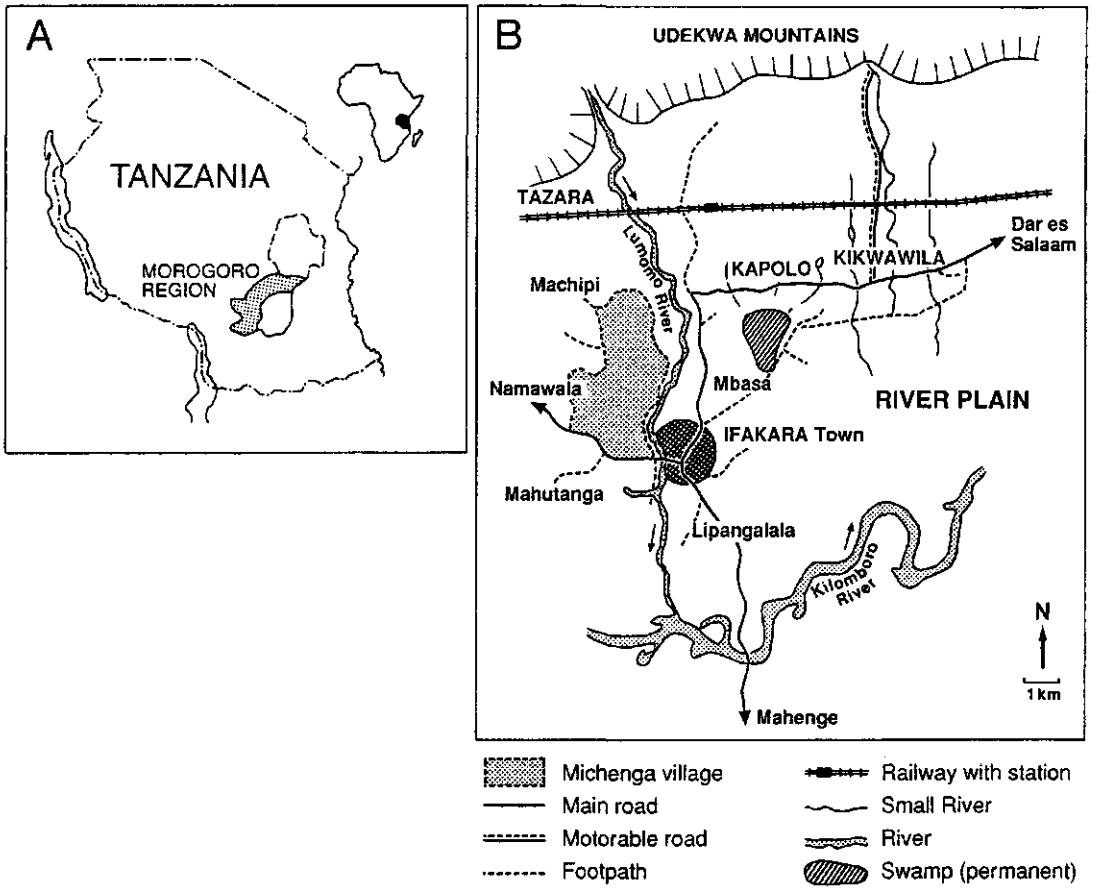

Fig. 1. A sketch map of Tanzania showing (a) Kilombero district (shaded area) in Morogoro region and (b) Ifakara agglomeration and the study village.

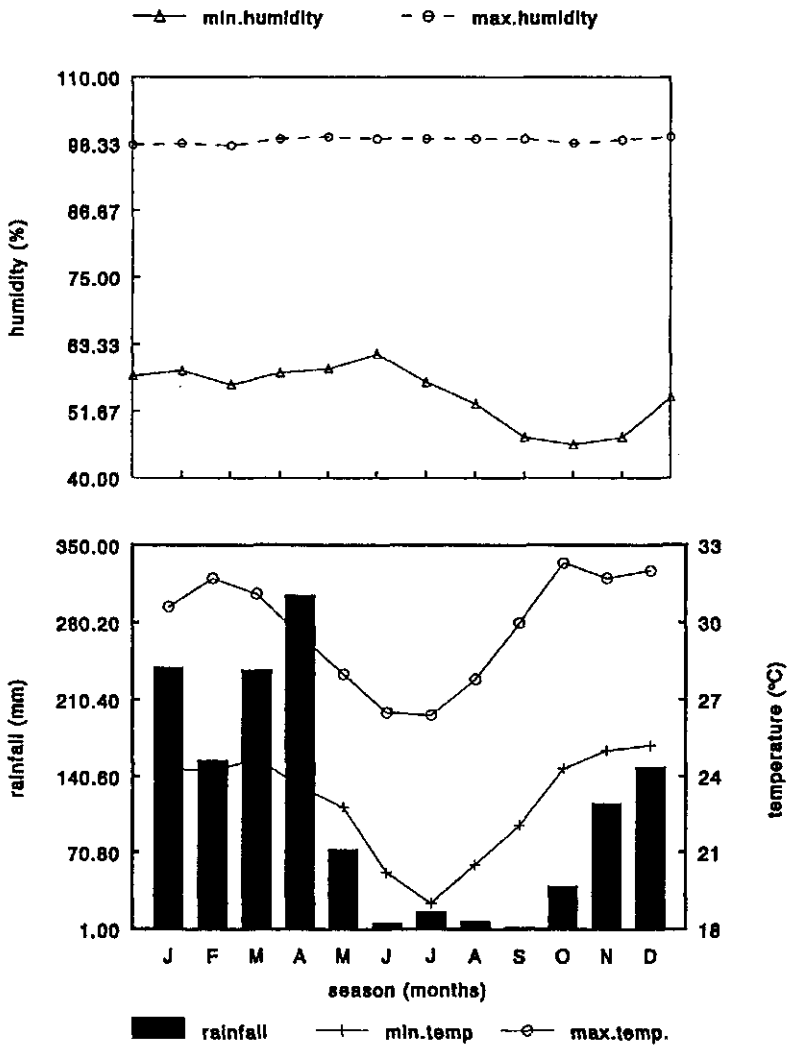

Fig. 2. Climatical measurements of rainfall, temperature (minimum and maximum) and relative humidity (%) at Katrin-Ifakara. Monthly mean values over five years (1987-1991).

Sampling of immatures

Standardised sampling of *An. gambiae* immatures is difficult because of the diversity and complexity of the immatures' habitats. There is continual variation in both the size and the number of habitats, such that quantitative estimates of either larval density or population size are subject to sampling errors. Of the many available larval sampling techniques, dipping is the most commonly used method. Dippers may vary in size and shape to suit the local needs and availability. Service (1976) gave a list of dippers developed and used in the field, with their advantages and disadvantages. The advantage of using a dipper is that it is easy to standardize the sampling procedure, for example the number of dips per site. The disadvantage of dipping is that larvae and pupae react to any movement or shadow on the water surface by diving and some mosquito larvae spend a long time submerged, and may be missed during sampling. Also in shallow sites dipping is difficult and may result in biased sampling.

The distribution of mosquito immatures in their breeding habitats is highly aggregated. Service (1976; 1985a) reported that in *An. gambiae s. l.* the spatial aggregation of immatures differs between instar stages. Because of this difference there are often large variations between the number of immatures caught in different samples. In view of this variability, large numbers of dips are taken in order to estimate the density of immatures per dip. In the present study standard dippers (or ladles) with a capacity of 125 ml and diameter of 8 cm were used.

Habitat characteristics and general breeding activity

From April to June 1991, water bodies within Michenga village were surveyed and probable *An. gambiae* breeding sites identified. Figure 3 shows a sketch map of Michenga village with the surveyed breeding sites. The sites varied in size and appearance and could be grouped into five categories, namely:

- Rice fields: rice fields flooded with rain water.
- Water holes: these were hand dug pits containing stagnant ground water for domestic use. Normally the water was clear and there was no emergent vegetation although some had hanging grass.
- Rain pools: temporary to semi-permanent, both natural or modified by man, filled with rain or seepage water.
- Borrow pits and ditches: borrow pits filled with rain water and natural or man-made ditches.
- Puddles and footprints: small water bodies formed as a result of human activities, for example puddles in maize farms and paths in swampy areas. (Sometimes there was overlap between puddle/footprint and the borrow pit/ditch types of breeding sites,

especially when the types were in the process of drying up, making it difficult to separate the two).

A total of 43 sites was visited daily over the sampling period, and sampled for the presence/absence of immatures. A team of two people with dippers plus trays measuring 24 x 18 x 6 cm., collected larvae and pupae for 10 minutes at each site. The number of anopheline larvae (irrespective of instar stage) and pupae was recorded. After counting, larvae were returned to the site and attempts were made to collect all the remaining pupae by taking a second round of checks after the normal sampling procedure to get an estimate of the total pupae production. Dipping for pupae continued until no pupae could be found. All pupae were brought back to the laboratory, allowed to emerge and adults were identified to species. Presence of culicine larvae and any other animals were also recorded. The water temperature of each site was measured with a mercury thermometer at a depth of 5 cm, daily in the morning (between 08.00 and 09.00 hours) and in the afternoon (between 14.00 and 15.00 hours). Each site was monitored as long as larvae or pupae were found, which was between 6 and 10 weeks. Wing lengths of emerged adult females were measured as described by Lyimo *et al.* (1992).

Immature development and survival

From the 43 water bodies surveyed, six breeding sites were selected for detailed studies. These sites consisted of one water hole, one rain pool, one borrow pit, two large ditches and one puddle. Dipping was done daily for a period of two weeks from April 26 to May 10, 1991. At each site ten dips were done with an interval of five minutes between dips, using the standard dipper. The number of larvae and pupae were counted separately for each stage in each dip. In order to estimate instar duration, wild females were allowed to lay eggs in the laboratory and newly hatched first instar larvae were introduced into plastic trays floated at one of the breeding sites (a large ditch). The trays (25 x 16 x 6 cm) had the bottoms cut out and replaced with a fine cotton cloth. Four replicates were set up with 25 larvae each. The trays were checked daily and larval counts and developmental stages recorded until all larvae had pupated. Survivorship of immature stages was estimated as described by Service (1973; 1977). In short, age distribution graphs were constructed by dividing the total number of each instar counted over the ten day period, by the instar duration and plotting the values against the median age in days of each stage. Survivorship curves were then fitted through the mid points of the histograms.

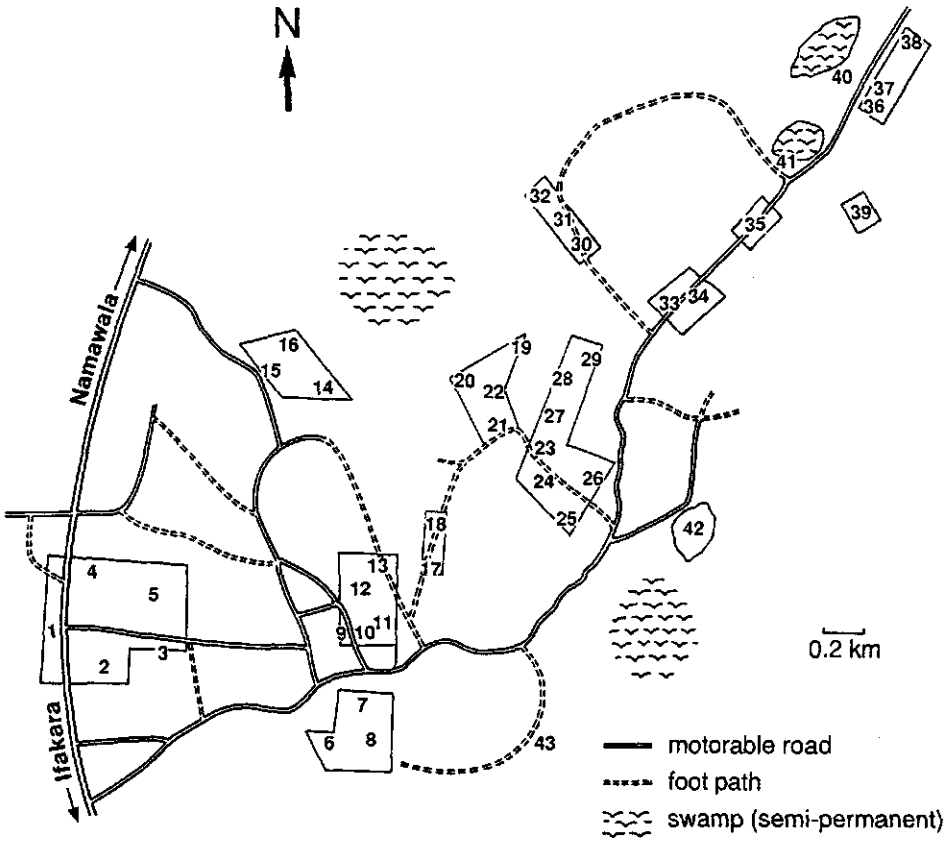

Fig. 3. A sketch map of Michenga village with surveyed breeding sites.

From the curves, life tables were then constructed and mortality of immatures estimated. For the construction of such tables it is assumed that the population size at the time of sampling is at equilibrium with the number of oviposition balancing eclosions and deaths in all stages.

Sibling species identification

The *An. gambiae* group was identified to sibling species level by the DNA probe technique. In brief, squash blots of males emerged from field collected pupae were analysed using synthetic DNA probes as described by Hill *et al.* (1991)

Results

The number of sites sampled and their distribution among the five categories is presented in Table 1. Also shown is the number of larvae and pupae collected. Seven sites out of 43 (16%) did not yield any larvae during the study period. These included 2 rice fields, 3 water holes and 2 rain pools. Both culicine and anopheline larvae occurred together in 22 sites, and 14 sites had anopheline larvae only.

Table 1: Type and number of breeding sites surveyed and the number of *An. gambiae* immatures collected

category	number surveyed	number with larvae	number[1] of larvae	number[1] of pupae
Rice fields	4	2	667	13
Water holes	8	7	4792	47
Rain pools	6	4	3762	251
Ditches/borrow pits	13	13	6812	458
Puddles/foot prints	12	12	6024	325

[1] Total number of larvae and pupae collected during the standardised sampling procedure.

Table 2: DNA probe results of adult males collected from different sites between April and June 1991.

Site	No. tested	No. positive for		Not determined
		gambiae	*arabiensis*	
1	6	5	1	0
4	17	9	8	0
5	13	7	3	3
6	30	24	5	1
7	3	2	0	1
8	14	11	2	1
9	7	5	2	0
12	6	4	2	0
13	4	2	1	1
16	3	1	1	1
20	1	0	0	1
21	39	32	5	2
25	21	14	7	0
26	6	6	0	0
29	6	4	2	0
31	1	0	1	0
32	3	1	2	0
33	3	1	2	0
37	1	1	0	0
39	2	2	0	0
Total	186	131	44	11

Mixing of the two subfamilies was observed in the larger breeding sites such as the water holes and the rain pools and occasionally in the smaller, very transient puddles and foot prints. Almost all anopheline pupae collected during the study period were of the *An. gambiae* complex. However, one *An. squamosus* Theobald and two *An. funestus* pupae were collected. The DNA probe analysis revealed that the ratio of *An. gambiae s.s* Giles and *An. arabiensis* Patton was approximately 75:25 and was consistent throughout the study period. The two sibling species occurred together in 18 out of 20 breeding sites where pupae were collected (Table 2).

Borrow pits and ditches formed the largest proportion of breeding sites, followed by puddles and foot-prints, water holes, rain pools and rice fields. There were abundant larvae in all sites positive for larvae. However, not all of them produced large numbers of pupae. Table 3 shows the final number of pupae collected i.e total pupae production. The rain pools produced on average the highest number of pupae per breeding site, followed by ditches/borrow pits, puddles/foot prints and rice fields . However, the ditches and borrow pits produced the highest proportion (52.5%) of the total pupae collected. In the first six weeks of the survey all sites contained immatures (Fig. 4). After ten weeks all other sites had dried out except the water holes, which continued to have larvae but no pupae of *An. gambiae*.

Table 3: Total pupal production of each type of breeding habitat.

category	number of pupae (%)	average/site
Rice fields	17 (0.9)	9
Water holes	60 (3.3)	12
Rain pools	347 (19.0)	116
Ditches/borrow pits	961 (52.5)	64
Puddles/foot prints	445 (24.3)	37
Total	1830 (100)	

A total of 1830 *An. gambiae s. l.* pupae were collected and, 358 female wing measurements were obtained from the emerged adults. No wing measurements could be made for females from the rice fields because of practical problems. The mean wing length of females collected from the water holes was not significantly different from those collected in rain pools. Females from these two categories had significantly longer wings than those from borrow pits/ditches and puddles/foot prints, which were different from each other (Table 4).

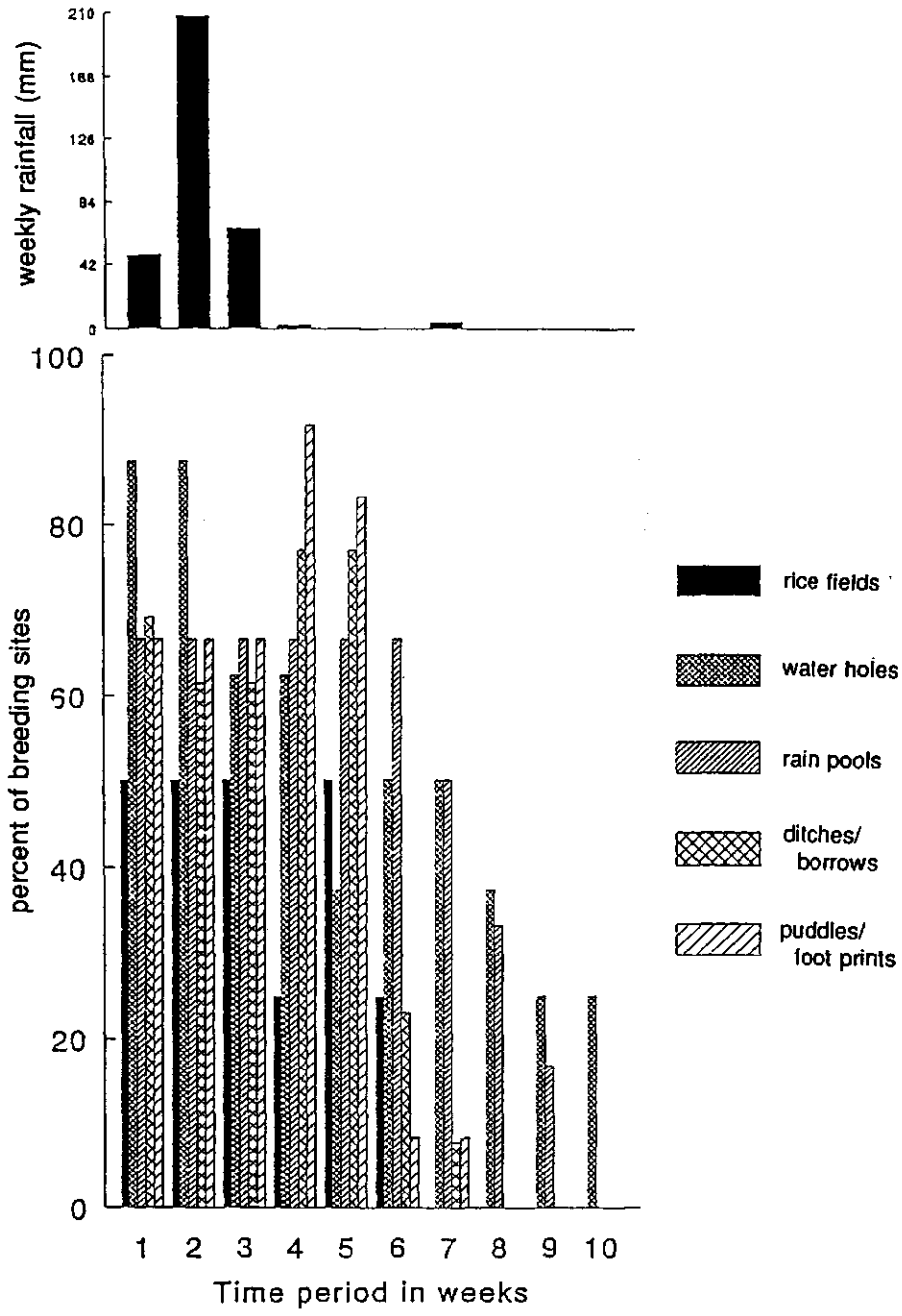

Fig. 4. Proportion of breeding sites by category found with larvae during the survey period. Also shown is the weekly total rainfall over the same period.

