EFFECTS OF PERMETHRIN-TREATED BEDNETS ON *Anopheles gambiae s.l.* IN THE GAMBIA

MARTHA L. QUIÑONES-PINZON, M.Sc.

**Submitted in fulfilment of the requirements
for the degree of Doctor of Philosophy
in the University of London**

**Vector Biology and Epidemiology Unit
Department of Medical Parasitology
London School of Hygiene and Tropical Medicine**

1996

ABSTRACT

A National Impregnated Bednet Programme (NIBP) was implemented in The Gambia covering all the villages with a primary health centre (PHC). The evaluation of the programme included epidemiology, entomology and social aspects. This thesis is part of the entomological evaluation.

Populations of *Anopheles gambiae s.l.* were found to be as long lived, abundant and infective in villages with permethrin-impregnated bednets as in villages with untreated bednets, confirming the lack of a "mass-killing effect", and the absence of a repellent effect of treated bednets at village level.

Movements of mosquitoes between PHC villages was assessed and found that overall, 17% (C.I. 11.02%-26.71%) were immigrant mosquitoes from neighbouring villages. This amount of movements between villages may explain the difference between The Gambia and other African countries in which a clear "mass-killing effect" has been seen after the introduction of impregnated bednets.

In the absence of a "mass-killing effect" the question arises of how protection against malaria in children is therefore achieved. Studies on the biting behaviour of the vector population were made to see if mosquitoes were diverted to bite outdoors, shifted the biting time, were prevented or delayed to feed or were diverted to bite other hosts rather than children. No evidence was found for a difference between treated and untreated villages in biting location (Indoors:Outdoors), mean biting time or human blood index of indoor resting mosquitoes. An indication was observed that the gonotrophic cycle length of *An. gambiae s.l.* was 2 days in untreated villages. No evidence of a change in the gonotrophic cycle length was found in the presence of treated bednets, although the number of mosquitoes were low to be conclusive. The density of indoor resting mosquitoes was significantly lower in the presence of treated bednets than in the presence of untreated ones, difference probably due to the excito-repellent effect of the insecticide. A study in which mosquito bloodmeals from children could be differentiated from bloodmeals from other hosts (adults and animals) was undertaken. A significant reduction in the proportion of bloodmeals that were apparently taken from children was found in the presence of treated bednets. However, it is not clear

whether these results are reliable. Assuming that they are, this could explain the way in which children are protected against malaria by the use of treated bednets.

A comparative study was carried out in two villages to see if entomological factors were part of the reasons that caused the NIBP not to be as effective in Zone 5 as in the other Zones. Some entomological differences were found between Zones 5 and 3 and those included: a higher proportion of *An. arabiensis* in Zone 5 (40%) than in Zone 3 (20%), a significantly higher frequency of the inversions j and d in the chromosome 2R in *An. gambiae s.s.*, an exophilic tendency of fed females in Zone 5 and lower densities but higher sporozoite rates, resulting in a higher EIR in Zone 5 than in Zone 3. Other parameters evaluated showed no significant difference including biting cycle, Indoor:Outdoor ratios, persistence of the insecticide in the bednets and no evidence was found that the treatment had a differential effect regarding the type of rooms. Levels of bednet usage were probably more significant than these entomological parameters as a cause of the observed differences between zones of the epidemiological impact of treated bednets.

The relative sampling efficiency of human landing collections were compared with light traps, exit traps and pyrethrum spray catches. In general there were few significant correlations between methods, due probably to night to night and house to house variation and a limited sampling in both duration and geographical extension.

DNA probes were compared with cytogenetics for the determination of the species of the *An. gambiae* complex. For the identification of *An. gambiae s.s.* and *An. arabiensis* DNA probes were shown to be as reliable as cytogenetics. However, a limitation was the variability between batches of probes. Also, the cross-reaction between the probes pAngss and pAnM14 made difficult the differentiation of *An. gambiae s.s.* and *An. melas*.

ACKNOWLEDGEMENTS

Many people contributed in various ways in this thesis. I want to thank them all, even if their names are not mentioned here.

First of all my gratitude goes to my supervisor Dr. Jo Lines for his guidance, invaluable help in all the phases of the thesis, criticism, patience and friendship. Dr. Madeleine Thomson encouraged me to do this project and I want to thank her for her support, ideas and stimulating discussions. The continuous support and encouragement of Professor Brian Greenwood is appreciated.

This thesis could not have been possible without the hard work of the Entomology team. I am indebted to Musa Jawara for his invaluable help in the field and in the laboratory and for his friendship. My thanks to John Adiamah for his help in the first part of the field work. The hard work of the field workers Fabacari Sanjang, Tumani Kuyateh, Eliman Jobe, Nding Cessay, Dembo Fatty, Ngansu Toure, Lamin Fadera, Omar Baldeh, Bacari Maneh, Lui Lope and the villagers assistants is highly appreciated.

Many thanks to Louise Groves, Wendy Macdowell, Chris Drakeley, Paul Emerson, Michelle Haywood and Dr. Olaf Muller for their participation in some of the studies and whose pieces of work constituted part of this thesis. I am most grateful to Proff. Mario Coluzzi, Mr. Gianni Petrangeli, Dr. Vicenzio Petrarca and Dr. Mariangela DiDeco for having me in their laboratory for training in cytogenetics.

My very special thanks to all the staff of the MRC Farafenni field station and in particular to Dr. Umberto D'Alessandro, Dr. Moses Aikins, Dr. Ben Olaleye and Dr. Kabiru Cham for those useful conversations about impregnated bednets and for their friendship. To Patricia Langerock, Steve Connor, Sara Connor, Brenda Aikins and Tanya Merchant for their friendship and support. To Batch Cham, Aida Secka, John Camara, Muhammed Hydara and the Farafenni drivers for their help in numerous ways. To Pamodou Njiae for his hospitality.

I am grateful to Laura New, Jo Armstrong-Shellemberg, Jo Morris and Steve Bennett for their help with statistics. To Professor Chris Curtis for his comments and corrections of the manuscript. Special thanks to Barbara Sawyer and Nigel Hill who were always there whenever mosquitoes or supplies were needed.

My sincere gratitude to my colleagues and friends in the Unit and in the Department who were very supportive in many ways, particularly in difficult moments. Especial thanks to James Montoya, Aura Aguirre, Claudia Romero, Nelson Salazar and Adriana Tami. Very special thanks to Pradya Somboon for those discussions about bednets, shared references and notes. To Paul Coleman and Sarah Cleaveland for trying to elucidate on the 'rabid-cows'.

I am most grateful to the WHO Special Programme for Research and Training in Tropical Diseases (TDR) and the MRC for their financial support. The kindness of the Sir Halley Stewart Trust in a difficult moment is appreciated.

Special thanks to Nelson Vanegas for his love, encouragement and help in many ways and to my family in Colombia for all their support.

I would like to dedicate this thesis to my son Felipe who arrived in the middle of the work bringing me lots of motivation and accompanied me during the analysis and writing-up.

TABLE OF CONTENTS

CHAPTER 2. ATTEMPTS TO FIND AN EFFECT OF PERMETHRIN-IMPREGNATED BEDNETS ON DENSITY, PAROUS RATES AND SPOROZOITE RATES ('MASS-KILLING EFFECT') OF *Anopheles gambiae s.l.* IN THE GAMBIA.

CHAPTER 3. EFFECT OF PERMETHRIN-TREATED BEDNETS ON MOVEMENTS OF *Anopheles gambiae s.l.* BETWEEN NEIGHBOURING VILLAGES

CHAPTER 4. EFFECT OF PERMETHRIN-TREATED BEDNETS ON BITING, EXITING BEHAVIOUR AND GONOTROPHIC CYCLE LENGTH OF *Anopheles gambiae s.l.* IN THE GAMBIA

CHAPTER 5. EFFECT OF PERMETHRIN-IMPREGNATED BEDNETS ON DIVERSION OF BITES OF *An. gambiae s.l.* FROM CHILDREN TO OTHER HOSTS

CHAPTER 6. SPECIES DETERMINATION OF THE *Anopheles gambiae* COMPLEX, CHROMOSOMAL POLYMORPHISM IN *Anopheles gambiae s.s.*, BEHAVIOUR AND IMPREGNATED BEDNETS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 General introduction

Pyrethroid-treated bednets are considered one of the most promising methods for malaria control nowadays. Compared with residual house spraying, impregnation of bednets with pyrethroids is an attractive alternative. The reasons for this include the relatively simple procedure and the fact that impregnation can be done with easily available equipment in areas with malaria transmission. One of the major advantages of this method is its potential to be incorporated into primary health care programmes. In the last decade, evaluations of insecticide-treated bednets have been carried out at different levels, with studies in the laboratory, in experimental huts and at village scale (Curtis *et at.*, 1991). Pyrethroid-treated bednets have been adopted as the main method for malaria control in China, where around 500 million people sleep under treated bednets (Bo *et al.*, 1995; Cheng *et al.*, 1995).

In Africa, where malaria is responsible for around 300-500 million clinical cases every year (WHO, 1995), control is difficult because of increasing resistance of *Plasmodium falciparum* to drugs. Vector control is hampered in a few places because of resistance of some vector populations to insecticides like DDT, but mainly because the infrastructure needed to sustain a spraying programme is lacking (Henderson, 1991). Under these circumstances the use of insecticide impregnated bednets is a reasonable approach as it could be carried out by the community and is appropriate to the endophagy, endophily, anthropophily and the late night biting activity of the *Anopheles gambiae* species complex and *An. funestus*, which are the main vectors in Africa. In other countries where the malaria vectors are not as antropophilic or endophilic as the African vectors, impregnated bednets have also been considered as a promising alternative to the methods currently carried out. For example, impregnated bednets have been shown to be effective in reducing morbidity for malaria in one trial in Guatemala (Richards *et al.*, 1993) where the vector *An. albimanus* has a zoophilic and exophilic tendency.

Several studies of treated bednets for control of malaria have been carried out in

different countries. However, many questions remain to be answered. The impact of the impregnation on the malaria vector populations is not predictable. In some cases reduction in density, longevity or sporozoite rates (mass-killing effect) has been found after the introduction of treated bednets into most of the houses of a village, while in other situations this has not been the case. The impact on malaria has also been variable in different areas (Bermejo and Veeken, 1992). Apart from differences in vector characteristics, this variability may be related to differences in the level of transmission, immune status and behaviour of the people. The evidence suggests that impregnated bednets are usually effective in reducing malaria-related morbidity (Choi *et al.*, 1995) although the effect on the prevalence of malaria infection has been variable.

Insecticide-impregnated bednets has been the subject of many extensive reviews (Rozendaal, 1989; Curtis *et al.*, 1991; Bermejo and Veeken, 1992; Sexton, 1994). This chapter focuses on the effects of treated bednets on malaria vector populations and on malaria, with emphasis in *Anopheles gambiae s.l.* and in the experience in The Gambia. The objectives of this thesis and its organization are also presented.

1.2. Effects of untreated bednets on malaria vector populations.

Bednets are used in many countries, mainly as a defence against mosquitoes. The purpose is to have a physical barrier to prevent mosquitoes from biting and to have an uninterrupted night's sleep. Bednets are used also for privacy, protection against cold, dust, snakes, insects in general, rats, etc., and even for 'beautifying' the room . (MacCormack and Snow, 1986; Rozendaal *et al.*, 1989; Aikins *et al.*, 1993).

A reduction in the numbers of blood-fed anophelines in rooms where untreated bednets are used has been observed in several studies (Lindsay *et al.*, 1989a; Port and Boreham, 1982; Charlwood, 1986). However complete protection against mosquito bites is not always achieved with untreated bednets for a variety of reasons: with use holes are torn, or the nets are not properly set, or have entry flaps, allowing mosquitoes to get in. As mosquitoes are attracted by the person sleeping under the bednet, even a small part of the

body in contact with the bednet will be enough for mosquitoes to bite (Curtis *et al.*, 1991). With intact untreated bednets as many as 19% (Port and Boreham, 1982) to 27% (Lines *et al.*, 1987) of anophelines entering an experimental hut to feed were blood fed the following morning, although it is not known what proportion fed on the occupant of the bednet.

1.3. Effects of untreated bednets on malaria.

Reduction in splenomegaly (Bradley *et al.*,1986) and parasite rates (Campbell *et al.*, 1987) were found in retrospective analysis in The Gambia between users of untreated bednets and children without bednets. In another study in which untreated bednets were given to people in 7 out of 16 villages (Snow *et al.*, 1988a), no significant reduction in splenomegaly, in fever associated with parasitaemia, in the prevalence of parasitaemia or in heavy parasitaemia was found among children in the villages with bednets. The explanation suggested by Snow *et al.* (1988a) for the results of the retrospective studies was that the proximity of children sleeping with and without bednets caused mosquitoes to be diverted to unprotected children, leading to an increase in malaria infections in the non users of bednets rather than, or as well as, a reduction in malaria morbidity in the users. However, in a recent study (D'Alessandro *et al.*,1995a), reduction of parasite and spleen rates and increase of the mean packed cell volume were found comparing children users of untreated bednets with children that sleep without a bednet. No difference was found in the proportion of children with high parasitaemia ($\geq 5000/\mu l$). Also, an inverse relationship was observed between malaria prevalence rates and bednet usage (untreated) in different areas in The Gambia (Thomson *et al.*, 1994).

Studies in other countries have shown similar results. In a study in India (Jana-Kara *et al.*, 1995) the proportion of positive slides, *P.falciparum* rates and monthly parasite index showed an increment in villages without bednets, while no such increase was observed in villages with untreated bednets. In Papua New Guinea (Genton *et al.*, 1994), lower parasite prevalences and spleen rates were found in people using their own untreated bednet, compared with non-bednet users. In this study the results of bednet users were compared

with those of non-users in houses without bednets to avoid bias due to the selection of the controls.

Untreated bednets therefore, may provide some protection against malaria. However, these (D'Alessandro *et al.*, 1995a; Jana-Kara *et al.*, 1995) and other studies with insecticide-impregnated bednets have shown a higher impact on malaria as described in the next section.

1.4. Insecticide-treatment of bednets.

1.4.1. Objectives of the impregnation of bednets.

Bednets are attractive to mosquitoes due to the carbon dioxide and odour of the sleepers under them, thus, insecticide-treated bednets act as a trap (Curtis *et al.*, 1991). Unlike intradomiciliary spraying with DDT, in which mortality of mosquitoes is expected to happen after blood-feeding, impregnated bednets tend to repel or kill mosquitoes before they bite protected sleepers. This difference is noted by the users and therefore, increases acceptability. Even, a person sleeping outside but in the same room as a treated bednet, has been found to receive fewer bites than if the bednet were untreated or if there were no bednet at all (Lines *et al.*, 1987).

The objectives of the impregnation of bednets with insecticide are two: first, to improve **personal protection** by overcoming the problems of damaged or badly used bednets. Second, to shorten the lifespan of the vector population (called the **'mass-killing effect'**). Mosquitoes would risk being killed by contact with the insecticide on the bednets in each gonotrophic cycle, hence reductions in the longevity, density and sporozoite rates in the populations are expected. In order for the treatment of bednets to be beneficial, one or other of these 2 effects is necessary.

1.4.2. Insecticides.

Pyrethroids are the insecticides of choice for treatment of bednets due to their low

toxicity to mammals and high toxicity against insects. They have proved to be very effective controlling insects pests of medical and veterinary importance (Zebra, 1988). Research on natural pyrethrins started in 1920's with the clarification of their structure. In 1930's pyrethrum house spraying was used against malaria in South Africa and in India with encouraging results (Busvine, 1993).

In the 50's the so-called 'first-generation' pyrethroids (e.g. allethrin, bioallethrin, tetramethrin) were found to be useful as domestic insecticides. In the 1960's and 1970's research was directed to look for more active compounds and to make them more photostable than their predecessors. Insecticides like permethrin and deltamethrin introduced a new era of photostable pyrethroids, often referred to as the 'second-generation' pyrethroids. New 'third-generation' pyrethroids have emerged mainly by purification of isomeric mixtures to concentrate the most active forms (cis) or substitutions within the alcohol and/or acid moieties (e.g. lambdacyhalothrin).

Pyrethroids act at the nerve membrane modifying the sodium channels, probably by impeding protein conformational changes at the lipid-protein interface. The neurophysiological consequences include repetitive firing, blockage of impulse conduction or of neuromuscular transmission, and spontaneous depolarization of the resting potential. The lethal activity of pyrethroids seems to involve action on both peripheral and central neurones, while the knock-down effect is probably produced by peripheral intoxication (Zebra, 1988).

Permethrin, deltamethrin, cyfluthrin and lambdacyhalothrin are the pyrethroids most frequently used for treatment of bednets. In the laboratory and studies in experimental huts other classes of insecticide have also been evaluated, mainly with the idea of using them as a mixture with pyrethroids to delay or prevent resistance (Miller *et al.*, 1991; Curtis *et al.*, 1996).

1.5. Effects of insecticide-treated bednets on malaria vector populations.

Insecticides cause diverse effects on the behaviour of insects. These include not only

neurotoxicity caused by the insecticide but also behavioural responses to the presence of the insecticide. Insecticides can stimulate or depress general locomotory behaviour of insects such as walking and flight. Insecticides often cause insects to become uncoordinated or even convulsive, and may therefore interfere with the normal patterns of reproduction, host seeking, dispersal, migration, and feeding in a very general way. Insects could respond to some insecticides by minimizing their contact with the toxic material (Haynes, 1988).

Insecticides in general, and pyrethroids on bednets in particular, have several effects on mosquito vectors. Inhibition of oocyst development of *Plasmodium* in the mosquito gut by pyrethroids has been found. Experiments exposing *Anopheles* mosquitoes to sublethal doses of pyrethroids on netting while feeding on mice infected with *Plasmodium yoelii* and membrane feeding on cultured *P. falciparum* gametocytes have shown a reduction in the proportion of individuals that became infected as well as on the number of oocysts per gut in those that were infected (Hill *et al.*, 1989; Hill and Ranford-Cartwright, 1993). This phenomenon may have an effect on malaria transmission but has not been investigated in the field.

The most important effect of insecticides for control of transmission is the reduction in the survival of the population. In addition, effects like repellency or deterrence could reduce man-vector contact, therefore reducing the risk of acquiring the infection. The finding of an insecticide-treated barrier over the source of blood, and in the room where they rest, could bring about changes in mosquitoes' biting and resting behaviour. One might expect that this could lead, for instance, either to diversion to biting unprotected people or animals, or to changes in the gonotrophic cycle. As well as these phenotypic effects, selection by the insecticide may also eventually cause a more permanent genotypic change such as physiological resistance (Vulule *et al.*, 1994 and 1996) and/or inherited behavioural changes.

The effects of insecticide-treated bednets on *Anopheles* mosquitoes have been described from studies in the laboratory, experimental huts and on the village scale (Curtis *et al.*, 1991). Mortality, repellency, deterrence and feeding inhibition as effects of excito-repellent insecticides have been described, as well as changes in biting and resting behaviour.

The use of experimental huts can help to distinguish these effects which are difficult to separate in observations in the field (Smith and Webley, 1968). Reduction in the number of mosquitoes and sporozoite rates due to changes in survival rates have been evaluated in village scale studies.

1.5.1. Effects in experimental hut studies

Experimental huts are built in a way that as far as possible resembles village huts, but also allows estimation of the number of mosquitoes that enter the huts, the number that exit, and the mortality. The design generally follows the description by Smith (1965): in two of the sides of the hut eaves are open to the outside so that wild mosquitoes can enter and exit the hut. On the other two sides screen verandah traps are placed to trap the mosquitoes in their way out (Figure 1.1).

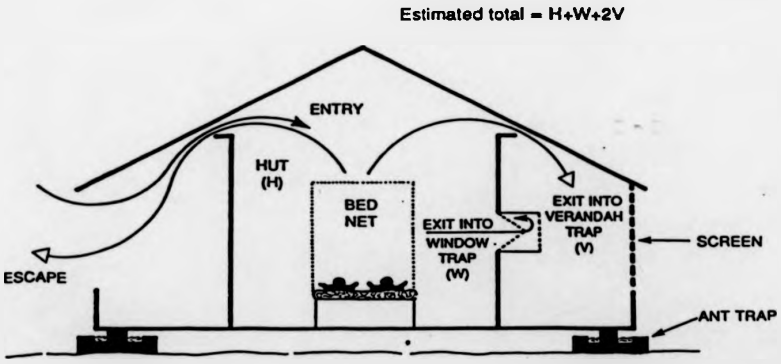

Figure 1.1. Diagram of an experimental hut showing a pair of sleepers under a bednet. Dark arrow shows how mosquitoes may enter the room via the open eave above the wall; open arrows show how mosquitoes may leave the room via the open eaves, either escaping or being trapped in the window trap or verandah trap. Diagram taken from Curtis *et al.* (1992) and Curtis et al. (1996).

To correct for possible bias in the direction of exiting, the verandah traps are alternated between the sides. In this way it is assumed that of the mosquitoes exiting through the eaves, half are caught and half succeed in escaping, so that the number collected in the verandahs can be multiplied by two to estimate the total that exited through the eaves. Huts are provided with ant traps to reduce the possibility of scavengers so that most of the mosquitoes that die in the hut can be found on the floor in the morning.

A summary of the results of experimental hut studies is presented in Table 1.1. The percentages presented are approximations based on comparisons between treated and untreated bednets, because in most of the studies the control used was an untreated bednet. In some studies (Lines *et al.*, 1987; Curtis *et al.*, 1992; Curtis *et al.*, 1996) controls with no-bednet were also used. In these studies however an untreated bednet was used too, so that this comparison can still be made. A series of experiments covering 4 years work in Tanzania, have recently been published (Curtis *et al.*, 1996). Different bednets and curtains materials, doses, formulations, and insecticides have been compared in these experiments. Only the averages by insecticide are presented in Table 1.1.

1.5.1.1. Deterrence

Deterrence has been defined as: "all possible causes that may prevent mosquitoes gaining access to houses treated with residual insecticides" (De Zulueta and Cullen, 1963). The number of mosquitoes that enter a hut is estimated by doubling the verandah trap catches and adding this to the numbers found inside the huts plus the exit traps (Smith, 1965).

Deterrence has been found in most of the studies with permethrin, with few exceptions. In the study by Pleass *et al.* (1993) deterrence was found only with the wash-resistant formulation (incorporating polystyrene into the emulsifiable concentrate) and not with the normal emulsifiable concentrate, contrasting with previous results found in the same Gambian experimental huts (Miller *et al.*, 1991; Lindsay *et al.*,1991 and 1992). No explanation was given for this contrast. Miller *et al.* (1991) found that the deterrent effect was lost after washing the net with local soap.

Table 1.1. Summary of the effects of insecticide-treated bednets on *Anopheles* populations in experimental hut studies. Results presented as approximate percent reduction or increase related to the percentages found with the untreated bednet used as control.

Effect: Det:increased deterrence, Exit:increased exiting, FI:feeding inhibition, Mort:increased mortality,

'ns':no significant difference,
' - ':not presented in the paper.

Insecticides: P:Permethrin, WR-P:Wash resistant permethrin, L:Lambdacyhalothrin, C:Cypermethrin, D:Deltamethrin, PM:Pyrimiphos-methyl, E:Etofenprox,

Anopheles	Insecticide, dosage in mg/m^2 (notes)	Det.	Exit	F.I.	Mort.	Reference
gambiae s.l.	P, 80	70%	60%	20%	17%	Darriet *et al.*(1984)
gambiae s.l.	P, 80	63%	66%	18%	18%	Carnevale *et al.*(1992)
funestus		68%	70%	13%	16%	
arabiensis	P, 200 (a)	20%	-	50%	50%	Lines *et al.*(1987)
	P, 200 (b)	ns	-	ns	50%	
	P, 200 (c)	57%	50%	60%	ns	
gambiae s.l	P, 200 (d)	50%	-	ns	ns	Curtis *et al.* (1992)
and *funestus*	D, 15 (d)	ns	-	70%	ns	
	L, 15 (d)	ns	-	ns	ns	
	P, 200 (e)	ns	-	80%	60%	
	D, 15 (e)	ns	-	ns	80%	
	L, 15 (e)	ns	-	ns	90%	
gambiae s.l	P, 200, 500 (d)	-	-	65%	ns	Curtis *et al.* (1996)
and *funestus*	D, 15, 25(d)	-	-	ns	45%	
	L, 5,10,15(d)	-	-	ns	41%	
	C, 30,50(d)	-	-	66%	ns	
	E, 200 (d)	-	-	ns	35%	
	P, 2% Oly(d)	-	-	92%	ns	
	P and D, 200,25 (d)(l)	-	-	57%	48%	
	P, 200, 500 (e)	-	-	85%	30%	
	D, 25, 3 (e)	-	-	76%	28%	
	L, 3, 5, 10 (e)	-	-	77%	32%	
	C, 50 (e)	-	-	77%	41%	
	E, 200 (e)	-	-	80%	26%	

Table 1.1. Continued..

Anopheles	Insecticide, dosage in mg/m² (notes)	Det.	Exit	F.I.	Mort.	Reference
gambiae s.l.	P, 500	60%	-	ns	30%	Miller *et al.*(1991)
	L, 25	ns	-	ns	50%	
	C, 100	ns	-	ns	50%	
	D, 25	ns	-	ns	50%	
	PM, 1000	ns	-	ns	50%	
gambiae s.l.	P (5)	20%	ns	30%	20%	Lindsay *et al.*(1991)
	P (50)	40%	ns	60%	20%	
	P (500)	60%	ns	90%	30%	
	L (25)	20%	ns	60%	30%	
gambiae s.l.	P (500)	Yes	-	50%	40%	Lindsay *et al.*(1992)
gambiae s.l.	P (500)	ns	ns	60%	30%	Pleass *et al.*(1993)
	WR-P (500)	25%				
sinensis	D (25) (f)	-	-	75%	95%	Li *et al.*(1987)
	D (25) (g)	-	-	14%	83%	
farauti	P (500)	ns	70%	-	88%	Ree (1988)
	P (200)	ns	72%	-	84%	
darlingi	P (500)	31%	-	36%	50%	Rozendaal *et al.*(1989)

(a): Experiment 1, comparison between treated and untreated bednets with holes;
(b): Experiment 1, comparison between treated and untreated intact bednets;
(c): Experiment 2, treated bednet with holes, compared with no bednet;
(d): Intact bednets, comparison with the untreated intact bednet;
(e): Bednets with holes, comparison with the untreated holed bednet;
(f): Bednets closed;
(g): Bednets open.
(h): Permethrin 2% w/w incorporated into the polyethylene fibre;
(I): 4mm mesh polyester bednets.

How mosquitoes are deterred from entering a room with an insecticide-treated bednet is not clear. It seems unlikely that deterrence is due to insecticide vapour, since pyrethroids exhibit a very low vapour pressure (Miller, 1990). Lindsay *et al.* (1991) evaluated a permethrin-free "blank" E.C. formulation and it was found that it caused the same deterrence as the formulation with the insecticide. Therefore, it was concluded that volatile components of the emulsifiable concentrate and not the permethrin itself were causing the deterrence. Smith and Webley (1968) explain deterrence with DDT as due to the build-up of deposit of the insecticide on the untreated overhanging eaves by an outflow of the insecticide from the hut, either as a dust or vapour. Experiments by Somboon (1993) showed that the insecticidal activity of lambdacyhalothrin was prevented by covering with tissue paper mosquitoes held in paper cups near an impregnated bednet. The tissue paper was intended to allow vapour to enter in contact with the mosquitoes but not dust. Although the amount of dust or vapour outside and inside the cups were not measured, it is likely that the paper had prevented dust from entering the cups. Although more experiments are necessary, these observations suggest the presence of insecticide on dust particles, and may explain why pyrethroids cause deterrence with such a low vapour pressure. However, little or no deterrence has been found with lambdacyhalothrin or other insecticides evaluated in experimental huts (deltamethrin, cypermethrin and pyrimiphos-methyl).