Correlation analysis of mean wing length with mean water temperatures gave a small but significant negative correlation coefficient (r = -0.397), df(2), P<0.05).

Natural densities

The simplified Mosquito Breeding Index (BI), Service (1976), was applied to the total immature counts, to compare population sizes between the six sites selected for detailed study.

The breeding index is given by:

$$BI = \frac{SA \times PD \times TLP}{(ND)^2}$$

where SA = surface area in square meters, PD = number of positive dips, TLP = logarithm of total number of immatures caught, and ND = total number of dips.

The two ditches and the rain pool had higher breeding indices, indicating that these sites had higher population densities (Table 5).

Table 4: Mean water temperature and mean wing length of female *An. gambiae* from different groups. Values in the same column with different letters are significantly different at P<0.001

category	mean temperature °C (range)	no. of measurements	mean wing length(mm) ± SD
Water holes	29.9 (27.0-36.0)	39	2.99 ± 0.209a
Rain pools	30.5 (24.0-38.0)	87	2.89 ± 0.189a
Ditches/burrow pits	30.5 (24.0-40.0)	140	2.80 ± 0.180b
Puddles/foot prints	31.5 (23.6-40.0)	92	2.65 ± 0.159c

Table 5: Characteristics of breeding sites selected for detailed studies.

Site	Type/size(cm)	Characteristics	Predators	BI[1] (all instars)	BI (pupae only)
4	water hole diam. 150 depth 95-145	manmade no emerging veg. hanging grass open-sunlight clay/sandy soil	tadpoles *Cx. tigripes* water beetles	0.05	0.02
6	puddle length 160-560 width 45-160 depth 1-15	tyre depression no grass some debris clay/sandy soil open-sunlight	tadpoles *Cx. spp.*	0.05	0.03
21	large ditch length 210-1000 width 95-450 depth 10-100	natural depression modified by cars grass at edges sandy soil open-sunlight	tadpoles Odonata spp Dolichopodidae adults	0.26	0.17
22	borrow pit diam. 110-190 depth 5-30	man made no vegetation sandy soil open sunlight	*Cx. tigripes* Odonata spp tadpoles	0.04	0.02
23	rain pool diam. 210-300 depth 10-40	natural depression no vegetation clay soil open sunlight	tadpoles *Cx. tigripes.* Odonata spp Dolichopodidae adults	0.16	0.09
34	large ditch length 200-635 width 91-250 depth 3-30	natural depression modified (path) grass at edges clay/cotton soil	tadpoles *Cx. tigripes.* Odonata spp Dolichopodidae adults	0.16	0.06

[1] breeding index (for explanation see text)

Table 6 shows the total number of immatures collected from the different sites during the two week period. Data from site number 6 (a puddle) and 34 (a ditch) were inconsistent and could not be analyzed further. The field experiments with floating trays gave instar durations of 1.2, 1.8, 1.5, 2.2, and 1.0 days for first, second, third, and fourth instars and pupae, respectively. The average duration from larval stage I to adult was 7.7 days. These values were assumed to represent those for wild mosquitoes in natural habitats and were used to obtain the age distribution graphs for all the other sites (Fig. 5a-d). From the distribution curves, mortality per instar was estimated following the procedure outlined by Service (1976) (Table 7 a-d). In the water hole and the rain pool sites, immatures suffered the highest mortalities at larval stage IV. The ditch site No. 21 showed a gradual increase in immature mortality reaching a maximum at the pupal stage, and only the borrow pit showed a higher mortality of larvae at stage II.

Table 6: Number of immature stages of *An. gambiae* counted in 100 dips in six selected sites

Site No.	Instar I	Instar II	Instar III	Instar IV	Pupae
4	1400	738	287	75	28
6	483	1018	310	155	93
21	1385	1421	549	61	1
22	389	280	80	37	14
23	424	301	191	70	30
34	71	423	261	37	5

Discussion

The proportion of sites within a category found with or without larvae suggests that for oviposition, females seem to select equally the smaller puddles and foot prints and the larger rain pools. The rain pools produced on average the highest number of pupae per site, but since only a few of these pools were present in the study area, they contributed less to the overall pupae production. The ditches and borrow pits contributed more to the overall production because a large number of these sites were found in the village and all of them contained larvae and pupae. The same applied to the puddles and footprints. Rice fields were the least productive.

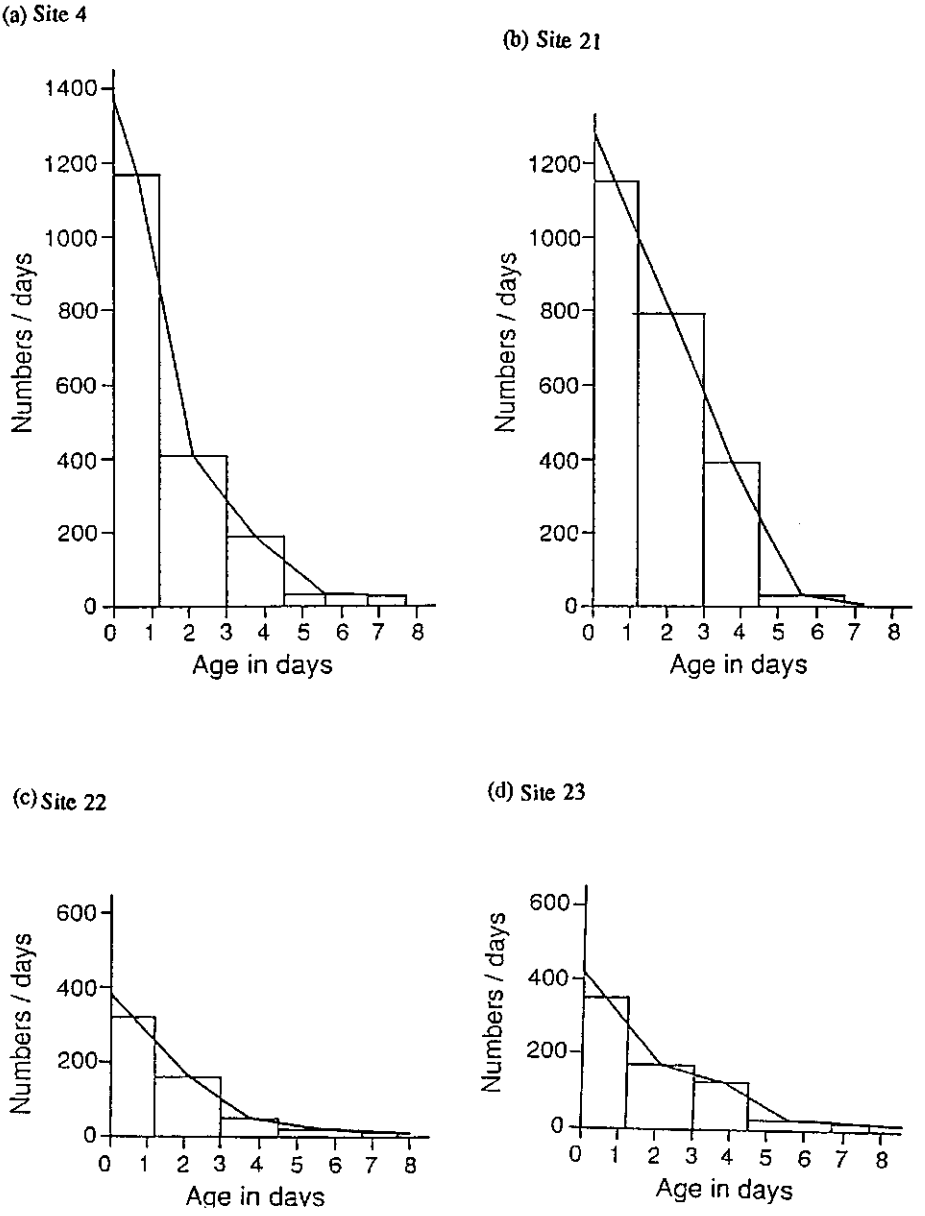

Fig. 5. Age distribution and survivorship curves for the immature stages of *An. gambiae s.l.* (a)water hole No.4, (b) ditch No.21, (c) borrow pit No.22, (d) rain pool No.23

Unlike studies elsewhere (Ejiofor & Okafor, 1985; Mukiama & Mwangi, 1989), in the Kilombero area, especially in Michenga village, there is no irrigation and the rice crop depends entirely on rainfall. Fields are planted early in the season and by the time they are flooded the rice has grown tall enough for the fields to be unsuitable for *An. gambiae* breeding. Chandler & Highton (1976) showed that *An. gambiae* does not breed in fields where the rice was more than 100 centimeters tall. For the few fields which could support breeding, mostly at the edges, larvae were probably more likely to suffer predation than in the other sites because of the diversity of aquatic fauna found in these fields, and therefore few pupae were produced. Thus, although the larger rain pools seemed to be the ideal breeding sites for *An. gambiae*, the smaller but more numerous breeding sites contributed more to the mosquito population in this area. It can be concluded that the transient water bodies such as ditches, borrow pits, puddles and footprints were the main *An. gambiae* breeding habitats in Michenga. This was also reflected in the seasonal fluctuations of adult mosquito densities in houses in this village. Higher densities occurred during the rainy season when temporary water bodies were in abundance. These results agree with those of a parallel study done in Namawala, another village in the Kilombero area (Jeroen Sytsma, MSc report, Wageningen University) and also with those of Biro (1987), who showed that temporary water bodies were more important *An. gambiae s. l.* breeding sites than rice fields and the larger, permanent water bodies.

It is well documented (Service, 1971; 1977; 1985a) that the immatures of *An. gambiae* are highly aggregated in their breeding sites and this may result in unequal sampling of instars. In the sites with gently sloping edges, for example the two ditches and the puddle (Table 5), proportionally more second than first instars were collected (Table 6). It may be that the first instars, being very small, tend to aggregate in relatively shallow areas along the edges where there is little likelihood of their being picked up by a ladle. In addition, the very short instar duration implies that they are present only briefly and are thus liable to be missed on sampling occasions. The mortality of immatures varied between stages and in different sites. On average, mortality was highest in the fourth stage, except in the borrow pit where mortality of young instars was highest. As observed in chapter 3 of this thesis, a combination of parasitism and predation may have contributed to the observed survivorship of the immatures. It is possible that whereas aquatic predators may kill all instars equally, surface predators which pick their prey from the surface of the water may select the older larger instars. An example of these are the dolichopodidae adults which were numerous in some sites in the present study. The parasites identified in dead larvae collected in one of the sites were the entomopathogenic fungi of the genus *Coelomomyces*, which normally kills older larvae and pupae (Federici, 1981). The estimated mortality of immatures in different sites was between 95% and 99%, which is similar to the range obtained in other studies for the *An. gambiae* complex in East Africa for the same types of breeding sites (Service, 1971; 1973; 1977).

Table 7: Instar mortalities of *An. gambiae s. l.* in Michenga

Water hole No.4

Instar	ti-1	Sti-1	Di	Di / Sti-1	1 - (Sti/Sti-1)$^{1/d}$
I	0	1175	223	0.190	0.161
II	1.2	952	571	0.560	0.399
III	3.0	381	206	0.541	0.405
IV	4.5	175	134	0.766	0.483
Pupae	6.7	41	9	0.220	0.220
Adults	7.7	32			

Rain pool No.23

I	0	404	146	0.361	0.312
II	1.2	258	118	0.457	0.288
III	3.0	140	50	0.357	0.255
IV	4.5	90	70	0.778	0.495
Pupae	6.7	20	2	0.100	0.100
Adults	7.7	18			

Borrow pit No. 22

I	0	373	63	0.169	0.143
II	1.2	310	207	0.668	0.458
III	3.0	103	36	0.350	0.249
IV	4.5	67	40	0.597	0.338
Pupae	6.7	27	7	0.259	0.259
Adults	7.7	20			

Ditch No. 21

I	0	1397	299	0.214	0.182
II	1.2	1098	463	0.422	0.416
III	3.0	635	357	0.562	0.423
IV	4.5	278	258	0.928	0.698
Pupae	6.7	20	20	1.000	1.000
Adults	7.7	0			

Legend: ti-1=age in days at beginning of instar,
Sti-1= No.entering instar, Di=deaths in instar,
Di/Sti-1= relative proportion dying in instar,
1-(Sti/Sti-1)$^{1/d}$ =proportion dying daily in instars
d*=instar duration in days (After Service, 1986)

Parasitism with *Coelomomyces* and other fungi, nematodes, and predation by adult dipteran, and coleopteran larvae been cited as the most important mortality factors. In the present study, dragonfly larvae, coleoptera, *Culex tigripes* larvae and dolichopodidae adults were found preying on immature mosquitoes.

Females breeding in large water bodies, for example in water holes and rain pools, had on average longer wings than those breeding in puddles or foot-prints. Several factors may have contributed to the observed differences. The water holes were deeper than the other sites (between 1-2 meters), and temperature fluctuations were more moderate than in the smaller bodies of water (Table 4). As a result of higher diurnal temperatures and wider fluctuations in the smaller types of breeding sites, larvae developed faster and eclose as small adults. The same effect of temperature on the wing length of adult mosquitoes has been reported both from laboratory studies (Hagstrum & Workman, 1971; Lyimo *et al.*, 1992) and in the field (Le Sueur & Sharp, 1991). Although temperature in the rain pools were equal to those in ditches, females emerging from the rain pools had larger wings. Rain pools with larger surface areas experienced less crowding , therefore competition for space as well as nutrients was reduced. In the puddles and footprints, crowding of immatures and competition for available food resources coupled with the high temperatures contributed to the observed smaller size of females emerging from these sites. No attempt was made to quantify the nutrient content of the breeding sites which might have affected immature development as well as size of the adults.

It is evident from this study that in the Kilombero area, the small but numerous temporary rain-fed water bodies are the main *An. gambiae* breeding sites, despite the fact that immature stages suffer high mortalities. The presence of such sites in large numbers during the rainy season ensured prolific breeding of the species. These sites also produced smaller females due to the high temperatures and crowding experienced in these sites.

Acknowledgement

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Chapter 5.

Some aspects of the adult bionomics of *Anopheles gambiae s.l.* in relation to malaria transmission in southeastern Tanzania.

Abstract

Adult females of the malaria vector *An. gambiae s.l.* were monitored for two years in a village setting. Density of females caught in light traps increased with the onset of the rainy season. The highest average number of females per light trap occurred towards the end of the rainy season in May. The females exhibited a nocturnal biting behaviour with peak biting occurring shortly after midnight and much earlier than previously described. Daily survival rate, estimated from the mean parous rate, was 0.84. The sporozoite rate was highest during the dry season. Entomological inoculation rate peaked at the same time as the peak mosquito density and the mean annual number of infective bites per person was estimated to be 548.

Introduction

The main vectors of malaria in Tanzania are species of the *Anopheles gambiae* complex and *An. funestus* (White *et al.*, 1972; Mnzava *et al.*, 1989). In the humid coastal and lacustrine areas the predominant vectors are *An. gambiae* s.s. and *An. funestus*, and malaria is holoendemic. In the dry and semi-arid areas, *An. arabiensis* predominates and malaria ranges from epidemic to hyperendemic (Mnzava & Kilama, 1986).

In the Kilombero area, southeastern part of the country, malaria is highly endemic (Freyvogel & Kihaule, 1968; Tanner, *et al.* 1986; Biro, 1987). A malaria study is being conducted in this area, aimed at defining markers for malaria transmission. Knowledge of the local malaria vectors was therefore required. The present study was undertaken as part of the major Kilombero Malaria Project to study the bionomics of malaria vectors in the area. In particular, the present study aimed at monitoring the seasonal densities, biting cycle, survival rate and sporozoite rate of *An. gambiae s.l.* in Michenga.

Materials and Methods

Study area.

The study was conducted in Michenga village, Kilombero district, southeast Tanzania. The area and its climatological features are described in detail in chapter 4 of this thesis.

Mosquito collection and processing.

Mosquito densities were monitored with standard CDC light traps hung inside bedrooms near people sleeping under bednets. The traps were run every two weeks in 38 houses from January 1990 to December 1991. All mosquitoes caught were brought back to the

Ifakara Centre laboratory, killed, sorted by species and counted. The *An. gambiae s. l.* females were kept dry on silica gel and stored at -20°C until required for sporozoite determination.

From February to August 1991, sub-samples of the *An. gambiae* females from the fortnightly light trap catches were dissected and their parity determined by the coiling or uncoiling of their ovarian tracheoles, as described by Detinova (1962). The most straightforward method of determining mosquito survival is by the mean parous rate (Davidson, 1955), i.e. the proportion of the females which have laid eggs at least once. The total number of parous females collected over several sampling occasions is divided by the total number of parous and nulliparous females in the sample to give the mean parous rate. This value then provides an estimate of the average survival rate per oviposition cycle. The method assumes that survival rate is independent of age and that all age classes are sampled equally in proportion to their relative density in the population. It is also assumed that recruitment to the population is constant, which is rarely the case.

An improved method for survival rate estimation was proposed by Birley and Rajagopalan (1981) and by Birley and Boorman (1982). The method makes the same assumptions as does the mean parous method, but in addition it assumes that daily variations in sample size are proportional to variation in the population density. The number of parous mosquitoes *(P)* in the population on any particular day t is equal to the total of nulliparous plus parous mosquitoes sampled one oviposition earlier, i.e $T_{(t-u)}$, multiplied by the survival rate S, where u is the duration of the oviposition cycle. Thus,

$$P_{(t)} = S \cdot T_{(t-u)}.$$

By treating the above equation as a linear regression through the origin, the survival rate S can be estimated. As the duration of the oviposition cycle is unknown calculations are repeated for several values of u. For each value, the residual sum of squares are calculated and presented as correlation index $R_{(u)}$. The $R_{(u)}$ ranges from 0 to 1 with maximum at u = 0, and decreases with increasing values of u. The second peak obtained across the range of values is taken as an estimate of the oviposition cycle length. Daily dissections for at least 30 days are sufficient for estimation of survival rate by this time series method. For the purpose of this study, dissections were done from 16 April to 15 May, 1991.

The biting cycle was investigated by all-night landing catches. Two huts, built about half a kilometre apart within the village, were used as catching stations. Two teams of two people each collected mosquitoes coming to bite inside the huts from 1800 to 0600 hours. Collections were done twice per week during the wet season, from March to June, 1991. Mosquitoes caught in each hour were kept separately in paper cups and brought back to the laboratory. The number of female *An. gambiae s.l.* caught each hour was recorded and dissected for parity. Wing lengths of the dissected females were measured as described by Lyimo *et al.* (1992).

Sporozoite detection in mosquitoes.

Heads and thoraxes of mosquitoes caught in the light traps were subjected to an ELISA specific for the $(NANP)_{40}$ repeat region of the circumsporozoite protein of *P. falciparum* (Campbell *et al.*, 1987). Positive controls of 1000, 500, 250, and 125 sporozoites were duplicated on the same plates, and the cut off point separating infected from uninfected samples was taken at 500 sporozoites.

Entomological inoculation rate

The entomological inoculation rate (EIR), which is the number of infective bites per man per night, was estimated as,

$$EIR = (ma) \times (sr),$$

where m is the relative density of vectors, a is the human biting habit, and sr is the sporozoite rate. The product ma was estimated from light trap catches. Lines *et al.* (1991) showed that light trap catches could be transformed to equivalent biting catches by multiplication by a factor of 1.5. In the Kilombero area the same relationship has been observed (Kilombero Malaria Project, unpublished). Therefore, the average number of mosquitoes per light trap was multiplied by a factor of 1.5 to give an equivalent man biting rate from which inoculation rates were calculated.

Results

Seasonal fluctuations

The most important man biting mosquitoes found in the area included *An. gambiae s.l.*, *An. funestus* and *Culex quinquefasciatus*. Other mosquito species caught were *An. coustani, An. squamosus, An. pharoensis, An. ziemanni,* and a number of *Aedes spp* and other *Culex spp.*

A total of 18,774 and 28,641 female *An. gambiae s. l.* were collected in 1990 and 1991 respectively. The monthly mean number of females per light trap, monthly total rainfall, and mean monthly minimum and maximum temperatures are given in Fig. 1. The number of females increased slowly at the onset of the wet season in January through March and then increased sharply to a major peak towards the end of the rainy season in May. During the dry season between June and December, the population remained at low densities.