1.5.1.2. Exiting

An increase in exiting of *An. gambiae s.l.* from huts with pyrethroid-treated bednets was found in experiments in Burkina Faso and Tanzania, but not in those in The Gambia (Lindsay *et al.*, 1991; Pleass *et al.*, 1993). Miller *et al.* (1991) did not mention exiting in their experiment. In the experiment in the Solomon Islands (Ree, 1988) *An. farauti* showed an increase in exiting of around 70%. Causes for this increase may include: a) repellency (by airborne action of pyrethroids at short range) causing exiting of mosquitoes which have already entered the hut (Miller *et al.*, 1991); b) irritability, causing the mosquito to leave the treated hut after having contacted the insecticide deposit. Irritated mosquitoes

found in verandah traps were shown to have picked up around 10 times less DDT than mosquitoes found dead in the experimental huts (Smith and Webley, 1968).

1.5.1.3. Feeding inhibition

Feeding inhibition has been found as one of the main effects of impregnated bednets. This effect has been found in most studies in experimental huts. Darriet (1984) found that the engorgement rate, defined as the ratio between the number of engorged females and the number of females caught, was reduced in *An. gambiae* by 20% and *An. funestus* by almost 10%.

Comparing intact treated and untreated bednets the reduction in feeding has been significant in some cases (Lindsay *et al.*, 1991 and 1992; Pleass *et al.*, 1993), but not in others (Lines *et al.*, 1987; Miller *et al.*, 1991, Curtis *et al.*, 1996). In the study of Miller *et al.*, (1991) around 40% of fed females contained animal blood, which means that they came to the huts to rest after feeding outside on animals. Whether or not this happened equally in huts with treated nets as well as in untreated ones is not mentioned in the paper.

When the comparison has been between torn bednets, the difference between treated and untreated bednets has been almost always significant (Lines *et al.*, 1987; Curtis *et al.*, 1992 and 1996). Torn bednets simulate better the situation found in the villages and the effect of the impregnation on feeding can be observed more clearly using torn bednets in experimental hut studies.

1.5.1.4. Mortality

Table 1.1 shows approximate percentages of significant increase in mortality compared with the untreated bednets. With permethrin, around 50% increase in mortality has been found in experiments in Tanzania (Lines *et al.*, 1987; Curtis *et al.*, 1992 and 1996), and around 30% in the ones in The Gambia (Miller *et al.*, 1991; Lindsay *et al.*, 1991, 1992; Pleass *et al.*, 1993). The highest increase in mortality (more than 80%) was recorded in the Solomon

Islands by Ree (1988). In general mortality has been higher with insecticides other than permethrin (Li *et al.*, 1987; Miller *et al.*, 1991; Curtis *et al.*, 1992). High mortality in the hut with the control untreated bednet (e.g. more than 50% mortality in huts with untreated bednets) has been found in the experiments of Lindsay *et al.* (1991), Lines *et al.* (1987), Miller *et al.* (1991) and Curtis *et al.* (1992). As mentioned by Curtis *et al.* (1992), this can be an artifact since mosquitoes that normally would search for a feed elsewhere, after being prevented from feeding by the bednet, are trapped and are forced to remain in the trap during the night increasing the chances of starvation. This can be important for example in the case of the experiment of Curtis *et al.* (1992) (experiment f in the table) where no significant difference in mortality was found between treated and the untreated bednet, but the difference was significant when the comparison was made with no-bednet.

1.5.1.5. Diversion

1.5.1.5.1. To unprotected people in the same room

The study by Lines *et al.* (1987) (experiment 2 in Table 1.1) was designed to determine whether or not an unprotected person would receive more bites than a person protected by a treated bednet in the same room. There was no evidence of diversion of mosquitoes, and in fact, the estimated number of bites received by the unprotected person was reduced compared with the control situation, in which either of the 2 sleepers were sleeping under a bednet. With an untreated bednet, by contrast, diversion from the protected to the unprotected sleeper was observed.

1.5.1.5.2. To neighbouring rooms or huts

Diversion to neighbouring rooms with untreated bednets might be expected if mosquitoes are deterred from entering rooms with treated bednets. However, when just one of the six experimental huts in The Gambia had a treated bednet, there was no evidence of an increase in the number of mosquitoes in the closest neighbouring untreated huts, in spite of the significant reduction in numbers of *An. gambiae* entering the hut with the treated

bednet (Lindsay *et al.*,1992).

Summarizing, the results in experimental huts have shown that permethrin-impregnated bednets have a good effect on personal protection either through deterrence or feeding inhibition. No evidence has been found for diversion of mosquitoes from a person under a treated bednet to either an unprotected person sleeping in the same room or to neighbouring untreated houses. Treated bednets greatly reduce the proportion of females feeding and surviving, which is the most important parameter for transmission. However, variable degrees of deterrence, feeding inhibition, mortality and exiting have been found between experiments in different areas and even in studies using the same experimental huts.

From these observations the following effects could be expected when treated bednets are used by whole communities: a) reduction in survival rates of the mosquito population due to the killing effect of the impregnated bednets and b) reduction in the man-vector contact due to deterrence and/or feeding inhibition. A summary of the findings in village scale trials is presented below.

1.5.2. Effects in village scale studies

1.5.2.1. The 'mass-killing-effect'.

The mass-killing effect can be defined as reduction of the vectorial capacity of the vector population due to the killing of mosquitoes by the insecticide. This effect might be expected at a village level with high usage of impregnated bednets, and could be observed as a reduction in the density, sporozoite rates and/or longevity of the local vector population. This is the most desirable effect because people would get protection against bites even when they were not under the bednets (e.g. in the early evening, early morning or sleeping without a bednet). The background and importance of this effect is presented in Chapter 2.

A summary of the results of field evaluations related to the mass-effect is

presented in Table 1.2. This summary is based on, and extends, a recent review by P. Somboon (1993). A direct comparison between studies is not straightforward because of the many differences between them (e.g. experimental design, methods of assessments used). The studies were categorised according to features of the design used for the evaluation: I) studies with a contemporary comparison area, ii) studies with base line data and iii) studies with replicate villages, and when the results were reported by village. These three classes are not mutually exclusive. The majority of the studies included base line data, most had a contemporary comparison area but few studies had replicate villages (Table 1.2).

1.5.2.1.1. Effect on density

Without exception all the studies evaluated density, comparing villages with and without and/or before and after introduction of impregnated bednets. Different sampling methods have been used. Pyrethroids cause deterrence and/or increase in exiting of mosquitoes as has been shown in the experimental hut studies (see section 1.4.1.). Therefore, bias in the assessments of density can occur when using, for example, only collections of mosquitoes resting indoors in the presence of treated bednets, as in the studies by Jambulingam *et al.*, (1989) and Jaenson *et al.*, (1994), or in the studies in China where mosquitoes were collected resting on treated bednets (Li *et al.*, 1989; Curtis *et al.*, 1991). However, in the Chinese studies, simultaneous collections were carried out outdoors on untreated bednets which allowed the assessment of density at a village level without bias by the treatment of the bednets.

Most of the studies in Table 1.2 used human-landing collections, and generally they were made indoors and outdoors. The reduction in density has usually been seen in both indoor and outdoor collections when an impact on density was observed. In the studies by Charlwood and Graves (1987) and Lindsay *et al.* (1993) outdoor and not indoor landing collections were used, which would avoid overestimating the effect of impregnated bednets on the indoor landing collections because of deterrence. In the study by Jana-Kara *et al.* (1995) only indoor landing collections were carried out, but this team used 3 methods of

Table 1.2. Summary comparison of the impact of insecticide-treated bednets on density (D), parous (P) and sporozoite rates (S) of the malaria vector populations in village scale studies. (Taken from Somboon (1993) and updated).

Country	Anopheles	Methodology				Impact on			Reference
		n	C	B	R	D	P	S	
The Gambia	*gambiae s.l.*	12	✔	✔	✔	ns	ns	ns	Lindsay *et al.*(1993)
		6	✔	✔	✔	ns	ns	ns	Thomson *et al.*(1995a)
Tanzania	*gambiae s.l.*	5	✔	✔	✔	+++	+++	+++	Magesa *et al.*(1991)
	funestus	5	✔	✔	✔	+++[1]			"
	arabiensis	1	X	✔	X	++[3]			Njau *et al.*(1993)
Burkina Faso	*gambiae s.l.*	1	X	✔	X	+++	+++	+++	Robert & Carnevale
	funestus	1	X	✔	X	ns	+++	++	(1991) and Carnevale *et al.*(1988)
Zaire	*gambiae s.l.*	3	✔	✔	X	+++	+++	+++	Karch *et al.*(1993)
Kenya	*gambiae s.l.*	6	✔	✔	X	+++	++[1]	++	Beach *et al.*(1993)
Guinea Bissau	*gambiae s.l.*	6	✔	✔	✔	+++[3]			Jaenson *et al.*(1994)
Cameroon	*gambiae s.l.*	1	X	✔	X	++[3]	+	ns	Le Goff *et al.*(1992)
	nili	1	X	✔	X	+++[3]	++	++	"
Papua New	*punctulatus*	8	✔	✔	✔	ns		++	Graves *et al.*(1987)
Guinea	*farauti*	1	X	✔	X	++	ns		Charlwood & Graves
	koliensis	1	X	✔	X	++[1]	+++		(1987)
India	*minimus*	12	✔	✔	X	+++			Jana-Kara *et al.*(1995)
	other species	12	✔	✔	X	ns			"
	culicifacies	2	✔	✔	X	++[1,2]		++[1]	Jambulingam *et al.*(1989)
Thailand	*minimus*	9	✔	✔	✔	ns	ns		Somboon *et al.*(1995)
Malaysia	*maculatus*	4	✔	✔	X	++[3]		+++[3]	Vythilingam *et al.* (1995)
The Solomon	*farauti*	4	✔	X	X	++	+		Kere (1992)
Island	*farauti*	4	✔	✔	X	++			Kere *et al.*(1993)
	punctulatus	4	✔	✔	X	ns			"
	punctulatus	1	X	✔	X	++[1]			Samarawickrema*et al.*(1992)
	farauti	4	✔	X	✔	+++		+++	Hii *et al.*(1993)
	farauti	3	✔	X	X		+++		Hii *et al.*(1995)

Table 1.2. Continued.

Country	Anopheles	Methodology n	C	B	R	Impact on D	P	S	Reference
China	*sinensis*	(4)	✔	✔	X	++(2)			Li *et al.*(1989)
	anthropophagus		✔	✔	X	+++(2)			"
	sinensis	(5)	✔	✔	X	+++(2)	+++		Yang *et al.*(1991;in
	antropophagus		✔	✔	X	+++(2)	+++		Curtis *et al.*(1991)
	dirus		✔	✔	X	+++(2)			Li (in Curtis *et al* 1991)
	sinensis and *anthropophagus*	45	✔	✔	✔	+++(2)	+++		Cheng *et al* (1995)

Key for methodology qualification: n= sample size, number of villages involved in the entomological evaluation
C=contemporary comparison area,
B=base line data,
R=replication.

Key for reported effects: ns: no significant,
+:slight,
++:moderate,
+++: strong.

(1) Studies in which study areas were not comparable with and without (before and after) intervention.
(2) Studies in which sampling methods may be biased by excito-repellent effect of the insecticide.
(3) Study with unclear presentation of the results (disagreement between figures for sporozoite rates and number of mosquitoes, significant reduction in density presented after intervention in a Table but not seen in the Figure)
(4) Around 40,000 people in the area.
(5) 30,374 people in the area.

collection in both treated and untreated villages: an unprotected collector, another under an untreated bednet and a third under a treated net. The bednets were raised about 8 cm off the ground. In this way they could assess not only personal protection given by the bednets but also the 'mass-effect' of widespread use of treated bednets in a whole village.

The selection of a room with an untreated bednet in treated villages ('sentinel rooms') for indoor mosquito collections is an approach that allows one to avoid the possible bias of using rooms with treated bednets (Githeko *et al.*, 1996). This approach has been used in Tanzania (Magesa *et al.*, 1991) and in The Gambia (Lindsay *et al.*, 1993; Thomson *et al.*, 1995a).

Other methods have been used to estimate changes in density and they include the use of light traps which have been placed indoors in 'sentinel rooms' in Tanzania (Magesa *et al.*, 1991) and in The Gambia (Lindsay *et al.*, 1993). As part of the same study in Tanzania correlation was shown between indoor human-landing and light traps collections (Lines *et al.*, 1991). Pit traps to collect outdoor resting mosquitoes have been used in Tanzania (Magesa *et al.*, 1991).

Another approach to estimate changes in density has been used in the studies in The Gambia (Lindsay *et al.*, 1989b; Thomson *et al.*, 1995a). Human fed females found resting indoors and in exit traps were utilised to estimate the human-biting density. The collections were carried out in 'sentinel rooms', thus avoiding the possible bias of using treated rooms.

As Table 1.2 shows, an effect on density has been found in most of the studies, except those in The Gambia, and those in Papua New Guinea on members of the *An. punctulatus* group. In India drastic reduction in density was found with the very antropophilic vector *An. minimus* after the introduction of deltamethrin-impregnated bednets, but not with other species of *Anopheles* or *Culex* (Jana-Kara *et al.*, 1995).

1.5.2.1.2. Effect on longevity

Besides reducing density, the killing of mosquitoes by the insecticide is

expected to reduce longevity of the population given that a proportion of females is killed in each gonotrophic cycle. Measuring a reduction in longevity can often be done more easily and with less bias than measurement of reduction in density (Garrett-Jones and Grab, 1964). About half of the studies presented in Table 1.2 have attempted to measure longevity. In the study of Magesa *et al.* (1991) in Tanzania, a reduction in the mean number of ovarian dilatations was observed in *An. gambiae s.l.* after the introduction of impregnated bednets. In the Solomon Islands, Hii *et al.* (1995) used time-series analysis to estimate the survival rate per gonotrophic cycle. A significant reduction in the expected infective life of *An. farauti* was found in the village with permethrin-impregnated bednets compared with the untreated and a DDT-sprayed village.

In the other studies presented in Table 1.2. parous rates have been used as an indication of longevity of the population of *Anopheles*. When large numbers of mosquitoes are dissected, and recruitment to the population is not changing dramatically, parous rates provide a reasonable estimate of longevity (Holmes and Birley, 1987). Reduction in parous rates have been observed in most of the studies except in The Gambia on *An. gambiae s.l.* (Lindsay *et al.*, 1993; Thomson *et al.*, 1995a) and Papua New Guinea on *An. farauti* (Charlwood and Graves, 1987).

1.5.2.1.3. Effect on sporozoite rates

A reduction in the sporozoite rates is expected partly because mosquitoes are prevented from feeding on infective people (personal protection), and may either bite non-human hosts or fail to feed at all. There may also be a direct effect of pyrethroids on the development of *Plasmodium* in mosquitoes (Hill and Ranford-Cartwright, 1993). However, the main reason to expect an effect on sporozoite rates is that mass-killing is expected to reduce longevity (see above). For example in Tanzania (Magesa *et al.*, 1991) there was little or no evidence for an increase in animal feeding, and multiple age grading showed that the effect on sporozoite rates could be entirely explained by a reduction in the survival rate per gonotrophic cycle. Sporozoite rates have been found to be reduced in all the studies where

this parameter was measured, except in those in The Gambia and in Cameroon on *An. gambiae*. In some studies the reduction in density was such that not enough mosquitoes could be collected to assess sporozoite rates (Jaenson *et al.*, 1994).

1.5.2.2. Effect on the gonotrophic cycle length

In Papua New Guinea, Charlwood and Graves (1987), observed that the effects of introducing permethrin-impregnated bednets in one hamlet were more complex than just the killing of mosquitoes or preventing them from feeding. Comparing before and after the introduction of bednets, besides a drop in the number of *An. farauti* collected landing on unprotected human baits and a drop in the human blood index, there was evidence for an increase in the length of the oviposition cycle from 2 to 3 days. The explanation suggested for the increase in the length of the oviposition cycle together with the change in the peak biting time (see section 1.5.2.3) was that the impregnated bednets prevented or delayed feeding by mosquitoes that have just laid eggs, so that they had to return to feed the following early evening.

In the Solomon Islands, a gonotrophic cycle length of 4 days for *An. farauti* No.1 was observed in the village with permethrin-impregnated bednets compared with a 3 day in the DDT-sprayed village and the untreated village (Hii *et al.*, 1995). Time series analysis was used to estimate the oviposition interval of the mosquito population.

Apart from these studies there have been no other reports of attempts to measure changes in the gonotrophic cycle length after the introduction of treated bednets.

1.5.2.3. Effect on the biting time

Changes in the biting time have been reported by Charlwood and Graves (1987) where a shift in the peak of biting time from post-midnight to pre-midnight was found for *An. farauti*. Njau *et al.* (1993) also found that the highest biting activity for *An. gambiae* was during the first hours of the night. The number of mosquitoes collected indoors dropped after

23:00 hours in houses with bednets treated with deltamethrin or lambdacyhalothrin, in contrast to the cycle observed in houses with no bednets. The effect of the insecticide on the biting cycle in this study cannot be explained in a similar way to the observations of Charlwood and Graves (see previous section 1.5.2.2.), since only some rooms in one village had treated bednets. Other studies have shown that the biting cycle of the anopheline mosquitoes remained unchanged in the presence of treated bednets (Zoulani *et al.*, 1994; Magesa *et al.*, 1991; Samarawickrema *et al.*, 1992)

1.5.2.4. Diversion to bite or rest outdoors

Reduction in entrance (deterrence) and increase in exiting of mosquitoes in rooms with a treated bednet have been shown in experimental hut studies (section 1.5.1). One could expect therefore that mosquitoes are diverted to bite and rest outdoors instead of indoors, particularly in cases when a 'mass-killing effect' has not been found. A change in the indoor/outdoor landing ratios would be expected in simultaneous human-landing collections, if the indoor collector is in a room with a treated bednet.

Man-landing collections indoors and outdoors simultaneously have been used as the method of assessment in most of the field studies in Table 1.2, the main objective being to measure changes in density. Because of this, indoor/outdoor ratios were not analysed in the majority of the studies. In the studies by Hii *et al.* (1993), Karch *et al.* (1993) and Beach *et al.* (1993) the indoor and outdoor collections were pooled in order to calculate the entomological inoculation rate. In the study by Graves *et al.* (1987) a great variation was found in the number collected related to insect population fluctuations caused by climatic factors, therefore the results of these collections were not analysed. In the study by Samarawickrema *et al.* (1992), the reduction of the *An. punctulatus* population outdoors was greater than that indoors in rooms with treated bednets, a surprising result tentatively explained by the authors as due to the few surviving mosquitoes biting more avidly indoors when excited by the insecticide.

Only in three studies has the question of diversion outdoors, either to bite or to

rest, been addressed. Magesa *et al.* (1991) carried out outdoor as well as indoor biting collections in the first 3 hours of the night and collections of resting mosquitoes in pit traps outdoors, Somboon (1993) and Robert and Carnevale (1991) analysed the indoor/outdoor human-landing ratios. No evidence has been found from these studies for diversion of mosquitoes to bite or rest outdoors.

1.5.2.5. Diversion to bite animals

Diversion to bite animals might be expected as an effect of impregnated bednets, particularly in species with zoophilic or not very strong anthropophilic tendencies. Few studies have attempted to assess this effect. Magesa *et al.* (1991) found, analysing the source of blood of small samples of mosquitoes resting in pit traps, that the great majority of feeds by *An. gambiae s.l.* were on humans, without any change after the introduction of the impregnated bednets. Other species (*An. funestus* and/or *An. rivulorum*) fed predominantly on animals both before and after the introduction of impregnated bednets. A reduction in the human blood index in mosquitoes resting indoors was found in one study in Papua New Guinea (Charlwood and Graves, 1987), suggesting an increase in the relative, if not the absolute rate of animal biting.

1.5.2.6. Overview of entomological effects

Summarising the effects of insecticide-impregnated bednets in village scale studies, the 'mass-killing effect' can be associated with behaviour of the malaria vector species. A clear 'mass-killing effect' has been seen in some countries, particularly when anthropophilic, endophagic and late bite malaria vector populations were present. For example in studies where the main vectors were *An. gambiae s.l.* (Africa), *An. anthropophagus* (China) and *An. minimus* (India). In other areas where the mosquito species were predominantly zoophilic e.g. *An. minimus* in Thailand, or where they were feeding on both animals and people, i.e. the *An. punctulatus* group in Papua New Guinea and species other than *An. minimus* in India, a 'mass-effect' has not been apparent. However, some exceptions

have been found. For example in The Gambia, despite the presence of anthropophilic *An. gambiae s.l.* populations, a number of studies covering different areas of the country, have found little or no evidence for a 'mass-killing' effect (more details in section 1.7.1) Preliminary results of trials currently carried out in Sierra Leone (E. Magbity, personal communication) also have shown little evidence for this effect on *An. gambiae s.l.*. On the other hand, in some situations in which the vector has a zoophilic tendency, treated bednets have shown to have a significant impact on density and parous rates as is the case of *An. sinensis* in China (Table 1.1). In Guatemala, in which the vector *An. albimanus* has a zoophilic and early biting tendency, a significant impact has been found in malaria transmission (Richards *et al.*, 1993). Whether a 'mass-killing effect' was observed after the introduction of treated bednets or any other effect is not known.

Other effects of pyrethroid-impregnated bednets include changes in behaviour (time, hosts, place of biting) or length in the gonotrophic cycle. These effects have been less often evaluated, but have been found in some studies. These effects can be of help in the understanding of how impregnated bednets work, particularly in cases where a 'mass-killing effect' is not found.

1.6. Effects of treated bednets on malaria

A summary of the results of village scale studies on prevalence (parasite rates), incidence (new cases after clearing pre-existing parasitaemia with chemotherapy) and clinical malaria (fever, high parasitaemia and splenomegaly) is presented in Table 1.3. Despite the differences between studies in design, study populations, seasonality and intensity of transmission and vector behaviour, most of the studies have reported a reduction in parasite rates. In areas of high endemicity (Tanzania, Burkina Faso, Cameroon, The Gambia), however, reduction in clinical malaria (fever, generally associated with high parasitaemia) has been more frequently found than effects on parasite rates (Table 1.3). The effect of treated bednets on mortality in children has been evaluated in The Gambia (Alonso *et al.*, 1991 and 1993; D'Alessandro *et al.*, 1995b), finding a significant reduction. This effect is

Table 1.3. Summary comparison of the impact of insecticide-treated bednets on malaria in village scale studies.

Country	Methodology				Impact on					Reference
	n	C	B	R	Prev.	Inc.	Clinical malaria FP	FHP	S	
The Gambia	16	✔	✔	✔	ns		+	+	+	Snow *et al.*(1988b)
	73	✔	✔	✔	+		+	+	+	Alonso *et al.*(1993)
	104	✔	✔	✔	ns			ns(1)	ns	D'Alessandro *et al.*(1995b)
					+(without area 5)			+(1)	ns	"
Tanzania	5	✔	✔	✔	+/-	+	+/-	+/-		Lyimo *et al.*(1991), Msuya & Curtis(1991)
	1	X	✔	X	+(2)					Njau *et al.*(1993)
	2	✔	✔	X		+				Stich *et al.*(1994)
Burkina Faso	1	X	✔	X	ns		+	+		Carnevale *et al.*(1988)
Zaire	3	✔	✔	X	+			+(1)		Karch *et al.*(1993)
Kenya	6	✔	✔	X	+	+	+			Beach *et al.*(1993)
Guinea Bissau	6	✔	✔	✔	+					Jaenson *et al.*(1994)
Cameroon	1	X	✔	X	ns			+(1)		Le Goff *et al.*(1992)
Papua New G	8	✔	✔	✔	+	+			ns	Graves *et al.*(1987)
India	3	✔	✔	X			+			Jana-Kara *et al.*(1995)
The Solomon Island	1	X	✔	X	+					Samarawickrema *et al.*(1992)
	43	✔	✔	X	+(3)					Kere *et al.*(1993)
	63	✔	X	X	ns					Hii *et al.*(1993)
China	43	✔	✔	✔	+					Li *et al.*(1989)
	(4)	✔	✔	✔	+					Yang *et al.*(1991;in Curtis *et al.*1991)
		✔	✔	✔	+		+			Cheng *et al*(1995)
Guatemala	5	✔	✔	X	+					Richards *et al.*(1993)

Key for methodology qualification: n= sample size, number of villages involved in the study, C, B, R=as in Table 1.2.

Key for impact on: Prev.= Prevalence of parasitaemia.
Inc.= Incidence of parasitaemia after clearing pre-existing parasitaemia.
Clinical malaria: FP=fever associated with parasitaemia, FHP=fever associated with high parasitaemia, S=splenomegaly.

Key for reported effects: ns: no significant reduction observed,
'+': significant reduction observed,
'+/-': reduction in the two estate villages but not in the other three (traditional) villages.

(1): High parasitaemia evaluated, notassociated with fever.
(2): Effect seen compared with no-bednets. No significant effect compared with untreated bednets.
(3): Maybe biased by chemotherapy.
(4): 30,374 people in the area.

currently being evaluated in Burkina Faso, Western Kenya, Coastal Kenya and Ghana. Although not yet formally reported, the trials in Coastal Kenya and Kenya are complete and the results are said to be encouraging (C. Lengeler, personal communication).

The finding of low or non-significant effects on parasite rates by impregnated bednets in areas of high parasitaemia could be explained by the relationship between infective bites and prevalence of parasitaemia (Figure 1.2). This figure shows the relationship between parasite rates and vectorial capacity based on data from the project at Garki, Nigeria (Molineaux and Gramiccia, 1980).

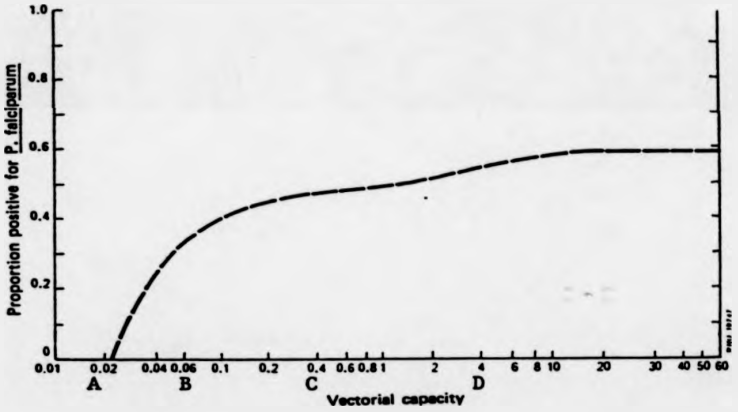

Figure 1.2. Yearly average crude parasite rate as function of yearly average vectorial capacity based on data from the project at Garki, Nigeria (after Molineaux and Gramiccia 1980). Taken from Rozendaal and Curtis (1989).

.As explained by Rozendaal and Curtis (1989), in areas of high endemicity people receive a high number of infective bites and those infections may persist concurrently for many days in the absence of drug treatment. In response to this high malaria challenge, a high degree of immunity is built up after early childhood. Under such conditions, a moderate reduction in the number of infective bites, for example a reduction from D to C,

may be insufficient to find a detectable reduction in the parasitaemia, since there would be few intervals during which all previous infections had been eliminated before the acquisition of new ones. In areas where there is generally a long interval between recovery from one malarial infection and acquisition of the next one, a strong relationship may exist between reduction in vectorial capacity and reduction in prevalence of infection, for example a reduction from C to B.

The reason for the greater effect of impregnated bednets on clinical malaria and mortality, than on parasitaemia, is not clear. In the first place, it is still unknown what triggers clinical malaria in a situation of high endemicity, where most of the people have already high levels of parasitaemia and only a small proportion suffer from fever and other malaria symptoms at any one time. Size of the inoculum of sporozoites (Greenwood, *et al.* 1991; Marsh, 1992) and strain-specific immunity (Lines and Armstrong, 1992) have been proposed as explanations. The first hypothesis assumes a relationship between inoculum size, parasite density and clinical malaria. However, there is little evidence for this relationship (Glynn *et al.*, 1995). Strain-specific immunity assumes that 'new strains' of the parasite, for which the immunity has not yet been developed, would result in clinical malaria. Impregnated bednets, by reducing the biting frequency, would reduce the probability of acquiring those 'new' strains, thereby, reducing the probability of the development of clinical malaria (Lines and Armstrong, 1992). Glynn *et al.* (1994) pointed out that by simple probability the protective efficacy of impregnated bednets is expected to be greater against the less common outcomes such as malaria with symptoms and severe symptoms rather than parasitaemia; also, other factors such as misclassification (e.g. definition of malaria), confounding factors (e.g. age or socio-economic status) and study design (cross-sectional or longitudinal studies) are possible explanations. But the question remains on what triggers the 'rare' outcomes (clinical malaria).

1.7. Malaria in The Gambia

Malaria is one of the major causes of mortality and morbidity in children in The Gambia. The transmission is seasonal, confined to the period during and just after the annual

rainy season which extends from June to November. *Plasmodium falciparum* is the dominant parasite and is responsible for nearly all serious malaria infections in children. *P. malariae* is also found especially in the dry season. It has been calculated that malaria is responsible for 4% of the deaths in infants and for 25% of those in children age 1 to 4 year. The overall malaria mortality rate in children under the age of 5 years was estimated as 10 per 1000 per year. Regarding morbidity, it has been estimated that children under the age of 7 in the rural areas suffer about one clinical attack of malaria per year on average (Greenwood *et al.*, 1987).

Control of malaria in The Gambia has been based mainly on presumptive treatment of attacks with chloroquine. Primary health care workers in the villages have been trained to recognize malaria symptoms and to treat them. However, according to one study, this has not resulted in reduction of malaria morbidity or mortality (Menon *et al.*, 1990).

The impact of chemoprophylaxis with Maloprim on morbidity in children has been studied with promising results. However, it is difficult to sustain this measure for a long period of time, as well as to implement it widely, and it may enhance the spread of drug resistant parasites (Greenwood *et al.*, 1988).

As a primary health care programme has been implemented in The Gambia, it is desirable that control of malaria constitutes part of this programme (MacCormack, 1991). Impregnation of bednets with insecticide is one of the methods that could relatively easily be carried out by local health workers. Compared to fortnightly administration of chemoprophylaxis, annual treatment of bednets would demand less effort from the village health workers. It was decided to evaluate permethrin-treated bednets for control of malaria in The Gambia at individual and village levels.

1.8. Impregnated bednets in The Gambia

The use of bednets impregnated with permethrin to control malaria has been studied since 1985 in different areas in The Gambia, with the intention of incorporating this activity into the primary health care system. The impact of impregnated bed-nets on malaria

has been evaluated progressively from individual to a national level:

The initial study during 1985 was carried out at an individual level, in one village, assessing the personal protection against malaria conferred by the use of impregnated bednets in children (Snow *et al.*, 1987a). A subsequent study was carried out in 1987 at a village level, where all the bednets in 7 villages were impregnated and 9 villages were kept as controls (Snow *et al.*, 1988b). The impact of permethrin-treated nets at village level on mortality in infants (< 1 year old) and children (1 to 4 years old), was evaluated in a trial which involved 17 villages for treatment, all part of the Primary Health Care system (PHC villages) and 56 control (non-PHC villages) during 1988-1989 (Alonso *et al.*, 1991). The criterion for a village to be part of the PHC programme is generally to have more than 400 inhabitants, although there are some exceptions. These large villages are surrounded by small, non-PHC, villages whose nets were not treated.

1.8.1. Entomological results of previous studies in The Gambia

The entomological evaluation in the first study was carried out in 8 pairs of treated and untreated rooms (Snow *et al.*, 1987b). In the second study, 2 pairs of treated and untreated villages were selected (Lindsay *et al.*, 1989b). The third was carried out in 6 pairs of treated and untreated villages, comparing both groups before and after treatment (Lindsay *et al.*, 1993). In the first two studies indoor resting densities in rooms with treated bednets were assessed by either room searches or pyrethrum spray catches (PSC) and searches of resting mosquitoes under treated bednets. Exit traps (ETC) were also used. In the third study 'sentinel rooms' (rooms with untreated bednets in treated villages) were used for the collections, which included PSC, ETC and outdoor landing catches.

The results were in summary:

Evidence of the excito-repellent effect of permethrin treated bednets has been found in the following observations:

a) A drastic reduction in all the studies in the number of females found resting inside the permethrin-treated nets.

b) A reduction in the number of unfeds resting in rooms with treated nets (study 1), and, in the number of rooms with gravid females (study 2).

c) A higher proportion of females in exit traps (studies 1 and 2).

Reduction in man-vector contact was estimated to be between 83% and 92% (Lindsay *et al.*, 1989b) based on the numbers of fed females of *An. gambiae s.l.* found in the morning in nets, rooms and exit traps and in their Human Blood Index. This estimate assumes a complete account of all mosquitoes that entered a room, whereas presumably only a fraction of exiting fed females are caught in the traps, the others leaving through routes other than the exit traps (i.e. eaves, doors etc.). In this way a bias in estimated efficacy of the net would be induced by the excito-repellent effect of the treated bednets.

However, although fewer females were found in rooms with a treated bednet, the proportion fed amongst them and their Human Blood Index, were not significantly different between treated and untreated rooms (Snow *et al.*, 1987b; Lindsay *et al.*, 1989b). This suggests that a similar proportion of females that entered succeeded in taking a blood meal as in rooms with untreated nets.

From these studies, it was not clear that a 'mass-killing effect' followed the impregnation of bednets at a village level, due mainly to the bias in the sampling methods that could reflect the excito-repellent effect of the insecticide in the first two studies. In the third study, in which 'sentinel rooms' were used, little evidence was found of an effect on density and on parous rates. However, sporozoite rates were reduced in both treated and untreated villages after the treatment of bednets (Lindsay *et al.*, 1993).

One of the hypotheses Lindsay *et al.* (1993) offered to explain the absence of a reduction in vector survival rates and sporozoite rates in treated villages is that mixture of mosquitoes between treated and neighbouring untreated villages was masking the 'mass-killing effect'.

A mark-release-recapture experiment was carried out in 1992, in three small non-PHC villages 1 - 1.4 Km apart, to estimate the rate of movements of *An. gambiae s.l.*

between villages. The methods used were similar to those described by Rawlings and Curtis (1981). It was calculated that 18.3 % (95% c.i. 12.3% - 26.3%) of mosquitoes collected in one village had come from another neighbouring village (Thomson *et al.*, 1995b). It was considered that these movements could have impaired the entomological evaluation of treated bednets in The Gambia, as the treatment of bednets was implemented in PHC villages, which are in most cases surrounded by small non-PHC villages which would be untreated.

1.8.2. Epidemiological results of previous studies in The Gambia

For the first study two hundred and five children were in the treated group and their bednets were impregnated with permethrin (500 mg/m^2). The bednets of the 184 controls were impregnated with a placebo. Splenomegaly, parasitaemia, packed cell volume, episodes of clinical malaria and incidence of deaths due to malaria were measured in both groups. Clinical episodes of malaria in children under impregnated bednets were reduced significantly compared with the controls. The number of children in this study was too small to measure any effect on mortality and no significant differences were found in the other parameters. No adverse side effects on the children were reported (Snow *et al.*, 1987a).

In the second study a bigger impact on malaria was expected than in the 1985 study, because of the possibility of a 'mass killing effect' of the impregnated bednets on the population of vectors. Splenomegaly, parasitaemia, packed cell volume and episodes of clinical malaria were evaluated in children 1 - 9 years old in both treated and placebo villages. A significant reduction in incidence rates of fever associated with parasitaemia and of fever associated with heavy parasitaemia were found in permethrin treated villages compared with the controls. Also, a significantly lower splenomegaly rate and a significantly higher mean Packed Cell Volume was found compared to the control villages. The prevalence of parasitaemia was similar in both groups of villages (Snow *et al.*, 1988b).

In the third study, during the pre-intervention period the overall all-cause child mortality rate was significantly higher in PHC villages than in non-PHC villages. The infant mortality rates were similar in both groups of villages. After the intervention, the all-cause

child mortality rate in PHC villages was 37% of that in the non-PHC villages. In the post-intervention period, the child malaria mortality rate in the treated villages was 30% of the untreated villages. There was no significant difference in the infant malaria mortality rate. Thus the overall mortality and malaria-specific mortality in children 1 to 4 years of age were reduced by 63% and 70% respectively in the treated villages. In the treated villages half of the children also received weekly chemoprophylaxis (maloprim) or placebo, which did not give a significant beneficial effect against mortality (Alonso *et al.*, 1991). However, clinical attacks of malaria were reduced by 95% in children who received chemoprophylaxis and slept under impregnated bednets but only 51% in those who slept under impregnated bednets alone (Greenwood, 1991).

1.8.3. National Impregnated Bednet Programme (NIBP)

Because of the encouraging epidemiological results of reduction of clinical attacks and mortality in children associated with the use of permethrin impregnated bed-nets in the previous studies, a large scale impregnation campaign was proposed as a National Impregnated Bednet Programme in The Gambia (NIBP).

The objectives were:

1. To introduce the impregnation of householders' own bednets into all Primary Health Care (PHC) villages over a period of two years.

2. To evaluate the impact of this programme on mortality in children.

3. To evaluate the impact of impregnated bednets on the outcome of pregnancy in primigravidae.

4. To monitor the effects of large scale use of insecticide-treated bednets on mosquito behaviour and sensitivity to pyrethroids.

5. To evaluate the cost effectiveness of insecticide-treated bednets and to investigate ways in which cost recovery could be accomplished.

The proportion of beds with a bednet was estimated to be 58% (95% C.I. 48% to 68%) in the country, in a census before the starting of the impregnation. The usage of bednets was higher in the Central region (76%) than in the Eastern or Western (51%) regions and it was higher among target groups such as infants, children less than 5 years and pregnant women than among the general population (D'Alessandro *et al.* 1994).

In the first year, 1992, bed-nets in 220 out of approximately 400 villages with a primary health care centre were impregnated with a mixture of 40 ml of an emulsion of permethrin made from 20 % E.C. in two litres of water to give a target dosage of 250 mg/m^2. The dipping was carried out between June and July, immediately before the start of the rainy season (K. Cham, personal communication). Figure 1.3 shows the dipping of a bednet in one of the PHC villages.

Figure 1.3. Women impregnating their bednets in the village of Brikamaba, Zone 3.

Five study zones were chosen, representing different geographic areas in the country for the evaluation of the programme (Figure 1.4). The study areas involved 104 villages with a population of approximately 113,000 including 18,000 children 1 to 4 years old.

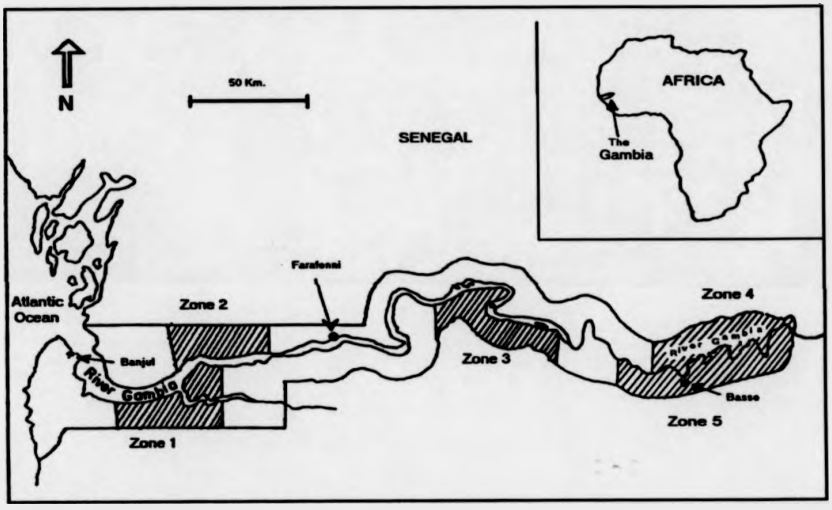

Figure 1.4. Map of The Gambia showing the 5 zones selected for the evaluation of the National Impregnated Bednet Programme.

The entomological evaluation was under the direction of Dr. M. Thomson. This involved studies in zones 2, 3 and 5 having different species composition. The main objectives were:

1. To evaluate the impact of permethrin-treated bednets on survival, sporozoite rates, biting cycle, changes to exophily, exophagy and/or zoophilic behaviour, and to estimate movements between neighbouring villages.

2. To monitor susceptibility to insecticides of the populations in the different zones.

The present study described in this thesis was part of the entomological evaluation of the NIBP.

1.9. Objectives of the study

1. To settle the question of whether or not there is a 'mass-killing effect' by permethrin impregnated bednets in The Gambia.

2. To estimate the amount of movements of mosquitoes between PHC villages and to determine if those movements are influenced by treated bednets.

3. To determine the effects of treated bednets on the behaviour of mosquitoes, including a possible shift of the time of biting, change of endophagic/endophilic habits to exophagic/exophilic, change in the gonotrophic cycle length, change of host from humans to animals, and change from biting children to other hosts.

4. To determine if differences in behaviour of the sibling species of the *An. gambiae* complex may affect the impact of treated bednets.

5. During the evaluation of the NIBP, epidemiological results showed that in one of the zones (zone 5) malaria mortality and morbidity were not reduced by the impregnated bednets. Included in this study was a search for any entomological difference between this area and the others that could explain the failure of the NIBP in this area.

1.10. Organization of the thesis

The thesis reports studies that were part of the entomological evaluation of the National Impregnated Bednet Programme (NIBP) in The Gambia. The aim of the thesis was to determine how impregnated bednets work in terms of their effects on mosquito behaviour.

Chapter 2 describes the results of a 20 village survey in pairs of treated and untreated villages and a cross-over study carried out to clarify whether or not permethrin-impregnated bednets had a 'mass-killing' effect on the populations of *Anopheles gambiae s.l.*.

Mark-release-recapture experiments were carried out to determine how treated bednets would affect movements of mosquitoes between neighbouring villages when one has treated bednets. The results are presented in Chapter 3.

Chapter 4 presents results related to biting time, biting place, and gonotrophic cycle length of the vector populations in the presence and absence of impregnated bednets. To determine whether or not impregnated bednets divert mosquitoes to other hosts (adults or animals), a cross-over study was carried out in two villages where children less than 10 years old were given anti-rabies vaccine to elevate their antibody levels so that blood meals in mosquitoes could be discriminated between those derived from children or from other hosts. The results are presented in Chapter 5.

Chapter 6 deals with a study carried out to compare the use of DNA probes with cytogenetics for identification of species of the *Anopheles gambiae* complex, and to see if there was any association between the different species of the *An. gambiae* complex and different polymorphic inversions in *An. gambiae s.s*, with infectivity and human-blood index.

The epidemiological impact of the NIBP was below expectation in one of the zones of evaluation (zone 5). The results of observations intended to determine whether or not there was an entomological factor involved in the lack of impact in that particular zone are presented in Chapter 7. Different methods of collection of mosquitoes were used and their sampling efficiency was compared within and between zones 3 and 5. The methods included man-landing collections, exit traps, spray catches and light traps. The results are presented in Chapter 7.

Finally, a general discussion and conclusions are presented in Chapter 8.

CHAPTER 2

ATTEMPTS TO FIND EFFECTS OF PERMETHRIN-IMPREGNATED BEDNETS ON DENSITY, PAROUS RATES AND SPOROZOITE RATES ('MASS-KILLING EFFECT') OF *Anopheles gambiae s.l.* IN THE GAMBIA.

2.1. INTRODUCTION

A major reduction in the vectorial capacity of the vector population is the most desirable effect when an insecticide is used for malaria control. Insecticide-treated bednets may be able to provide not only personal protection but also a reduction in the vectorial capacity of the vector population. The degree of this reduction depends on which entomological parameter is most affected: human-vector contact (i.e. mosquito densities and the frequency of feeding on humans) or survivorship of the population of vectors. According to Macdonald's model (Dye, 1986), a given percentage reduction in survivorship gives by far the greatest impact on the vectorial capacity.

The scale on which a given control strategy must be carried out depends on how it operates. For example, house spraying of residual insecticides is effective mainly because of its impact on the survivorship of the vector populations. To achieve this, a high rate of coverage of sprayed houses is necessary over a large area (Onori *et al.*, 1993). With repellents, by contrast, access to individual human hosts is affected, and there is little or no impact on survivorship of the population; in this case individual use is equally effective and it is not necessary to have coverage over a large scale.

In some studies of insecticide-impregnated bednets, a clear 'mass-killing effect' has been found, with observed reductions in survivorship, sporozoite rates and density of the mosquito vector populations at a village level (Magesa *et al.*, 1991; Karch *et al.*, 1993), or a dramatic reduction in biting density (Jana-Kara *et al.*, 1995; Kere *et al.*, 1993). In other situations, by contrast, little evidence for such an effect has been seen (Somboon *et al.*, 1995; Charlwood and Graves, 1987; Lindsay *et al.*, 1993).

In The Gambia, initial epidemiological results suggested that malaria protection improved as coverage with impregnated bednets increased. When individual bednets were impregnated with permethrin or placebo, and used by less than 10% of the children in one village, children sleeping under treated bednets had fewer clinical episodes of malaria but other parameters such as splenomegaly, parasitaemia and packed cell volume (PCV) were not altered significantly (Snow *et al.*, 1987a). In a subsequent community-level study involving 16 villages, where all the bednets in 7 villages were impregnated, a significantly lower acquired spleen rate and a significantly higher mean PCV were found in children in the treated villages in addition to a reduction of clinical cases (Snow *et al.*, 1988b). This apparent improvement in malaria protection when the coverage of impregnated bednets increased was consistent with the hypothesis of a mass-killing effect on the vector populations.

Entomological observations in both studies involved collections of indoor resting mosquitoes (collected in bednets and by knockdown spray catches) and exit trap collections. The results in the first study showed significantly fewer females resting in bednets, fewer unfed females resting in treated rooms, and a higher percentage of females exiting treated rooms (Snow *et al.*, 1987b) measured as ETC/ETC+PSC, where ETC=No. in exit traps and PSC=No. in pyrethrum-spray catches. In the second study, when the impregnation of bednets was at a village level, collections were again carried out in treated rooms in treated villages and in untreated rooms in untreated villages, and this time parous rates, the human blood index (HBI) and sporozoite rates were also evaluated. As in the previous study, a significant reduction in the numbers of fed females resting in rooms with a treated bednet was found. The reduction in human-vector contact was estimated to be over 90%. However this calculation was based on the number of fed females of *An. gambiae s.l.* collected inside and exiting from treated and untreated rooms, together with their human blood index (Lindsay *et al.*, 1989b). This estimate assumed a complete account of all mosquitoes that entered and fed in a room, whereas it is likely that only a fraction of exiting females were caught in the traps, the others leaving through routes other than the exit traps (eaves, doors etc). In this way a bias in the estimated efficacy of the impregnated bednets could be induced by the excito-

repellent effect of the treated bednets. No significant difference was found in parous rates, sporozoite rates or human blood index.

In a subsequent study (Lindsay *et al.*, 1993), also conducted on a village scale, untreated ("sentinel") rooms in treated villages were used for the entomological collections. The use of sentinel rooms avoided the above-mentioned bias caused by the excito-repellent effect of the insecticide when spray catches and exit traps are used. Little evidence of a mass effect was found; there was no indication that treated bednets caused a decrease in density, measured by light traps and spray catches, and the HBI and sporozoite rates were similar in treated and untreated villages. Reductions in the sporozoite rate and in mosquito numbers were found in treated villages after the intervention, but these changes were noted also in villages without treated bednets. However, the possibility of a mass killing effect was not ruled out completely because movements of mosquitoes between treated and neighbouring untreated villages might have masked the mass-killing effect of the insecticide, thus accounting for reductions in mosquito numbers. However, in the post-intervention year a lower rainfall than in the previous year was observed, and this could also have accounted for the reduction in mosquito numbers.

Knowledge of whether impregnated bednets can be expected to produce a 'mass-killing effect' at the village level under local conditions is important for the planning of implementation programmes of malaria control. If a 'mass-killing effect' occurs then, as with DDT house spraying, programs encouraging high coverage are likely to be most effective. On the other hand, if only individual protection is given by the use of impregnated bednets, different strategies could be employed, for example encouraging target groups especially at risk of malaria to acquire and impregnate their bednets.

In order to clarify whether or not permethrin-impregnated bednets have a 'mass-killing effect' in The Gambia, two further entomological studies have been carried out. The first involved a survey of 10 treated and 10 untreated villages, the second, a cross-over study in two villages. Longevity, biting and resting density and sporozoite rates were assessed.

2.2. MATERIALS AND METHODS

2.2.1. Twenty village survey:

Collections were carried out in villages in the central area of The Gambia, between 200 and 280 Km from the Atlantic coast, in July and October, 1992. Twenty primary health care (PHC) villages were selected from amongst these used for epidemiological evaluation of the Gambian National Impregnated Bednet Programme (NIBP) (D'Alessandro *et al.*, 1995b). Ten of these villages had bednets that had been treated between 10 June to 10 July with permethrin at a target dose of 250 mg/m². Pairs of treated and untreated villages were matched by proximity. Locations of the study villages are shown in Figure 2.1.

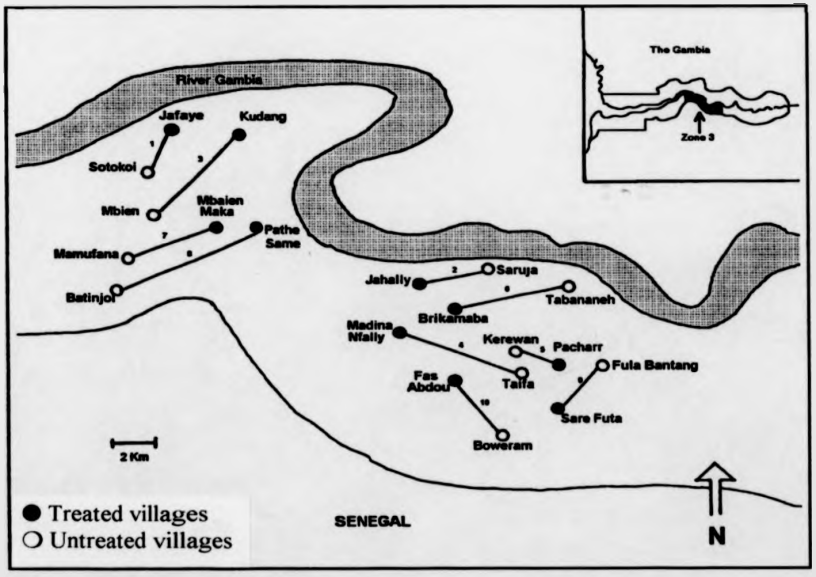

Figure 2.1. Map of the study villages in the 10 pairs of villages survey.

A single human-landing collection was carried out in each village between the 9th and 30th July, 1992. Two teams of 8 collectors were recruited from villages in the study area. One MRC supervisor was assigned to each team. One team worked in a house in a treated village while the other worked on the same night in a house in its paired untreated village. The collectors worked in pairs, two indoors and two outdoors for consecutive periods of 3 hours, from 19:00h to 07:00h. They exchanged places every hour. After 3 hours they were relieved by the other 4 collectors who worked in the same way. All mosquitoes landing on them were collected and placed in a paper cup with a pad of sugar solution.

On the morning after the human-landing collections had been done, pyrethrum spray catches were carried out using an aerosol of 0.3% d-allethrin and 0.1% d-phenothrin (Target®, Reckitt & Colman) in 5 rooms in each village. The teams worked alternately in treated and untreated villages. In addition, in the same ten pairs of villages, mosquitoes were collected in 4 exit traps set on one night in each village during October 1992. Figure 2.2 shows photographs of the human landing and exit trap collections.

Unfed mosquitoes of the *An. gambiae* complex collected in human-landing catches and in exit traps were dissected for parity determinations from the ovarian tracheoles. To avoid possible bias, the slides for parity determinations were coded and scored without knowledge of the village of collection .

Heads and thoraces of *An. gambiae s.l.* mosquitoes were assayed by ELISA (Wirtz *et al.*, 1987) to determine the presence of *P. falciparum* circumsporozoite protein. Cytogenetic examination was carried out to identify the species of a sample of *An. gambiae s.l.* collected in the study area (Coluzzi *et al.*, 1979).

2.2.2. Cross-over study

The cross-over study was carried out in two villages, Madina and Jakoto in the Niamina District, 180 km from the coast, in October 1993. The main objective was to determine changes in the gonotrophic cycle length using mark-release recapture, the results of which will be reported in Chapter 4. However, the sampling routines (spray catches and

NUMEROUS ORIGINALS IN COLOUR

Figure 2.2. Human landing (above) and exit trap (below) collections in the 20 villages survey.

exit traps) also allowed measurements of changes in density, parous rates and sporozoite rates, and these results are presented here.

At the beginning of the study, the objective and procedures of the study were explained to the residents in each village and, through the elders, their consent was obtained. The people in Madina were given new permethrin-impregnated bednets, one for each bed in the village. Bednets were purchased locally, made of light cotton netting and dipped in permethrin, target dose 500 mg/m², at the MRC field station in Farafenni to avoid contamination of mattresses as far as possible. The people in Madina were asked to use the new impregnated bednets for 2 weeks but to keep their own untreated ones in a safe place. In the other village, Jakoto, people were asked to continue using their own bednets, which were unimpregnated. Mosquitoes were collected in both villages for 11 days using pyrethrum spray catches in 10 rooms every day. Five exit traps were set daily in each village. After 2 weeks, the impregnated bednets were collected from Madina and the people began using their own untreated bednets again. The people in the village of Jakoto were then provided with new bednets which were dipped in the village, and dried on their mattresses. People were asked to use them instead of their own untreated bednets. The collection of mosquitoes continued for another 2 weeks in the same way as before. At the end of the study, permethrin-impregnated bednets were given to both villages.

Unfed mosquitoes were dissected for parity determination every day and the percentage with circumsporozoite protein in heads and thoraces was determined by ELISA (Wirtz *et al.*, 1987). Palps of a sample of *An. gambiae s.l.* were dissected and measured for species determination from the palpal ratio (Coluzzi, 1964). Females with a palpal ratio of 0.81 or more were classified as *An. melas* and those with a lower ratio as fresh-water *An. gambiae* (Bryan, 1980).

2.3. RESULTS

2.3.1. Twenty village survey.

2.3.1.1. Density

The total number of *An. gambiae s.l.* collected by human-landing collections in treated villages (Table 2.1) was correlated with that in the corresponding untreated village (r=0.899; t=5.8; df=8; P=<0.001) thus validating the paired design. The difference between treated and untreated villages in the total number of *An. gambiae s.l.* collected in human-landing collections was not significant in a paired t-test after log-transformation of the numbers collected (t=0.19, df=9, P=0.848) (Table 2.1). Significantly fewer *An. gambiae s.l.* were found resting in rooms with treated bednets (geometric mean 4.91/room) than in rooms with untreated bednets (geom. mean 10.52/room) (Wilcoxon signed rank test with geometric means by pair of villages P=0.009) (Table 2.1). Since no such difference was seen in human-landing collections this was presumably due to the excito-repellency effect of permethrin. There were no significant differences between village groups in the proportions of females in each gonotrophic stage (unfed, feds, gravids) or in the sex ratio of *An. gambiae s.l.* resting indoors in the morning (Table 2.2). The number of unfeds resting indoors was very low in both treated and untreated villages.

2.3.1.2. Parous rates

Parous rates from the samples collected in human-landing catches (1 month after bednet treatment) were very variable in both treated and untreated villages (Table 2.3). In some cases the sample sizes were very small. A t-test of the differences was not significant (t=1.35, P=0.21). In the samples collected in exit traps, parous rates were also variable. However, in exit traps the parous rates of *Anopheles gambiae s.l.* collected in exit traps 4 months after treatment were significantly lower in treated than in untreated villages (t=2.44, P=0.035) (Table 2.3).

Table 2.1. Total number of *Anopheles gambiae s.l.* collected in human-landing collections (HLC) from 19:00 h to 7:00 h indoors and outdoors and geometric mean (GM) of mosquitoes resting indoors obtained using pyrethrum spray catches (PSC) in pairs of villages with treated or untreated bednets, (n) number of rooms.