The biting cycle

An. gambiae s. l. females started biting at 19.00 hours, and increased gradually to attain a peak between 23.00 and 02.00 hours. The largest hourly proportion of females biting was between 01.00 and 02.00 hours. Afterwards, the number of females biting decreased

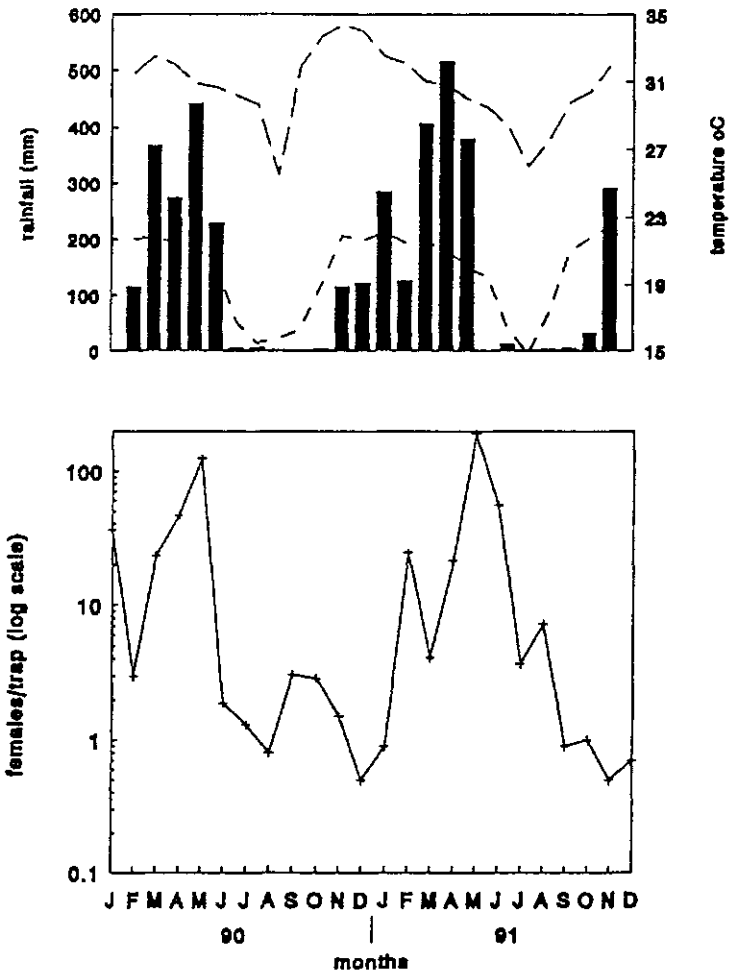

Fig. 1. Monthly mean numbers of *An. gambiae s. l.* females collected per light trap (log scale) in Michenga. Also shown is the monthly total rainfall, monthly minimum and maximum temperature.

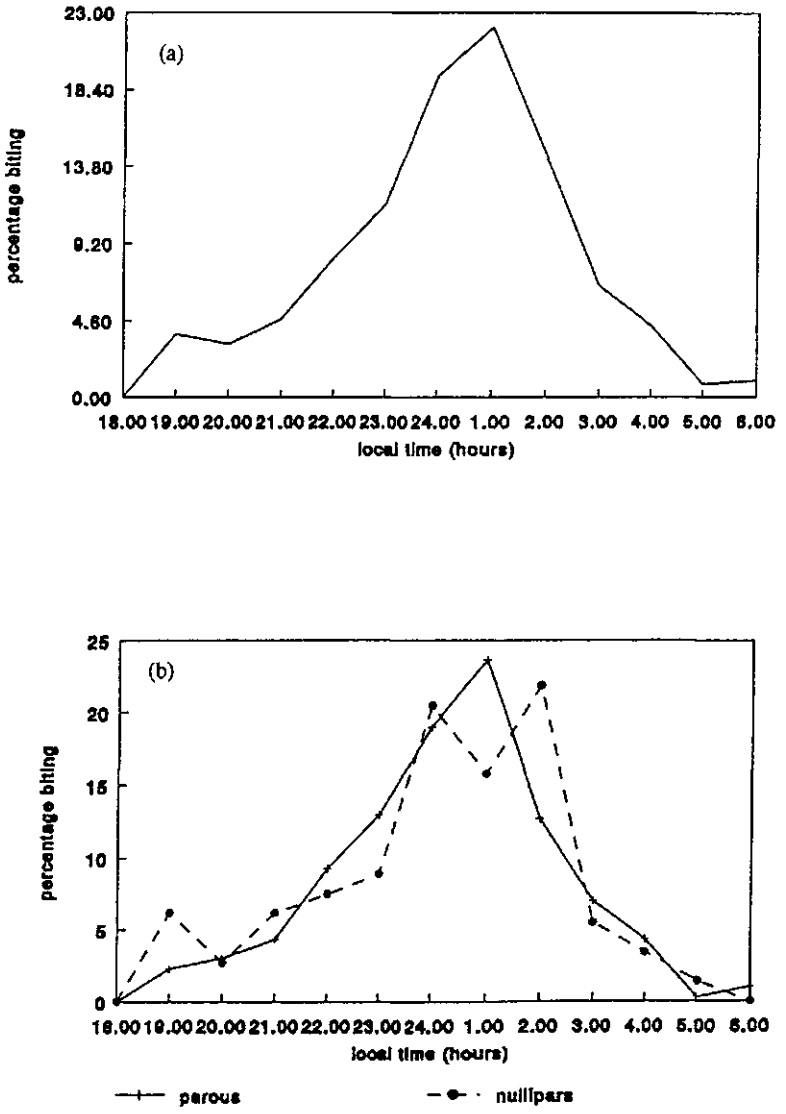

Fig. 2. Hourly biting activity of *An. gambiae s. l.* (a) total females (b) nulliparous (dashed line) and parous (solid line).

steadily to a minimum at sunrise approximately 06.00 hours (Figure 2a). When females were separated according parity, a slightly different pattern was noted. The young nulliparous females showed a low peak at 19.00 hours and two higher peaks between 23.00 and 3.00 hours. The first of the larger peaks occurred at midnight followed by a drop one hour later, and the second peak was observed at 2.00 hours. Biting activity of the parous females showed a steady increase with a peak at 1.00 hours (Figure 2b). There was no difference in the wing lengths of females caught hourly (analysis of variance, F-value = 15.53,(df=11) P>0.05).

Table 1. Estimates of survival rate from time series for *An. gambiae s.l.*

Time delay in days (u)	Survival rate (s)	Correlation index (R_u)
0	0.42	0.83
1	0.42	0.59
2	0.41	0.56
3	0.41	0.52
4	0.41	0.45
5	0.40	0.29
6	0.40	0.37

Survival rate estimations

Dissection for parity of 2810 *An. gambiae* females was done during the 1991 wet season, and fortnightly parous rates determined (Fig. 3). The parous rate fluctuated throughout the season, but parity was notably lower at the peak of the rainy season. The mean parous rate during the period of observation was 0.49.

Estimated survival values and cross correlations calculated for time-lag of 0-6 days are presented in Table 1. Significant cross correlation values were evident from day 1 to day 4 but no major peak could be identified from the series. As a result, the length of the oviposition cycle could not be estimated. Provisionally, survival rate per oviposition cycle was estimated assuming a 3-day oviposition cycle (Gillies & Wilkes, 1965), and from the

mean parous rate (0.49). The daily survival rate was therefore estimated to be 0.836. From the daily survival rate the expectation of infective life of the vector v could be estimated as:

$$v = \frac{p^n}{-\log_e p} = \frac{0.836^{10}}{-\log_e 0.836} = 0.93 \text{ days},$$

where n is duration of the extrinsic cycle of the parasite, and is taken as 10 days for *Plasmodium falciparum* (Molineaux & Gramiccia, 1980), and p is the survival rate per day (Macdonald, 1952).

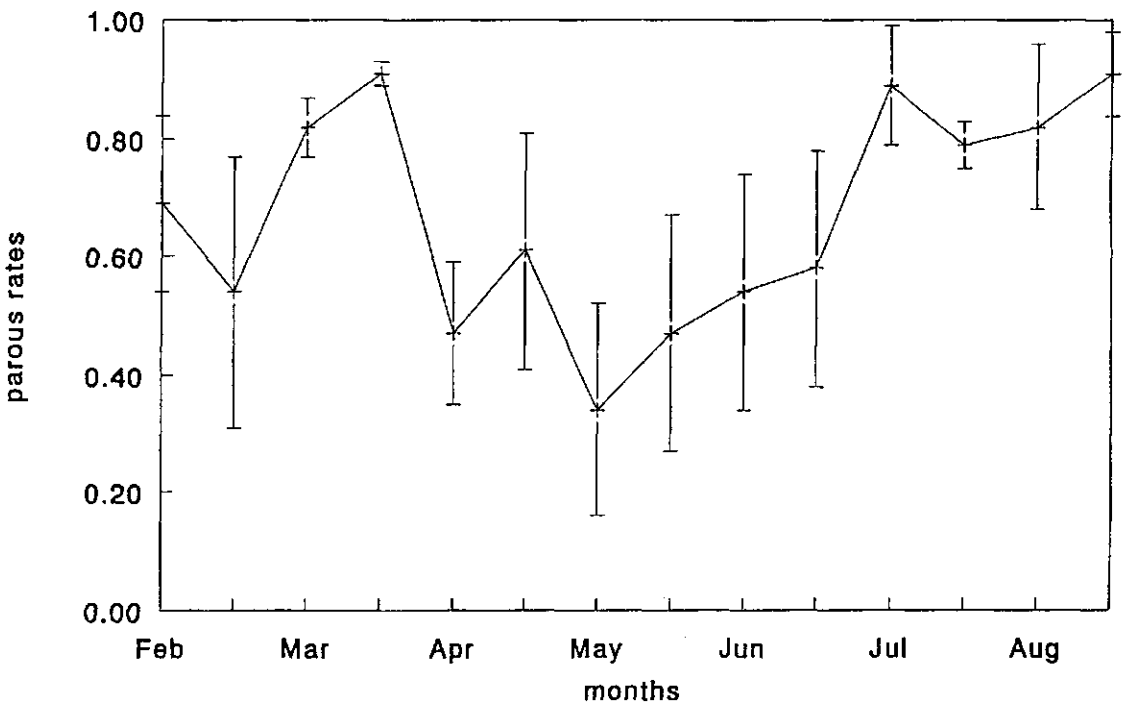

Fig. 3. Parous rates of female *An. gambiae s. l.* in Michenga. Values represent fortnightly means with standard deviations (vertical bars).

The sporozoite rate

A total of 2868 and 3200 *An. gambiae* females were processed for sporozoite determination in 1990 and 1991 respectively. The data from the two years were pooled together and the monthly sporozoite rates are shown in figure 4a. Few mosquitoes were positive for sporozoites in the first half of the year. The highest number of sporozoite positive females was observed in July and rates remained high during the dry season between July and December. The sporozoite data were analysed further to look at the seasonal relationship between sporozoite rate and mosquito density. The data were grouped into four seasons, January-March, April-June, July-September and October-December, and analysed using a logistic regression method. The sporozoite rates increased as density of mosquitoes decreased, resulting in high sporozoite rates during the dry season. The rate of increase in mosquito density also had a negative effect on the sporozoite rate (Table 2).

Table 2. Analysis of regression table for seasonal relationship between female mosquito density and sporozoite rates in *An. gambiae s.l.*

Source	Df	Chi-square	Prob.	Estimate
Intercept	1	1107.89	<0.00001	-2.8045
Density	1	12.64	<0.00001	-0.0006
Rate of Increase	1	589.02	<0.00001	-1.6747
Season	3	485.22	<0.00001	

Monthly entomological inoculation rates by *An. gambiae s. l.* (Fig. 4b), showed two peaks, an early peak in January and a major extended peak between April and July. The mean annual entomological inoculation rate for the area was estimated at 548 infective bites per person.

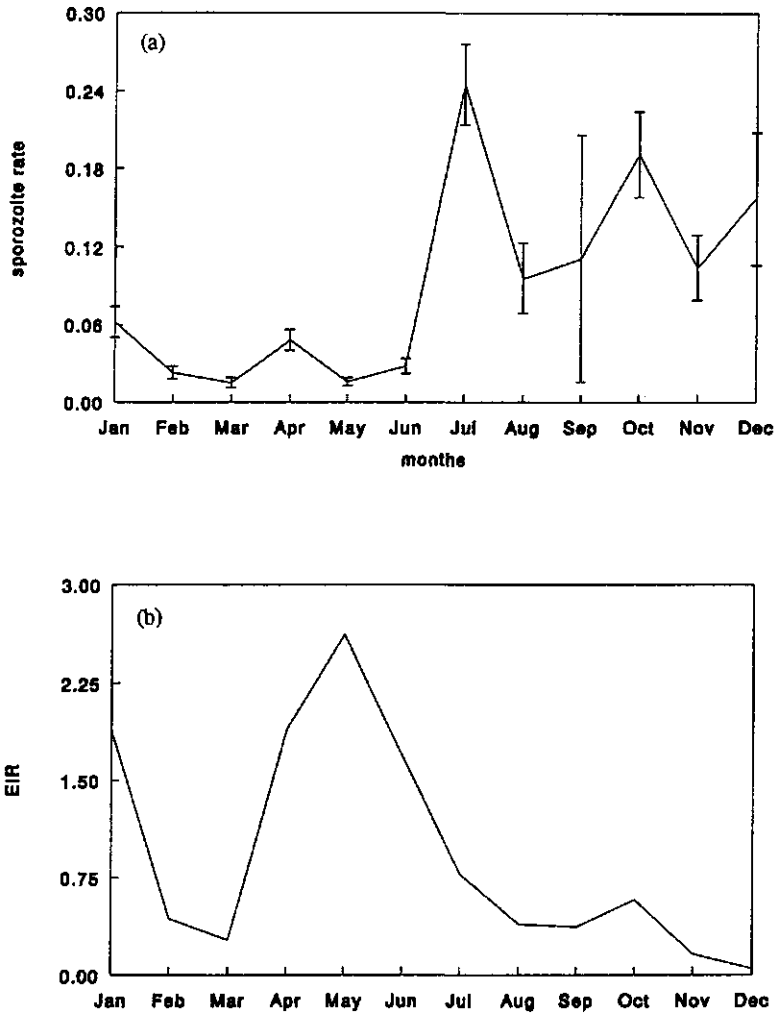

Fig. 4. The sporozoite rates (a) and entomological inoculation rates (b) in *An. gambiae s. l.* in Michenga. Value are monthly means over two years. In (a), the vertical bars denote S.E. of the means.

Discussion

Seasonal variation in density of *An gambiae s. l.* followed the rainfall pattern of the area; the short rains in November/December were followed by a small peak in density of females caught in light traps later in January, and highest densities were caught during the main peak of rainfall in May. The delay in population build up was caused solely by the instability of the breeding sites created at the onset of the rains. Because of the dryness of the soil and the irregular torrential rainfall, these sites tended to dry up before the larvae managed to complete their development, and were sometimes washed away by the rainstorms. Later in the season the soil was saturated as a result of continuous rainfall and the favoured breeding sites of the species became abundant and stable. This resulted in high numbers of emerging mosquitoes as was evident from the decrease in parous rates (Fig. 3). Density decreased sharply after the rainy season, probably due to 1) decreased emergence as temporary sites dry up, killing the immatures, and 2) a decrease in general breeding. The 'classical' density pattern observed in the present study was similar to that observed by other workers in areas free from irrigation (Haddow, 1942; White, 1974; Joshi *et al.*, 1975; Aniedu, 1992). Because *An. gambiae* is almost entirely a temporary rain-pool breeder, it depends on the local pattern of rainfall for breeding. During the dry season the population was maintained at lower densities mainly because breeding was limited to the large permanent bodies of water such as water holes and small sites created around swamps. These types of breeding sites are known to be less favourable sites for *An. gambiae* and survival of immatures is reduced (Christie, 1959).

Studies on the biting activity of *An. gambiae* females showed that this mosquito is a nocturnal feeder, and any biting before sunset and after sunrise was negligible (Haddow & Ssenkubuge, 1973). The peak biting time varied widely and sometimes different patterns were observed on subsequent nights. The findings of the present study agree with other studies conducted in East Africa which showed that biting was intense in the hours after midnight (Gillies, 1957; Smith, 1961; Chandler *et al.*, 1975). However, the peak time of biting was much earlier than previously described. Previous studies, except those of Gillies, involved other members of the complex, probably *An. quadriannulatus* and *An. arabiensis* (Zahar, 1985), and these members may differ in their biting activity. However, as the present study collected data during a single season, the observed activity should not be regarded as the 'typical' pattern and more intensive studies with collections done both indoors and outdoors should be undertaken. Moreover, the exact knowledge of the biting period is of interest especially now that the area is planned for control trials using insecticide impregnated bednets, which aim at protecting people while sleeping. The pattern observed between nulliparous and parous females is of interest and needs further investigation. We speculate that the different biting pattern between nulliparous and the parous females is caused by part of the nulliparous females resting indoors, (which include

those failing to feed the previous night and the pre-gravid proportion taking a second meal). These constitute the early biting peak of the nulliparous, whereas the later peak is from those emigrating from outdoors. The parous females do not show this early peak because they enter the houses later, i.e. after oviposition.

The data collected for time series analysis were incoherent, indicating that the population at the time of sampling was unstable. This discrepancy was probably due to a number of reasons. The population could have been dominated by day to day disturbances with variable recruitment. Our sampling method could have introduced a sampling bias. For example trap position could result in samples of different age structure depending on whether they were close to or far from a breeding site (Charlwood *et al.* 1985). It is possible that our samples had a deficit of parous females thus giving a low estimate of the survival rate. The pre-gravid individuals could not be distinguished by the method used to determine parity. These were included in the nulliparous group. Differential dispersal in different age groups could also be a source of error.

The mean parous rate method is often claimed to be inadequate in estimating the survival rate because it assumes constant recruitment. Normally there are considerable fluctuations in recruitment, and the mean parous rate therefore overestimates the survival rate. However, as sampling was extended through the season, the mean parous rate was found sufficient for estimating the survival rate in this study. The daily survival rate obtained here was much lower than those reported for *An. gambiae* in the dry season by other workers in Tanzania (Gillies & Wilkes, 1965; Garrett-Jones *et al.*, 1972). Possibly, survival of *An. gambiae* may differ between the dry and the wet season. The parous rates (Fig. 3) suggest such a possibility, being higher in the dry season. Nevertheless, confirmation of this phenomenon is necessary.

There was a strong seasonal effect on sporozoite rates, with highest sporozoite rates being observed during the dry season. This difference was caused by the difference in growth rates of the population. During the wet season, the population was composed of young females which were more likely to be sporozoite negative, whereas the dry season population had older females with higher possibility of being infected. This concept is also supported by the observed effects of density of females as well as the growth rate of the population, as sporozoite rates decreased with increased density or increased growth rate of the population. However, the presence of infected mosquitoes throughout the year means that in this area *An. gambiae* maintains perennial transmission of malaria parasites (Fig. 4)

Although higher sporozoite rates were observed during the dry season, the EIR did not follow the same pattern. The probability of getting infective bites was higher towards the end of the rainy season in May and June. This was caused by the large numbers of females biting during this time of the year. In Michenga village, the maximum number of bites per person per night remains above 100 during the rainy season, thus the chances of

being infected were higher compared to the dry season. No data on survival of *An. gambiae s.l.* were available for the dry season, but the persistence of the vector throughout the year and the high inoculation rate observed may account for the non-seasonality and high endemicity of malaria in the area.

Acknowledgements

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Chapter 6.

Seasonal variation in adult wing length of *Anopheles gambiae s.l.*

Abstract

Populations of *Anopheles gambiae s.l.* from three localities in two districts in Tanzania were studied to determine the extent of size variation in adult females. The mean wing lengths between the three populations were significantly different and showed a high degree of variation with coefficients of variation between 18% and 30%. In the two localities where seasonal changes were studied, the mean wing lengths varied seasonally and were negatively correlated with air temperature.

Introduction

Many species of mosquito show considerable variation in adult size. This variation may reflect larval conditions during development, which depends on the type of larval habitat of the mosquitoes (Reisen *et al.*, 1984; Fish, 1985; Haramis, 1985; Nasci, 1986b). In general those that breed in temporary habitats are subject to greater stress such as crowding, insufficient food and relatively high temperatures, and show a larger amount of variation in adult size than insects occupying more permanent habitats (Haramis, 1983; 1985; Fish, 1985; Nasci, 1987).