Date	Pair (a)	Days after impregnation	VILLAGES WITH TREATED NETS		VILLAGES WITH UNTREATED NETS	
			HLC	PSC	HLC	PSC
			Total	GM (n)	Total	GM (n)
09/07/92	1	21	470	27.20 (5)	737	114.96 (5)
10/07/92	2	10	150	16.80 (4)	31	26.49 (4)
15/07/92	3	24	210	5.00 (5)	84	17.96 (5)
17/07/92	4	8	77	2.63 (3)	38	8.20 (5)
21/07/92	5	30	44	4.26 (5)	57	6.09 (5)
22/07/92	6	14	44	1.98(5)	140	9.50 (5)
24/07/92	7	32	112	8.45 (5)	155	18.26 (5)
28/07/92	8	34	28	2.10 (5)	29	7.70 (5)
29/07/92	9	20	13	1.99 (5)	15	1.05 (5)
31/07/92	10	20	5	2.57 (5)	7	2.46 (5)
Total			**1158**	**4.91 (47)**	**1293**	**10.52 (49)**

(a): Pairs of villages (treated - untreated): (1) Jafaye - Sotokoi, (2) Jahally - Saruja, (3) Kudang - Mbien, (4) Madina Nfally - Taifa, (5) Pacharr - Kerewan, (6) Brikamaba - Tabananeh, (7) Mbaien Maka - Mamufana, (8) Pathe Same - Batinjol, (9) Sare Futa - Fula Bantang, (10) Fas Abdou - Boweram.

Table 2.2. Indoor resting collections of female *Anopheles gambiae s.l.* classified by gonotrophic stage (U:unfed, F:fed, G:gravid) as percent of total females in pairs of villages with treated or untreated bednets.

Date	Pair	VILLAGES WITH TREATED BEDNETS				VILLAGES WITH UNTREATED BEDNETS			
		%U	%F	%G	Total	%U	%F	%G	Total
09/07/92	1	43.9	55.5	0.7	155	39.8	58.9	12.4	645
10/07/92	2	3.7	72.9	23.4	107	3.6	82.6	13.4	138
15/07/92	3	0.0	74.2	25.8	31	1.1	89.3	9.7	93
17/07/92	4	0.0	100	0.0	10	1.3	17.9	80.8	78
21/07/92	5	0.0	86.7	13.3	30	0.0	71.7	28.3	46
22/07/92	6	0.0	82.8	17.2	29	0.0	82.2	17.8	73
24/07/92	7	0.0	78.6	21.4	56	3.0	79.0	18.0	100
28/07/92	8	0.0	69.2	30.8	39	0.0	57.3	42.7	82
29/07/92	9	0.0	100	0.0	11	12.5	75.0	12.5	8
31/07/92	10	0.0	72.2	27.8	18	0.0	83.3	16.7	18
	Total	**14.8**	**70.4**	**14.8**	**486**	**20.9**	**64.9**	**14.2**	**1281**

Wilcoxon signed rank test of the proportions in treated and untreated villages:
Unfed P=0.44; Feds P=0.50; Gravids P=0.50

Table 2.3. Parous rates of *Anopheles gambiae s.l.* from human-landing and exit trap collections in pairs of villages with treated or untreated bednets.

a) From human-landing collections

		VILLAGES WITH TREATED BEDNETS		VILLAGES WITH UNTREATED BEDNETS	
Date	**Pair**	**% parous**	**n**	**% parous**	**n**
09/07/92	1	69.23	104	35.71	98
10/07/92	2	91.25	80	72.72	22
15/07/92	3	66.31	95	71.15	52
16/07/92	4	86.48	37	60.00	30
20/07/92	5	80.00	35	95.55	45
21/07/92	6	100.00	21	70.17	114
23/07/92	7	31.31	99	37.30	126
27/07/92	8	56.52	23	65.21	23
28/07/92	9	100.00	8	66.00	12
30/07/92	10	33.33	6	50.00	2
Totals		**67.52**	**508**	**57.25**	**524**

b) From exit-traps

		VILLAGES WITH TREATED BEDNETS		VILLAGES WITH UNTREATED BEDNETS	
Date	**Pair**	**% parous**	**n**	**% parous**	**n**
29/09/92	5	64.15	53	73.47	49
30/09/92	1	37.27	110	54.72	106
01/10/92	2	74.36	78	72.88	59
02/10/92	4	80.00	10	87.18	39
03/10/92	7	23.81	21	60.00	35
05/10/92	8	24.14	58	45.45	11
06/10/92	3	40.43	94	61.76	34
07/10/92	6	53.85	104	75.68	74
08/10/92	9	33.33	6	53.85	13
13/10/92	9	94.12	119	72.12	104
15/10/92	7	71.74	46	69.23	13
16/10/92	4	76.67	30	90.00	30
Totals		**58.16**	**724**	**69.14**	**567**

2.3.1.3. Sporozoite rates

A total of 2 out of 640 (0.31%) and 11 out of 1344 (0.82%) mosquitoes were found to be ELISA positive from the treated and untreated villages respectively. This difference is not significant (Fisher's exact test P=0.245).

2.3.2. Cross-over study

2.3.2.1. Density

The numbers of *An. gambiae s.l.* collected in exit traps in the presence and absence of treated bednets are shown in Figure 2.3. Results of a paired t-test on log-transformed data showed that the difference in density in the presence and absence of treated bednets was not significant (t=1.253, df=18, P=0.226).

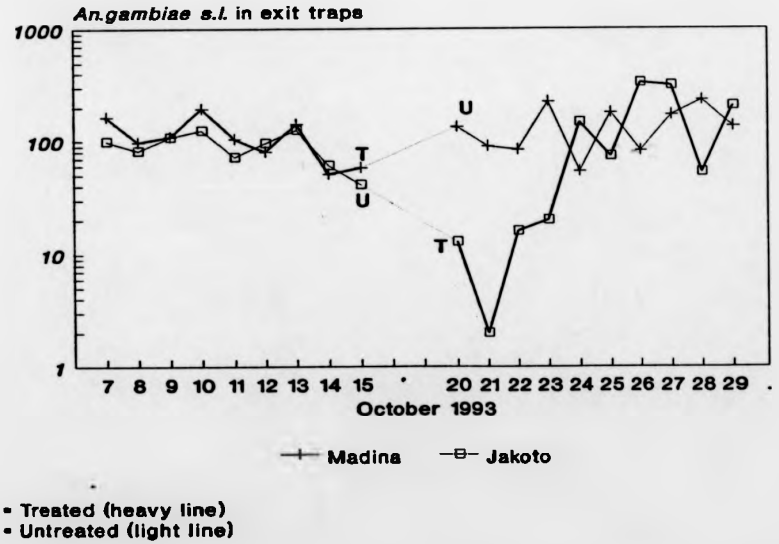

Figure 2.3. Number of females of *An. gambiae s.l.* collected in exit traps in the presence and absence of treated bednets.

The number of females collected in exit traps was very similar in both villages during the first part of the study. However, soon after the cross-over, exit trap densities declined in the treated, but not in the untreated village, suggesting that the traps had been contaminated during the dipping process. Thus, they were washed 3 days after the cross-over and the number of mosquitoes collected in traps in the treated village, Jakoto, then increased again.

As in the 20 village study, the numbers of mosquitoes collected by morning pyrethrum spray catches were lower when treated bednets were present than when they were absent. When bednets were treated, the number of *An. gambiae s.l.* collected resting indoors was, on average, a quarter of the number found when they were untreated. This change in the indoor resting density after the treatment was seen in both villages (Fig. 2.4). A paired t-test showed the difference to be significant (t=8.574, df=17, P=<0.001).

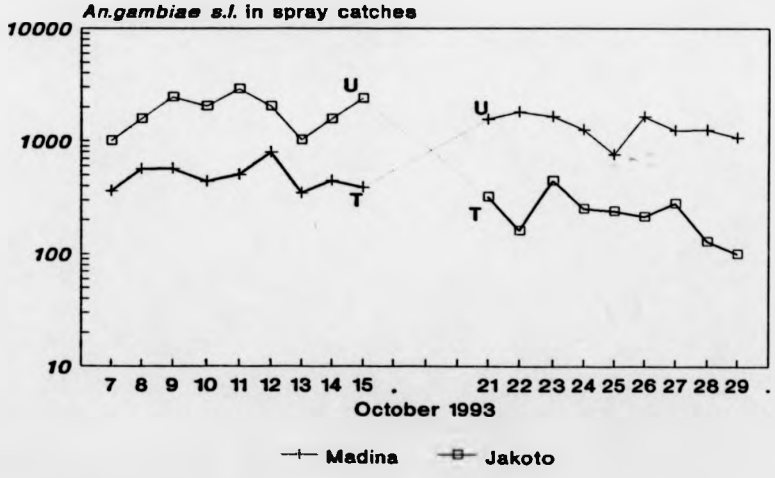

Figure 2.4. Number of females of *An. gambiae s.l.* collected resting indoors by pyrethrum spray catches in the presence and absence of treated bednets.

2.3.2.2. Parous rates

The parous rates observed in the cross-over study are shown in Figure 2.5. A similar pattern was seen in the presence or absence of treated bednets throughout the study, although greater variation was found in the second part of the sampling period. There was no significant difference between parous rates in the presence or absence of treated bednets (paired t-test t=1.05, df=17, P=0.308).

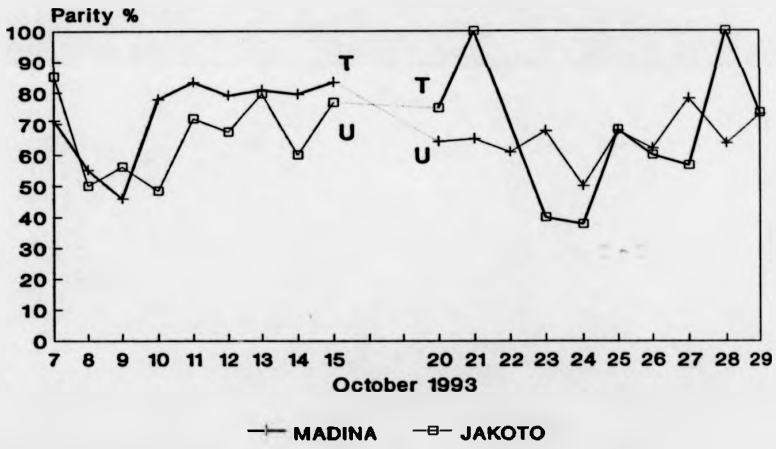

Figure 2.5. Parous rates of females of *An. gambiae s.l.* in the presence and absence of treated bednets.

2.3.2.3. Sporozoite rates

Sporozoite rates were not significantly different in the presence or absence of treated bednets. Results of a χ^2 test are shown in Table 2.4.

Table 2.4. Sporozoite rates of *Anopheles gambiae s.l.* in the cross-over study, October 1993.

Village	Treated	Mosquitoes		Rate	χ^2	P value
		Tested	Positives			
Madina	Yes	2376	24	0.0101		
					0.21	0.649
Jakoto	No	2376	20	0.0084		
Madina	No	2534	14	0.0055		
					0.03	0.989
Jakoto	Yes	2546	15	0.0059		
Overall	Yes	4922	39	0.0079		
					0.21	0.646
Overall	No	4910	34	0.0069		

2.3.3. Species identification

In the area of the 20 village study, 96.1% (n=154) of the *An. gambiae s.l.* were characterised by cytogenetics as *An. gambiae s.s.* Savanna type (Coluzzi *et al.*, 1985), the remaining 3.9% being *An. arabiensis*. In the cross over study, according to the palpal ratio, 82.4% (n=321) of the females in the two study villages were either *An. gambiae s.s* or *An. arabiensis*, while 17.6% were identified as *An. melas*.

2.4. DISCUSSION

Entomological assessment of vector control in a manner that is representative of a particular area or situation is complicated by local environmental differences between villages or areas, and by normal variations in recruitment to, and density of, mosquito populations. Ways to cope with this problem include the use of study designs that exclude sources of variability or that allow for variations in relevant factors (like rainfall, temperature etc.) in the analysis. The usual approach is to sample at regular intervals in small groups of villages with and without the intervention (Lindsay *et al*, 1993; Thomson *et al*., 1995a). The studies presented in this chapter illustrate two different approaches to comparisons of mosquito populations in villages with treated and untreated bednets. In the first one, single observations were made in as many replicate pairs of villages as possible, and in the second, repeated observations were made in two villages in a cross-over design, which allowed control not only of local differences but also of differences over time.

The paired village study should have demonstrated conclusively whether or not there were consistent differences in density, parous rate or sporozoite rates between the villages with treated and untreated bednets. For logistic reasons only a single observation was made in each pair, raising the possibility that the results obtained were not representative of the general situation in those villages because of some particular event that occurred that night in the particular house chosen for the observation. However, this effect could be nullified by inclusion of either more than one observation in the same pairs of villages or more pairs of villages.

The correlation in human landing collections between the pairs of villages could have been related either to the proximity of the two villages in each pair, and/or to the fact that the catches in each pair were carried out on the same night. The 5 pairs of villages with higher numbers of mosquitoes were nearer the river than the ones with fewer mosquitoes, as noted in other parts of The Gambia (Lindsay *et al.*, 1993; Thomson *et al.*, 1994).

To investigate the power of the twenty villages study, a calculation was made of how large the difference between treated and untreated villages would have had to have been

significant, given the observed variance between villages and the same overall mean. The answer was approximately two fold for both density and parous rates. Such differences have been described in Burkina Faso where the density of *An. gambiae s.l.* was reduced from 69.4 to 27.3 mosquitoes/night and the parous rate from 27% to 19% in the second year after the introduction of deltamethrin-treated bednets (Robert and Carnevale, 1991). A more drastic reduction was found in the biting rate in Zaire, from 17.3 to 0.9 mosquitoes/night after the introduction of deltamethrin-impregnated bednets. For the parous rate a reduction was reported from 79.7% to 50.3% (Karch *et al.*, 1993). If this approach is used in future studies it would be preferable to sample in more pairs of villages, or sample more than once in each pair.

The cross-over study was a more sensitive test because local differences between the villages were controlled by the design of the study. One possible source of bias with this design is the carry over of the effect of the insecticide-impregnated bednets and contamination of the rooms with insecticide in the first village. In this case, entomological parameters might be affected for some days after removing the treated bednets. A gap of some days between the cross-over of treated bednets was used to attempt to overcome the possible carry-over effect. As in the paired village study, no evidence of a 'mass-killing effect' was found in the cross-over study. No significant difference was observed in the parameters measured (density, parous rates and sporozoite rates).

One possible explanation of the fact that parity rates were lower in villages with treated bednets in exit trap collections but not in human landing collections is that nullipars may be more easily inhibited from feeding through a treated bednet, or more easily induced to exit, than parous females. However, since the two sets of collections were made 3 months apart it is also possible that the difference was due to behavioural differences associated with changes in the inter-intra specific composition of the two *An. gambiae s.l.* populations.

Absence of a 'mass-killing effect' following the use of insecticide-impregnated bednets has been found in other countries including Thailand (Somboon *et al.*, 1995) and has been explained by the exophagic, zoophilic and early evening biting habits of the vectors

found in that country. However, in The Gambia, *An. gambiae s.l.* is endophagic, anthropophilic and has late biting behaviour. Furthermore, in other countries with the same malaria vectors (i.e. Tanzania, Zaire and Burkina Faso) a mass-killing effect has been found. How can the difference between The Gambia and the other countries with the same mosquito vector be explained?

One possibility is that the effect was masked by movements of mosquitoes between treated and untreated villages, as proposed by Lindsay *et al.* (1993). Results of two mark-release recapture experiments regarding this question are presented in Chapter 3.

Another possible explanation is that mosquitoes are not generally killed by treated bednets in The Gambia, perhaps because the material of the bednets in The Gambia is different from the ones used both in previous experimental hut studies where killing of mosquitoes has been measured (Lindsay *et al.*, 1991; Miller *et al.*, 1991) and in countries where a mass-killing effect following insecticide-impregnation of bednets at a community level has been seen (Magesa *et al.*, 1991; Karch *et al.*, 1993). In The Gambia synthetic sheeting, synthetic netting and cotton sheeting are the most common materials for bednets (Aikins *et al.*, 1993), while in the experimental hut studies and in the village trials in Tanzania for example, standard nylon bednets have been used. In a recent experimental hut trial, low mortality was found when Gambian bednets were used (2 nylon netting, 2 synthetic sheeting and 1 synthetic muslin netting) compared with previous results using the same experimental huts with imported nylon bednets (Nagle, 1994).

Although the results of resistance tests confirm the susceptibility of the different populations of *An. gambiae s.l.* (M. Jawara, personal communication), the possibility that the vector in The Gambia may be slightly more tolerant to permethrin than populations in other countries cannot be completely ruled out.

The results of the studies presented here indicate that malaria vectors in The Gambia are generally as abundant, long-lived, and as likely to be infected in villages with treated bednets as in villages with untreated bednets. The clear reduction in the density of the resting indoor population in rooms with a treated bednet reflects the excito-repellency of the

insecticide. Mosquitoes entered treated rooms in similar densities and a similar proportion of freshly fed mosquitoes was found resting indoors in treated and untreated rooms. This means that, somehow, mosquitoes are managing to feed and survive in treated villages.

This study confirms that in The Gambia the protection against malaria seen in children using impregnated bednets must be attributed to personal protection rather than to a 'mass-killing effect' at a village level. The occurrence of individual protection of permethrin-treated bednets and the absence of a 'mass-killing effect' at a community level makes it justifiable to direct intervention programmes to high risk target groups like children and pregnant women rather than whole communities. Coordinated programmes with other organizations working with the same target groups could facilitate the use of impregnated bednets.

2.5. SUMMARY

In The Gambia, the use of permethrin-impregnated bednets has led to a reduction in morbidity and mortality for malaria in children. However, no clear evidence has been found for a 'mass-killing effect' on the population of mosquito vectors as a result of this intervention. Two further entomological studies to investigate this phenomenon have been carried out: a 20 village survey in which pairs of villages with treated and untreated bednets were selected, and a cross-over study in two villages in which treated bednets were switched between villages after a period of 2 weeks, and were then removed and introduced to a second village for another 2 weeks. Longevity, biting and resting density of the malaria vector population and sporozoite rates were assessed in both studies.

Malaria vectors were found to be as abundant, long-lived, and as likely to be infective in villages with treated bednets as in those with untreated bednets. However, a clear reduction in the density of the indoor-resting population of mosquitoes in rooms with treated bednets was found, which presumably reflects the excito-repellency of the insecticide. Mosquitoes entered treated rooms in similar densities and a similar proportion of freshly fed mosquitoes was found resting indoors in treated and untreated rooms.

This study confirms that, in The Gambia, the protection against death and morbidity from malaria seen in children using impregnated bednets must be due to personal protection rather than to a 'mass effect' on the mosquito vector population at a village level.

CHAPTER 3

EFFECT OF PERMETHRIN-TREATED BEDNETS ON MOVEMENTS OF *Anopheles gambiae s.l.* BETWEEN NEIGHBOURING VILLAGES

3.1. INTRODUCTION

Movement between treated and untreated villages was proposed by Lindsay *et al.* (1993) as one possible explanation of the lack of evidence for a 'mass-killing effect' by permethrin-impregnated bednets on populations of *An. gambiae s.l.* in villages in The Gambia. For example, in a large-scale village trial of permethrin-treated bednets (Lindsay *et al.*, 1993) vector densities and sporozoite rates fell in all the treated villages, but this was also true for the untreated ones. It is possible that this was the result of less rain in the post-intervention year, 1989, relative to the pre-intervention year, 1988. Alternatively, it was also proposed that mosquito movements between treated and untreated villages, which were often less than 1.25 Km apart, might account for the lack of observed differences in mosquito density and longevity between treated and untreated villages. In other words, it was suggested that treatment of bednets had a 'mass-killing effect', but that mosquito movements caused this to be shared between villages, and hence masked.

Besides the possibility that any effect on density, longevity and sporozoite rates was diluted by migrants from untreated villages, it was also possible that bednet treatment caused increased emigration of mosquitoes from the treated villages to neighbouring untreated ones, making transmission of malaria worse in those villages.

Mark-release recapture studies on mosquitoes have been used with different purposes, e.g. to determine population size, adult survivorship and movements or dispersion of mosquitoes (Service, 1993). *An. gambiae s.l.* has been shown to have the ability to disperse considerable distances. Flight range of *An. gambiae s.l.* was studied by Gillies (1961) using mark-release recapture experiments in Tanzania. Laboratory reared mosquitoes were marked with two radioisotopes in the larval phase or by topical application of paint to adults. The

mean flight range of females released in the centre of the sampling area was 1.03 Km and that of females released in the periphery was 1.57 Km. The maximum range of flight was 3.62 Km. The flight range was related to the distribution and the number of houses.

A mark-release recapture experiment showed that such movements between Gambian villages do take place (Thomson *et al.*, 1995b). In a study involving 3 non-primary health care (non-PHC) villages, the proportion of mosquitoes in one village that came from a neighbouring village 1-1.5 Km away was calculated to be 17.2% (95% C.I. 12.2% and 22.4%) for mosquitoes released blood-fed from bednet collections and 20.1% (95% C.I. 14.7% and 25.3%) for releases of unfeds from exit trap collections.

The results are presented here of two mark-release recapture experiments. An initial experiment was carried out in 1992 with the objective of determining the amount of movement between primary health care (PHC) villages involved in the National Impregnated Bednet Programme, and to see whether or not there was any effect of the treatment on movement, given that the bednets in one of the villages were treated. The second experiment was carried out in 1993 with the main objective of determining the length of the gonotrophic cycle (see Chapter 4), but information about movements between villages was also obtained.

3.2. MATERIALS AND METHODS

3.2.1. Experiment in 1992

Four rounds of mark-release-recapture were carried out in three villages: Brikamaba (treated PHC village), Saruja (untreated PHC village), and Wellingara (untreated non PHC village) (Figure 3.1). The former two villages were part of the National Impregnated Bednet Programme. The villages are 2 - 2.3 Km apart. The first two rounds each comprised 12 consecutive days' sampling and the last two rounds 18 consecutive days.

Releases of fed females are usually preferred in mark-release experiments, partly because in this condition females are robust and easy to manipulate, and partly because it is easy to infer whether or not recaptured females have oviposited between release and recapture. Because of the deterrence and repellency effects however, it was difficult to collect

sufficient numbers of fed females for release from the treated village (Brikamaba). As an alternative, unfed females were therefore collected in exit traps. To avoid more manipulation they were marked and released as unfeds. Fourteen exit traps were set in each village for collections of live unfed females. Only the unfed females were marked and released in each village.

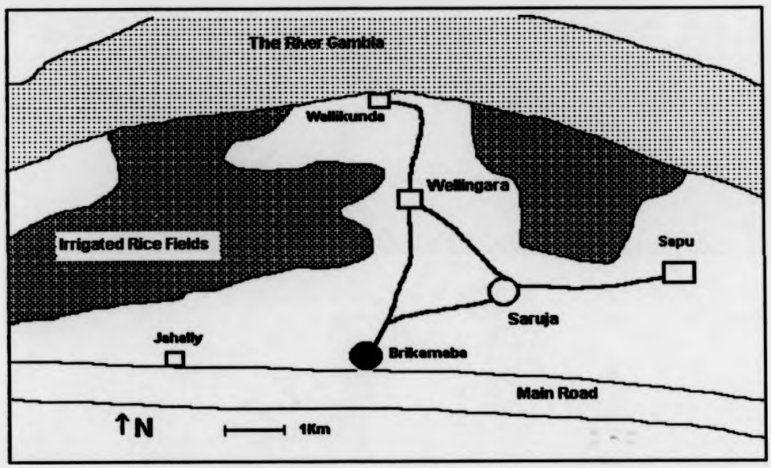

Figure 3.1 Sketch map showing the location of the villages involved in the mark-release recapture experiment in 1992.

In the first two rounds, two different colours were used in each village: one on the first day and the other on all subsequent days. In the last round of the experiment three different colours were used in each village, a different colour on each successive day in cycles of three days (Table 3.1). The rationale for this was that, as we were releasing unfed females, it was impossible to be sure from the stomach condition of a recaptured female whether she had oviposited between release and recapture. This was important as females that have visited breeding sites to oviposit are presumably more likely to be recaptured in a different village than females that have not yet oviposited since being released. Using a

different colour on three successive days was intended to enable identification of those females that had probably been recaptured the day after release, without having had the chance to leave the village of release to oviposit. For this, the colour and gonotrophic stage of the recapture were taken into account, for example: a recapture in spray catches of a fed female marked with the colour used the previous day was considered as having been recaptured the day after release.

In the last two rounds, additional collections and releases were made of fed females found resting in bednets in the morning. This was in order to test the possibility of a 'memorized' home range in *An. gambiae s.l.*, as has been described for *An. farauti* in Papua New Guinea (Charlwood *et al.*, 1988) and *A. cantans* in England (Renshaw *et al.*, 1994), and to increase the number of releases and thus the number of recaptures for the estimates of movements between villages. Collections were carried out only in the untreated villages (Saruja and Wellingara) because, in the treated village (Brikamaba) fed females resting in bednets were not found because of the presence of the insecticide. For these releases of fed females, three further colours were used in each of the untreated villages, one on each successive day. The first day mosquitoes were collected, marked and released in the same village. The second day, mosquitoes were displaced from the village of collection to the other village. The third day mosquitoes were moved in a car after collection but released in the same village of collection. The 3-day cycle was continued till the end of the experiment. This part of the work was carried out with the assistance of P. Emerson, and a more detailed report was produced (P. Emerson, 1992).

Pyrethrum spray catches were made for collections of recaptures, spraying in 10 rooms per village, in different compounds every day. Attempts were made to collect mosquitoes resting outdoors using large jars placed in shady places in the pairs of villages; however after the first week no *An. gambiae* mosquitoes were collected resting in these jars. Therefore, three 'Muirhead Thomson' pit traps (dimensions: 1.5 x 1.5 x 1.5 m) were dug in each of the three villages for outdoor resting collections (Service, 1993). The pits were dug in 3 different compounds near animal shelters. Collections of mosquitoes resting in the pits were carried out every morning.

An uninhabited room in a compound located in the centre of each village was used as a "village-laboratory". All the mosquitoes collected (in exit traps, bednets, pits and spray catches) were taken there to be anaesthetized with ether, counted and checked for recaptures with a U.V. lamp. The unmarked mosquitoes were transferred to marking cups until recovery from the anaesthetic and marked using fluorescent powders (Fiesta Daylight Colours, SWADA, Stratford, London). Groups of no more than fifteen at a time were marked by the 'dust storm method' (Curtis and Rawlings, 1980) using paper cups and released. Figure 3.2 shows the marking procedure. The recaptures were checked again in the field station under a microscope.

3.2.2. Experiment in 1993

Two villages were selected for this experiment, Madina and Jakoto in the Niamina District, 180 km from the coast. A detailed description of the study area is given in Thomson *et al.* (1995b). Permethrin-impregnated bednets (500mg/m^2) were given to the villages in a cross-over with one village having treated bednets only for the first two weeks, the other having them only for the last 2 weeks of the work. The methodology of the impregnation, distribution and cross-over of the impregnated bednets is described in Chapter 2.

Mark-release recapture procedures were carried out twice in each study village, once during the two weeks before the cross-over of bednets and a second time during the two weeks after it. Resting fed *An. gambiae s.l.* were collected daily from approximately 600 untreated bednets sited in both the untreated study village and in 4 neighbouring, untreated villages: Barokunda, Bkfula, Bkmandinka, Ebria and on some occasions in Yoroya. The location of the villages is shown in Figure 3.3. During the first six days of each experiment half the females collected were transferred to Jakoto whilst the other half were transferred to Madina for marking and releasing in each village. Checking for recaptures, marking and releasing took place in a designated hut in the centre of each village.

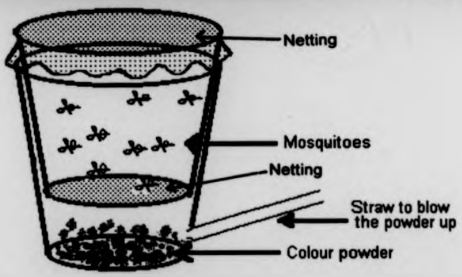

Figure 3.2 Marking of mosquitoes with the fluorescent powder. Above, photograph of the moment in which the colour powder is being blown to mark mosquitoes in the paper cup. Below, diagram showing the system used for marking.