Members of the *Anopheles gambiae* complex, the malaria vector in most of sub-Saharan Africa, breed in different kinds of habitat, but mostly in temporary small pools. For such a mosquito a wide variation in adult size is expected, and Gillies and De Meillon (1968) reported the range of wing length for this species to be 2.8-4.4 mm. Since their time no studies have looked at size variation in this mosquito. Recently, Lyimo *et al.* (1992) showed that in a laboratory colony the adult body size of *An. gambiae* was affected by the temperature and density at which the larvae were reared. In the field, *An. gambiae s. l.* breeding sites change seasonally depending on the rainfall (Aniedu, 1992; chapter 4 of this thesis). This means, therefore, that different populations experience diverse environmental constraints and might show variation in adult size. For this reason and for the purpose of the present thesis, this study was carried out to examine the extent and pattern of adult size variation in field populations of *An. gambiae s. l.*

Materials and Methods

Mosquitoes were collected from three villages in two districts of mainland Tanzania. The main part of the study was carried out in Michenga and Namawala villages, (about 36 km apart) in Kilombero district, south eastern Tanzania. Here, the main rainy season extends from March to May and the lesser one from November to December. The mean annual

temperature is 26°C, with cooler months between June and September and warmer months between October and May. Meteorological data for Kilombero were obtained from the Kilombero Agricultural Training Institute, which is situated adjacent to Michenga village.

Additional mosquitoes were collected from Kisiwani village in Muheza district. Muheza is about 600 km from Ifakara town in north eastern Tanzania. This area experiences similar weather conditions with mean temperature of 26 °C and annual rainfall of 925 mm, with a perennial high humidity. The rainy season extends from December to May (White *et al.*, 1972).

Host seeking females were collected by light-traps inside houses from January to December 1990 in Namawala and from January 1990 to December 1991 in Michenga. Catches were made every two weeks in 30 houses in each village from 22.00 to 02.00 h for the first six months. In later collections 10 houses were sampled from 20.00 to 06.00 h fortnightly. At Kisiwani, single collections were carried out in 10 houses between April 15 and 21, 1990. The mosquitoes were sorted to species and stored on silica gel until required for measurement. From the monthly catches, 100 *An.gambiae s. l.* females were randomly sub-sampled when mosquito densities were high or all *An. gambiae* females were processed when mosquito densities were low. One wing of each specimen was removed and mounted onto a clean glass slide. The wing length was then measured from the distal end of the alula to the tip, excluding the fringe scales, using a dissecting microscope fitted with a camera lucida. Wing length was chosen as a unit of comparison because wing length correlates positively with dry body weight in many mosquitoes including *An. gambiae* (Christophers, 1960; Haramis, 1983; chapter 2 of this thesis).

Analysis of variance (ANOVA) was used to detect differences in mean wing lengths among populations. Size variation between populations were compared by coefficient of variation (CV) and G_i, (a measure of skewness) was used to detect any departure from normality within the populations. Collections from the month of April 1990 were used for spacial size comparison of the three populations, while seasonal variations were analysed for Namawala and Michenga populations only.

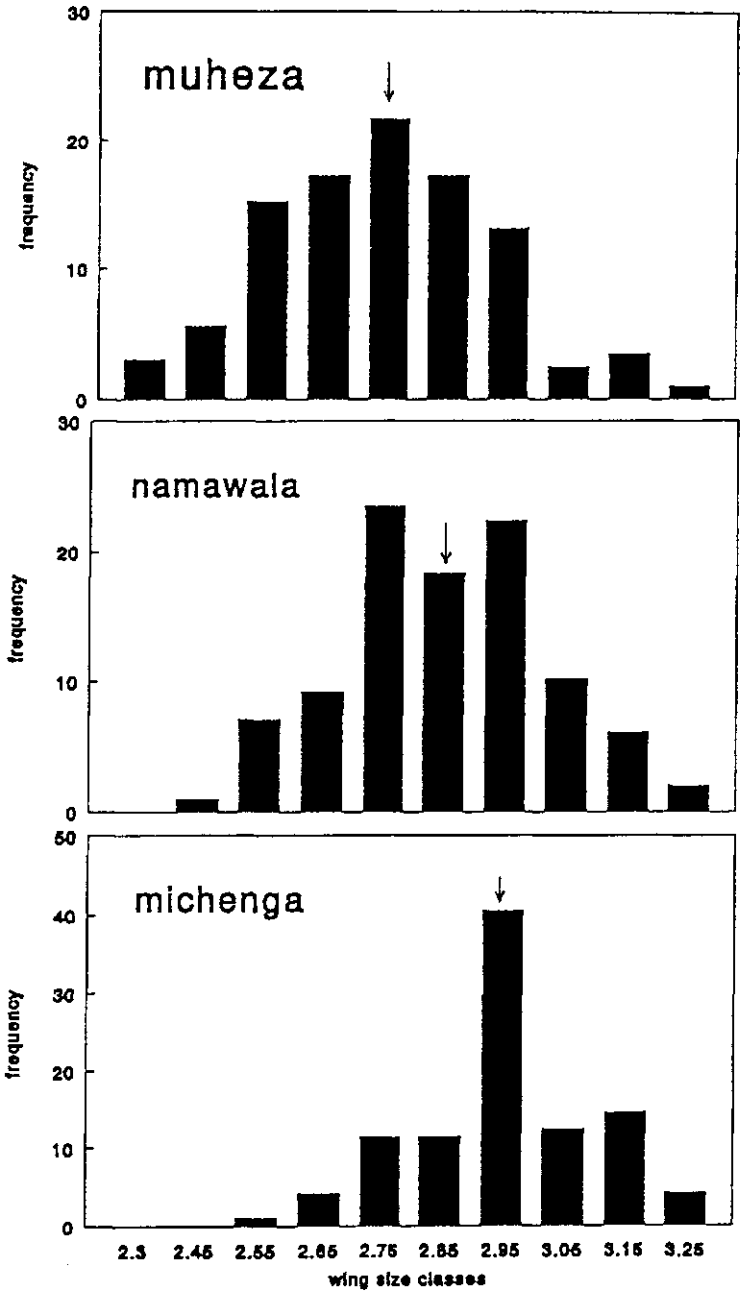

Figure 1. Wing length frequency distributions for female *An. gambiae s.l.* collected from three different localities between 15 and 21 April, 1990. Arrows indicate location of the mean.

Results

The wing lengths of the females ranged from 2.17 mm to 3.68 mm with means between 2.70 mm and 2.98 mm. The mean wing lengths of females collected at the same period in April from the three different localities were significantly different (ANOVA test, F = 48.63, P<0.0001) (Fig. 1, Table 1). Females collected in Muheza were smaller and more variable than those collected in the two villages in Kilombero. The coefficients of variation for the three populations were high. The wing size distribution of mosquitoes collected in Muheza was positively skewed, while that of Michenga and Namawala showed a negative skewness. However, the G^1 values of the three populations were not significant. Females collected from Michenga in April 1990 were not significantly different in wing size from those collected the same month in 1991.

Table 1: Summary of ANOVA for size of female *An. gambiae s. l.* collected in April, 1990 from three different localities. [1] G_i not significant at P=0.05. Column figures with different letters are significantly different (P<0.001)

Locality/year	N	winglength (mm) mean ± SD	Coefficient of variation	Gi
Muheza/ 1990	100	2.70 ± 0.20a	30.57	0.1613 ns[1]
Namawala/1990	100	2.84 ± 0.18b	23.53	-0.1135 ns
Michenga/1990	100	2.93 ± 0.17c	21.31	-0.1879 ns
Michenga/1991	100	2.98 ± 0.16c	18.16	-0.2417 ns

Table 2 summarises the monthly variations in wing sizes for Michenga and Namawala populations. The monthly mean wing lengths of the females showed minor fluctuations from January to June, increased to a maximum in August and decreased again from September to December, and were negatively correlated to the monthly minimum and maximum air temperatures (Fig. 2, Table 3). Rainfall seems to have no apparent effect on the size of the mosquitoes.

Table 2. Summary of the variation in monthly mean wing lengths for An. gambiae s. l. collected in two villages in Kilombero district.

Village	Month/year	n	wing length ± sd (mm)	C.V	G_i
Namawala	January/90	100	2.91 ± 0.27	22.58	-0.934
	February/90	100	2.83 ± 0.31	23.35	0.012
	March/90	100	2.90 ± 0.24	18.84	-0.211
	April/90	100	2.84 ± 0.18	23.53	0.114
	May/90	100	2.90 ± 0.42	24.84	-0.107
	June/90	100	2.93 ± 0.35	22.45	0.207
	July/90	100	2.97 ± 0.35	21.12	0.106
	August/90	100	3.04 ± 0.31	18.38	-0.210
	September/90	100	2.97 ± 0.27	18.63	-0.247
	October/90	100	2.90 ± 0.30	21.31	-0.370
	November/90	100	2.93 ± 0.34	22.92	0.018
	December/90	100	2.91 ± 0.34	22.46	-0.047
Michenga	January/90	100	2.95 ± 0.18	21.29	-0.257
	February/90	70	2.91 ± 0.38	20.94	-0.251
	March/90	100	2.87 ± 0.17	22.23	-0.158
	April/90	100	2.93 ± 0.17	21.31	-0.188
	May/90	58	2.92 ± 0.46	19.30	-0.059
	June/90	100	2.82 ± 0.20	26.66	0.413
	July/90	100	2.89 ± 0.46	21.20	0.033
	August/90	72	3.02 ± 0.23	25.84	-0.306
	October/90	100	2.83 ± 0.30	22.24	0.368
	November/90	100	2.86 ± 0.31	24.49	-0.367
	December/90	27	2.78 ± 0.16	22.46	0.602
	January/91	100	2.89 ± 0.17	21.84	-0.309
	February/91	100	2.92 ± 0.19	22.40	-0.180
	March/91	100	2.73 ± 0.16	24.67	-0.444
	April/91	100	2.98 ± 0.16	18.16	-0.242
	May/91	100	2.97 ± 0.17	19.08	0.187
	June/91	100	2.90 ± 0.31	22.22	-0.169
	July/91	100	2.96 ± 0.21	23.95	-0.091
	August/91	100	3.08 ± 0.20	20.49	-0.091
	September/91	20	2.89 ± 0.12	21.43	-1.106
	October/91	17	2.80 ± 0.17	nd	nd
	November/91	14	2.83 ± 0.12	nd	nd
	December/91	9	2.93 ± 0.02	nd	nd

nd = not done (sample too small)

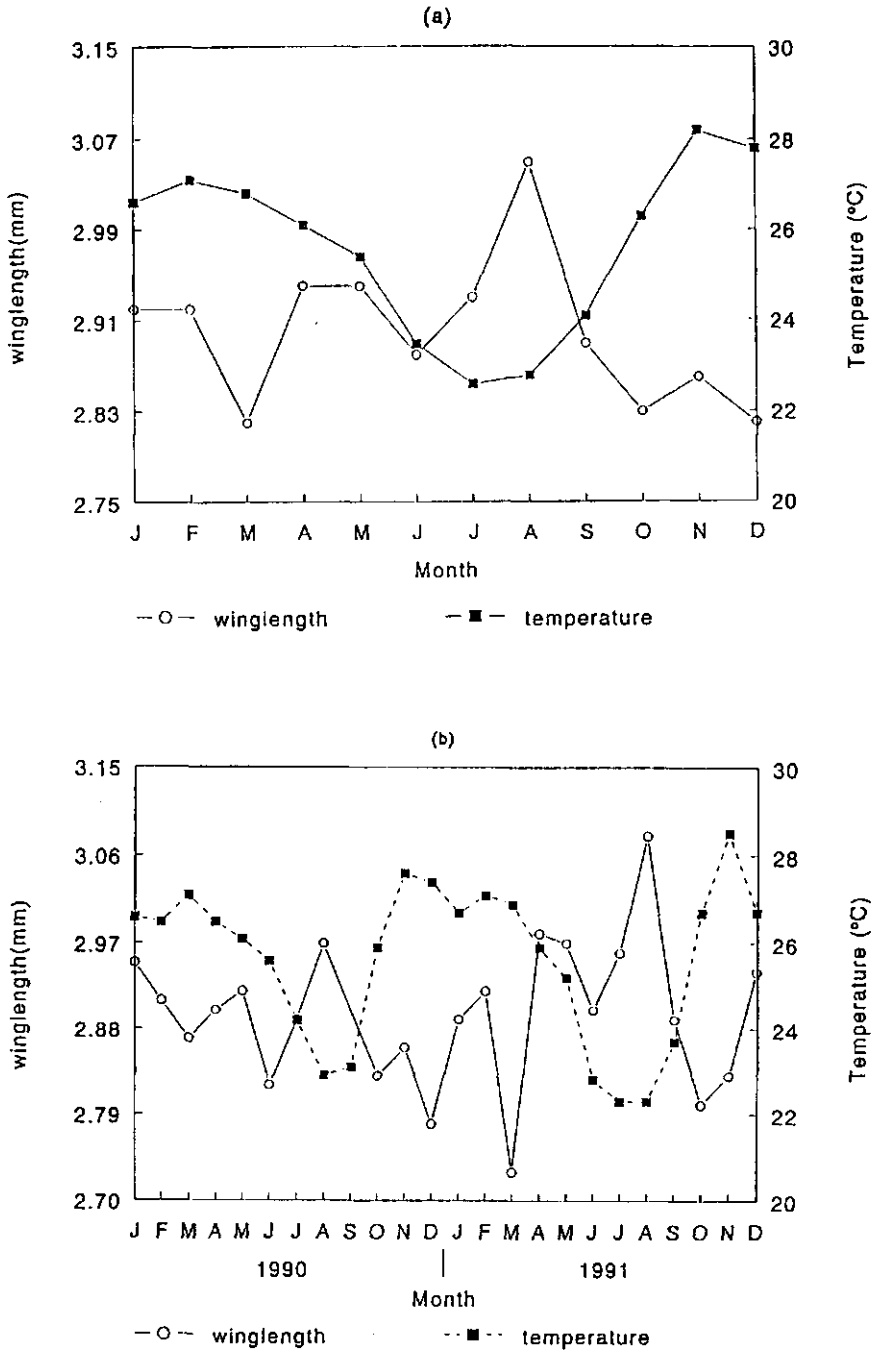

Fig. 2. Relationship between monthly mean wing lengths of female *An. gambiae s.l.* and monthly mean air temperatures for (a) Namawala, January through December 1990 and (b) Michenga, January 1990 through December 1991.

Table 3. Summary of the correlation analysis of monthly minimum and maximum temperatures and rainfall on monthly mean wing lengths

Source	df	sum of squares	F ratio	Prob>F	Slope
Min.temp.	1	0.2291	5.412	0.0201	-0.008
max.temp	1	0.9035	21.339	<0.0001	-0.026
mean rain	1	0.0598	1.411	0.2350	-0.002

Discussion

The adult females collected from the three different localities varied significantly in size. In all three localities the coefficient of variation was high, between 18% and 30%. This was as expected for a species like *An. gambiae* which breeds in a variety of habitats, and is therefore subjected to different stress conditions during its development. The April populations from the three localities showed a nonsignificant degree of departure from normality. However, the observed trends could vary from time to time as was observed in the monthly variations for the Michenga and Namawala populations. As *An. gambiae s.l.* breeds in temporary rain pools as well as in larger habitats such as water holes and ponds (Service, 1976; Gillies & Coetzee, 1987; chapter 4 in this thesis), wide variations in microhabitats of the immatures is expected, which in turn result in diverse adult populations as was observed in the present study.

The monthly mean wing lengths of female *An. gambiae* varied with air temperatures and were negatively correlated. The association observed between mean wing length and temperature in the present study was similar to that observed by Le Sueur and Sharp (1991) in *An merus* and by Bock and Milby (1981) in *Cx. tarsalis* as well as Day *et al.* (1990) in *Cx. nigripalpus,* who showed that wing size decreased with increasing temperatures. This is probably caused by increased metabolic requirement over food procurement by the larvae during the hot months. Among other things, the size of an emerging adult depends on the amount and quality of food (Carpenter, 1983). Food availability and acquisition may vary in different habitats depending on the degree of competition. As *An. gambiae* breeds mostly in temporary rain pools, these habitats tend to dry quickly and concentrate mosquito larvae. The intense competition for food and space resulting from this concentration leads to emergence of individually variable adults (Day *et al.*, 1990).

The mean wing lengths of females collected from the two Kilombero villages fluctuated widely during the rainy season, although rain itself seemed to have no effect on the mean size of the mosquitoes. At most, the rain affected the size of the adults indirectly by creating variable breeding sites, and as was observed in chapter 4 of the present thesis, these different sites produce differently sized females. During the rainy season small as well as large breeding sites are available and as a result there is more variation in adult size, but on the average females are smaller. Whereas, in the dry season most adults emerge from larger sites, and the lower water temperature during this time reduces larval developmental time. This means more time for feeding. Survival of mosquito immatures in the larger sites is low (Christie, 1959), so few larvae with less competition, which give rise to larger adults. These studies show that there is a wide variation in adult size in *An. gambiae* and that this variation is caused by different enviromental factors experienced by the immature stages during development. Temperature, and competition for nutrients and space may be the main contributors to this variability.

Acknowledgements

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Part III. EFFECTS OF ADULT SIZE ON FEMALE CHARACTERISTICS

Chapter 7.

Effects of adult body size on fecundity and the pre-gravid rate of *Anopheles gambiae s. l.* females in Tanzania.

Abstract

The influence of adult body size on the pre-gravid state and fecundity was studied in *Anopheles gambiae* females hand caught inside houses and virgin females collected as pupae. Blood fed mosquitoes were kept for two to three days before dissection. Those females which did not develop eggs were classified as pre-gravid, and examined for insemination. The number of mature eggs in those mosquitoes which became gravid were counted. Virgin females were blood fed and kept for egg maturation in the laboratory. Wing lengths of females were measured to determine the size of the mosquitoes. The overall pre-gravid rate in the resting *An. gambiae* population was found to be 21% and, of these, 66% had been inseminated. In the newly emerged females pre-gravid rate was 92.6%. The mean wing length of wild females which became gravid was significantly larger than those which remained pre-gravid. There was a positive correlation between fecundity and wing length. Smaller females tended to require two or three blood meals to facilitate completion of the first gonotrophic cycle. The critical size permitting oviposition from the first bloodmeal was a wing length of 3.00 mm.

Introduction

The tendency for part of mosquito populations of different species to take more than one blood meal in their first oviposition cycle has been reported by many workers. The cause of this tendency has not been well established and is believed to be associated with non-insemination of the females (Roy, 1940; Rao, 1947; Jaswant & Mohan, 1951). Gillies (1954a) termed this condition 'pre-gravid' and the proportion of such females in a population, the pre-gravid rate.

As regards members of the *An. gambiae* complex in Africa, Muirhead-Thomson (1948) found that a single bloodmeal was sufficient for egg maturation in all fertilised *An. melas* Theobald around Lagos, Nigeria. Gillies (1954b, 1955) working in Muheza, Tanzania, observed that a proportion of the blood fed *An. gambiae* females could not produce mature eggs after a single meal, and of this proportion, 26% were fertilised. Similarly in Burkina Faso, Adam *et al.* (1960) found that the majority of pre-gravid anophelines were not inseminated. In contrast, Hocking & MacInnes (1948) mentioned a tendency for fertilised female *An. gambiae* and *An. funestus* Giles to take multiple blood meals before they could produce their first batch of mature eggs.

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It seems unlikely, therefore, that non-insemination is the only explanation of pre-gravidness. Reasons why some inseminated female mosquitoes ingest multiple bloodmeals in a single gonotrophic cycle must be attributed to other factors.

Laboratory studies have shown that small adult female mosquitoes from larvae stressed during development may not develop eggs after their first bloodmeal (Reisen, 1975). Briegel (1990) working with four *Anopheles* species including *An. gambiae*, observed that there was a threshold of about 1.5 calories of ingested blood needed for initiation of oogenesis. He also noted in *An. albimanus* Wiedemann and *An. gambiae*, that females which started egg maturation after one bloodmeal were significantly larger than those which did not.

A number of studies have shown that the number of eggs produced by a female mosquito is related to its size (Bock & Milby, 1981; Steinwascher, 1982; Packer & Corbet, 1989). Reisen (1975) compared *An. stephensi* Liston adults from larvae cultured at different densities, and found that larger females produced more eggs. Also in the laboratory, Briegel (1990) reported a positive correlation of female size with fecundity of *Anopheles spp.* and Akoh *et al.* (1992) found the same for *Culex quinquefasciatus* Say.