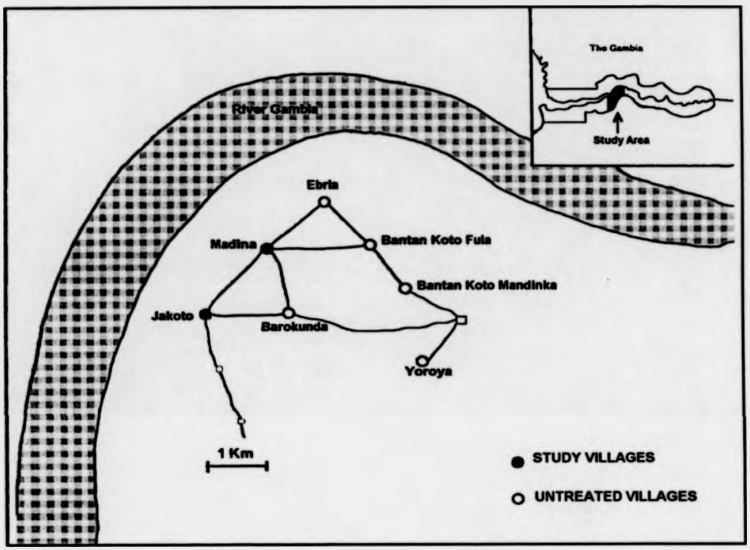

Figure 3.3 Sketch map showing the location of the villages involved in the mark-release recapture experiment in 1993.

Mosquitoes were marked using fluorescent powders (Fiesta Daylight Colours, SWADA, Stratford, London) according to the method described in Thomson *et al.* (1995b). The colours used were: Red (R), Blue (B), Green (G), Orange (O) and Magenta (M); in addition, powders were mixed to produce a further 7 colours: Purple (P) (1 B+2 R), Turquoise (T) (1 G+ 2 B), Dark Blue (DB) (1 M+ 1 B), Light Blue (LB) (1 Yellow + 1 B), Dark Green (DG) (1 G + 1 R + 1 B), Yellowish (Y) (1 O + 1 R + 1 G) and Bright Green (BG) (1 Yellow + 1 G), all of which could be distinguished with certainty under a dissecting microscope. Of these 12 colours, 6 were used in each village, the colour being changed every day for six consecutive days. Thus, the colour of a recaptured mosquito indicated the village

and day of its release. With this marking routine, females that were collected the first day after release could be identified with confidence and were excluded from the estimates of recapture rates and movements between villages.

For recaptures, mosquitoes were collected in both villages for 11 days using pyrethrum spray catches in 10 rooms and five exit traps in each village. These mosquitoes were checked initially in the village for marks using a U.V. lamp; those identified as probably or possibly marked were checked later under a microscope in order to confirm their colour. Date and village, method of collection, colour and gonotrophic stage were recorded for each recapture.

3.2.3. Definition of Indices

3.2.3.1. Recapture rate: Number recaptured by all sampling methods in all villages/Number released

3.2.3.2. Movement index: Estimations of the proportion of mosquitoes (marked and unmarked) in a village which had moved from another village were calculated following the method described in Rawlings *et al.* (1981). To estimate the proportion of mosquitoes in village A that came from village B, calculations were made of:

I) The number of marked mosquitoes found in A that had been released in B, as a proportion of the total collected in A.

II) The number collected marked in B that had been released in B, as a proportion of the total collected in that village.

III) The estimate of the proportion of the population in A that had come from B, calculated as I/II. Thus the proportion of marked mosquitoes found to have moved to A from B is corrected for the fact that a much larger proportion of unmarked mosquitoes will also have moved to A from B.

IV) 95% Exact confidence limits for the ratio of two proportions were calculated as the confidence limits for an Odds Ratio (Dawson-Saunders and Trapp, 1994) using the

program StatXact.

3.3. RESULTS

Tables 3.1 and 3.2 show the number of *An. gambiae s.l.* marked and released in the experiments in 1992 and 1993 respectively. The number of *An. gambiae s.l.* recaptured and collected by the different methods and the number of recaptures by village of release are shown in Tables 3.3 and 3.4 for the experiments in 1992 and 1993 respectively.

For the experiment in 1992 a total of 182 mosquitoes were recaptured. Twenty five were considered to have been recaptured the day after release taking into account the colour and gonotrophic stage of the recapture. For example, a recapture in spray catch of a fed female with a colour used the day before, was assumed to have been released the previous day. This was not entirely satisfactory, since a 3 day cycle of colours did not allow us to differentiate recaptures after 1 day from those after 4 days, and possibly even 7 days after release. Nonetheless, these mosquitoes were excluded from the calculation of recapture rates and estimation of movements between villages. A complete list of all recaptures in this experiment with information about villages and dates of release and recapture, colour, and gonotrophic stage, is presented in Appendix 1.

For the experiment in 1993 a total of 148 mosquitoes were recaptured, 26 of which were discounted as they were considered as having been collected the day after release. As a different colour was used every day, these "first day recaptures" could be unambiguously identified. A list of all recaptures in this experiment is presented in Appendix 2.

3.3.1. Recapture rates

Recapture rates are presented in Table 3.5. For the experiment in 1992, the total number of mosquitoes released was 36643, of which 0.42% were recaptured. The overall recapture rate from releases of fed females collected in bednets (0.61%) was higher than releases of unfeds collected in exit traps (0.38%) (χ^2=5.78; p=0.016).

Table 3.1. Number of *An.gambiae s.l.* females marked and released in Brikamaba, Saruja and Wellingara and colour used every day. ETC: unfed females from exit traps, BNC: fed females from bednet collections. Colours: M:Magenta, B:Blue, Y:Yelow, G:Green, R:Red, O:Orange, PU:Purple, TU:Turquoise, PE:Peach, LI:Lime, DB:Dark blue, LB:Light blue, BG:Bright green, DG: Dark green.

Date	Released from ETC						Released from BNC			
	Bri	Col	Sar	Col	Well	Col	Sar	Col	Well	Col
04/08/92	64	M	127	Y	180	R				
05/08/92	28	B	113	G	136	O				
06/08/92	29	B	102	G	157	O				
07/08/92	43	B	151	G	275	O				
08/08/92	15	B	134	G	237	O				
09/08/92	41	B	129	G	185	O				
10/08/92	41	B	113	G	91	O				
11/08/92	32	M	148	Y	100	R				
12/08/92	35	M	106	Y	134	R				
13/08/92	36	B	83	Y	139	R				
14/08/92	29	B	107	G	131	O				
18/08/92	26	M								
19/08/92	26	B			122	O				
20/08/92	24	B	70	G	103	O				
21/08/92	61	B	202	G	160	O				
22/08/92	85	B	239	G	123	O				
23/08/92	70	B	243	G	52	O				
24/08/92	63	B	156	G	126	O				
25/08/92	42	B	56	G	80	O				
26/08/92	49	B	152	G	96	O				
27/08/92	31	B	62	G	65	O				
28/08/92	208	B	202	G	88	O				
01/09/92	29	B	126	G	97	O	52	PU	15	R
02/09/92	8	B	76	G	70	O	77	TU	15	M
03/09/92	154	B	163	G	95	O	72	PE	82	LI
04/09/92	38	B	87	G	78	O	87	PU	78	R
05/09/92	101	B	271	G	224	O	123	TU	126	M
06/09/92	109	B	276	G	144	O	109	PE	170	LI
07/09/92	127	B	203	G	212	O	116	PU	139	R
08/09/92	63	B	404	G	183	O	196	TU	314	M
09/09/92	131	B	992	G	537	O	191	PE	210	LI
10/09/92	354	B	845	G	482	O	243	PU	126	R
11/09/92	168	B	531	G	712	O	182	TU	271	M
12/09/92	244	B	572	G	311	O	113	PE	147	LI

Table 3.1. Continued.

Date	Released from ETC						Released from BNC			
	Bri	Col	Sar	Col	Well	Col	Sar	Col	Well	Col
13/09/92	270	B	687	G	279	O	197	PU	141	R
14/09/92	335	B	545	G	256	O	155	TU	116	M
15/09/92	342	B	357	G	223	O	82	PE	104	LI
28/09/92	171	B	297	G	153	O	109	PU	155	R
29/09/92	182	DB	306	BG	168	RO	142	TU	101	M
30/09/92	173	LB	144	DG	200	Y	198	PE	144	LI
01/10/92	164	B	189	G	146	O	129	PU	130	R
02/10/92	107	DB	193	BG	219	RO	207	TU	134	M
03/10/92	49	LB	60	DG	124	Y	109	PE	129	LI
04/10/92	192	B	171	G	156	O	98	PU	147	R
05/10/92	124	DB	134	BG	97	RO	104	TU	58	M
06/10/92	203	LB	232	DG	188	Y	129	PE	144	LI
07/10/92	298	B	300	G	213	O	50	PU	73	R
08/10/92	95	DB	137	BG	203	RO	144	TU	154	M
09/10/92	177	LB	250	DG	203	Y	121	PE	62	LI
10/10/92	178	B	191	G	223	O	126	PU	96	R
11/10/92	385	DB	172	BG	306	RO	95	TU	81	M
12/10/92	150	LB	265	DG	104	Y	92	PE	68	LI
13/10/92	238	B	257	G	180	O	97	PU	61	R
14/10/92	160	DB	331	BG	189	RO	61	TU	62	M
TOTAL	6597		12459		9755		4006		3853	

Table 3.2. Number of *Anopheles gambiae s.l.* marked by colour (Col.: initial of the colour, see text for notation) released, collected in pyrethrum spray catches (PSC) and exit traps (ETC) and total number of recaptures per day (REC).

	Madina - Treated				Jakoto - Untreated				REC
Date	**Col**	**Released**	**PSC**	**ETC**	**Col**	**Released**	**PSC**	**ETC**	
05/10/92	BG	481			M	542			
06/10/92	R	574			G	737			
07/10/92	B	697	362	164	LB	562	160	99	1
08/10/92	O	612	563	98	Y	749	1563	82	3
09/10/92	P	838	571	109	DB	745	2461	108	6
10/10/92	T	770	439	195	DG	1027	2032	124	8
11/10/92			503	104			2890	72	8
12/10/92			793	80			2044	96	5
13/10/92			346	141			1021	126	5
14/10/92			445	51			1570	61	2
15/10/92			387	58			2379	41	1
	Jakoto - Treated				**Madina - Untreated**				
19/10/92	M	913			BG	641			
20/10/92	G	1077		13	R	538		134	0
21/10/92	LB	1248	320	2	B	835	1558	90	5
22/10/92	Y	1363	160	16	O	749	1801	83	13
23/10/92	DB	1372	443	20	P	1001	1649	223	9
24/10/92	DG	1544	250	147	T	933	1254	54	16
25/10/92			240	73			760	178	22
26/10/92			215	331			1644	82	23
27/10/92			281	312			1247	171	15
28/10/92			130	53			1251	231	4
29/10/92			101	206			1069	135	2
Totals		11489	6549	2173		9059	28353	2190	148

Table 3.3. Number of mosquitoes recaptured in 1992 from Brikamaba, Saruja and Wellingara, released unfeds from exit traps and feds from bednet collections, village of recapture and total collected by pyrethrum-spray catches (PSC), exit traps (ETC), resting in bednets (BNC) and in pit traps (PITS).

Village of recapture	Recaptures released in: Bri.	Sar.	Well.	Total collected by: PSC	ETC	BNC	PITS
a)Unfed releases							
Recaptured in Brikamaba	53	0	0				
Recaptured in Saruja	0	5	3				
Recaptured in Wellingara	1	4	43				
				2596	10256	-	586
				7457	18706	5424	1375
				6964	14199	5342	1209
b)Fed releases							
Recaptured in Brikamaba	-	4	1				
Recaptured in Saruja	-	15	6				
Recaptured in Wellingara	-	4	18				
Overall	54	32	71	17017	43161	10766	3170

Table 3.4. Number of mosquitoes recaptured in 1993 from Jakoto and Madina when treated (T) and untreated (U), village of recapture and total collected by pyrethrum-spray catches (PSC), exit traps (ETC) and resting in bednets (BNC).

Village of recapture	No. of recaptures which had been released in:		No.Collected by:		
	Jakoto	Madina	PSC	ETC	BNC
Jakoto-U	19	1	16120	809	2271
Madina-T	2	1	4409	1000	-
Barokunda	0	1			3095
Bkfula	4	1			2585
Bkmandinka	0	0			2695
Ebria	1	0			4977
Yoroya	1	0			320
Jakoto-T	8	4	2140	1173	-
Madina-U	24	41	12233	1381	3009
Barokunda	4	0			5510
Bkfula	2	3			3001
Bkmandinka	2	0			3548
Ebria	1	2			7189
Yoroya	0	0			918
Overall	68	54	34902	4363	39199

Table 3.5. Recapture rates (%) of *Anopheles gambiae s.l.* mosquitoes released in the experiments in 1992 and in 1993. In parentheses are numbers recaptured/numbers released.

Village of release	Condition of the bednets	
	Treated	**Untreated**
I) Experiment in 1992		
a) Unfed releases:		
Brikamaba	0.82 (54/6597) 95% C.I. 0.60-1.08	
Saruja		0.07 (9/12379) 95% C.I. 0.03-0.12
Wellingara		0.47 (46/9810) 95% C.I. 0.31-0.45
b) Fed releases:		
Saruja		0.58 (23/3944) 95% C.I. 0.35-0.82
Wellingara		0.64 (25/3913) 95% C.I. 0.39-0.89
II) Experiment in 1993		
Jakoto	0.55 (41/7417) 95% C.I. 0.38-0.72	0.62 (27/4362) 95% C.I. 0.39-0.85
Madina	0.10 (4/3972) 95% C.I. 0.002-0.2	1.06 (50/4697) 95% C.I. 0.77-1.36

There was no significant difference in the recapture rates from fed females released in the two untreated villages. However, from the unfed females (exit trap releases) there were significant differences between all the villages. The treated village, Brikamaba, showed the highest recapture rate, followed by the untreated non-PHC village, Wellingara. The lowest recapture rate was in the PHC untreated village, Saruja.

In the 1993 experiment, the total number of mosquitoes released was 20448, of which 0.60% were recaptured. Lower recapture rates were found in the presence of treated bednets in both villages (Jakoto and Madina), however, the difference was significant only in Madina (Table 3.5). In Jakoto the confidence limits overlap for the recapture rates in the presence and absence of treated bednets.

3.3.2. Movements between villages

Table 3.6 presents estimates of the proportion of *An. gambiae s.l.* in one village that come from another, together with their confidence limits.

In the 1992 experiment, a total of 23 mosquitoes were recaptured in a village other than the one of release, while 134 were recaptured in the village of release. Significant differences were found between villages in the estimates of immigrant females. The estimated proportion of females in Wellingara and Brikamaba that came from Saruja were higher (47.57% and 49.05% in Wellingara and Brikamaba respectively) than the proportion of immigrants moving from Wellingara or Brikamaba (Table 3.6). This is due not so much to a greater number from Saruja having been found in the other 2 villages, as to the lower proportion of marked females released and recaptured in Saruja (6.1% compared to 22.0% and 39.4% in Wellingara and Brikamaba respectively). This is because the latter proportion forms the denominator for the estimate of immigrants (column 3 divided by column 4 in Table 3.6). The overall estimate of the movement index is 17.2% (95% C.I. 11% - 26.7%), which means that, overall, approximately 17.2% of mosquitoes in any given village had moved from a neighbouring one.

Table 3.6. Estimations of proportions of immigrant mosquitoes in village populations, i.e. the proportion of *Anopheles gambiae s.l.* in one village (B) that came from another village (A). (see definition in text).

Village of release (A)	Village of recapture (B)	Number that were released in A, then recaptured in B (1)	Total number collected in village B (2)	Number that were released in A, then recaptured in A (3)	Total No. collected in village A (4)	% in B which came from A (½+3/4) (95% exact C.I.)
a)Experiment in 1992						
Saruja	Wellingara	8	27714	20	32962	47.57 (18.12-112.7)
Saruja	Brikamaba	4	13438	20	32962	49.05 (12.19-146.4)
Wellingara	Saruja	9	32962	61	27714*	12.40 (5.40-25.09)
Wellingara	Brikamaba	1	13438	61	27714	3.38 (0.08-19.52)
Brikamaba	Saruja	0	32962	53	13438	0.00 (0.00-2.36)
Brikamaba	Wellingara	1	27714	53	13438	0.91 (0.02-5.30)
Overall		23	148228	134	148228	17.16 (11.02-26.71)*
b)Experiment in 1993						
Jakoto-T	Other vill.	33	36789	8	3313	37.14 (16.78-93.03)
Jakoto-U	Other vill.	8	19091	19	19200	42.34 (16.02-101.30)
Madina-T	Other vill.	3	32872	1	5409	49.36 (3.96-453.2)
Madina-U	Other vill.	9	23479	41	16623	15.54 (6.63-32.41)
Treated	Other vill.	36	69661	9	8722	50.08 (23.65-118.2)
Untreated	Other vill.	17	42570	60	35823	23.80 (13.02-41.36)

*: Confidence limits approximated using a Binomial Distribution.

In the experiment in 1993, a total of 122 mosquitoes were recaptured, 69 in the village of release and 53 in a different village. The proportion of females in the other villages coming from Jakoto was similar in both cases, when Jakoto had treated or untreated bednets. The proportion of mosquitoes in the other villages estimated to have come from Madina when this village was treated (49.36%) was higher than when Madina was untreated (15.54%) (Table 3.6). However, this difference was not statistically significant since the confidence limits overlap. Overall, there is little or no evidence that treatment affected emigration from a village.

On the assumption that mosquitoes can recognize a 'home village' we might expect that mosquitoes would show greater dispersion when they are released in a different village than when they are released in the village where they were recaptured. This phenomenon has been described with *An. farauti* in Papua New Guinea and with *A. cantans* in England and interpreted as evidence for memorized homing behaviour. If this is the case for *An. gambiae s.l.* in the area of this experiment, it was expected that fewer recaptures would be found in the village of release when mosquitoes were displaced. However, there was no significant difference in the proportions recaptured between mosquitoes released where they were captured and those released in another village (χ^2=1.17, P=0.28)(Table 3.7).

Table 3.7. Number of recaptures when fed females were released in the same village of recapture, or transferred to be released in another village.

		Recaptured in:		
		Village of release	**Other village**	**Total**
Released in:	Village of collection	16	5	21
	Other village	17	10	27
	Total	33	15	48

3.4. DISCUSSION

In the 1992 experiment, recapture rates ranged between 0.07% to 0.82% for unfed releases, while recapture rates for releases of fed females showed less variability between villages and were overall higher than when unfed females were released (Table 3.5). Comparing recapture rates between villages, the lower recapture rate found in Saruja could have been due to handling of large numbers of unfed mosquitoes. The marking procedure took longer in Saruja than in the other villages, and mosquitoes were crowded in the cups for a longer period of time. In the experiment in 1993 in which all releases were fed females, recapture rates varied from 0.1% to 1%. The overall recapture rates were lower than 1% for both experiments. The recapture rates found in these experiments were similar to those in previous studies with *An. gambiae s.l.*. Gillies (1961) recorded a rate of 0.77% from releases of teneral laboratory bred females in Tanzania. Releases of wild caught females carried out by Thomson *et al.* (1995b) in The Gambia, in the same villages as the 1993 experiment reported in this chapter, gave recapture rates of 1.2% and 1.4% respectively for fed and unfed *An. gambiae s.l.* released.

Repellency of permethrin-impregnated bednets at a village level could have been reflected in finding lower recapture rates in villages with treated bednets. However, the evidence was not consistent. In the experiment in 1992 the treated village (Brikamaba) had actually the highest recapture rate. Lower recapture rates were found in the presence of treated bednets in the experiment in 1993, however, the difference was not consistent since it was significant only in one village (Madina).

A more clear indication that repellency of permethrin-impregnated bednets at village level could be taking place, would be to find a reduction in density in villages with treated bednets. However, there has been no evidence for such a reduction in villages in The Gambia (Lindsay *et al.*, 1993, Thomson *et al.*, 1995a, Chapter 2). Therefore, it is unlikely that permethrin-impregnated bednets are repelling mosquitoes at a village level.

There was no evidence for homing behaviour of females of *An. gambiae s.l.*. The proportion of *An. gambiae s.l.* recaptured in their 'home' village or in 'another' village was not

significantly different when releases were made in their home village or females were transferred to be released in a different village. Evidence for such 'homing behaviour' has been found with other species. *An. farauti* had longer oviposition cycle and the recapture rate was lower when they were displaced and released in a different village other than the one of collection (Charlwood *et al.*, 1988). The explanation suggested was that displaced mosquitoes were disorientated and showed a tendency for greater dispersal. *A. cantans* showed a tendency actually to return to the wood of collection when released elsewhere (Renshaw *et al.*, 1994). Neither of these two tendencies were found with *An. gambiae s.l.* in the experiment presented in this Chapter.

This result validates the use of *An. gambiae s.l.* females captured in one village and released in another village for mark-release and recapture experiments in The Gambia. Transference of females from different villages to be released in another village was used in the experiment of Thomson *et al.* (1995b) and in the experiment in 1993 presented in this Chapter. This transference of females can be helpful in mark-release-recapture studies when is difficult to collect in one village enough live females for release, for example when the village has treated bednets.

A consistent pattern was not found in movements of mosquitoes from or to treated villages. In the experiment in 1992, the estimates of immigration implied movements of mosquitoes predominantly from Saruja to the other two villages and from Wellingara to Saruja. There was no evidence that mosquitoes from untreated villages (Saruja and Wellingara) were moving less to the treated village (Brikamaba); similar proportions were found in Brikamaba coming from Saruja and in Saruja coming from Wellingara. However, lower amount of movement from Brikamaba to the other two villages were estimated (Table 3.6). These results, together with the higher recapture rate in the treated village would suggest that the effect of the treatment, if any, is a tendency for females to stay in the village. However, it is important to consider that releases from Brikamaba were only with unfed females and they are more sensitive to manipulation and that the confidence intervals were wide and in most cases overlapping. In the experiment in 1993, no consistent evidence for any effect of permethrin-impregnated bednets on movements of mosquitoes between villages

was found. No significant differences were found for the pairs of villages in the presence or absence of treated bednets - the confidence limits overlap in all cases.

Overall, the results from both experiments do not suggest that permethrin-impregnated bednets affect movements between villages. The difference in recapture rates when the villages had treated bednets and when they did not, were influenced by the lower collection of mosquitoes, particularly indoor resting, in the presence of treated bednets. The possibility that sampling efficiency may vary in this way between villages is taken into account by the method of estimating movements. It is therefore unlikely to be biased by, for example, the excito-repellency effect of permethrin, when indoor resting mosquitoes are collected. However, the estimates have very wide confidence limits in most cases.

One important factor to consider for the interpretation of movements of mosquitoes between villages in The Gambia is that it is difficult to find a breeding place in the villages, the most probable breeding places being outside. The villages used for the experiment in 1992 are surrounded by rice fields, located approximately 1 Km away. Although there has not been any larval survey, rice fields are likely to be the most important breeding places in that area (Lindsay *et al.*, 1991). In the area for the experiment in 1993, swamps along the Gambia river are considered the most probable breeding places (Thomson *et al.*, 1995b). In both cases, therefore, most females must go to outside the village to oviposit and must then fly to another village for the next blood meal. The result of the 'homing' experiment did not give any evidence for 'recognition' of a particular village after oviposition (Emerson, 1992). Apparently, mosquitoes go to a village to take their next blood meal without regard to the village that they came from. When breeding places are inside or very close to the villages, less movement between villages could be expected, as is the case in villages in Tanzania where the most common breeding places are small puddles, streams and rice fields within and near villages (K. Njunwa, personal communication). This may be why in Tanzania, in a similar mark-release recapture experiment, only 1.5% (3/204) of recaptures were in a village other than the one of release (Njunwa, 1993), while this proportion in The Gambia was 27.2% (76/279) for the experiments presented in this chapter and 26.5% (98/370) in the experiment of Thomson *et al.* (1995b).

The estimates of the proportion of immigrant mosquitoes confirm previous estimates in The Gambia (Thomson *et al.*, 1995b). These movements of mosquitoes between villages are important for the interpretation of measures of parity, density and sporozoite rates. The effect of a vector control intervention could be diluted if mosquitoes are exchanged with neighbouring villages which are not included in the intervention. However, it is not clear exactly how this measure of exchange of mosquitoes between villages is to be compared with parameters like parity, density or sporozoite rates. In other words, how much exchange between villages (as measured in this way) would be necessary to obscure a given degree of 'mass killing' within treated villages? Attempts at the construction of a mathematical model are being made (J. Lines, personal communication). As a consequence of the preliminary work for the model it has been realised that the amount of mosquito movement required to mask a 'mass-killing' effect on the parous rate or on vector abundance, must be higher than that required to mask changes in the sporozoite rate. This is because older mosquitoes are more likely to have moved between villages.

3.5. SUMMARY

Movements of mosquitoes between treated and untreated neighbouring villages could mask an impact of permethrin-impregnated bednets on density, longevity and sporozoite rates (the 'mass-killing effect'). Also, the treatment could cause an increase in emigration of mosquitoes from treated villages.

Two mark-release recapture experiments were carried out. The aim of the first one in 1992 was to determine the rate of movements between primary health care (PHC) villages involved in the National Impregnated Bednet Programme, and to see whether or not there was any effect of the treatment on movements. Bednets in one of the villages were treated. The second experiment was carried out in 1993 with the main objective of determining the length of the gonotrophic cycle, but information about movements between villages was also obtained.

Around 152000 *An. gambiae s.l.* females were marked and released and 279 were recaptured in the two experiments. The recapture rates were slightly lower than 1% as found in previous studies with the same species. No significantly lower recapture rates were found in the presence of treated bednets as would be expected if there is repellency at the village level. In one of the treated villages the recapture rate was actually higher than in the untreated villages.

No evidence was found for any effect of permethrin-treated bednets on movements between villages. Although significant differences were found between villages in the estimates of immigrant mosquitoes, a consistent pattern that could link the treatment of the bednets with large or small amounts of movement from or to villages with treated bednets was not seen. However, confidence limits were wide in most cases.

The overall estimated proportion of immigrant mosquitoes in the villages in the first experiment was 17.2% (95% C.I. 11.02-26.71), similar to previous estimates from The Gambia. In the second experiment the proportion of mosquitoes found in other villages (untreated) that came from a treated village was 50.08% (95% C.I. 23.65-118.2) and from an untreated village was 23.8% (95% C.I. 13.02-41.36). The results from both experiments confirm previous results in non-PHC villages about the interchange of mosquitoes between villages in The Gambia.

CHAPTER 4

EFFECT OF PERMETHRIN-TREATED BEDNETS ON BITING, EXITING BEHAVIOUR AND GONOTROPHIC CYCLE LENGTH OF *Anopheles gambiae s.l.* IN THE GAMBIA.

4.1. INTRODUCTION

Insecticide-impregnated bednets could have several effects on mosquito populations. Exposure to treated bednets might reduce mosquito survival and thus lower sporozoite rates. In addition, insecticide-impregnated bednets may have an important effect on mosquito behaviour. Behavioural effects on *Anopheles* mosquitoes have been described in studies carried out in the laboratory, experimental huts and at village scale. Feeding inhibition of mosquitoes exposed to treated bednets has been observed both in the laboratory and in experimental hut studies. For example, no mosquitoes fed on an arm pressed against a 200mg/m² permethrin-impregnated bednet (Hossain and Curtis, 1989). In experimental hut studies, a reduction in the proportion of mosquitoes feeding has been found in Burkina Faso (Darriet *et al.*, 1984, Carnevale *et al.*, 1992), Tanzania (Lines *et al.*, 1987, Curtis *et al.*, 1992), The Gambia (Lindsay *et al.*, 1991 and 1992, Pleass *et al.*, 1993), China (Li, 1987) and Suriname (Rozendaal, 1989). Deterrency (a reduction in the number of mosquitoes entering a room) has been found in various experimental hut studies (Darriet *et al.*, 1984, Miller *et al.*, 1991). An increase in the proportion of mosquitoes exiting from experimental huts containing permethrin-treated bednets was found in experiments in Burkina Faso (Darriet *et al.*, 1984) and Tanzania (Lines *et al.*, 1987), but not in The Gambia (Lindsay *et al.*, 1991; Pleass *et al.*, 1993) (see Table 1.1 in Chapter 1).