This paper reports the results of laboratory studies on the relationships between female adult body size and the tendency to take multiple bloodmeals before the first oviposition. We have also investigated the relationship between body size and fecundity in a natural population of *An. gambiae s. l.* in rural Tanzania.

Materials and Methods

Study area.

The study was carried out in Michenga village, Kilombero district, southeastern Tanzania. The village lies in the Kilombero river plain at an average altitude of 270m. The area has two rainy seasons, the main season extending from March through May and the lesser one occurring in November. Annual rainfall averages 1200 mm with an average temperature of 26 °C. During the main rainy seasons much of the village is flooded, providing ample breeding sites for *An. gambiae* and *An. funestus*, the important malaria vectors. Consequently malaria is holoendemic, the prevalence of parasitaemia in 0-4 year olds being around 80%.

Mosquito collection and processing

Indoor resting mosquitoes

Blood fed mosquitoes were collected by hand inside houses using aspirators and torches. Weekly collections were conducted from 28th January to 6th March and 24th April to 26th June 1991. Mosquitoes were brought back to the laboratory, immobilised by cooling,

sorted by species and their abdominal condition noted. Only fully bloodfed *An. gambiae s. l.* females were selected for further processing. These females were kept individually in vials (6 cm x 2 cm diameter) with a wet filter paper at the bottom of the vial to facilitate oviposition and a cotton wick soaked in 10% glucose as the sugar source at the top. Vials were kept in a constant temperature chamber at 27°C and relative humidity of 90% for 2 days. Eggs laid during this period were counted under a dissecting microscope. All the females were dissected on the third day and any retained eggs were also counted. Those females which did not develop eggs, and were found to be nulliparous on dissection, were classified as pre-gravid and the stage of follicle development was noted. The spermatheca of each female mosquito was examined for insemination. One wing of each *An. gambiae s. l.* female was glued onto a glass slide and the length measured from the distal end of the allula to the wing tip, excluding the fringe scales, using a dissecting microscope fitted with a camera lucida.

Newly emerged female mosquitoes

Pupae were collected from field sites over a period of three weeks towards the end of the main rainy season. The adult mosquitoes were allowed to emerge in the laboratory at the Ifakara Centre and the virgin females were offered a human bloodmeal on the second day after emergence. Fully fed *An. gambiae s. l.* females were kept individually as in the first study. After two days gravid females were dissected and the number of mature eggs counted. The non-gravid females were offered a second bloodmeal and kept for another two days. The same procedure was repeated for the third meal. Female wing lengths were measured as described above.

Results

The indoor resting mosquitoes.

For the 852 indoor-resting *An. gambiae s. l.* females assessed, the mean wing length was 2.92 mm (SD=0.006), range 2.34 mm - 3.62 mm. The proportion of females which became gravid after blood feeding was 78.7%, leaving the overall pre-gravid rate as 21.3%. The proportion of pre-gravid females fluctuated widely over the study period with relatively high pre-gravid rates during April-June after the onset of the main rainy season in March (Table 1). However, the pre-gravid rate and total rainfall the same week or one week before were not significantly correlated.

Table 1: Rainfall and weekly catches of blood fed *An.gambiae s.l* collected resting indoors in Michenga. Also shown are the proportions which became gravid and those remaining pre-gravid

Week after January 1	Weekly rainfall(mm)	Number collected	Number gravid (%)	Number pre-gravid (%)
5 (January)	52.6	122	115 (94.3)	7 (5.7)
6 (February)	117.2	38	32 (84.2)	6 (15.8)
7	5.4	58	54 (93.1)	4 (6.9)
8	5.8	69	64 (92.8)	5 (7.2)
9	40.1	35	32 (91.4)	3 (8.6)
10 (March)	0.0	24	24 (100.0)	0 (0.0)
17 (April)	17.3	69	50 (72.5)	19 (27.5)
18 (May)	137.5	33	31 (93.9)	2 (6.1)
19	96.2	142	45 (31.7)	97 (68.3)
20	84.3	51	42 (82.4)	9 (17.6)
21	60.5	48	38 (79.2)	10 (20.8)
22	0.0	49	39 (79.6)	10 (20.4)
23 (June)	0.0	57	47 (82.5)	10 (17.5)
25	0.0	36	35 (97.2)	1 (2.8)
26	0.0	30	30 (100.0)	0 (0.0)
Total	529.9	861	678	183

The wing lengths were classified into 10 size classes and the proportional distributions of the gravid and the pre-gravid females are presented in Figure 1. The mean wing length of females which became gravid was significantly larger (2.93 mm, S.E.= 0.007) than that of the pre-gravid females (2.87 mm, S.E.=0.0.013); t = 16.05, P<0.001, 850 d.f. (Table 2). Variations in wing length over the weeks followed the same pattern, with pre-gravids having consistently shorter wings than the gravids (Figure 2).

The insemination rate of the pregravid females of *An. gambiae s. l.*, assessed by examination of the spermathecae for the presence of sperm, was 73/111 (63.7%) inseminated. All pre-gravid females had follicles at the resting stage, with a few yolk granules deposited around the oocyte nucleus i.e Christophers' stage IIb (Clements, 1963).

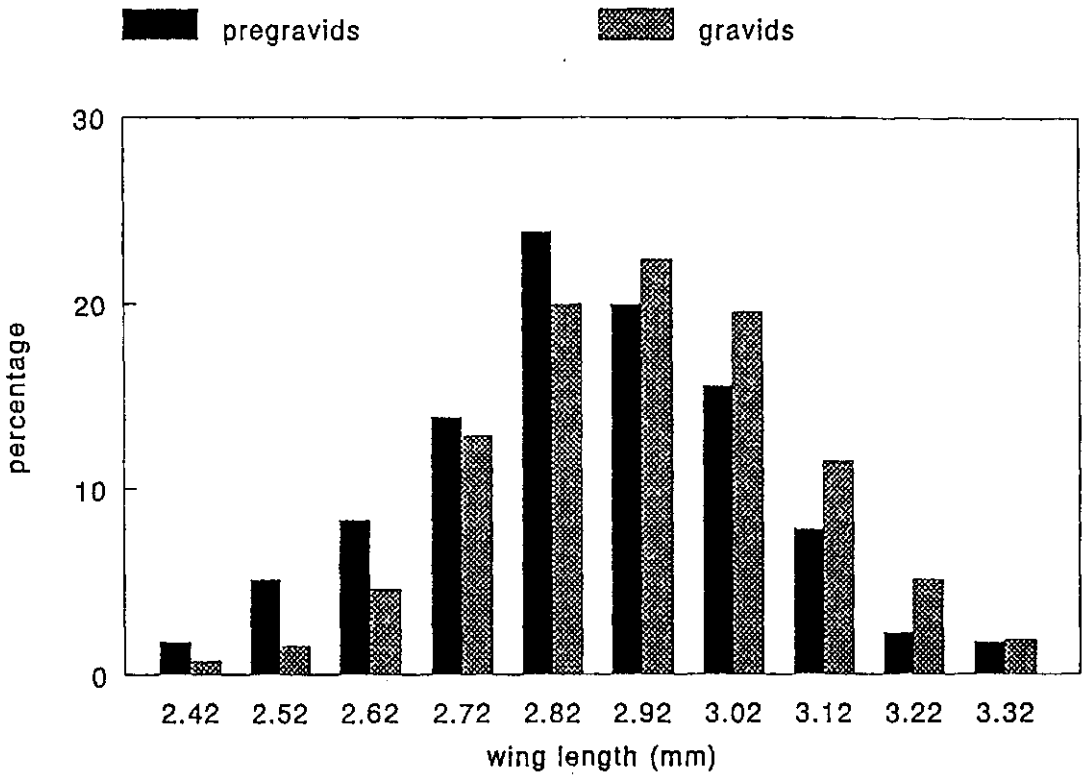

Fig. 1. Frequency distribution of wing size classes for the gravid and pre-gravid females of the indoor resting population of *Anopheles gambiae s.l.*

Table 2: Mean wing length of gravid and pre-gravid *An. gambiae s.l.* in the resting and emerging populations. Different letters in the same column designate significant differences (P<0.01; t-test)

	Resting population		Emerging population	
State	n	wing length(mm) mean ± S.E	n	wing length(mm) mean ± S.E
Gravid	672	2.93 ± 0.007a	6	3.18 ± 0.051a
Pregravid	180	2.87 ± 0.013b	72	2.83 ± 0.025b
Total	852		78	

Among the *An. gambiae s. l.* collected from indoor resting sites, the mean number of mature eggs per gravid female was 150 (range 66 - 290). Spearman rank correlation analysis between the number of mature eggs and the wing length of the females showed a significant positive relationship, with a correlation coefficient r = 0.518, (F-value =143.09, P<0.0001). The regression equation is Y = 113.93X-187 (Figure 3).

The newly emerged mosquitoes.

A total of 136 females of *An. gambiae s. l.* emerged successfully from 264 pupae collected. Their mean wing length was 2.84 ± 0.013 mm and, after blood feeding, the overall pre-gravid rate was 92%. As shown in Table 2, the mean wing length of the females which became gravid after one bloodmeal was significantly larger than of those which did not (t = 10.24, p<0.001,76 d.f.). The mean number of mature eggs per gravid female was 111 (range 48-178).

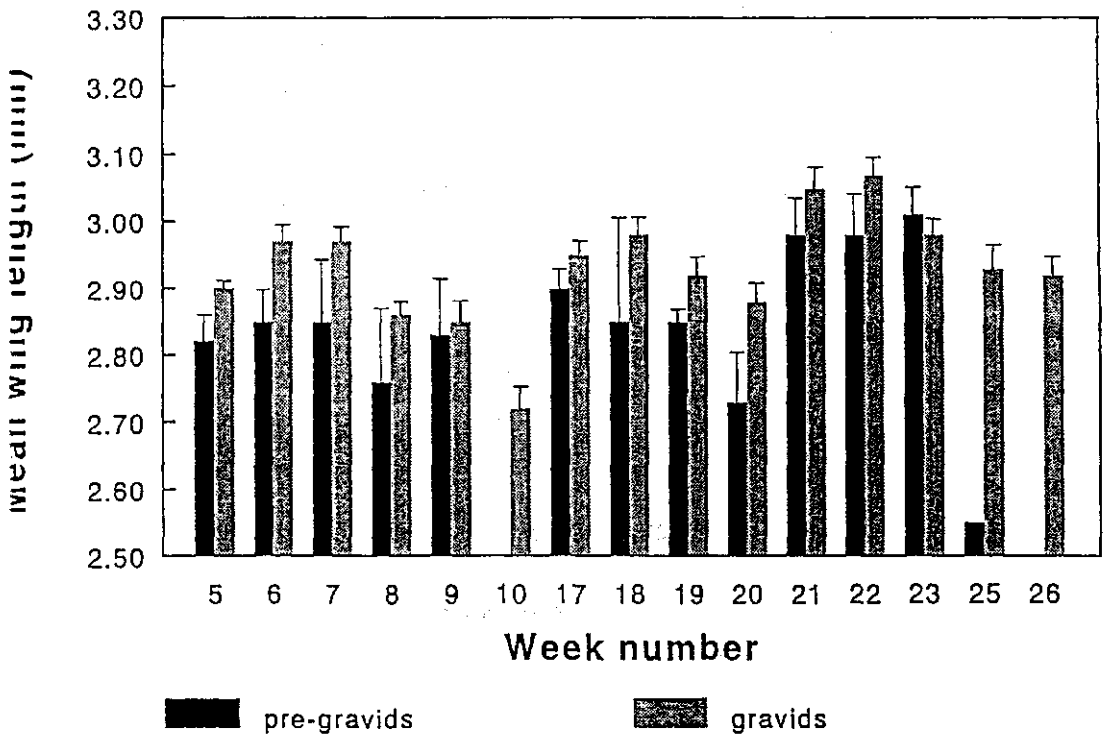

Fig. 2. Weekly mean wing length of gravid and pre-gravid females of *Anopheles gambiae s.l.* caught resting indoors. Vertical bars denote the standard error of the means.

Table 3 shows the number of females requiring one, two or three bloodmeals in order to develop mature eggs, the mean wing lengths of the three groups and their mean number of mature eggs. *An. gambiae* females which produced eggs after only one bloodmeal had wings longer than or equal to 3.00 mm.

Females requiring three bloodmeals had the smallest wing length, but these were not significantly different from those requiring two bloodmeals ($t = 0.946$, $p>0.1$, 25 d.f.) for completion of the first gonotrophic cycle. Females that required two or three bloodmeals to produce eggs, were of lower fecundity. A Spearman rank correlation analysis of wing lengths with the size of egg batch showed a significantly positive correlation ($R=0.602$, F-value = 12.91, $P<0.01$). (Fig. 3).

Table 3: Mean wing length of females laying eggs after one, two or three meals. Different letters in the same column designate significant differences ($P<0.05$; t-test)

Number of meals	Total fed	Number which became gravid	Mean number mature eggs ± s.e.	Mean wing length (mm) ± s.e.
One	78	6(7.7%)	122 ± 8.8a	3.18 ± 0.051a
Two	51	22(43.1%)	109 ± 7.7ab	2.89 ± 0.035b
Three	10	5(50.0%)	91 ± 6.0b	2.82 ± 0.066b

Discussion

Although the pre-gravid rates increased during the long rains when more breeding sites were available, there was no significant relationship between rainfall and pre-gravid rate. The insemination rate in the pre-gravid group was high, indicating that non-fertilisation was not the main cause of the pre-gravid state.

The mean wing length of the pre-gravid females was smaller than that of those which became gravid. Some of the collected adult mosquitoes which became gravid had no doubt been through a pre-gravid state and were caught after their second blood meal. Such individuals could not be distinguished by the methods used in the first study. This would explain the larger difference between wing length of gravid and pre-gravid females

observed in the newly emerged females and the greater pre-gravid rate among the latter. Laboratory studies have shown that in some mosquito species, females with a wing length of less than 2.90 mm cannot develop eggs on their first blood meal, but those with a mean size equal to or larger than 3.03 mm can (Briegel, 1990). Results from the present study agree with those of the laboratory study. Considering the results from the newly emerged group it is evident that most of the field mosquitoes need two to three meals before they are able to produce their first mature egg batch, the earlier meals being used for maternal energy supplementation.

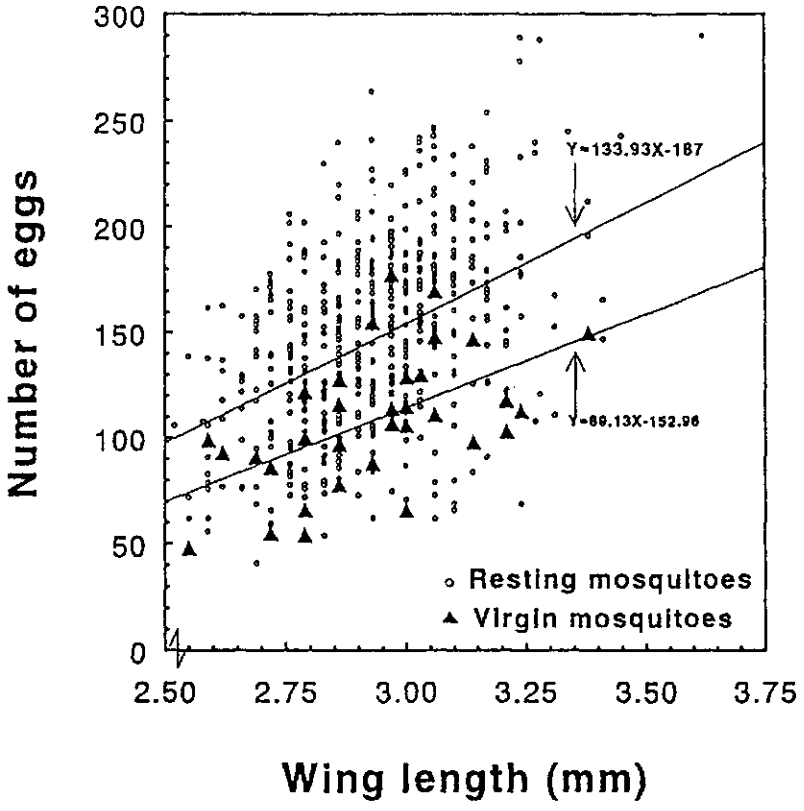

Fig. 3. Scatter diagram of the number of mature eggs in relation to wing length: circles represent the resting females, and triangles represent virgin females emerged from wild collected pupae.

The number of eggs developed was positively correlated with wing length. Various studies have shown the same relationship in other anophelines (Reisen, 1975; Briegel, 1990) and in culicines (Colless & Chellaphah, 1960; Bock & Milby, 1981; Steinwascher, 1982; Packer & Corbet, 1989; Akoh *et al.*, 1992). Many factors are known to influence the number of eggs developed by female mosquitoes. The quality of a blood meal may result in development of few follicles, and the age of the female is also important (Clements, 1963; Akoh *et al.*, 1992). These factors were not investigated in the present study.

In conclusion, this study showed that the size of adult females influences the number of blood meals required to complete the first gonotrophic cycle and the number of eggs which will mature. These findings are important in the population dynamics of this mosquito and in its role as a disease vector. Smaller females take longer to start reproduction and also produce fewer offspring. The smaller females, with a more frequent blood feeding behaviour, will most likely have a greater chance of picking up the malaria parasite at an early age. But they also run a greater risk of being killed by any mosquito control measures being used in houses, including self protective activities by the host due to their frequent host seeking behaviour.

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Chapter 8.

Relationship between adult body size and survival as measured by parity in field populations of *Anopheles gambiae sensu lato.*

Abstract

Newly emerged and host seeking female *Anopheles gambiae* Giles were studied in Michenga village in south east Tanzania to find out the relationship between size and parity. The host seeking females were dissected for parity by the observation of the ovarian tracheole condition and wing lengths were measured in both populations. Newly emerged females from field collected pupae had significantly shorter wings than the host seeking mosquitoes. There was no difference in mean wing size between the host seeking nulliparous and parous females. Results presented in this paper indicate that females with shorter wings die early in adult life.

Introduction

Body size in adult mosquitoes varies greatly within populations (Feinsod & Spielman, 1980; Bock & Milby, 1981; Haramis, 1985; Fish, 1985). Variation in body size influences parameters such as reproduction and survival that are important for the success of the mosquitoes (Steinwascher, 1982; Siddiqui *et al.*, 1976). Body size may also affect the ability of a vector to transmit diseases (Takahashi, 1976; Grimstad & Haramis, 1984; Lyimo & Koella, 1992). For many mosquito species, and in particular for the well-studied genus *Aedes*, it was found that mean parity rates among large individuals were higher than among the smaller ones and larger individuals were thought to be more successful in obtaining blood meals and survived longer (Haramis, 1983; Nasci, 1986a; 1986b; 1987). Other studies, however, failed to demonstrate the advantage of large size. For example, Hawley (1985) found that in *Aedes sierrensis* above a certain optimal size there was a negative correlation between adult size and survivorship. On the other hand, Walker *et al.*, (1987) showed that size has no effect on survival of *Ae. triseriatus* and *Ae. hendersoni* as measured in a mark-release-recapture study. Further, Landry *et al.* (1988) did not find that increased size was advantageous to survival in *Ae. triseriatus*. Within the anophelines, Kitthawee *et al.* (1990) showed that in laboratory reared *An. dirus*, larger females survived longer than smaller ones, and Kittayapong *et al.* (1992) found that host seeking parous *Anopheles maculatus* had significantly longer wings than nulliparous ones. By using parity as a measure of age, they concluded that larger females survived longer than smaller ones. Nasci (1987) reported significantly higher parous rates in the largest size class in *An. crucians*.

An. gambiae breeds in temporary habitats and exhibits a wide range of body sizes (Gillies & De Meillon, 1968; chapter 5 in this thesis). However, it is not known if this

variation in adult size influences characteristics that affect disease transmission of this vector, such as blood feeding success and survival through the sporogonic cycle of the *Plasmodium* parasites. To become parous, a mosquito must survive long enough to find at least one bloodmeal and to lay eggs: parity rates are therefore used as a measure of survival and/or feeding success (Garrett-Jones, 1964; Service, 1976). The objective of this study was to determine the relationship between body size and parity in field populations of the *An. gambiae s. l.* and relate this to survival.