Other behavioural changes on exposure to treated bednets were noted in a village-scale study undertaken in Papua New Guinea (Charlwood and Graves, 1987). Comparisons made before and after the introduction of permethrin-impregnated bednets in one village showed a reduction in the human blood index of mosquitoes collected resting indoors, implying a diversion to animal feeding. In addition, a shift away from a post-midnight peak towards a pre-midnight peak of biting activity was described for *An. farauti* and *An.*

koliensis; there was also a disruption in the regularity and an increase in the duration of the oviposition cycle of *An. farauti*. The interpretation given to the latter finding was that mosquitoes were diverted outside by the presence of the impregnated bednets, and thus prevented from feeding on the same night as oviposition. Diverted females had to return to feed the following night, biting in the early evening, thus increasing the length of their gonotrophic cycle. Changes in the biting pattern of *An. arabiensis* were also found after the use of impregnated bednets with deltamethrin and lambda-cyhalothrin compared with controls with no bednets in Tanzania (Njau *et al.*, 1993). The explanation for this shift in biting time is probably different from the one proposed by Charlwood and Graves (1987) since, in this case, the contrast was seen between treated and untreated houses within the same village. In the Solomon Islands a change from 3 to 4 days in the duration of the gonotrophic cycle length of *An. farauti* was found in a village with permethrin-treated bednets, compared with a DDT-sprayed village and an untreated village (Hii *et al.*, 1995).

In The Gambia, permethrin-impregnated bednets have been shown to reduce morbidity and mortality due to malaria in children (Snow *et al.*, 1987a; Snow *et al.*, 1988b; Alonso *et al.*, 1993). However, these epidemiological findings have not been associated with the kind of 'mass-killing effect' that has been described in other countries e.g. Tanzania (Magesa *et al.*, 1991) and Zaire (Karch *et al.*, 1993) where clear reductions in density, parous rates and sporozoite rates were found after the introduction of insecticide treated bednets. In The Gambia, mosquitoes of the *Anopheles gambiae* species complex have been generally found to be as abundant, as long-lived and as likely to be infected in villages with treated bednets as in villages with untreated ones (Lindsay *et al.*, 1993; Thomson *et al.*, 1995a; Chapter 2). However, changes in behaviour may have taken place which have allowed mosquitoes to survive in villages with treated bednets.

In this chapter the results of two studies are reported in which attempts have been made to detect changes in the behaviour of *An. gambiae s.l.* as a result of impregnation of bednets organized on a village scale. The effects of treated bednets on mosquito densities, parous rates and sporozoite rates in these villages are presented in Chapter 2. One study involved a survey of biting and exiting behaviour of *An. gambiae s.l.* in twenty villages, half

of which used treated bednets. The second study investigated the effect of insecticide-impregnated bednets on the length of the gonotrophic cycle using a mark-release recapture technique and a cross-over design.

4.2. MATERIALS AND METHODS

4.2.1. Twenty villages survey - biting and resting behaviour:

This study was undertaken mainly during a 3-week period in July 1992 in 20 villages with primary health care system in the centre of The Gambia. Details of the study area and the collection methods used are given in Chapter 2. Villages with treated bednets were paired with their nearest untreated control village. All-night, human-landing collections were carried out on one occasion in each village, indoors and outdoors simultaneously, between 19:00h and 07:00h. Pyrethrum spray catches were carried out on the morning after the human-landing collections in 5 rooms in each village. An ELISA was used to determine the Human Blood Index (HBI) of the fed mosquitoes.

Further collections were made in these villages in October 1992, when 3 exit traps were set in each village for one night in rooms with treated bednets in treated villages and in rooms without a treated bednet in untreated villages.

Cytogenetic studies were carried out to identify a sub-sample of mosquitoes from the study area (Coluzzi *et al.*, 1979).

4.2.2. Mark-release recapture experiment for the gonotrophic cycle study:

The methodology for this experiment is presented in Chapter 3. Briefly a cross-over study was carried out in two villages, Madina and Jakoto. Permethrin-impregnated bednets (500 mg/m^2) were given to the people of Madina village during the first two weeks, while in Jakoto the people continued using their own unimpregnated bednets. After two weeks this was reversed: new impregnated bednets were given to the people in Jakoto, the impregnated bednets were collected from Madina, and the people there were asked to use their own

unimpregnated bednets again.

Mark-release recapture procedures were described in Chapter 3. Briefly, fed *An. gambiae s.l.* females were collected resting in untreated bednets in the study villages (when they had untreated bednets) and in 4 neighbouring untreated villages. Half of the females were transferred to and released in Jakoto and the other half were released in Madina. Releases continued over 6 consecutive days, a different colour being used every day and in the two villages (12 colours in all), thus, the colour of a recaptured mosquito indicated the village and day of its release. After the cross-over of bednets the experiment was repeated. Mosquitoes were collected in both study villages using pyrethrum spray catches and exit trap collections during the 4 weeks of the study.

Palps of a sample of *An. gambiae s.l.* were dissected and measured for species determination according to the palpal ratio (Coluzzi, 1964). Specimens with a ratio of 0.81 and above were ascribed to *An. melas* and those with a ratio less than 0.81 to *An. gambiae* (Bryan, 1980).

4.3. RESULTS

4.3.1. Indoor and Outdoor human-landing collections

Ratios between the numbers of *An. gambiae s.l.* collected landing on human baits indoors or on human baits outdoors are presented in Table 4.1. The ratios showed wide variation, ranging from 0.6 to 2.5 in treated villages and from 0.5 to 8.3 in untreated villages. Overall ratios in villages with treated and untreated bednets did not differ significantly (Wilcoxon signed rank test P=0.064), and the pooled ratios (1.45 and 1.39) were remarkably similar.

Table 4.1. Indoor:Outdoor ratio of females of *Anopheles gambiae s.l.* collected in all night human landing collections in the 20 villages survey.

Date	Pair	TREATED VILLAGES I:O Ratio (n)	UNTREATED VILLAGES I:O Ratio (n)
09/07/92	1	1.29 (470)	1.17 (737)
10/07/92	2	1.31 (150)	3.43 (31)
15/07/92	3	2.50 (210)	8.33 (84)
17/07/92	4	1.96 (77)	2.17 (38)
21/07/92	5	0.69 (44)	2.56 (57)
22/07/92	6	0.76 (44)	1.22 (140)
24/07/92	7	2.16 (112)	1.28 (155)
28/07/92	8	0.87 (28)	1.42 (29)
29/07/92	9	0.63 (13)	0.50 (15)
31/07/92	10	0.00 (5)	1.33 (7)
Overall		**1.45 (1158)**	**1.39 (1293)**

Pairs of villages (treated - untreated): (1) Jafaye - Sotokoi, (2) Jahally - Saruja, (3) Kudang -Mbien, (4) Madina Nfally - Taifa, (5) Pacharr - Kerewan, (6) Brikamaba - Tabananeh, (7) Mbaien Maka - Mamufana, (8) Pathe Same - Batinjol, (9) Sare Futa - Fula Bantang, (10) Fas Abdou - Boweram.

4.3.2. Biting cycle

Hourly geometric mean numbers of *An. gambiae s.l.* collected in human landing collections indoors and outdoors are shown in Figure 4.1. The mean biting time was calculated for each village (Table 4.2) by assuming that each mosquito was collected in the middle of the period i.e, all mosquitoes collected between 2am and 3am were assumed to have been collected at 2:30am. The times were then weighted by the numbers biting in each hourly period. The tenth pair of villages was excluded from this calculation since only 7 and 5 mosquitoes were collected respectively. No significant difference was found in the analysis of variance between the mean biting times of mosquitoes caught in villages with treated

bednets or villages with untreated bednets, or between those caught indoors or outdoors (Table 4.3). However, there were significant differences between the mean biting times in different pairs of villages.

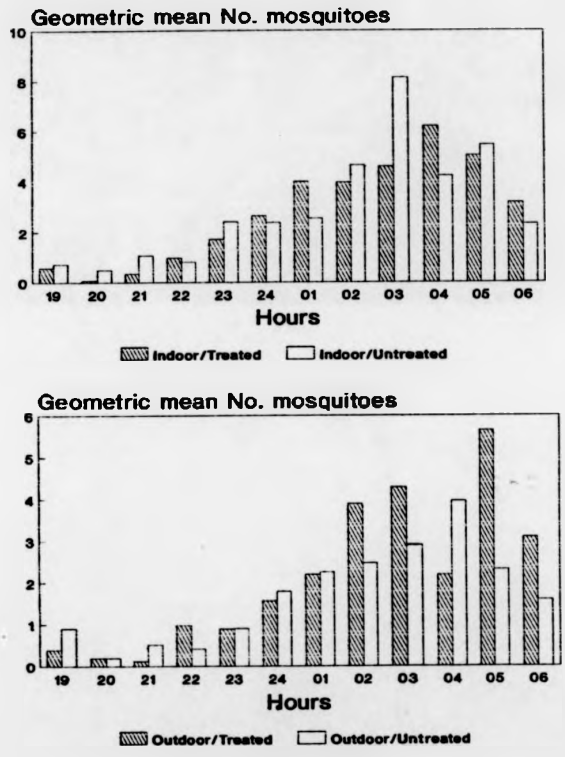

Figure 4.1. Hourly geometric mean numbers of *An. gambiae s.l.* collected by human-landing indoors and outdoors in the ten pairs of villages survey.

Table 4.2. Estimated mean biting time of *Anopheles gambiae s.l.* indoors and outdoors in pairs of treated and untreated villages. Number of mosquitoes in parentheses.

Date	Pair	TREATED VILLAGES		UNTREATED VILLAGES	
		Indoor	Outdoor	Indoor	Outdoor
09/07/92	1	3:05 (256)	2:53 (205)	2:54 (397)	2:58 (347)
10/07/92	2	3:23 (85)	3:28 (65)	1:47 (24)	1:47 (7)
15/07/92	3	3:15 (150)	3:12 (60)	2:38 (75)	1:50 (9)
17/07/92	4	4:31 (51)	4:14 (26)	3:25 (26)	4:40 (12)
21/07/92	5	2:53 (18)	2:19 (26)	3:11 (41)	3:00 (16)
22/07/92	6	2:30 (19)	3:30 (25)	2:27 (77)	3:10 (63)
24/07/92	7	3:21 (80)	3:16 (37)	3:25 (87)	2:59 (68)
28/07/92	8	2:49 (13)	3:10 (15)	2:37 (17)	12:20 (12)
29/07/92	9	2:42 (5)	3:15 (8)	4:19 (5)	4:06 (10)
Overall		**3:16 (686)**	**3:08 (467)**	**2:53 (749)**	**2:58 (544)**

Table 4.3. Analysis of variance of the biting times of mosquitoes in relation to presence or absence of treated bednets in the villages, place where caught (indoors/outdoors), and village pair.

Source of variation	DF	Sum of Squares	Mean Square	F	P
Treatment	1	13.472	13.473	2.00	0.157
Place	1	0.050	0.050	0.01	0.931
Pair	8	173.224	21.653	3.21	0.001
Treatment*Place	1	1.265	1.265	0.19	0.664
Treatment*Pair	8	97.759	12.219	1.81	0.069
Place*Pair	8	38.973	4.871	0.72	0.671
Error	2410	16233.9	6.736		
Total	2437	16623.5			

4.3.3. Host choice

The results of the human blood index (HBI) of mosquitoes resting indoors in villages with and without treated bednets are shown in Table 4.4. The overall HBI observed in the pairs with treated bednets was lower than in the villages with untreated bednets. However, the difference was not statistically significant (Mantel-Haenszel Chi square =1.47, P=0.225). Thus, although there were fewer females resting indoors in villages with treated bednets than in villages with untreated bednets, there was no significant difference in the proportion that had fed on humans and remained resting indoors.

Table 4.4. Human blood index (HBI) of *Anopheles gambiae s.l.* collected resting indoors in pairs of treated and untreated villages.

Date	Pair	TREATED VILLAGES % HBI (n)	UNTREATED VILLAGES % HBI (n)
15/07/92	3	52.17 (23)	56.25 (48)
17/07/92	4	28.57 (7)	0 (1)
21/07/92	5	56.25 (16)	80.00 (20)
22/07/92	6	54.55 (11)	84.31 (51)
24/07/92	7	83.33 (36)	65.22 (46)
28/07/92	8	61.90 (21)	81.08 (37)
29/07/92	9	0 (1)	20.00 (5)
31/07/92	10	60.00 (10)	80.00 (10)
Overall		**62.40 (125)**	**71.10 (218)**

4.3.4. Exiting

The mean number of females of *An. gambiae s.l.* collected in exit traps and the proportion of unfeds, feds and gravids is shown in Table 4.5. No evidence for an effect of exposure to treated bednets was found in the mean numbers of females collected (Wilcoxon sign rank test P=0.426) or in the proportions by gonotrophic stage (unfeds P=0.426; feds P=0.475 and gravids P=0.812).

Table 4.5. *Anopheles gambiae s.l.* collected in exit traps in pairs of villages with treated or untreated bednets. Percent collected by gonothrophic stage: unfeds (UF), feds (FF) and gravids (GR) and geometric mean of mosquitoes collected per trap (GM). In parentheses total of mosquitoes.

		TREATED VILLAGES					UNTREATED VILLAGES				
Date	**Pair**	**%UF**	**%FF**	**%GR**	**GM**	**(n)**	**%UF**	**%FF**	**%GR**	**GM**	**(n)**
29/09/92	5	100	0	0	1.8	(61)	100	0	0	1.9	(75)
30/09/92	1	99.4	0	0.6	28.8	(165)	96.8	1.6	1.5	84.2	(856)
02/10/92	4	58.8	17.7	23.5	3.6	(13)	78.0	22.0	0	11.0	(50)
03/10/92	7	100	0	0	7.4	(24)	97.6	0	2.4	6.0	(42)
06/10/92	3	99.0	1.0	0	14.5	(102)	90.7	4.7	4.7	19.4	(43)
08/10/92	9	63.6	9.1	27.3	3.0	(6)	0	0	0	0	
10/10/92	10	100	0	0	0.4	(1)	25.0	50.0	25.0	1.3	(4)
13/10/92	9	93.6	4.7	1.8	22.4	(170)	100	0	0	11.8	(119)
15/10/92	7	96.7	3.3	0	3.2	(19)	91.7	8.3	0	0.9	(12)
16/10/92	4	84.0	10.0	5.0	4.5	(20)	89.7	8.6	1.7	12.4	(58)
Overall		**95.4**	**2.7**	**2.0**	**6.5**	**(602)**	**95.8**	**2.8**	**1.4**	**5.8**	**(1259)**

4.3.5. Gonotrophic cycle length

A total of 39199 fed mosquitoes were collected from bednet collections during the entire study in the various villages. Of these, 11489 were marked and released in a village with treated bednets and 9059 were marked and released in a village with untreated bednets. In spray catches and exit traps 6549 and 2173 mosquitoes were collected respectively in the two release villages when treated bednets were present and 28353 and 2190 when untreated bednets were present. Details are presented in Table 4.6.

The total number of recaptures was 148, of which 26 were collected the day following their release, as shown by their colour. These comprised 23 gravid, 1 fed and 2 unfed females. These mosquitoes had probably not completed a cycle since being released as feds, and therefore they were not taken into account in estimating the gonotrophic cycle length. Of the remaining 122, a total of 93 females were recaptured either unfed or fed, and 29 were gravids. Table 4.7 shows the number of recaptures by day after release, by gonotrophic stage, and according to whether or not bednets in the village of recapture were treated.

Since the releases were all fed females, the fed recaptures provide the most direct information on the duration of the gonotrophic cycle. Recaptures of fed females showed a clear two day periodicity, at least in untreated villages. The majority of fed recaptures were found on day 2, and another peak took place on day 4 after release. The situation is less clear for unfed females, perhaps because of the small numbers of recaptures, but the peaks of recapture of gravid females were on days 1 and 3 after release. Together, these results imply a 2 day gonotrophic cycle of *An. gambiae s.l.* in villages with untreated bednets. The mean day of recapture was calculated to be 3.47 for untreated villages.

Unfortunately, there were few recaptures when the villages used treated bednets because pyrethrum spray catches sampled fewer mosquitoes resting indoors in the presence of treated bednets. However, the highest number of recaptures of fed females in the villages in the presence of treated bednets was again on day 2 after release. The mean day of recapture was calculated to be 3.27, which is little different from that estimated for untreated villages.

Table 4.6. Number of *Anopheles gambiae s.l.* marked by colour (Col.: initial of the colour, see text for notation) released, collected in pyrethrum spray catches (PSC) and exit traps (ETC) and total number of recaptures per day (REC).

	Madina - Treated				Jakoto - Untreated				REC
Date	**Col**	**Released**	**PSC**	**ETC**	**Col**	**Released**	**PSC**	**ETC**	
05/10/92	BG	481			M	542			
06/10/92	R	574			G	737			
07/10/92	B	697	362	164	LB	562	160	99	1
08/10/92	O	612	563	98	Y	749	1563	82	3
09/10/92	P	838	571	109	DB	745	2461	108	6
10/10/92	T	770	439	195	DG	1027	2032	124	8
11/10/92			503	104			2890	72	8
12/10/92			793	80			2044	96	5
13/10/92			346	141			1021	126	5
14/10/92			445	51			1570	61	2
15/10/92			387	58			2379	41	1
	Jakoto - Treated				Madina - Untreated				
19/10/92	M	913			BG	641			
20/10/92	G	1077		13	R	538		134	0
21/10/92	LB	1248	320	2	B	835	1558	90	5
22/10/92	Y	1363	160	16	O	749	1801	83	13
23/10/92	DB	1372	443	20	P	1001	1649	223	9
24/10/92	DG	1544	250	147	T	933	1254	54	16
25/10/92			240	73			760	178	22
26/10/92			215	331			1644	82	23
27/10/92			281	312			1247	171	15
28/10/92			130	53			1251	231	4
29/10/92			101	206			1069	135	2
Totals		11489	6549	2173		9059	28353	2190	148

Table 4.7. Recaptures by day after release in villages during periods of use of treated and untreated bednets, according to their gonotrophic stage unfed (UF), fed (FF) or gravid (GR).

Days after release	Recaptured in a treated village			Recaptured in an untreated village		
	UF	FF	GR	UF	FF	GR
1		1	6	2		17
2		5		2	36	3
3	1	2	1	1	6	15
4	1	1		1	16	4
5	1		1	2	5	4
6					5	1
7		1			4	
8		1			1	
12					1	

4.3.6. Species identification

In both studies the main species identified was *An. gambiae s.s.* In the area of the 10 pairs of villages, 96.1% (n=154) of those identified by cytogenetics were *An. gambiae s.s.* savanna type, the remaining 3.9% were *An. arabiensis*. In the cross-over study, according to the palpal ratio, 85.0% (n=321) of the *An. gambiae s.l.* in the two study villages were fresh water *An. gambiae* (either *An. gambiae s.s* or *An. arabiensis)* while 15.0% were identified as *An. melas*. For the recaptures, 93.5% of 109 were fresh water *An. gambiae*, a significantly higher proportion than the samples from the villages (χ^2=4.57, P=0.032). Therefore, the results of the gonotrophic cycle length are relevant only to fresh water *An. gambiae* species.

4.4. DISCUSSION

The results of the studies show no evidence of a change in biting behaviour in terms of place, time and host choice or in the length of the gonotrophic cycle of *An. gambiae s.l.* in villages with permethrin-treated bednets in The Gambia. As the collections were made soon after the impregnation of bednets, our measures would have detected an immediate phenotypic effect of the insecticide on the biting behaviour of the mosquitoes and not changes selected genetically.

Deterrence has been found in the presence of impregnated bednets in experimental hut studies (Darriet *et al.*, 1984, Lines *et al.*, 1987, Lindsay *et al.*, 1991; Miller *et al.*, 1991), but no evidence for this was found from the night landing collections in the present study. This contrasts with the deterrence observed in experimental hut studies, including those in The Gambia (Lindsay *et al.*, 1991; Miller *et al.*, 1991).

Indoor/outdoor biting ratios were similar in rooms with treated bednets to those in rooms with untreated bednets, which indicates that mosquitoes were entering rooms with treated bednets and those without, at a similar rate. Similar results were described in Burkina Faso (Robert and Carnevale, 1991). A change in the ratio of biting outdoors:indoors of *An. farauti* from 1:1.17 to 1:0.47 was found in the Solomon Islands after house spraying with

DDT (Taylor, 1975). The change in this case was considered not to be due to the temporary repellent effect of the insecticide, but due to selection in favour of an outside and early biting population.

The fact that in rooms with treated bednets there was a reduction in the density of indoor resting but not of human biting mosquitoes confirms the excito-repellency of the insecticide (Darriet *et al.*, 1984; Lines *et al.*, 1987; Rozendaal *et al.*,1989). As the presence or absence of a treated bednet had no significant effect on the indoor/outdoor biting ratio, the excito-repellent effect must occur after mosquitoes have entered a room with a treated bednet and perhaps even after they have tried to bite through the impregnated bednet. This would imply that any unprotected person sitting in a room with a treated bednet would receive the same number of bites as if he or she were in a room with an untreated bednet. This result contrasts with observations in experimental huts in Tanzania (Lines *et al.*, 1987), where it was shown that the presence of a permethrin-impregnated bednet reduced biting on an unprotected person in the same room. Room size may be one important factor to be considered when comparing the results from experimental hut studies with collections in rooms in villages. The size of the rooms used in experimental huts, including those in The Gambia (1.80m long x 1.8m wide), are generally smaller than that of any Gambian village room.

An increase in the exiting rate of mosquitoes in rooms with a treated bednet has been described in several studies in experimental huts (Darriet *et al.*, 1984; Lines *et al.*, 1987) and in field evaluations (Snow *et al.* 1987b; Charlwood and Dagoro, 1987; Lindsay *et al.*, 1989b). If mosquitoes entered rooms with treated bednets at similar rate to those with untreated bednets, as was shown by the human-landing collections, yet were found resting in treated rooms in the morning at lower densities (Chapter 2), then one would have expected to find higher densities in the exit trap collections in rooms with treated bednets than in rooms with untreated bednets. However, no significant differences were found in the density of exiting mosquitoes or in the proportion of unfed, fed or gravid mosquitoes exiting rooms with treated or untreated bednets. The majority of mosquitoes exiting were unfed females. Perhaps, mosquitoes left rooms with treated bednets through other openings such as eaves,

holes on the walls etc or were killed by the insecticide and were not collected in the exit traps.

The biting pattern and mean biting time of the population of *Anopheles* in the 10 pairs of villages were similar to those described previously for *Anopheles gambiae s.l* in West Africa (Gillies and De Meillon, 1968; Lindsay *et al.*, 1989). Differences between villages with treated and untreated bednets were not significant. Similar results were described in a study carried out in Congo (Zoulani *et al.*, 1994) in rooms with bednets impregnated with deltamethrin (25mg/m^2 or 12.5mg/m^2). No change in the biting cycle was observed.

Despite the small sample size for the analysis of the human blood index in this study (Table 4.4), no evidence was found for a significant difference between villages with treated or untreated bednets. This result is in agreement with previous results in The Gambia by Lindsay *et al.* (1991 and 1993) and Thomson *et al.* (1995a). The possibility remains that mosquitoes were diverted to bite animals and rest outdoors due to the excito-repellent effect of the insecticide. Unfortunately, collections of outdoor resting mosquitoes were not carried out in this study. In Tanzania, no differences were found in the source of blood of *An. gambiae s.l.* collected resting outdoors in pit traps before and after introduction of treated bednets (Magesa *et al.*, 1991); the majority were human fed.

The higher frequencies of recaptures on days 2 and 4 in untreated villages indicate that the gonotrophic cycle of *An. gambiae* in The Gambia is 2 days. A similar cycle duration has been reported for other countries in Africa (e.g. Muirhead Thomson, 1947), although a 3-day period between successive feeds has been reported equally frequently (Gillies and Coetzee, 1987). No evidence of any change in the gonotrophic cycle length in the presence of treated bednets was found in this study, although the number of recaptures was low when the villages had treated bednets.

Several studies on permethrin-treated bednets in The Gambia have demonstrated that the densities and sporozoite rates of *An. gambiae s.l.* are similar in villages with treated and untreated bednets, indicating that survival rates are unaffected (Lindsay *et al.* 1991 and 1993; Thomson *et al.*, 1995a; Chapter 2). Indoor resting density is reduced, presumably by

the excito-repellent effect of the insecticide. The results presented here show that biting behaviour and probably also the gonotrophic cycle length are unchanged. In particular, the proportion of females feeding on human rather than non-human hosts is not reduced, at least amongst those resting indoors. Nevertheless, there is ample evidence that children in villages with permethrin-treated bednets are protected against malaria (e.g. Alonso *et al.*, 1993, D'Alessandro *et al.*, 1995b). If there are equal numbers of infective mosquitoes in villages with treated and untreated bednets, and these are biting humans in a similar proportion, how then are children protected?. One possibility is that mosquitoes are diverted to bite other hosts including adults instead of children, adults being more exposed on account of their sleeping habits. Results of a study to test this hypothesis are presented in Chapter 5.

4.5. SUMMARY

Permethrin-impregnated bednets protect children against malaria in The Gambia. However, no effect on the density, sporozoite and parous rates of the mosquito vector population has been found and only a reduction in the numbers of mosquitoes resting indoors in rooms with treated bednets in the morning has been observed. A possible explanation for this paradox is that exposure to treated bednets leads to changes in vector behaviour such as a shift in biting time, a diversion to biting outdoors instead of indoors, to biting animals instead of humans, or to an increase in the length of the gonotrophic cycle. The results of two studies which have explored these possibilities are presented in this chapter. In the first study, biting and exiting behaviour was studied in 10 pairs of villages, half of which had permethrin-treated bednets. In the second study, a mark-release recapture experiment was carried out to investigate the possible influence of treated bednets on the gonotrophic cycle length.

No significant difference was found between villages with treated and untreated bednets in the indoor/outdoor ratio of human biting, in mean biting times or in human blood indices of females found resting indoors in the mornings. The proportion of unfed, fed or gravid females related to the total of females collected in exit traps and the geometric mean numbers of exiting mosquitoes showed no significant differences between rooms with treated and untreated bednets. Indications for a gonotrophic cycle length of 2 days were found. No evidence for any change in the gonotrophic cycle length in relation to exposure to treated bednets was found, although the number of recaptures was low in the villages with treated bednets.

Since equal numbers of infective mosquitoes have been found in villages with treated and untreated bednets and no changes in mosquito behaviour have been found, the question remain on how children are protected against malaria by treated bednets.

CHAPTER 5

EFFECT OF PERMETHRIN IMPREGNATED BEDNETS IN DIVERTING BITES OF *An. gambiae s.l.* FROM CHILDREN TO OTHER HOSTS

5.1. INTRODUCTION

The use of permethrin-impregnated bednets has been shown to reduce morbidity and mortality due to malaria in children in The Gambia (Snow *et al.*, 1987a; Snow *et al.*, 1988b; Alonso *et al.*, 1993; D'Alessandro *et al.*, 1995b). However, these epidemiological findings have not been associated with the kind of 'mass-killing effect' on the mosquito vector population that has been described in other African countries, for example in Tanzania (Magesa *et al.*, 1991) or in Zaire (Karch *et al.*, 1993). In these countries, survivorship, sporozoite rates and density of the mosquito vector populations were reduced by village-wide use of insecticide-impregnated bednets.

Previous entomological studies in The Gambia (Lindsay *et al.*, 1989, 1993; Thomson *et al.*, 1995a, Chapter 2), suggested that impregnation of bednets protected children not by reducing the abundance of infective vectors in treated villages but by preventing these vectors from feeding on the children. However, the human-blood index of indoor resting mosquitoes was found in the same studies not to change significantly in the presence of treated bednets.

Neither was there any evidence for a change caused by the treatment of bednets in the time of night or location (indoors vs. outdoors) of biting (Lindsay *et al*, 1993; Thomson *et al.*, 1995a, Chapter 4), nor any major change in the gonotrophic cycle length of *An. gambiae s.l.* in villages with permethrin-treated bednets in The Gambia (Chapter 4). The only consistent difference in all these village-scale studies has been the reduction of the indoor resting population of vectors in rooms with treated bednets, which can be explained by the excito-repellent effect of the insecticide.

This suggests that epidemiological protection is mediated in The Gambia purely by the "personal protection" effect of the bednets. However, this in turn raises the question of

whether the mosquitoes, that would otherwise have bitten the protected children, failed to feed altogether, or instead fed on other hosts (adults and/or animals).

A cross-over study was carried out in two villages to look for evidence that the proportion of bites on children were reduced in association with the use of impregnated bednets, and to determine whether mosquitoes were diverted to adults and/or animals.

5.2. MATERIALS AND METHODS

Rabies antibodies were used as markers in order to differentiate between children and adults in mosquito blood meals. Two similar villages were selected: Sinchu-Njabo and Mbai-Niake, near Farafenni, approximately 200 Km from the Atlantic coast. The characteristics of the villages in number of rooms, children, adults, and animals are presented in Table 5.1. All the children, 1 to 10 years old, in both villages were vaccinated against rabies during the dry season (April-July) in 1994. They received three doses of the vaccine.

A cross-over of permethrin-impregnated bednets was carried out in the two villages over 5 weeks of the rainy season (August-September) of 1994. New permethrin-impregnated bednets were provided to the people in Mbai-Niake, one for each bed in the village. The bednets were dipped in permethrin emulsion to give a target dose of 500 mg/m^2. This was done at the MRC field station at Farafenni rather than in the village in order to avoid major contamination of the rooms. Indoor resting mosquitoes were collected in 5 rooms every morning by spray catches (PSC) using an aerosol containing Tetramethrin 0.10% w/w, d-Allethrin 0.10% w/w, Dichlorvos 0.50% w/w and Permethrin 0.02% w/w (BOP^R) in both villages. Only rooms in which both children and adults were sleeping were selected for the collections. Five rooms were sampled daily, a different set being sampled every day in a 3 or 4 day cycle. Exit traps were set in 3 rooms in each village selected by the presence of a window and in which both adults and children were sleeping. Mosquitoes were collected from the exit traps every morning. Five pits were dug in each village between rooms and animal shelters, for daily collections of outdoor resting mosquitoes. Collections were

continued until around a thousand fed females had been collected in each village. This took 3 weeks in the village with treated bednets and 2 weeks in the village with untreated bednets. Thus, at the end of week 2, new impregnated bednets were given to the people in Sinchu-Njabo and collections of mosquitoes were carried out as before. At the end of week 3 the impregnated bednets were collected from Mbai-Niake and the people there used their own unimpregnated ones for the following weeks of the study. At the end of the study the permethrin-impregnated bednets were given to both villages and the adults were vaccinated against rabies.

Table 5.1. Characteristics of the study villages.

	Mbay-Niake	**Sinchu-Njabo**
Compounds	5	6
No. rooms	36	35
No. beds	54	52
No. children (<10)	25	41
Total No. inhabitants	77	86
Rooms in which children and adults sleep together	16	23
Animals in the village		
Cows	6	10
Horses	8	8
Sheeps	24	12
Goats	20	52
Donkeys	0	3

The mosquitoes collected were transferred to the laboratory in Farafenni where *An. gambiae s.l.* were sorted from other mosquitoes and fed females of *An. gambiae s.l.* were separated. The stomachs of the fed females were dissected into 20 µl phosphate buffered saline (PBS pH 7.2) and added to an eppendorf tube containing 80 µl PBS. Samples were spun at 18,000g in an Eppendorf centrifuge. The supernatant was removed and stored in liquid nitrogen until assayed by ELISA to detect anti-rabies antibodies. Positive controls were processed in the same way, around 10 per day, after feeding *An. gambiae s.l.* females with blood from an adult vaccinated against rabies, using a membrane feeding system. For the negative controls, females were fed with blood from an un-vaccinated adult. The positive control of the kit was also used on each plate.

The methods of processing the samples and detecting the presence of anti-rabies antibodies in mosquito bloodmeals were standardised and carried out by C. Drakeley. A modified Platelia$^{(R)}$ Rabies Kit from Diagnostics Pasteur was used for the detection of vaccine-induced rabies virus anti-glycoprotein antibodies.

The ELISA procedure for the anti-rabies antibodies was: plates were removed from the foil packaging and the wells washed twice with washing buffer (Tris NaCl pH 7.4, 1% Tween-20, 0.01% sodium merthiolate). In each well 200 ul of blocking buffer (0.5% casein, 1.0% Tween-20) was added and the plates were incubated in a moist box at 37°C for one hour. The blocking buffer was removed and 40ul/well of prepared mosquito control or sample was added to each well. The plates were incubated at 37°C for two hours in a moist box. The samples were removed by inversion and the plates washed 4 times with washing buffer. Goat anti-human Ig HRPO (Southern Biotechnology) (100ul/well) diluted 1:5000 in diluent (0.5% boiled casein, 0.025% Tween-20) was then added and the plates were incubated at 37°C for two hours in a moist box. The plates were washed 5 times with washing buffer. Substrate solution (O.P.D. 0.2mg/ml in 0.05M citric acid/sodium citrate pH 5.6, 0.03% H_2O_2, 0.01% sodium merthiolate) was added (100ul/well) and the colour reaction allowed to develop in the dark at room temperature. The reaction was stopped after 30 minutes by addition of 50ul/well 4N H_2SO_4.

The plates were then read visually and at 492nm using a Titertek Multiscan plate reader. The optical densities (OD) values were log-transformed, and the median found for each plate; the median was then subtracted from each individual test value, and the results ranked over the whole experiment. Three criteria were used to determine positivity: I) direct visual reading, ii) transformed values above the 85th percentile, iii) transformed values above the 75th percentile. This procedure was adopted because of a plate to plate variation found in the negative controls. Figure 5.1 shows the variation in the median OD values of the negative controls by plate, the values after transforming and subtracting the median plate and the 75th and 85th percentiles. Table 5.2 shows the proportion "positive" using the three different criteria. Similar results were given with any of these criteria comparing the proportion positive in the presence and absence of treated bednets in the villages. The 85th percentile was chosen arbitrarily for the analysis. Some samples were tested in duplicate on the same plate. They always gave the same classification as positive or negative, whichever criterion for the cut-off was applied.

Attempts to determine the human blood index by ELISA (Burkot *et al.*, 1981) were carried out without satisfactory results: fewer than expected positives for human blood were found in the samples, which was attributed to the way the samples were prepared for the anti-rabies ELISA. A PCR method was therefore developed and standardised by M. Haywood in the L.S.H.T.M. to discriminate between human and non-human blood meals in a sub-sample of 914 bloodmeals selected at random. PCR for cytochrome b DNA was used to discriminate between mammals and other hosts (Irwin *et al.*, 1991) as the source of blood. Since non-mammalian bloodmeals must have been rare, this stage in effect acted as a check on sample DNA quality. Following hybridization to a cytochrome specific probe samples were visually scored in 5 categories (0, 1, 2, 3, and 4) depending on the intensity of the reaction. The samples with a score of 2 or higher were considered to contain enough good quality DNA to be used for a subsequent testing with a β-globin gene PCR (Saiki *et al.*, 1985; Bauer *et al.*, 1991) which would identify samples with human blood. The PCR product was hybridised to a β-globin specific probe.

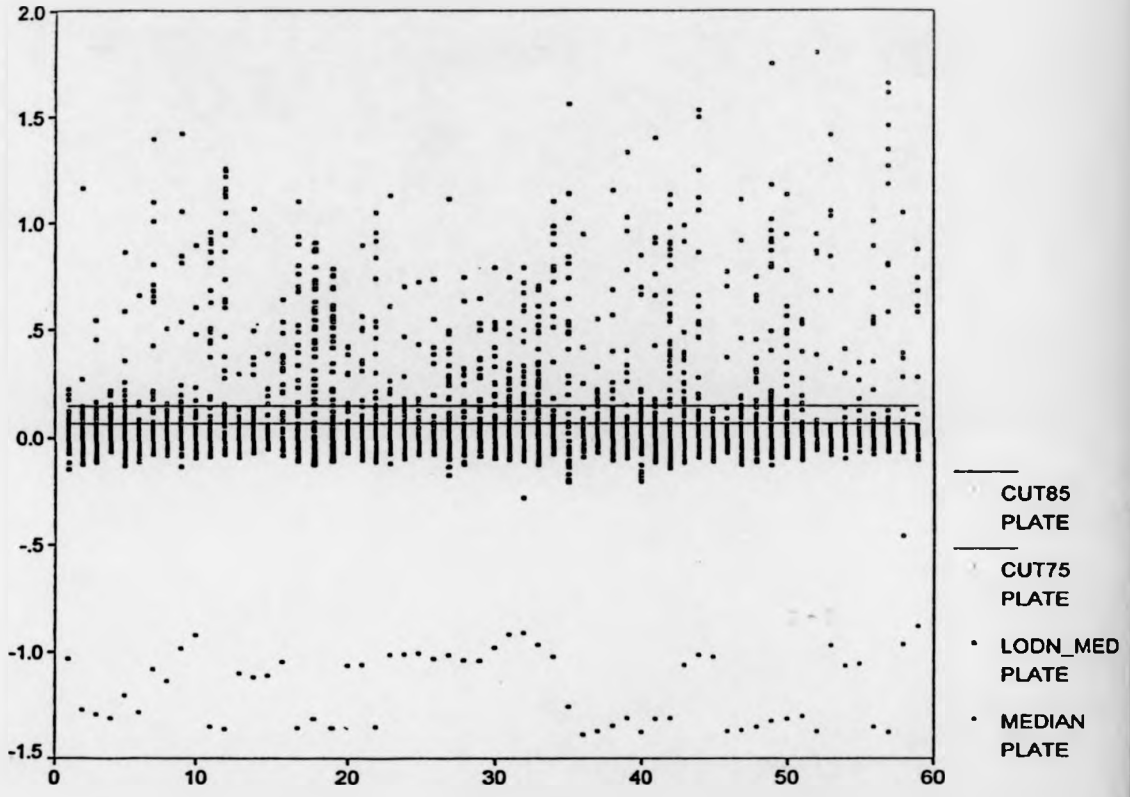

Figure 5.1. Optical density readings (log-transformed and having substracted the plate median) in the anti-rabies ELISA plates (LODN_MED PLATE), median of the negative controls in each plate (MEDIAN PLATE) and 75th (CUT75 PLATE) and 85th (CUT85 PLATE) percentiles as cut-off.

Table 5.2. Proportion of anti-rabies positives according to different criteria: The optical densities (OD) values were log-transformed, and the median found for each plate; the median was then subtracted from each individual test value, and the results ranked over the whole experiment. Three criteria were used to determine positivity: I) direct visual reading, ii) transformed values above the 85th percentile, iii) transformed values above the 75th percentile. Proportion "positive" according to Village, Collection (Indoors=PSC and ETC; Outdoors=PIT traps) and whether the bednets were treated or untreated.

Village	Collection	Criteria	Treated	Untreated	
Mbai-Niake	Indoors		n=109	n=870	
		Visual	7.3%	24.3%	**
		>85%	9.2%	30.8%	**
		>75%	23.9%	37.0%	**
Mbai-Niake	Outdoors		n=783	n=385	
		Visual	0.9%	9.4%	**
		>85%	5.9%	12.5%	**
		>75%	20.8%	21.6%	ns
Sinchu-Njabo	Indoors		n=319	n=510	
		Visual	3.4%	23.3%	**
		>85%	6.9%	28.0%	**
		>75%	19.7%	35.7%	**
Sinchu-Njabo	Outdoors		n=811	n=414	
		Visual	1.4%	1.7%	ns
		>85%	3.7%	6.0%	ns
		>75%	12.7%	16.7%	ns

(**)=P<0.01 by a χ^2
(ns)= not significantly different

5.3. RESULTS

A total of 4,010 fed females were collected in the study. During 2 weeks of sampling around 2,000 fed females were collected in the villages when they had untreated bednets. It took 3 weeks of sampling to collect a similar number of fed females when the villages had treated bednets. The difference in time of sampling was due to the lower density of mosquitoes resting indoors in the presence of treated bednets as shown in Figure 5.2. In the presence of treated bednets, indoor resting density was reduced, as seen previously (see Chapters 2 and 4) and explained by the excito-repellent effect of the insecticide. The number of females resting outdoors was not affected by the presence of treated bednets in the houses.

The number of fed *An. gambiae s.l.* tested and considered positive for anti-rabies antibodies (criterion 85th percentile) discriminated by date, method of collection and treatment condition of the bednets in the villages is presented in Table 5.3. Figure 5.3 shows the weekly proportions of fed *An. gambiae s.l.* positive for anti-rabies antibodies (criterion 85th percentile) by place of collection (indoors/outdoors) in the presence and absence of treated bednets. In the females collected indoors (PSC+ETC), a significant reduction of bloodmeals with rabies antibodies was seen in both villages associated with the presence of treated bednets (Table 5.2). The overall reduction was from 34.3% to 7.5%, i.e. 4.5 times fewer bloodmeals with anti-rabies antibodies in the presence of treated bednets. In the samples collected outdoors (PIT) the proportion of bloodmeals positive was significantly lower in the presence of treated bednets in Mbai-Niake (5.9% and 12.5% in the presence of treated and untreated bednets respectively). This difference was in the same direction but not significant in Sinchu-Njabo (3.7% and 6.0% in the presence of treated and untreated bednets respectively).

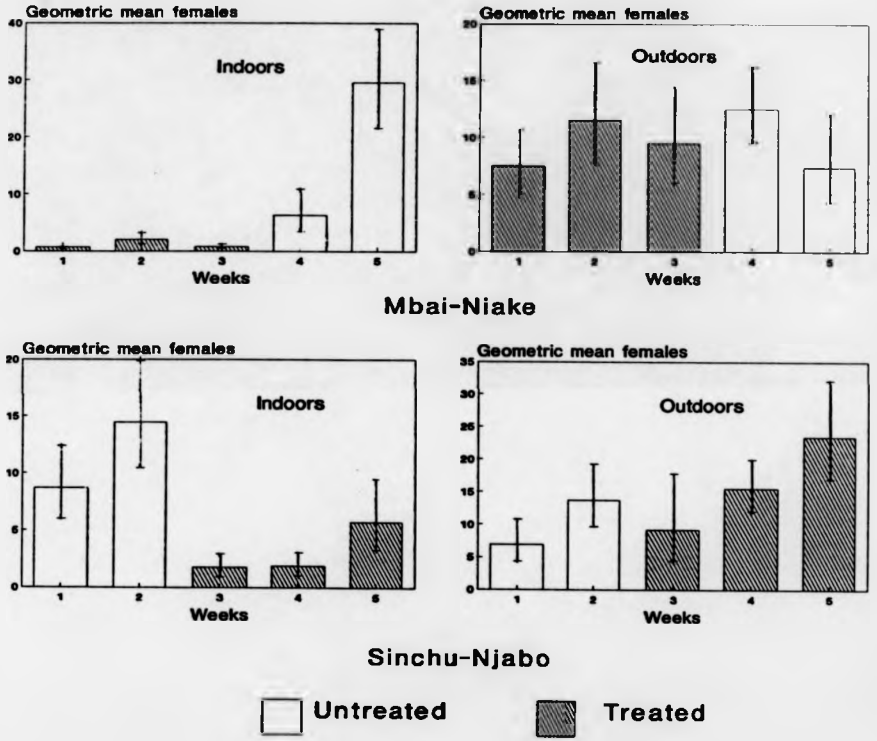

Figure 5.2. Geometric mean numbers of *An. gambiae s.l.* collected resting indoors by pyrethrum spray catches and resting outdoors in pit traps in the presence and absence of treated bednets in the villages of Sinchu-Njabo and Mbai-Niake.

Table 5.3. Number (n) of fed females tested and number of positives (Pos) for anti-rabies antibodies ELISA (criterion >85%), by date and method of collection (PSC:pyrethrum-spray catches; ETC:exit traps; PIT:pit traps).

Village: Mbai-Niake							
		Collection method					
	Date	**PSC**		**ETC**		**PITS**	
		n	**Pos**	**n**	**Pos**	**n**	**Pos**
Treated							
	08/08	4	0	0	0	6	0
	09/08	5	1	0	0	68	3
	10/08	1	0	1	0	32	0
	11/08	5	0	0	0	24	1
	12/08	5	3	0	0	46	1
	15/08	15	0	0	0	69	6
	16/08	7	0	0	0	24	0
	17/08	6	0	0	0	53	3
	18/08	8	1	0	0	118	5
	19/08	17	3	1	0	42	2
	22/08	4	0	0	0	34	6
	23/08	2	0	2	0	40	1
	24/08	7	0	1	1	69	4
	25/08	0	0	0	0	0	0
	26/08	11	0	0	0	47	3
	29/08	4	0	3	1	111	11
Total treated		**101**	**8**	**8**	**2**	**783**	**46**
Untreated							
	30/08	20	11	0	0	29	3
	31/08	44	30	0	0	71	4
	01/09	82	12	0	0	51	4
	02/09	18	8	0	0	32	4
	05/09	82	27	1	1	74	3
	06/09	44	15	0	0	51	10
	07/09	89	66	6	1	33	10
	08/09	302	96	2	1	44	10
Total untreated		**681**	**265**	**9**	**3**	**385**	**48**

Table 5.3. Continued....

Village: Sinchu-Njabo							
		Collection method					
	Date	PSC		ETC		PITS	
		n	Pos	n	Pos	n	Pos
Untreated							
	08/08	35	15	0	0	5	1
	09/08	34	11	0	0	55	2
	10/08	37	5	1	1	23	0
	11/08	20	10	0	0	22	1
	12/08	55	5	3	2	42	2
	15/08	71	8	4	2	42	1
	16/08	76	34	0	0	50	2
	17/08	88	34	3	1	42	6
	18/08	36	5	2	0	89	8
	19/08	37	8	2	2	34	2
Total untreated		**496**	**135**	**14**	**8**	**414**	**25**
Treated	22/08	0	0	0	0	0	0
	23/08	13	0	6	1	66	3
	24/08	12	1	8	1	92	4
	25/08	8	1	0	0	4	0
	26/08	5	0	0	0	73	3
	29/08	19	4	10	0	98	3
	30/08	4	0	2	2	25	1
	31/08	10	0	3	0	81	2
	01/09	24	1	1	0	49	2
	02/09	10	2	4	0	19	0
	05/09	42	0	0	0	77	1
	06/09	37	1	5	2	101	3
	07/09	38	5	12	1	85	8
Total treated		**260**	**15**	**59**	**7**	**811**	**30**

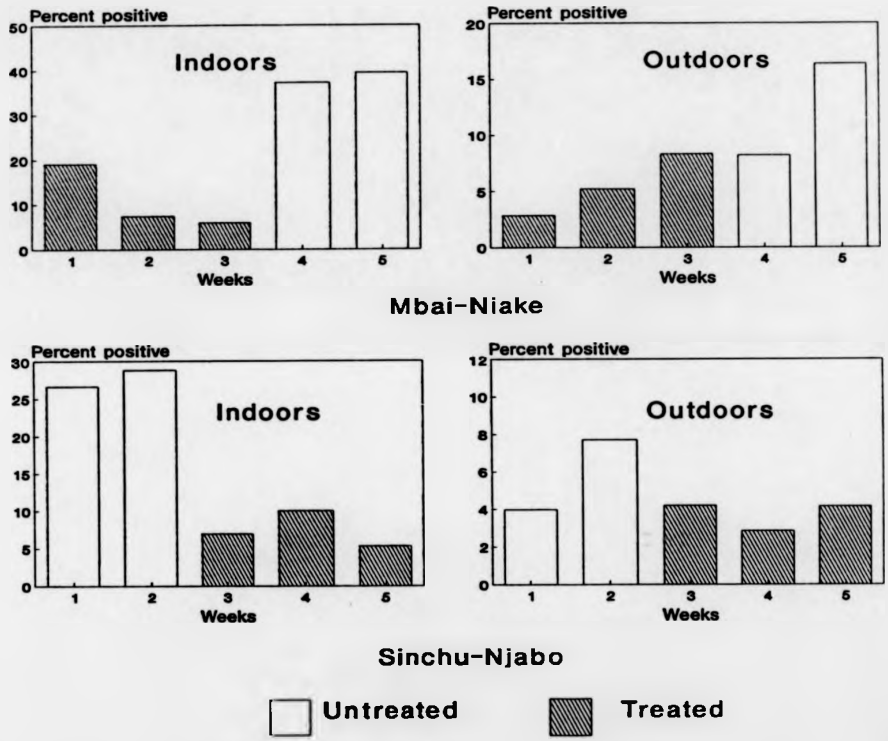

Figure 5.3. Proportion of positive females for anti-rabies antibodies by ELISA in the presence and absence of treated bednets in the villages of Sinchu-Njabo and Mbai-Niake.

The number of specimens tested and positive for mammalian (cytochrome DNA) and human (β-globin) blood is presented in Table 5.4. No significant difference was found in either village between the HBI of indoor resting females collected in the presence or the absence of treated bednets. In the samples resting outdoors (PIT) a just significantly lower human blood index (HBI) was found in Mbai-Niake in the presence of treated bednets (χ^2=3.93, P=0.047). In Sinchu-Njabo the difference was not quite significant (Fisher's exact test P=0.055) but the observed HBI was lower when the village had untreated bednets. In other words, the HBI was lower in both villages during the first half of the experiment.

Table 5.4. Number of specimens positive for mammalian (cytochrome b DNA) and human (β-globin DNA gene) blood by method of collection and treatment condition of the study villages. Shaded the first part of the cross-over study.

Collection	Village	Treatment	Tested	Mammals	Humans	HBI%
PSC	Mbai-Niake	Treated	30	4	3	75.0
PSC	Mbai-Niake	Untreated	269	132	107	81.1
PSC	Sinchu-Njabo	Untreated	191	30	21	70.0
PSC	Sinchu-Njabo	Treated	65	14	9	64.3
PIT	Mbai-Niake	Treated	106	36	17	47.2
PIT	Mbai-Niake	Untreated	83	25	19	76.0
PIT	Sinchu-Njabo	Untreated	55	18	10	55.6
PIT	Sinchu-Njabo	Treated	115	54	44	81.5

An apparent anomaly arose when combining the results from the anti-rabies ELISA and the DNA human test (Table 5.5). A noticeable proportion (32%) of anti-rabies positives mosquitoes appeared negative for the human β-globin gene. In other words, the ELISA results implied these bloodmeals were from vaccinated children while the DNA results implied they were from non-human hosts. Indeed the rabies ELISA positivity rate was little higher among samples subsequently identified as human (47.4%) than it was among those identified as non-human (42.4%).

Table 5.5. Combined results from the anti-rabies ELISA and the β-globin PCR.

				β-globin probe	
Village	**Treatment**	**Method**	**Anti-rabies**	**Positive**	**Negative**
Mbai-Niake	Treated	PSC	Positive	0	0
			Negative	3	1
	Untreated	PSC	Positive	76	22
			Negative	31	3
Sinchu-Njabo	Treated	PSC	Positive	0	1
			Negative	9	4
	Untreated	PSC	Positive	13	7
			Negative	8	2
Mbai-Niake	Treated	PIT	Positive	1	2
			Negative	16	17
	Untreated	PIT	Positive	12	2
			Negative	7	4
Sinchu-Njabo	Treated	PIT	Positive	5	1
			Negative	39	9
	Untreated	PIT	Positive	2	0
			Negative	8	8
Overall			**Positive**	**109**	**35**
			Negative	**121**	**48**

5.4. DISCUSSION

Results of entomological evaluations in the village-scale trials in The Gambia (Lindsay *et al.*, 1989, 1993; Thomson *et al.*, 1995a; Chapter 2) imply that personal protection rather than a mass-killing effect on the population of *An. gambiae s.l.* is the route by which treated bednets work in The Gambia. Yet feeding success, as measured by the percentage fed amongst females in spray catches and exit traps, has not been reduced, nor was there any significant reduction in indoor human blood index. It seems that there are just as many mosquitoes (and infective mosquitoes) in villages with treated bednets as in villages with untreated bednets and that they are biting people just as much. It is therefore not immediately clear how children are protected against malaria in villages with treated bednets.

The study presented in this Chapter intended to look for evidence for a reduction of bites on children associated with treated bednets, by discriminating mosquito bloodmeals taken on children and from those taken on adults and animals.

Different methods have been used to determine the source of human feeds in mosquitoes. ABO blood grouping and haptoglobin typing have been used to study selective feeding between groups of mothers and children in Kenya and The Gambia (Bryan and Smalley, 1978; Boreham *et al.*, 1978). Both methods were used in a study in The Gambia in which host size and frequency of feeding were directly related (Port *et al.*, 1980). People who differ in blood group and who sleep together can be selected for such studies. To monitor the feeding patterns of mosquitoes at a village level, as was the purpose of the present study, this approach would have restricted the sample size. A more accurate method which allows identification of the person from which the bloodmeal has been taken is by DNA fingerprinting (Coulson *et al.*, 1990). This method has been used in experimental hut studies with insecticide-impregnated bednets in Tanzania and in The Gambia (Gokool *et al.*, 1993; Tami-Hirsch, 1994). Although DNA fingerprinting provides the most detailed information about the source of the mosquito bloodmeal, it is expensive and time consuming. Also, the major limitation of this method is that only around 35% of the mosquito bloodmeals have so far been found to be readable. The approach used in the study presented in this Chapter

should allow discrimination between mosquito blood meals from children and adults at a village level with an ELISA. Raising the antibody levels in one group of interest was necessary with a vaccine, which gave protection against rabies to the people in the villages. One of the limitations seen before the study was the cost of the ELISA kits. Another approach recently described is an ELISA for the measurement of the aminopropeptide of human procollagen type I which is at a different level in adults and children (Bodker *et al.*, 1995). This has not yet been evaluated in the field.

The results of the anti-rabies ELISA, assuming that the proportion of positives reflects the proportion of bites taken from children, are consistent with the hypothesis that in the presence of treated bednets, the proportion of mosquito bites on children are reduced. This reduction was clear in samples of females resting indoors. For outdoor resting females, reduction in the proportion of bloodmeals from children was observed in both villages in the presence of treated bednets, however only in one village was the difference significant.

The HBI results agreed with previous reports: no significant difference was found in the HBI in the presence or absence of treated bednets in indoor resting females, as in previous studies in The Gambia (Lindsay *et al.*, 1993, Thomson *et al.*, 1995a, Chapter 4). The observed HBI in pits showed an increase over time in both villages, although the difference was significant only in one village. This difference was not associated with the presence or absence of treated bednets. Since the increase in both villages coincide with the second part of the study, seasonal differences in species composition between *An. gambiae s.s.* and *An. melas* for example, could have been reflected in these results.

In other words, from both sets of results, looking at them independently and assuming that the anti-rabies ELISA was assessing bloodmeals from children and the PCR was assessing HBI, we could conclude that the proportion of bites of *An. gambiae s.l.* females on children was reduced in the presence of treated bednets. No evidence was found for an increase in bloodmeals from animals.

However, as the discrimination between human and non-human blood meals was carried out after the anti-rabies ELISA, the finding of "rabies-positives" in "non-human"

bloodmeals calls for an explanation.

The following possibilities were considered:

a) False negatives in the human DNA detection.

Most (63%) of the non-human bloodmeals that were "positive" for anti-rabies antibodies were found in samples from indoor resting females in one village (Mbai-Niake) in the presence of untreated bednets (Table 5.5). It is conceivable that those bloodmeals were from humans. However, it is very unlikely that the β-globin test failed to worked considering that the DNA was in good condition according to the cytochrome b results (M. Haywood, personal communication).