Materials and Methods

Study area

The study site, Michenga village, is situated near Ifakara town (8° 10'S, 36° 38'E) in the Kilombero valley in southeastern Tanzania. The village lies on the Kilombero river plain at an altitude of 270m. The area experiences two rainy seasons, the main rains extending from March through May and the shorter rains occurring in November. The annual rainfall averages 1200 mm and the average temperature is around 26 °C. The area is holoendemic for malaria, and the main vectors are *An. gambiae s. l.* and *An. funestus*.

Mosquito collection and processing

Host seeking mosquitoes were collected by light traps placed inside houses near people sleeping under bednets (Lines *et al.*, 1991). Mosquitoes were collected from 20.00 to 02.00 hours daily from April 16 to June 5, 1991 and then once a week from June 28 to August 15, 1991. Collections were brought back to the laboratory and sorted by species. Female *An. gambiae s. l.* were dissected and their parity was determined by the state of their ovarian tracheoles as described by Detinova (1962). One wing of each female was mounted on a glass slide and the length measured from the distal end of the alula to the tip, excluding the scales, using a dissecting microscope fitted with a camera lucida.

A daily census of 43 breeding sites was carried out from April 24 to July 11, 1991 (chapter 4). Two collectors searched for pupae in these sites and all pupae found were collected, brought back to the laboratory and allowed to emerge at a normal room temperature. One day after they had emerged, adults were killed and sorted by species and sex. *An. gambiae* females were selected for further processing. The wing length of each female was measured by the same method as for the host seeking females.

Results

A total of 1830 pupae were collected and from the emerged 1380 adults, 358 female wings were measured. The wing lengths of 2272 host-seeking females were measured. Wing lengths of the newly emerged females was 2.28-3.44 mm (mean = 2.80, S.E = 0.005) and of host seeking females from 2.38-3.68 mm (mean = 2.97, S.E = 0.005 for the nulliparous and 2.98, S.E = 0.006 for the parous mosquitoes). The wing lengths were grouped into 11 size classes by steps of 0.10 mm, starting with 2.28 mm. and the wing length proportional distribution of the newly emerged and host seeking nulliparous and parous females examined (Fig. 1).

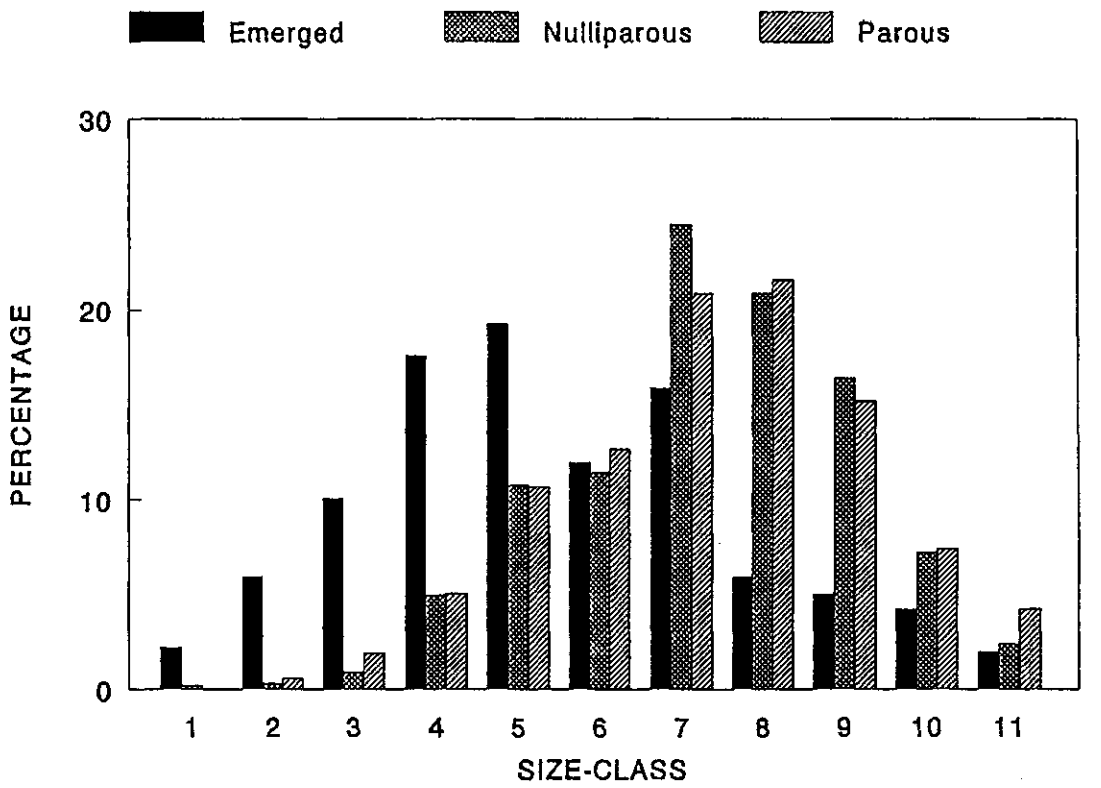

Fig. 1: Frequency distribution of wing size for the newly emerged, nulliparous and parous female *Anopheles gambiae s. l.* Size classes are in steps of 0.10 mm, starting with 2.28mm.

The distributions shifted from a positive skewness for the newly emerged females (G_1 = 0.317, P< 0.05, n=358) to a negative skewness for the two groups of host seeking females (G_1 = -0.192, P>0.05, n=350; and G_f = -0.277, P<0.05, n=350) for the nulliparous and the parous females respectively. There was an increase in wing length over time for the host seeking nulliparous and parous females (Fig. 2). Analysis of covariance was performed with week as a covariant to determine differences between the three age groups. The increase in wing size with time was significant and the differences between the groups were also significant (Table 1). However, the difference between groups was due to the difference between the newly emerged population and the host seeking populations. Therefore, comparisons were performed between mean wing lengths of the newly emerged and host seeking females, and between nulliparous and parous females. The mean wing length of the newly emerged females was significantly smaller than that of the host seeking females (t= 216, p<0.0001). There was no significant difference in mean wing length between the nulliparous and the parous females in the host seeking population (t= 0.259, p>0.10).

Table 1: Analysis of covariance results for the emerged, nulliparous and parous female *An. gambiae s.l.*

Source	DF	Sum of squares	F ratio	P
week	1	5.3822	173.3843	<0.0001
parity group	2	10.4983	169.0984	<0.0001
error	2627	82.0130		

Based on other results (chapter 7 of this thesis), it was concluded that the first gonotrophic cycle of most females of *An. gambiae* in Michenga lasts for at least 4 - 5 days. It was assumed therefore, that parous females caught at a particular time represented to a large extent the nulliparous females of the previous week. Based on this assumption, mean wing length of nulliparous females of each week was compared with the mean wing length of parous females one week later. Table 2 shows the results of these comparisons, indicating that the size of the weekly 'cohorts' was not significantly different.

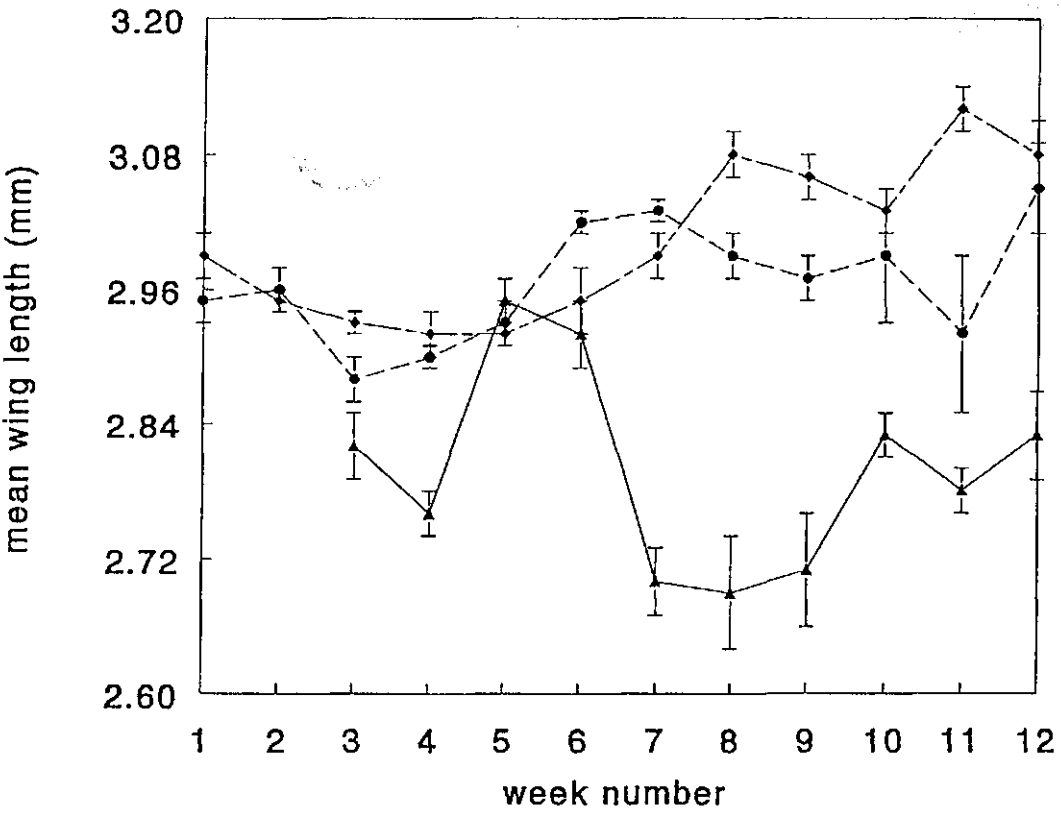

Fig. 2: Weekly mean wing length for the newly emerged (—▲—), nulliparous (--•--) and parous (··◆··) female *Anopheles gambiae s. l.* Vertical bars denote the 95% confidence interval.

There was an increase in wing size of parous females towards the end of the sampling period, but this increase was not statistically significant.

Discussion

The mean wing length of the newly emerged females was significantly smaller than that of the host seeking females. These results agree with those of Nasci (1986b) who found in *Ae. aegypti*, that adults emerged from field collected pupae had shorter wings compared to the host seeking females. The different pattern in wing size distribution between the

newly emerged females and the host seeking females in the present study is caused by a deficit of small individuals in the latter group.

Table 2: Difference between mean wing lengths of *An. gambiae* nulliparous females and that of parous females one week later.

Weeks compared	Difference(mm)	P-value
1-2	0.00	0.93
2-3	0.03	0.10
3-4	-0.04	0.14
5-6	-0.02	0.51
6-7	0.03	0.58
7-8	-0.05	0.12
8-9	-0.07	0.11
9-10	-0.06	0.16
10-11	-0.13	0.04
11-12	-0.16	0.12

It means, therefore, that the mortality of smaller individuals was taking place early after emergence. Possibly the smaller females with insufficient energy reserves were unable to fly from their breeding sites to the feeding grounds. Such an effect of size was shown by Terzian & Stahler (1949) in *An. quadrimaculatus* and also by Klowden *et al.* (1988) in *Ae. aegypti*. The first sugar or blood meal is very important for mosquito survival. In *Ae. communis*, small females which emerged from field pupae had a reduced survival, especially when they were unable to get an early sugar meal (Andersson, 1992).

The present study did not demonstrate any difference between average wing size of nulliparous and parous females in the host seeking population. Other studies of host seeking populations of different species have shown different results. For example, Nasci (1986a) found in 2 of 4 species examined that parous females had significantly longer wing lengths than nulliparous females, but not in the other two. Kitthawee *et al.* (1990) showed increased survival in larger females of laboratory reared *An. dirus*, but could not show the same relationship in host seeking females of that species (Kitthawee, *et al.*

1992), possibly because newly emerged individuals were not included in the study.

A better method of looking at size-survivorship relationship is by mark-release-recapture studies of different sized mosquitoes of known age. Such a study was not possible during the present project because of time limitations and mosquito breeding problems. Normally a large number of mosquitoes needs to be released to obtain meaningful results, which was not possible. Nevertheless, the results of this study indicate that smaller mosquitoes may have a decreased survival and/or blood feeding success, and that looking at the question of size-survivorship relationship by examination of the host seeking population alone might overlook the effect of size on longevity of mosquitoes, especially for the tropical species which have a much shorter nulliparous phase than those of temperate zones.

Acknowledgement

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Chapter 9.

Relationship between body size of adult *Anopheles gambiae s.l.* and infection with the malaria parasite *Plasmodium falciparum*

Abstract

The influence of adult female body size of *Anopheles gambiae s. l.* on development of midgut and salivary gland infections by the parasite *Plasmodium falciparum* was investigated in a field study carried out in Tanzania. The proportion of mosquitoes infected during a blood meal was independent of size. However, the number of oocysts harbored by infected mosquitoes increased with size of the mosquito. The proportion of mosquitoes with sporozoites, and thus potentially infective to humans, was highest in intermediate-sized mosquitoes, whereas the largest and smallest mosquitoes were less likely to have sporozoites. This pattern is interpreted as a combination of high survival rate of large, uninfected mosquitoes and of low survival rate of mosquitoes infected with many oocysts.

Introduction

Variability within mosquito populations of factors affecting malaria transmission has received only little attention, although theoretical studies have shown that it affects the pattern of transmission (Dye & Hasibeder, 1986; Kingsolver, 1987; Koella, 1991). One of the factors contributing to variability in transmission may be body size of the mosquito vector. Considerable variation in body size within populations has been observed for many mosquito species (Fish, 1985), including *Anopheles gambiae* (Gillies & De Meillon, 1968), the main vector of malaria in Africa. In addition, body size has been found to affect transmission of several viral diseases. Small individuals of *Culex tritaeniorhynchus* transmit Japanese encephalitis virus (Takahashi, 1976) and West Nile virus (Baqar *et al.*, 1980) at higher rates than larger individuals. Grimstad & Haramis (1984) have shown that nutritionally deprived larvae of *Aedes triseriatus* developed into small adults that transmitted La Crosse virus at higher rates than larger, well-nourished individuals.

The effect of body size on malaria transmission has only rarely been studied. Kitthawee *et al.* (1990) artificially fed four size classes of laboratory reared *Anopheles dirus* with *Plasmodium falciparum* and showed that the largest size class developed the highest number of oocysts, though the proportion of infected mosquitoes was independent of size. In contrast, two earlier studies found no relationship between body weight of *Aedes aegypti* and susceptibility to *Plasmodium gallinaceum* (Hovanitz, 1947) or between

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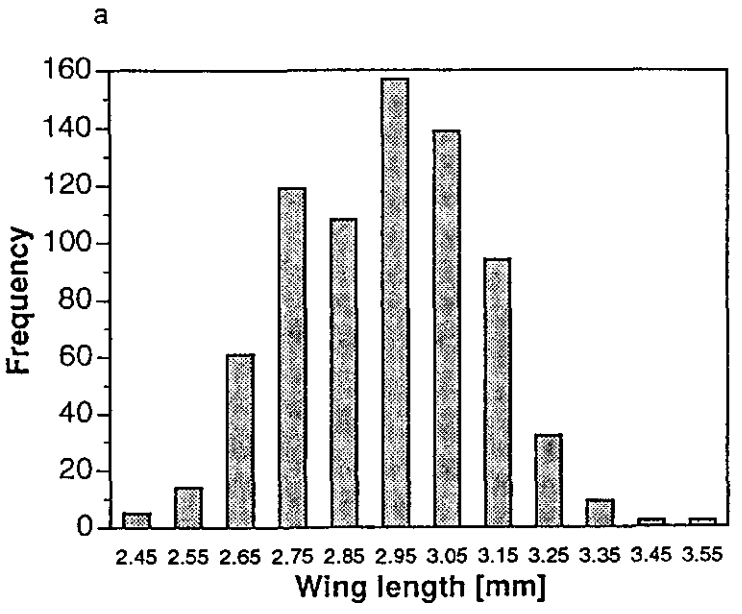
wing length of *Anopheles stephensi* and the number of oocysts of *Plasmodium yoelii nigeriensis* (Ichimori, 1989).
In the present study, we examine the relationship between adult size of the vector *An. gambiae* and natural infections with *P. falciparum*. We examine oocysts as a measure of infection from humans to mosquitoes as well as sporozoites as a measure of infection from mosquitoes to humans.

Material and Methods

The study was carried out in Michenga, Kilombero District, southeastern Tanzania. The area is holo- to hyperendemic for malaria with peak transmission in June and July. The main malaria species is *P. falciparum*, occurring in about 95% of all infections, and its main vectors are *An. gambiae* and *An. funestus*. The study area is described in detail by Tanner *et al.* (1987) and Biro (1987).

An. gambiae s. l. were sampled from five houses in July and the beginning of August, 1990. Female mosquitoes were caught as they were resting on the walls inside houses in the early morning. We assumed that blood-fed mosquitoes had fed only on humans because no animals were kept in the houses.
For oocyst detection freshly fed female mosquitoes were selected. They were fed on a 10% glucose solution for five days, then their midguts were dissected and examined for oocysts. For sporozoite detection mosquitoes were killed immediately after capture. Their heads and thoraxes were investigated with an ELISA specific for the $(NANP)_{40}$ repeat region of the circumsporozoite protein of *Plasmodium falciparum* (Campbell *et al.*, 1987). The cut-off value for the optical density separating infected from uninfected mosquitoes was set to the optical density of a control well on the same plate containing 500 sporozoites, which allows a compromise between the number of false positives and false negatives (N. Weiss, unpublished data).
The size of a mosquito was measured as the length of its wing, which correlated with dry weight (E. Lyimo, unpublished data). Wings were measured to the nearest 0.01mm from the distal end of the alula to the tip, excluding the fringe scales using an ocular micrometer.

For the analysis of the proportions of mosquitoes with oocysts and with sporozoites, the wing lengths were grouped into eight size classes. The cut-off values between classes were determined so that the number of mosquitoes in each class was similar. The proportions of infected mosquitoes in each size class were compared to a uniform distribution with a chi-square analysis.



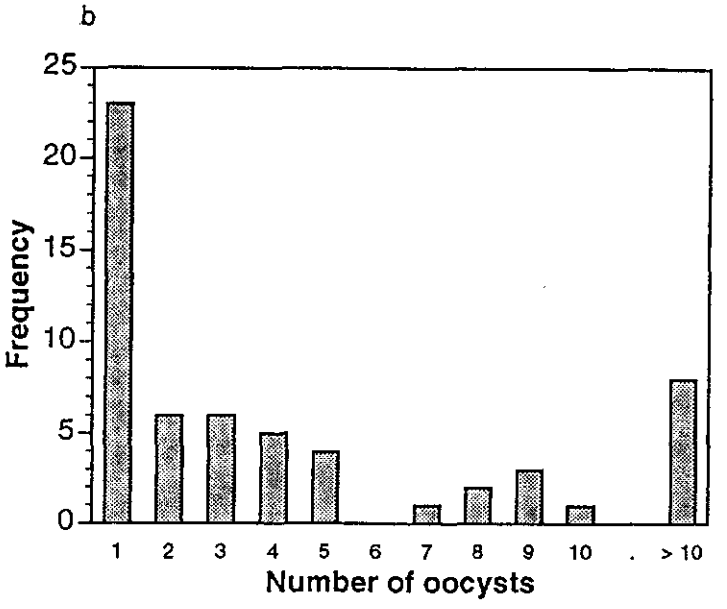


Fig. 1. Frequency distributions of (a) wing lengths and (b) of oocyst numbers in infected mosquitoes. In (a) the wing lengths are grouped into 0.1mm intervals and each interval is labelled with its midpoint.

Because the distribution of oocysts was non-Gaussian, the relationship between number of oocysts and wing length was analysed with a chi-square analysis of trend (Fleiss, 1981). Only infected mosquitoes were used for this analysis. So that size classes had a sufficient number of mosquitoes, four new size classes were formed by combining adjacent classes mentioned above. Because of the procedures described above, oocyst infection was measured as an incidence, i.e. as the proportion of mosquitoes that acquired a new infection during a blood meal, whereas sporozoite infection was measured as a prevalence, i.e. the proportion of mosquitoes that harbored sporozoites at a given time. Thus the two proportions are not directly comparable.

Results

A total of 324 mosquitoes were dissected for oocysts and 425 investigated for sporozoites. The wing lengths varied from 2.41 to 3.52 mm (Fig. 1a) with a mean of 2.92 mm and a standard deviation of 0.175mm. 18.2% of the mosquitoes were infected with oocysts and in the infected mosquitoes the number of oocysts ranged from 1 to 49 (Fig. 1b) with a mean of 5.3 and a median of 2.5. Of the infected mosquitoes 38.9% had only one oocyst. The proportion of mosquitoes with sporozoites was 28.1%.

The proportion of infected mosquitoes was independent of size (Fig. 2a; Chi-square = 5.355, df=7, $p>0.5$). In contrast, the proportion of infected mosquitoes with two or more oocysts increased with wing length (Fig. 2b). Whereas only 37.5% of the mosquitoes in the smallest size-class had more than one oocyst, 88.9% of mosquitoes in the largest size-class did. For every 0.1mm of increase in wing length, an increase of 11% in infection rate was observed (Chi square = 6.858, df=1, $p<0.01$) with no indication of non-linearity (Chi square = 0.204, df=2, $p>0.5$).

The proportion of mosquitoes with sporozoites increased from 19.5% in the smallest mosquitoes to 41.1% in intermediate size-classes and dropped again to 7.1% in the largest mosquitoes (Fig. 3). The differences in sporozoite infection rates between size-classes were statistically significant (Chi square = 22.6; df=7; $p<0.01$).

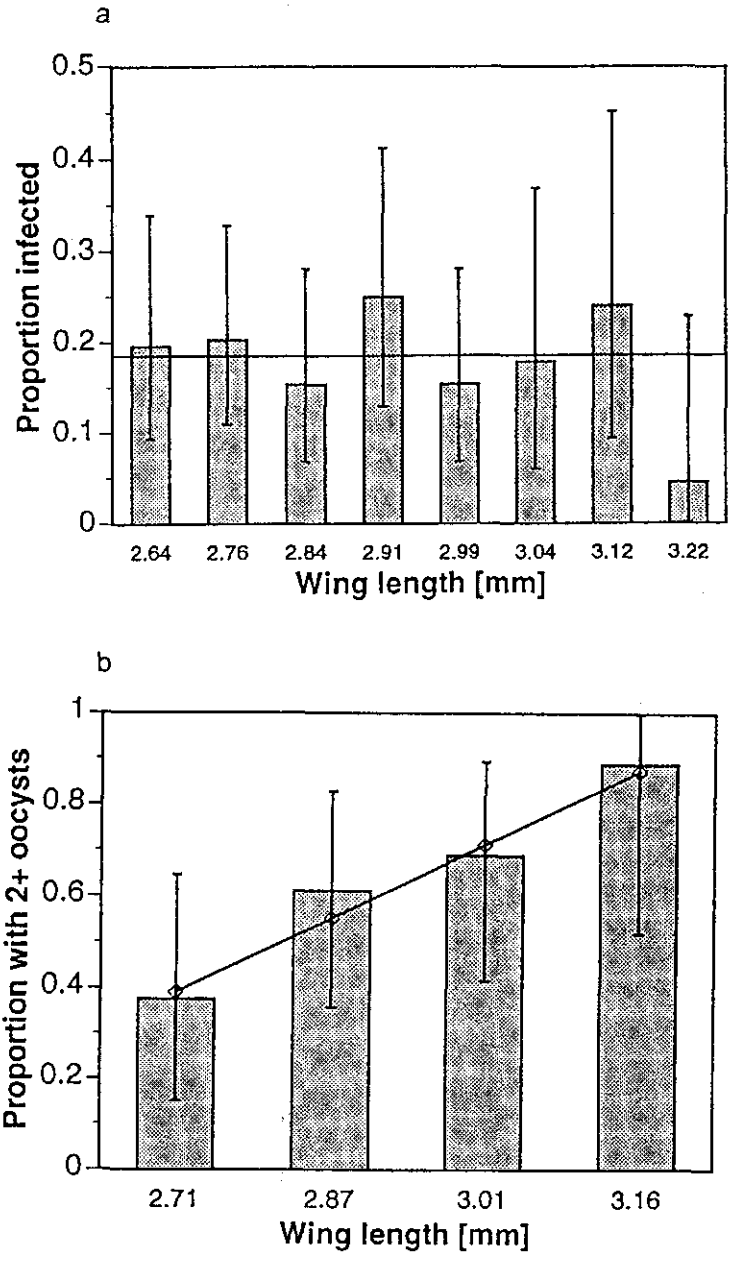


Fig. 2. Association between infection with oocysts and wing length. The x-axis is labelled with the mean wing length of the mosquitoes in each category. (a) Proportions of mosquitoes infected in eight size classes. The vertical lines denote 95% confidence intervals. The horizontal, dashed line denotes the overall proportion of infected mosquitoes. (b) Proportions of the infected mosquitoes with two or more oocysts in four size classes. The measured proportions are shown as bars, the 95% confidence intervals as vertical lines. The proportions predicted by an analysis of trend are shown as connected points.

Discussion

Adult size of *An. gambiae* affected key factors of transmission of the malaria parasite *P. falciparum*. Although the risk of becoming infected during a bloodmeal was independent of size, mosquitoes tended to develop many oocysts only if they were large. This pattern is similar to that observed in laboratory-reared mosquitoes (Kitthawee *et al.*, 1990) and might be due to differences in size of the bloodmeal. Size of the bloodmeal is positively correlated with body size in several mosquito species (Reisen, 1975; Ichimori, 1989; Kitthawee *et al.*, 1990), including *An. gambiae* (P. Billingsley, unpublished data). For the low gametocyte densities encountered in human infections this leads to positive correlations between size of the mosquito, number of ingested gametocytes and number of oocysts (Carter & Graves, 1988; T. Ponnudurai, pers. comm.).

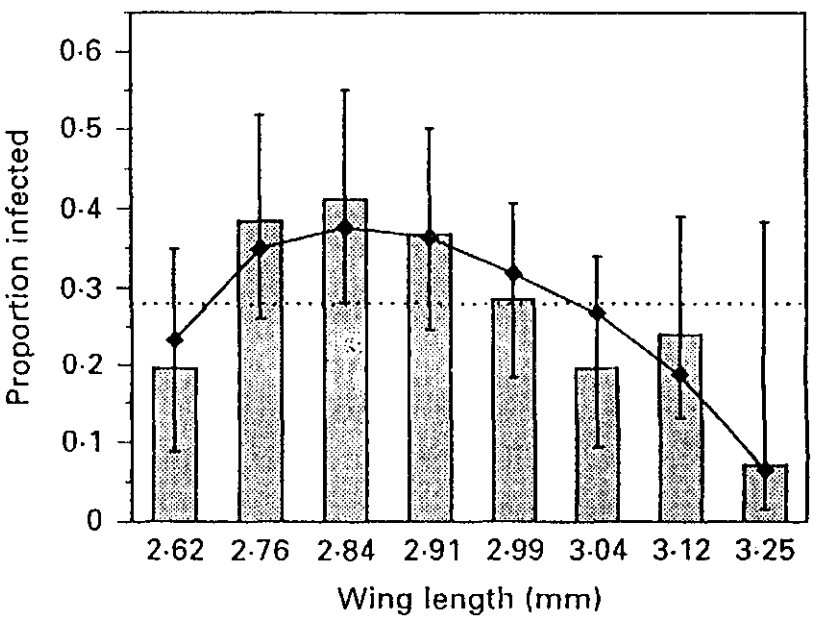


Fig. 3. Proportion of mosquitoes infected with sporozoite in eight size classes. The x-axis is labelled with the mean wing length of the mosquitoes in each category. The vertical lines denote 95% confidence intervals. The horizontal, dashed line denotes the overall proportion of infected mosquitoes.

Intermediate-sized mosquitoes were most likely to harbor sporozoites. This result is contrary to the expectation. Because adult survival generally increases with body size (Nasci, 1986; Packer & Corbet, 1989; Landry *et al.*, 1988; Haramis, 1985), the proportion of mosquitoes surviving the parasite's incubation period is expected to be highest in the largest mosquitoes, which would lead to the highest prevalence of sporozoites in the largest mosquitoes (Macdonald, 1958). The fact that prevalence decreased in the largest mosquitoes thus implies increased mortality in large mosquitoes. We could speculate that this increased mortality was due to the large number of oocysts found in the large mosquitoes. Although investigations of the pathogenicity of malaria parasites in mosquitoes are not conclusive, studies by Gad *et al.*, (1979) and by Klein *et al.*,(1982) have shown that survival is lowered in infected mosquitoes, in particular in those mosquitoes harboring more than ten oocysts (Klein *et al.*, 1986).

Whatever the processes leading to the observed patterns, mosquito size has a strong effect on the number of gametocytes that develop into oocysts and sporozoites. Thus, mosquito size might influence the rate at which malaria parasites are transmitted.

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Chapter 10.

General discussion and conclusions

The various aspect of my studies have been discussed in detail in the previous chapters. In this chapter only the main findings of my studies in relation to the biology of *An. gambiae s. l.* and malaria transmission will be discussed. A simplified flow of events throughout the life cycle of an anopheline mosquito is illustrated in Fig. 1.

Factors affecting larval development and size of adults

Larval density and temperature were identified as the major factors affecting larval development and eventually the size of adults. Temperature is known to affect development by accordingly increasing or decreasing metabolic rates of larvae (Hagstrum & Workman, 1971). Larval density affects development rate by causing competition for nutrients, space and sometimes by production of growth retardant factors (Dye, 1984; Carpenter, 1983; Suleman, 1982). In the present studies (chapters 2 and 3), rearing larvae at high temperatures increased larval development rate and resulted in small adults, while low temperatures gave the opposite results. High larval densities as opposed to low or medium densities also increased development rate and decreased adult size (Fig. 1).

The interaction between larval density and temperature modified developmental processes differently at various temperatures. In chapter 2, for example, when food was in ample supply, low (24 °C) or high (30 °C) temperatures with higher density of larvae led to an increased growth rate, wing length and dry weight of adults, but at the intermediate temperature growth rate of larvae, adult wing length and dry weight decreased with an increase in density of larvae. If high density leads to increased competition, the pattern observed at the intermediate temperature (27°C) is expected. An explanation for the opposing pattern observed at lower and higher temperatures is not apparent. It may be assumed that, at limiting conditions, large rapidly growing larvae have a competitive advantage over small, slow growing ones. The high mortality of larvae reduce competition for nutrients, consequently, surviving larvae develop fast and emerging adults are large. In contrast, at lower density small and large larvae survive and the emerging adults are mixed or small. At limited food supply (chapter 3) any increase in larval density decreased larval development rate, survival and size of adults (refer Fig. 1, left side).

In the field however, the situation was more complex. Temperatures varied between daytime and nighttime and from day to day. Larval densities also vary substantially. *An. gambiae s. l.* was found breeding in a variety of habitats, ranging from very small foot

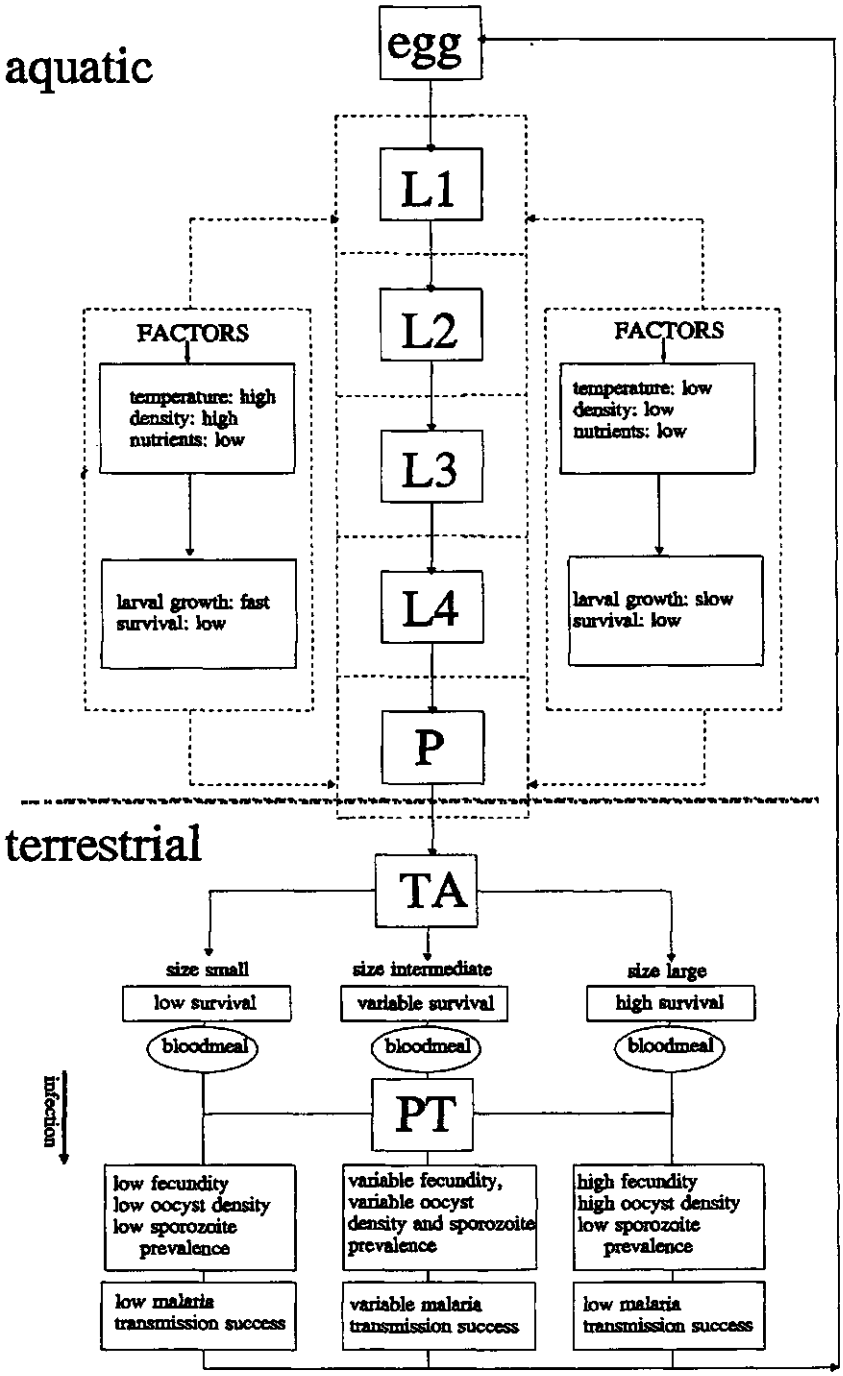


Fig. 1. Summary of events taking place throughout the life cycle of a mosquito. L1-L4 = larval stages 1-4, P = pupae, TA = teneral adults, PT = post teneral adults,

prints to large rain pools and water holes (chapter 4). The nature of these habitats differed in terms of the density of larvae present and temperature fluctuations experienced. The larger breeding sites experienced less temperature fluctuations with lower mean temperatures, less crowding and therefore less competition, and so produced on the average large adults. In contrast, the small breeding sites had smaller surface areas, experienced wider temperature fluctuations with high mean temperatures and produced on the average smaller mosquitoes. Nevertheless, between the two extremes there was a range of intermediate events, consequently the adult population was composed of individuals from the whole range of different sites. The seasonal variation in size of females observed in this species was probably caused by the diversity of breeding sites available at different seasons coupled with changes in temperature. Temporary small bodies of water were abundant during the rainy season and temperatures were high, so breeding of *An. gambiae* was prolific. At the end of the rainy season mostly larger breeding sites such as water holes were available. The drop in temperature between June and August (the cool dry season) meant cooler breeding sites, with a relatively slow growth of larvae resulting in larger mosquitoes. During the hot dry season (September to November) breeding of *An. gambiae* is very limited and restricted to isolated small sites created mainly by human activities near streams and swamps and these sites experience high temperatures.

Effect of size on adult female characteristics

Small females required two or more blood meals to mature their first batch of eggs. Field observations (Boreham & Garret-Jones, 1973; Burkot *et al.*, 1988) as well as laboratory studies (Briegel, 1990) have shown that female *Anopheles* take multiple blood meals. Possibly *Anopheles gambiae* is no exception, especially as this mosquito has the ability to eclose as small undernourished adults. The need for multiple blood meals, as was observed in chapter 7, shows that smaller females delayed initiation of reproduction. These females also had a low fecundity, which resulted in a low reproductive efficiency.

Although it was difficult to show a size-survivorship relationship among the host-seeking population, the observed differences between the newly emerged population and the host-seeking population indicated that survivorship of small females was relatively less compared to larger females. The combination of low fecundity and low survivorship demonstrates decreased fitness of the smaller mosquitoes.

Considering the seasonal variations in female size found in chapter 6, and the differences in fecundity and survival resulting from size variations (chapters 7 and 8), the *An. gambiae* population will be expected to exhibit seasonal differences in survival and fecundity. Whether this was true in the present study, is difficult to tell because it was not possible to follow cohorts of newly emerged females in the field. Nevertheless, from the parity rate measurements (chapter 5), increased survival rate in July and August

accompanied the increase in mean wing size of the females. Possibly, these females were responsible for maintaining the population through the dry season. In order to confirm this event, a more detailed study extended over the four seasons will be necessary.

The risk of infection with malaria parasites during a bloodmeal was independent of mosquito size, that is, a mosquito feeding on an infective host was equally likely to pick up infection regardless of its size. Yet, large females developed more oocysts than the smaller ones (chapter 9). As survival increases with size one would expect the prevalence of females with sporozoites to be higher in large sized females. Contrary to this, the intermediate sized group had the highest proportion of sporozoite positive females. This observation is puzzling. My speculations are that larger infected females were not surviving long enough for the parasite to complete its development, probably due to the higher load of oocysts developed in this group. There is as yet no direct evidence that high parasite load is detrimental to survival but the present results indicate that this might be the case. Alternatively, the larger and more robust females were able to arrest development of the oocysts to sporozoites, thus, not all oocyst infections resulted into sporozoite gland infections.

Effect of female size on malaria transmission

The transmission of malaria is governed by the course of infection in the human host and the mosquito vector. For a mosquito to be able to transmit malaria, it needs to bite at least twice, first to pick up an infection, and second to pass the infection to another human host. Also it needs to survive long enough for the parasite to complete development in the vector (10-12 days in the case of *P. falciparum*). Thus, the basic model of malaria transmission, the Ross-Macdonald model (Macdonald, 1957) describes the basic reproductive number R_o, the number of secondary infections resulting from a single case as

$$R_o = \frac{ma^2 b_1 b_2 e^{-\mu T}}{\mu r}$$

where m = the number of mosquitoes per human host
a = the biting rate of the mosquitoes on the human host
b_1= the infectiousness of human hosts to the mosquitoes
b_2= the susceptibility of the humans
μ = the mortality of adult mosquitoes
T = the incubation period of the parasite in the mosquito
r = the rate of recovery of infected humans.

In this model transmission of malaria is favoured by a high density of mosquitoes, high biting rates, and highly susceptible human hosts and mosquito vectors. Transmission is

hindered by high mortality of mosquito vectors and quick recovery of the human host. Following from the above equation, the inoculation rate (h) of the vector can be summarised as

$$h = ma^2e^{-\mu T}\frac{y}{\mu + ay}$$

where y is the prevalence of malaria in the human population and the other parameters are as described above. Therefore, a good vector will be present in high densities, bite frequently and most important, live long enough after being infected for the parasite to develop in the vector and be transmitted to a new host.

Results of my study showed that small sized females take extra blood meals to produce mature eggs. This tendency subjects them to frequent contacts with their host thus increasing their biting activity and also their chances of picking up malaria parasites. But these mosquitoes have lower chances of survival, which reduces their probability of transmitting the parasite to another host. From chapter 9, it was inferred that parasite 'induced' mortality increased with increasing size. Consequently, these opposing processes will tend to eliminate the smaller females as well as the larger females. Although these variations in individual parameters in relation to size will tend to modify the inoculation rate accordingly, however, in both parameters i.e biting and mortality rates, size dependent effects seem important only at the extremes of the size distribution. These extremely large or small females form a very small proportion of the mosquito population. Therefore, the effects of adult mosquito size on the overall transmission of malaria will be negligible. Below, a theoretical model is described which supports this hypothesis.

Simulation of the results

In order to check the assumption that the effect of mosquito adult size on malaria transmission is negligible, an attempt was made to simulate the malaria transmission with the model of Koella (1991), which is a modification of the Ross-Macdonald model. It is modified to incorporate variability in biting and survival rates in relation to mosquito size and illustrated in appendix 1. I looked at the contribution of individual size classes to transmission, and the total transmission as a function of size.
The functions I chose were:

(i) size distribution $\Phi(s)$ of mosquitoes, Gaussian with mean s and variance σ

$$\phi(s) = \frac{1}{\sqrt{2\pi\sigma^2}}\exp\left[-\frac{s-\bar{s}^2}{2\sigma^2}\right] \quad \ldots\ldots\ldots\ldots\ldots(1)$$

(ii) biting rate a, decreases with increasing size

$$a(s) = a_1 - \frac{a_1 - a_o}{1 + [\frac{s_a}{s}]^n} \quad \ldots\ldots\ldots\ldots\ldots (2)$$

where a_o and a_l are the lower and upper biting rate values, n in the number of mosquitoes and s is size of females.

(iii) natural mortality μ decreases with increasing size

$$\mu(s) = \mu_o + (\mu_1 - \mu_o)\ \exp\{-\ln(2)\frac{s - s_o}{s_1 - s_o}\} \quad \ldots\ldots\ldots\ldots (3)$$

where μ_0 and μ_1 are the lower and upper mortality values, s_o and s_1 are the lower and higher size limits.

(iv) parasite induced mortality α increases with increasing size

$$\alpha(s) = 0, \text{ when } s < s_1$$
$$\alpha(s) = p(s - s_1)^k, \text{when } s \geq s_1 \quad \ldots\ldots\ldots\ldots (4)$$

where p is the survival probability within a size class
Fig. 2 represents the functions chosen, and their variation in relation to size as postulated in my study population. The parameters were estimated to include values observed from the field, thus female size was assumed to vary from 2.0 to 4.0 mm, biting rate between 0.5 and 1.5 and daily mortality rate between 0.1 and 0.2. Adding these functions to the Ross-Macdonald equation gives the inoculation rate h for a given size class as,

$$h(s) = ma^2 e^{-(\mu+\alpha)\tau} \frac{y}{\mu + \alpha + ay} \quad \ldots\ldots\ldots\ldots (5)$$

where y is the prevalence of malaria infection in the human population

The total inoculation rate H is the sum of the individual h's, weighted by the frequency of the respective size class:

$$H = \int \phi(s) h(s) ds \quad \ldots\ldots\ldots\ldots (6)$$

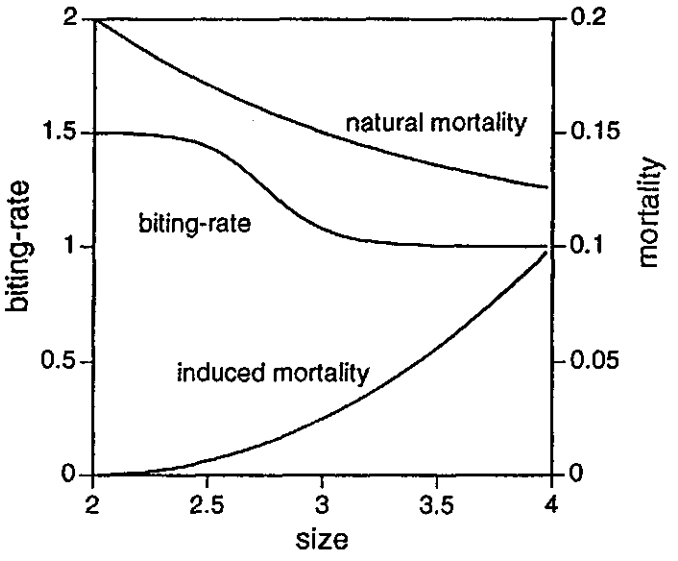


Fig. 2. Variation in biting rate, natural mortality and parasite induced mortality in relation to size of mosquitoes

Since H depends on the prevalence y, and y in turn is calculated from Ross-Macdonald equation as,

$$y = \frac{H}{r + H} \quad \ldots\ldots\ldots\ldots (7)$$

and thus depends on H, I found the inoculation rate interactively by repeatedly utilising equation 6 and 7 until an equilibrium was reached.

Fig. 3 shows the solution of the standard Ross-Macdonald equations where all parameters are independent of size. The total transmission as a function of size follows a Gaussian distribution. Figures 4a-c show the solutions of the Ross-Macdonald equations modified to account for biting rates (according to equation 2), natural mortality (equation

3) and induced mortality (equation 4) respectively. Increased biting rate by small mosquitoes results in an increased inoculation rate of the smaller mosquitoes (Fig. 4a). Increased natural mortality of smaller mosquitoes, results in a decreased inoculation rate of smaller mosquitoes (Fig. 4b) and, increased parasite induced mortality results in decreased

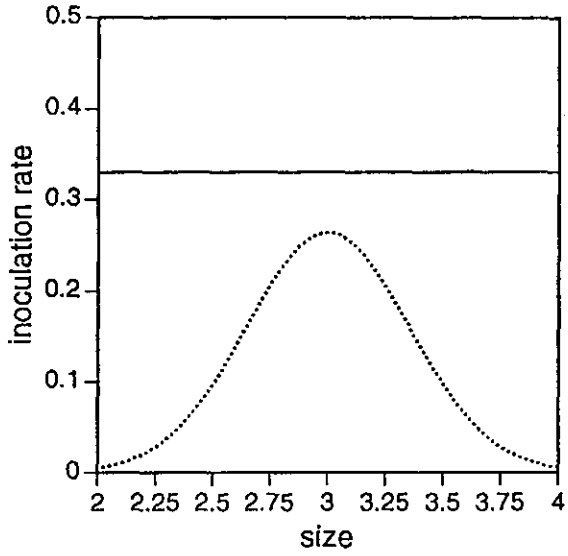


Fig. 3. Solution of the Ross-Macdonald equations. The solid line shows that the inoculation rate h(s) is independent of any given size while the dotted line shows the frequency distribution of inoculation rates, $h(s)\Phi(s)$, i.e. the contribution of mosquitoes of a given size to total transmission. The parameters are $a=1.2$, $\mu=0.1$, and $\alpha=0$.

inoculation rates of the larger mosquitoes (Fig. 4c). The contribution of each size class to total transmission shows a slight difference from the original distribution. Figure 4d shows the solution of the equations taking into account the effect of size on all parameters. Inoculation rate as a function of size seems higher in smaller mosquitoes. Each individual parameter has a large effect on the inoculation rate of mosquitoes within a given size, h(s).

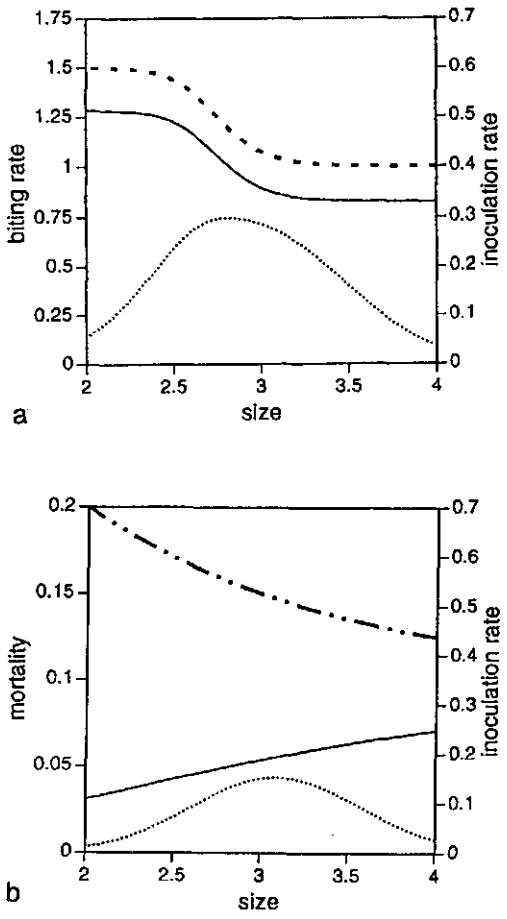
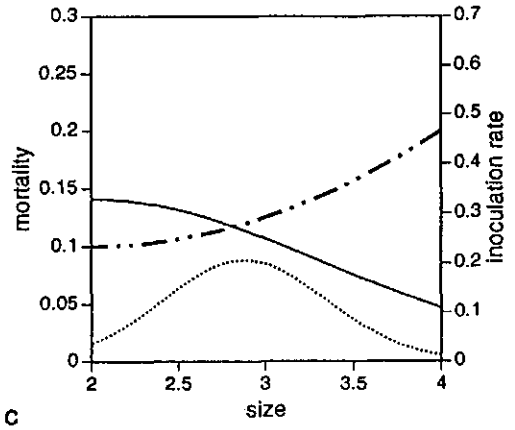


Fig.4. Solution of the Ross-Macdonald equations, modified to take account of an association between (a) biting rate of the mosquitoes and their size according to equation (2). The dashed line shows the biting rate as a function of size, a(s). Parameters a_o=1.0, a_1=1.5, s_a= 2.75, n=10. The solid line shows inoculation rate as a function of size, h(s), and the dotted line shows the contribution of each size to total transmission, h(s)Φ(s). Mortality is constant, with μ=0.1 and α=0. (b) natural mortality of the mosquitoes and their size according to equation (3). The dash-dotted line shows mortality as a function of size, μ(s). Parameters μ_o=0.1, μ_1=0.2, s_o=2, s'=3. The solid line shows inoculation rate as a function of size, h(s), and the dotted line shows the contribution of each size to total transmission, h(s)Φ(s). Biting rate is constant with a=1, and α=0.



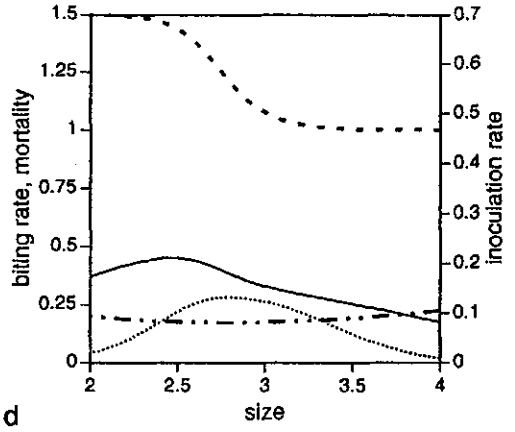


Fig 4 continued..(c) induced mortality by the parasite, with additional assumption that large mosquitoes are harmed more than small mosquitoes. The dash-dotted line shows the induced mortality as a function of size, $\alpha(s)$. Parameters p=0.05, s_1=2, s_o=2, k=2. The solid line shows inoculation rate as a function of size, h(s), and the dotted line shows the contribution of each size to total transmission, $h(s)\Phi(s)$. Biting rate is constant with a=1, and μ=0.1. (d) an association between size and the interaction between all parameters. The dash-dotted line shows the mortality as a function of size, $\mu(s) + \alpha(s)$, and the dashed line shows the biting rate as a function of size. The parameters are chosen as in the previous graphs. The solid line shows inoculation rate as a function of size, h(s), and the dotted line shows the contribution of each size to total transmission, $h(s)\Phi(s)$.

Nevertheless, the effects of 'natural' mortality and 'induced' mortality act on the opposite ends of the distribution, resulting in intermediate sized mosquitoes contributing most to total inoculation rates. Because most of the effects are at the tail ends of the size distribution, and these form only a small proportion of the population, the contributions of the individual inoculation rates as a function of size, $h(s)\,\Phi(s)$, of mosquitoes to transmission remains more or less Gaussian distributed. Thus, in the field normally occurring variations in mosquito size seem to have no effect on the overall transmission of malaria.

Conclusions

The *An. gambiae* population in the study area experiences relatively large size variations caused by environmental conditions. Climate (rainfall and temperature) and nutritional factors each contribute to these variations. From my studies, it emerged that the smaller individuals of the adult population die early in adult life, probably due to nutritional stress. Of the surviving adults, the smaller females need two or more blood meals to complete the first gonotrophic cycle. This increases the biting rates and probably their chances of becoming infected by malaria parasites. These mosquitoes, however, develop fewer oocysts than the larger ones. The larger mosquitoes seem to suffer from the high parasite load, and as shown by the model above, the smaller and larger females contribute relatively less to malaria transmission because of their small number in the total vector population.

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Appendix I.

Malaria transmission model of Koella (1991)

The standard Ross-Macdonald model of malaria transmission, (which is the commonly used model in malaria epidemiology),was modified to allow for different mosquito parameters in this case, biting and mortality rates depending on their size. This is a slight modification of the model incorporating variability described by Koella (1991).

With the subscript i to donate mosquitoes belonging to the same size class and with the mosquito dynamics assumed to be at equilibrium, the model can be written as

$$\dot{y} = (1 - y)\sum m_i\, a_i\, w_i\, b_2 \; - \; ry$$

$$\dot{v} = a_i\, b_1\, y\, (1 - v_i - w_i)(1 - e^{-\mu_i T}) - \mu_i v = 0$$

$$\dot{w} = a_i\, b_i\, y\, (1 - v_i - w_i) e^{-\mu_i T} - \mu_i w = 0$$

where, y is the prevalence of infection in human population, a is the biting rate of the mosquito vector and b_2 is the infectiousness of the mosquitoes to humans, v is the proportion of mosquitoes which are infected but not infective, b_1 is the infectiousness of humans to mosquitoes, w is the proportion of infective mosquitoes, μ is the mortality rate of mosquitoes, and T the incubation period of the parasite in mosquitoes. By manipulation of the second and third equations the proportion of infective mosquitoes in a given size class can be written as

$$w_i = \frac{a_i\, b_i\, y\, e^{-\mu_i T}}{\mu_i + a_i\, b_i\, y}$$

To estimate the function of size variability on the basic reproductive number R_0, it was assumed that the variations in biting rate and in mortality were small and were wrote as

$$\mu(s) = \bar{\mu} + \delta(s)$$

and

$$a(s) = \bar{a} + \varepsilon(s)$$

This allows the terms exp(-μ(s)T) to be approximated as

$$e^{\mu(s)T} \approx e^{-\mu T}\ 1 - T\delta + (\frac{T^2}{2})\ \delta^2$$

and the term in the denominator to be approximated as

$$\frac{1}{u(s) + a(s)b_1y} \approx \frac{1}{u + ab_1y}\ 1 - \frac{\delta + \varepsilon b_1y}{u + ab_1y} + (\frac{\delta + \varepsilon b_1y}{u + ab_1y})^2$$

Combining these approximations together with the definition of variance and covariance allow the total inoculation rate to be calculated as

$$\int m(s)\ a(s)\ w(s)\ b_2 ds = ma^2b_1b_2e^{-uT}\frac{y}{u + ab_1y}\quad 1 + V_a[\frac{1}{a} - \frac{b_1y}{u + ab_1y}]^2 + V_u(\frac{T}{2} + \frac{1}{u + ab_1y})^2 + \frac{T^2}{4} + Cov_{au}\ \frac{b_1y}{u + ab_1y} - \frac{2}{a}\ T + \frac{1}{u + ab_1y}$$

where V_a and V_μ denotes the variance of a and μ, and $COV_{a\mu}$ denote the covariance between a and μ.

The basic reproductive value is obtained by introducing this equation into the first equation and calculating the condition under which a small prevalence δy will increase in frequency. This leads to

$$R_0 = \bar{R}_0\ 1 + \frac{V_a}{a^2} + \frac{V_u}{u^2}[1 + uT + \frac{(uT)^2}{2}] - 2\frac{COV_{au}}{au}[1 + uT]$$

where

$$\bar{R}_0 = \frac{ma^2b_1b_2e^{-uT}}{ur}$$

is the basic reproductive rate calculated with the mean parameter values.

These equations show that , if biting rate and mortality rate vary independently with size, the variability in female size will increase the endemicity and stability of malaria. If the parameters covary, which will be the case if, for example, biting is risky and higher biting rates leads to higher mortality, the effects of increasing variability are lower and might even be cancelled out by the covariance between the parameters.

Appendix II

Glossary of some mosquito entomological terms used in this thesis

Endophilic: Indoor liking- mosquitoes which prefer human dwellings than outdoor for resting.

Exophilic: Outdoor liking- mosquitoes which prefer outdoor shelters as opposed to indoor habitats.

Endophagic: Indoor feeding- mosquitoes which prefer to feed inside houses especially at night.

Exophagic: Outdoor feeding- mosquitoes which prefer to feed out of doors.

Pre-gravid: a pregravid mosquito is the one which fails to develop eggs after its first blood meal.

Pre-gravid rate: This is the proportion of individuals in a population which fail to develop eggs on their first blood meal.

Indoor resting mosquitoes: Population of mosquitoes attracted indoors for feeding and rests indoors for blood digestion.

Host seeking mosquitoes: is the population of hungry mosquitoes activily searching for a host to get a blood meal.

Curriculum vitae

Ms. Edith Onesmus Kirenga Lyimo was born on 17 August 1957 in Marangu, Moshi Tanzania. She attended primary education at Marangu and secondary education in Dar es Salaam and Mtwara where she completed her 'A' levels in 1977. In 1979 she joined the University of Dar es Salaam and obtained a Bachelor of Science degree majoring in zoology and chemistry. After university education she joined the Tanzanian National Institute for Medical Research as a research scientist III, where she was involved in studies of the malaria vectors resting behavior and insecticide resistance in *Culex quinquefasciatus* for two years. She obtained her MSc degree in Medical Entomology and Parasitology at the University of Jos, Nigeria in 1985 and her research topic was on breeding of *Culex quinquefasciatus*. Back in her Institution as a research scientist II, she obtained a joint research grant from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) to do trials in malaria control by use of impregnated bednets in Muheza district, where she was the team leader between 1988 and 1989. In January 1989 she attended a three months advanced course in Medical and Veterinary vector control methods at Imperial College, Silwood Park after which she joined the Kilombero Malaria Project whose studies constitute this thesis. She has published eight papers on malaria and filariasis vectors and participated in teaching at the Vector Control Training Centre in Tanga, Tanzania and the WHO International Malariology courses.

PROPOSITIONS

1. Knowledge of variability in mosquito vector parameters is important in understanding malaria transmission. However, knowing the variability alone is not enough, but the relationship between parameters and how they vary and/or co-vary in relation to each other is essential.

   This thesis.

2. The *Anopheles gambiae s.l.* population in the Kilombero area maintains a body size range which ensures the survival of the species in this area.

   This thesis.

3. The development rate of the aquatic stages of mosquitoes is so dependent on abiotic and biotic characteristics of the environment that the larvae occupy, that it is impossible to generalise, even within a confined geographic area.

   This thesis.

4. The high pre-gravid rate found in *Anopheles gambiae s.l.* makes it difficult to estimate the survival rate of the females of this species with precision because the pre-gravid females stay longer in the nulliparous state than the females that do not undergo a pre-gravid phase.

   This thesis.

5. *Anopheles gambiae s.l.* appears to select oviposition sites randomly, but recent findings in several *Culex* species suggest that chemical cues may direct the gravid *Anopheles* female to specific sites.

   This thesis.
   Millar, J.G., Chaney, J.D. & Mulla, M.S. (1992) J. Am. Mosq. Control Assoc. 8: 11-17.
   Beehler, J.W., Millar, J.G. & Mulla, M.S. (1993) J. Chem. Ecol. 19: 635-644.

6. Arbitrary measures of heterogeneity are tempting and very popular, but their ability to reflect the relevant properties of the system of interest is unclear and questionable.

   Kolasa, J. & C.D. Rollo (1991). The Heterogeneity of Heterogeneity: A Glossary. *in* Ecological Heterogeneity (J. Kolasa and S.T.A. Pickett, eds.), Ecological Studies 86, Springer-Verlag New York Inc., New York.

7. To say that a disease depends on certain factors is not to say much, until we can also form an estimate as to how largely each factor influences the whole result.

   Ross, R. (1911). The prevention of malaria. Murray, London. Pg. 651.

8. Overall development programmes can have an impact on transmission of malaria. A strong cross-sectoral approach is therefore required in order to lessen the potential burden of disease on the very people this development seeks to help.

   Gwadz, R.W. (1991). Malaria and development in Africa. A cross-sectoral approach. AAAS, Washington DC.

9. External support to health projects in developing countries often leads to problems once such support comes to an end, as most of these projects are not sustainable.

10. There is nothing like 'a finger in the dyke' for malaria control in the sub-Saharan Africa. What is needed are 'fingers'.

11. It is too bad the mosquito is such a pain - (not to mention the itch) - its life cycle is quite fascinating.

Barnard, B. (1991). Agricultural Research, USDA-ARS, Beltsville, MD.

Propositions with the thesis "The bionomics of the malaria mosquito *Anopheles gambiae sensu lato* in Southeast Tanzania - adult size variation and its effect on female fecundity, survival and malaria transmission" by Edith O.K. Lyimo.

Wageningen, December 1, 1993.