The results of the HBI assessed by the β-globin test fit the general expectations: higher HBI in samples from PSC than from PITS, as expected according to results of other studies with *An. gambiae s.l.* in which the frequency of human bloodmeals in PSC is higher than in outdoor resting populations (Boreham and Port, 1982).

Further experiments are currently being carried out by M. Haywood to try to identify a particular animal species (cow, goat and sheep) as the source of blood in the samples negative for the human β-globin.

b) False negatives in both human DNA and anti-rabies antibody detection.

Only 34% of the samples tested for the cytochrome *b* gene gave positive results (Table 5.4), when almost all the bloodmeals were expected to be from mammals, since these are the most probable source of blood for mosquitoes. Regarding the anti-rabies ELISA, an important limitation in the interpretation of the results is that the mosquito bloodmeal controls (positives and negatives) did not work, necessitating the use of an arbitrary cut-off.

An association was observed between the cytochrome probe positivity and the anti-rabies ELISA positivity (Table 5.6), which indicates that samples with good quality DNA were likely to have been "positive" for the anti-rabies ELISA.

Table 5.6. Association between the quality of the cytochrome *b* reaction and positivity of the anti-rabies ELISA.

	ELISA results	
	Negative (<75%)	**Positive (>85%)**
Cytochrome *b*		
1	88	10
2	115	30
3	80	87
4	13	39
Total	296	166

χ^2=96.24, d.f.=3, P<0.001

The simplest interpretation of these results is that both tests were measuring, at least in part, the quality of the samples.

If this is true, it would be reasonable to expect that "positives" for anti-rabies antibodies would be aggregated on particular days of collection or on one of the villages. Significantly higher positivity (χ^2=37.41, P<0.001) was found in Mbai-Niake (17.3%) than in Sinchu-Njabo (10.7%).

This hypothesis however would not explain the association between the presence of treated bednets and reduction in the proportion positive for anti-rabies antibodies, consistently found in both villages. There is no apparent reason why in the presence of treated bednets samples would be better preserved, given that each team of collectors worked in the same village throughout the study (in the presence and absence of treated bednets).

c) False positives in the anti-rabies antibody detection.

Non-specific binding has been reported by a study of rabies serology in unvaccinated UK dogs using the Platelia® anti-rabies ELISA (Cleaveland, 1996). The non-specific binding for dog sera was confirmed in a trial replacing the Platelia® coated plates with saturated Nunc plates with BSA and skim milk. Those findings were consistent with other studies in which non-specificity of the Platelia® system has been observed in sera from dogs but not humans. The explanation given by Cleaveland (1996) is that dogs have higher concentration of immunoglobulin than humans and this may contribute to aggregations of Fc fragments formed during thawing of frozen serum. It has been demonstrated that positive reactions were caused by non-specific binding of large molecular weight fragments recognized by anti-dog IgG. The most reactive UK sera were those from clinically sick dogs that had elevated globulin levels. The Platelia® anti-rabies ELISA results in the same study showed low variability and high repeatability in replicated samples as in the present case. This ELISA was therefore considered by Cleaveland (1996) as inappropriate for measurements or detection of rabies antibody in canine sera.

It is unlikely that dogs were the hosts for the mosquito bloodmeals, since no dogs were recorded in the study villages (Table 5.1). However, a similar non-specific binding could have occurred with other animal bloodmeals. False positives in serum from cows and horses is accepted in the manufacturers' handbook for the Platelia® ELISA.

This possibility could explain the presence of animal bloodmeals positive for the rabies ELISA. With some reservations it could explain both the anti-rabies results and the human blood index results. The weakness of this explanation is that it implies a much higher frequency of "false positives" in animal bloodmeals (35/83=42%) than in the results of Cleaveland (21/94=22% of unvaccinated UK dogs). Besides, if we assume that for bloodmeals of human origin the ELISA worked well, the estimated proportion of bloodmeals from children in the indoor resting samples in untreated villages is around 32% (approx. 279/863). This proportion is higher than previous reports of Port *et al.*, (1980) and Boreham and Port (1982) in which the proportion of bloodmeals from children was estimated to be

around 22% and 11.7% respectively, in controlled studies of mothers and children sleeping together.

Because of the reservations about the reliability of the results of this study, conclusions cannot be made. If we assume that i) the "positives" in the anti-rabies ELISA of human bloodmeals accurately reflected the frequency of bites on children, ii) the PCR accurately reflected the frequency of bites on humans and animals, then we must accept the possibility of non-specific binding to explain the finding of rabies positives in non-human bloodmeals as well (see page 133) and could then conclude that a reduction in the proportion of mosquito bites from children was caused by permethrin-treated bednets in The Gambia. The population of malaria vectors could then survive at the expense of other hosts, including adult humans. This could explain why the human-blood and sporozoite rates have no significant change when bednets in a village are treated. Adults go to bed at later times (Port *et al.*, 1980) and wake up earlier than children, presumably this makes them more exposed to mosquito bites. If we accept this argument, then it implies that this is the first evidence of any kind that treated bednets divert mosquitoes from one person to another. Given the importance of this point, the ambiguity of the data and the availability of alternative explanations we cannot make any such clear conclusion.

Diversion to animals however cannot be ruled out entirely. Although this effect was seen in only one of the two villages involved in this study, and may not be associated with the presence of treated bednets, it is reasonable to think that if diversion of mosquito bites from children is occurring due to the presence of treated bednets in the rooms, hungry mosquitoes may have been driven outdoors to search for meals and bite animals, if they are available. Diversion to animals, however, would depend on the availability of animals in the villages as well as on the degree of anthropophily of the vector species concerned.

The question of diversion of mosquito bites by treated bednets is of interest partly for our general understanding of how treated bednets work, but also for ethical reasons, especially in programmes where some, but not all, people in a given village have their bednets impregnated. In experimental hut studies, no evidence has been found for diversion from a person sleeping under a treated bednet to an unprotected person sleeping in the same room (Lines *et al.*, 1987). Also, no evidence has been found for diversion from a hut with a treated bednet to a neighbouring room with an untreated bednet (Lindsay *et al.*, 1992). Even

diversion from a treated to untreated villages has been considered as one of the possibilities, but no evidence for this was found (see Chapter 3).

If the assumption of false positives in the anti-rabies ELISA with animal sera is accepted, the results of the study presented here can be interpreted as suggesting that permethrin-impregnated bednets divert mosquitoes from protected children to other hosts including adults. Without changes in sporozoite rates in the vector population, the risk of malaria infection in adults would increase. However, the degree of immunity developed in adults would counteract the risk that they would suffer significantly increase of disease.

Lines (1996) suggested that the population-level effects of treated bednets can be understood as a balance between killing of the mosquitos and diversion of bites to either unprotected people or animals. In The Gambia, killing of mosquitoes by impregnated bednets seems to be less important than in other countries such as Tanzania and Zaire where killing was clearly a major effect of the treated bednets (Table 1.2 and Chapter 2). In the Gambia, diversion of bites from protected people to other hosts seems to be more important than killing.

5.5. SUMMARY

A cross-over study was carried out in two villages to look for evidence that the proportion of bites on children were reduced in association with the use of impregnated bednets, and to determine whether mosquitoes was diverted to adults and/or animals.

In both villages all the children were vaccinated against rabies. Blood-fed females of *An. gambiae s.l.* were collected by pyrethrum spray catches, exit traps and in pits outdoors. Antibodies against rabies glycoprotein were detected in the bloodmeals using a modified ELISA kit. The discrimination between human and non-human bloodmeals was done with around 25% of the samples by PCR.

A significant reduction in the proportion of "positive" bloodmeals for the anti-rabies ELISA was observed in the presence of treated bednets. No evidence was found for an increase in bloodmeals from animals.

However, the reliability of the results of this study was hampered by two major problems: First, the mosquito bloodmeal controls (positives and negatives) for the ELISA did not work, necessitating the use of an arbitrary cut-off. Second, a surprisingly high proportion (32%) of anti-rabies positives mosquitoes, which were presumed to be from vaccinated children, appeared negative for the human PCR. Some possibilities were considered to explain these findings.

Assuming that the antirabies ELISA "positives" reflect mosquito bites from children, the results of the study could be interpreted as suggesting that permethrin-impregnated bednets divert mosquitoes from protected children to other hosts including adults.

CHAPTER 6

IDENTIFICATION OF SPECIES OF THE *Anopheles gambiae* COMPLEX, CHROMOSOMAL POLYMORPHISM IN *Anopheles gambiae s.s.*, BEHAVIOUR AND IMPREGNATED BEDNETS.

6.1. INTRODUCTION

Anopheles gambiae s.s., *An. arabiensis*, and *An. melas* are the malaria vector species found in The Gambia (Bryan, 1979). These species have differences in their behaviour, geographical distribution, seasonality and role in malaria transmission (White, 1974). *An. gambiae s.s.* is the most anthropophilic and endophilic species of the complex, *An. arabiensis* shows a wide range of feeding and resting patterns, being generally more exophilic and zoophilic than *An. gambiae s.s.*, and *An. melas* shows a marked exophily and zoophagy. *An. gambiae s.s.* typically occurs in forest and humid savanna areas although its range extends into the arid savanna areas. *An. arabiensis* on the other hand is rare or absent in humid savanna areas, at least during the rainy season, but is prevalent in arid savanna areas. In areas where both *An. gambiae s.s.* and *An. arabiensis* are sympatric, shifts in seasonal prevalence of both species are observed with an increase in the relative frequency of *An. arabiensis* during the dry season. *An. melas* is a salt-water breeder found near the coast. *An. gambiae s.s.* and *An. arabiensis* are recognized as the main vectors of malaria in most of the areas. *An. melas* can play an important role under local conditions (White, 1974).

Differences in behaviour and geographical distribution have been found not only between species of the *An. gambiae* complex, but also within species, between carriers of particular chromosomal inversions. In northern Nigeria during the Garki project, a non-random exposure to the insecticide sprayed indoors was found between female mosquitoes carrying different inversions. For *An. gambiae s.s.* the chromosomal arrangements more often found in man biting collections outdoors were the same as those that tend to be associated with humid environments (carriers of the 2Rd inversion) while the arrangements favoured in dry environments (carriers of the 2Rb - 2La inversions) were those found more frequently

in indoor collections, resting in bednets and in rooms (Coluzzi *et al.*, 1979). This phenomenon of differences in behaviour between carriers of different chromosomal inversions and therefore differential exposure to the insecticide in the houses was proposed as a possible explanation for the failure to reduce the malaria vectorial capacity below the critical level by house spraying with propoxur (Coluzzi, 1984).

Intraspecific variations in infectivity with *P. falciparum* have also been found associated with chromosomal polymorphism. *An. gambiae s.s.* carriers of the standard form of the inversion 2La were associated with at least two times higher sporozoite infection rates in Kenya (Petrarca and Beier, 1992). Incidentally, the same inversion was found associated with 2 esterase loci that were at the same time associated with the ability of mosquitoes to encapsulate ookinetes of *P. cynomolgi*. The phenotypes of esterase associated with refractoriness of *An. gambiae* were carriers of the inverted 2La region. Although these results contrasted with the findings in Kenya, there is a possibility that they could still reflect the same underlying gene (Crews-Oyen *et al.*, 1993). This study strongly suggested that the inversion 2La was maintaining the linkage between the esterase genes and the *Plasmodium*-susceptibility locus.

In The Gambia, two chromosomal forms have been described for *An. gambiae s.s.*, the main vector species for the country: the 'Savanna form', which is typical of dry-savanna freshwater inland zones, with a high frequency of inversions 2Rb and 2La, and the 'Bissau form', which is found in more humid, coastal areas and southern zones, and is characterized by a high frequency of the 2Rd inversion and low frequency of 2Rb and 2La. In the central area of the country, where both forms coexist, the hybridization frequency was found to be lower than expected according to Hardy-Weinberg ratios, which suggested an incipient speciation process (Bryan *et al.*, 1982, Coluzzi, 1984).

Cytotaxonomy has been used as the standard method of identification, having the advantage of being less expensive and based on laboratory facilities that are more easily available than other techniques. More importantly, chromosomal analysis provides unique additional information on polymorphic chromosomal inversions, which might constitute a

very useful guide to epidemiologically important heterogeneities in vector populations (Coluzzi, 1984). However, this method of identification requires intensive training and the number of specimens per day that can be identified is limited. The use of the DNA probes should allow a larger number of specimens to be dealt with, improving the identification process for routine collections. Non-radioactive DNA probes have been developed and successfully used in the laboratory for the identification of specimens of the *An. gambiae* complex (Hill *et al.*, 1992, 1994a). A comparison with a standard method for their use with field specimens is necessary.

Differences in behaviour between species of the *An. gambiae* species complex, and between cytotypes of *An. gambiae s.s.* play an important role in transmission of malaria, and could also be of importance if the species have a differential response to the insecticide-impregnated bednet programme in The Gambia. One objective of this study was to determine the sporozoite rates and human blood index of the species of the *An. gambiae* complex and of the different cytotypes of *An. gambiae s.s.* found in The Gambia. A second objective was to identify differences in behaviour that could influence the effect of impregnated bednets. A third objective was to compare the use of DNA probes with cytogenetics for the identification of the species of the *An. gambiae* complex. Unfortunately, each of these objectives was achieved only to a limited degree.

6.2. MATERIALS AND METHODS

The study was carried out in the village of Balingho, near Farafenni (200 Km from the Atlantic coast). This village was selected because previous reports from the area (Bryan *et al.*, 1987; Bryan *et al.*, 1982) showed the presence of the three species of the *An. gambiae* species complex and also the presence of the two cytotypes 'Savanna' and 'Bissau' (populations 1 and 2 in Bryan *et al.*, 1982) of *An. gambiae s.s.*.

Females of the *An. gambiae* species complex were collected in five situations: resting inside bednets in the morning (BNC), resting indoors by pyrethrum spray catches (PSC) in the afternoon around 5pm, exit traps (ETC) set in the evenings and collected in the mornings,

outdoor resting mosquitoes collected in pit traps (PIT) and animal baited net (ANC) with a calf set at night and collections made in the morning. Figure 6.1 shows the pit trap collection. A sample of 300 mosquitoes from each method of collection was intended to be analysed. Collections were carried out during the rainy season in 1993, between August and October. Females caught in BNC, PIT and ANC were mostly already blood-fed, therefore they were held in the insectary until the afternoon when they were half-gravid and suitable for cytogenetics. Females from PSC were transferred immediately to the laboratory in Farafenni to be processed since most of the them were half-gravid. Most of the females from ETC were unfed, hence they were transferred to the insectary and fed using a membrane feeding system.

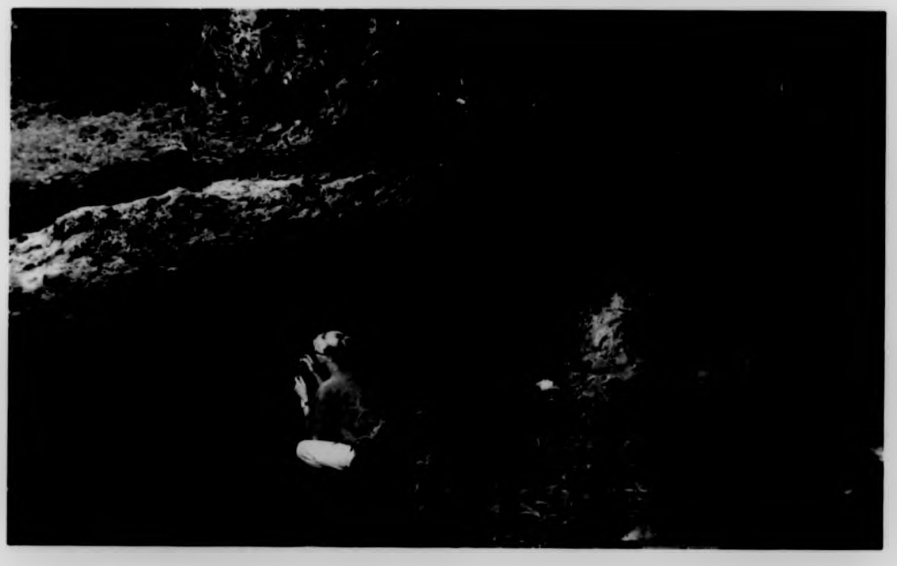

Figure 6.1. Collection of *An. gambiae s.l.* resting outdoors in pit traps in the village of Balingho.

In the laboratory *An. gambiae s.l.* females were selected and dissected, the information being recorded for each individual female. Heads were preserved in isopropanol for identification by DNA probes (Hill *et al.*, 1994a). Squash blots were performed on nylon filters as described by Hill *et al.* (1991) in the laboratory in Farafenni. A known *An. gambiae s.s.* and *An. arabiensis*, from colonies at the L.S.H.&T.M., on each filter acted as positive controls. Hybridizations with the probes Angsl, Angss and Anml4 were carried out by W. Macdowall for her MSc project (Macdowall, 1994), following the procedures of Hill *et al.* (1992 and 1994b). Identifications were made using the key of Hill and Crampton (1994a), shown in Table 6.1.

Table 6.1. A key to the hybridization of the probes used to identify female *An. gambiae* (Hill and Crampton, 1994a). +:intensity of the reaction.

	Probes		
Species	pAngsl	pAngss	pAnM14
An. gambiae s.s.	++	++	+
An. arabiensis	++	--	+
An. melas	++	++	+++

Thoraces were preserved dry to determine sporozoite positivity by ELISA (Wirtz *et al.*, 1987).

The blood in the stomach of females collected fed was squashed onto a filter paper to identify the source of blood (Burkot *et al.*, 1981).

The rest of the abdomen, the part with the ovaries, was put in Carnoy's fixative (3 parts of ethanol: 1 part glacial acetic acid) and stored at -20C until needed. Polytene chromosomes from the ovarian nurse cells were prepared using the method described by

Coluzzi (1968) and modified by Hunt (1973). The species were identified by microscopic examination of the specific band sequences on the X-chromosome and autosomes, and the inversion karyotypes of *An. gambiae s.s.* in the autosomes were scored following the nomenclature of Coluzzi *et al.* (1979).

The recorded karyotype frequencies were compared to the ones expected from the Hardy-Weinberg equilibrium by using Wright's F statistic (Brown, 1970):

$$F= [4a \times c - b^2] \div [(2a + b)\times(2c + b)]$$

were a and c are the absolute frequencies of the homozygous classes and b is the absolute frequency of the heterozygotes. Significant ($P<0.05$) deviation from the Hardy-Weinberg predicted value is obtained when $|F|>1.96/\sqrt{n}$, where n is the sample size. An F value greater than zero indicates deficiency of heterozygotes, while a value lower than zero indicates excess.

6.3. RESULTS

6.3.1. Comparison between DNA probes and cytogenetics for identification of the species

Twelve filters were prepared with a total of 1086 mosquitoes collected. All of them were processed for identification by DNA probes. A total of 317 slides of polytene chromosomes were prepared from which 155 (48.9%) were readable and the species identified by the chromosome banding pattern. A total of 149 females of the *An. gambiae* species complex were identified by both methods and the results are shown in Table 6.2. The percent of agreement between the two methods was 68.45%. Comparing the results and taking cytogenetics as the 'gold standard' method, DNA probes showed the highest sensitivity and specificity (100% and 96.9% respectively) for the identification of *An. arabiensis*. The main difficulty with the DNA probes was found in the discrimination between *An. gambiae s.s.* and *An. melas*. For *An. gambiae s.s.* the sensitivity of the DNA probes was high (93.4%) but the specificity was only 52.3% (60% of *An. melas* were identified as *An. gambiae s.s.*). This was due mainly to the lack of positive control for *An. melas*. Because of the cross-

reaction between the probe for *An. gambiae s.s.* with *An. melas*, whenever the filter for *An. melas* did not show clear results, all the *An. melas* mosquitoes were wrongly identified as *An. gambiae s.s.*. Because of this, the sensitivity of the *An. melas* probe was low (38.6%), but its specificity was high (98.73%).

Table 6.2. Comparison between DNA probes and cytogenetics. Number of females positive for the DNA probes pAngsl (*for An.gambiae s.l.*), pAngss (for *An.gambiae s.s.*) and pAnM14 (for *An.melas*), and interpretation of the results for identification of the species by the probes: (a)= positive for pAngsl and pAngss minus positive for pAnM14; (b)= positive for pAngsl minus positive for the other two; (c)= positive for both pAngsl and for pAnM14.

Species by	**Positive for the probes**			**Interpretation of DNA probes**		
Cytogenetics	**pAngsl**	**pAngss**	**pAnM14**	***gambiae s.s***	***arabiensis***	***melas***
				(a)	**(b)**	**(c)**
gambiae s.s	61	58	1	57	3	1
arabiensis	18	0	0	0	18	0
melas	70	69	27	42	1	27
Total	149	127	28	99	22	28

Looking at the filters that showed good resolution for *An. melas*, the 3 best included 76 mosquitoes identified also by cytogenetics (30 *An. gambiae s.s.*, 11 *An. arabiensis* and 35 *An. melas*). Despite the absence of a positive control for *An. melas*, the agreement between the two methods of identification was 84.2%, and the specificity of the *An. gambiae s.s.* probe and the sensitivity of the *An. melas* were 80.4% and 74% respectively.

Considering only the identification of *An. gambiae s.s.* and *An. arabiensis*, 78 mosquitoes were identified by cytogenetics: 61 *An. gambiae* and 18 *An. arabiensis*. The sensitivity and specificity of the identification with the probe for *An. gambiae s.s.* were high: 93.44% and 100% respectively. Three *An. gambiae* were identified as *An arabiensis* and 1

as *An. melas*. For *An. arabiensis* also the sensitivity and specificity were high, 100% and 96.6% respectively.

6.3.2. Relative frequency of species

Table 6.3 shows the number of females collected by the different methods and identified by DNA probes. From BNC, PSC and ETC the target of around 300 females of the *An. gambiae* complex were achieved. However, from PIT traps only 152 females could be collected. Animal baited traps could be used on only two occasions, and only 11 females of the *An. gambiae* complex were collected. *An. gambiae s.s.* and *An. melas* were the most abundant species for all the methods of collection (85.2% overall). Because of the mis-identification between *An. gambiae s.s.* and *An. melas*, they cannot be confidently separated by method of collection. Assuming a similar probability for each species to be caught in any method of collection, the expected number of females and the χ^2 were calculated (small figures in the Table). A significant difference was found between species by method of collection, the main contribution to the χ^2 being the difference between observed and expected numbers of *An. melas* in pit traps, in which higher numbers than expected were observed.

Table 6.4 shows the same information as in Table 6.3 but with the sample identified by cytogenetics. *An. gambiae s.s.* and *An. melas* were found in similar proportions overall, *An. gambiae s.s.* being predominant indoors in BNC, PSC and ETC while *An. melas* was predominant outdoors in PIT and ANC. As with the DNA identifications by method of collection (Table 6.3), a significant difference was found between species identified by cytogenetics with respect to method of collection. The excess of *An. melas* and the deficiency of *An. gambiae s.s.* in PIT and ANC were the main contribution to the χ^2.

Only the sample identified by cytogenetics can be confidently reported by method of collection, and for infectivity and human blood positivity. This was due to the confusion between *An. gambiae s.s.* and *An. melas* with the DNA probes as mentioned above. This factor reduced the sample size and prevented more precise conclusions about the behaviour

of the sibling species of the *An. gambiae* complex.

Table 6.3. Number of females of the *Anopheles gambiae* species complex identified by DNA probes with respect to method of collection: BNC=resting in bednets, PSC=resting indoors using pyrethrum spray, ETC=in exit traps, PIT=outdoor resting collected in pit traps, ANC=animal baited net. Expected (e) figures were calculated from the marginal totals.

Method		*An.gambiae s.s.* and *An.melas*	*An.arabiensis*	*An.melas*	Total	$\sum(o-e)^2/e$
BNC	o	266 (83.4%)	41 (12.9%)	12 (3.8%)	319	
	e	252.9	42.6	23.5		
	$(o-e)^2/e$	0.68	0.06	5.63		6.36
PSC	o	258 (79.9%)	36 (11.1%)	29 (9.0%)	323	
	e	256.1	43.1	23.8		
	$(o-e)^2/e$	0.01	1.18	1.14		2.33
ETC	o	218 (77.6%)	55 (19.6%)	8 (2.8%)	281	
	e	222.8	37.5	20.7		
	$(o-e)^2/e$	0.10	8.15	7.79		16.04
PIT	o	109 (71.7%)	12 (7.9%)	31 (20.7%)	152	
	e	120.5	20.3	11.2		
	$(o-e)^2/e$	1.10	3.40	35.0		39.5
ANC	o	10 (90.9%)	1 (9.1%)	0 (0%)	11	
	e	8.72	1.47	0.81		
	$(o-e)^2/e$	0.19	0.15	0.81		1.15
Total		861 (79.3%)	145 (13.4%)	80 (7.4%)	1086	
					χ^2=	65.39
					df=8, P<0.001	

Table 6.4. Number of females of the *Anopheles gambiae* species complex identified by cytogenetics with respect to method of collection: BNC=resting in bednets, PSC=resting indoors using pyrethrum spray, ETC=in exit traps, PIT=outdoor resting collected in pit traps, ANC=animal baited net. Expected (e) figures were calculated from the marginal totals.

Method		*An.gambiae s.s.*	*An.arabiensis*	*An.melas*	Total	$\sum(o-e)^2/e$
BNC	o	26 (59.1%)	5 (11.4%)	13 (29.5%)	44	
	e	18.45	5.11	20.44		
	$(o-e)^2/e$	3.09	0	2.71		5.80
PSC	o	18 (46.1%)	8 (20.5%)	13 (33.3%)	39	
	e	16.35	4.53	18.12		
	$(o-e)^2/e$	0.17	2.66	1.44		4.27
ETC	o	15 (68.2%)	3 (13.6%)	4 (18.2%)	22	
	e	9.23	2.55	10.22		
	$(o-e)^2/e$	3.61	0.08	3.79		7.48
PIT	o	6 (13.9%)	2 (4.6%)	35 (81.4%)	43	
	e	18.03	4.99	19.97		
	$(o-e)^2/e$	8.03	1.79	11.30		21.13
ANC	o	0 (0%)	0 (0%)	7 (100%)	7	
	e	2.94	0.81	3.25		
	$(o-e)^2/e$	2.94	0.81	4.32		8.07
Total		65 (41.9%)	18 (11.6%)	72 (46.5%)	155	
					χ^2=	46.74
					df=8, P<0.001	

6.3.3. Cytotypes of *An. gambiae s.s*

Three inversions were found in *An. gambiae s.s.*: 2Rb, 2Rd and 2La. The majority (71.2%) of the individuals were carriers of the 2Rb inversion in either homozygote or heterozygote form and 2Rd standard homozygote (Table 6.5). No significant difference was found in the frequency of the inversions 2Rb and 2Rd by method of collection (Table 6.6). The inversion 2La showed significantly higher frequency in BNC and ETC than in PSC and PIT.

Table 6.5. Number of the different arrangements of the inversions 2Rb, 2Rd and 2La found in *An. gambiae s.s.* (+/+:standard homozygote, +/-:heterozygote, -/-:inverted homozygote, U=undetermined).

Inversions:	2Rb	2Rd	2La	Number observed
	-/-	+/+	-/-	14
	+/-	+/+	-/-	10
	-/-	+/+	U	6
	-/-	+/+	+/-	5
	+/-	+/+	+/-	3
	+/+	+/+	+/-	3
	+/+	+/-	+/+	3
	+/-	+/+	+/+	2
	+/+	-/-	+/+	2
	+/+	-/-	U	2
	-/-	+/-	-/-	1
	-/-	+/+	+/+	1
	+/-	+/-	+/-	1
	+/+	+/-	+/-	1
	+/+	+/+	-/-	1
	+/+	+/+	+/+	1
	+/+	+/+	U	1
	+/-	+/+	U	1
	U	U	-/-	1
Total:				59

Table 6.6. Number of *An. gambiae s.s.* found with the different karyotypes for the inversions 2Rb, 2Rd and 2La and inversion frequency with respect to method of collection.

	Method of collection					
Karyotype	**BNC**	**PSC**	**ETC**	**PIT**	χ^2	**P**
2Rb+/+	5	6	2	1		
2Rb+/-	8	2	4	3		
2Rb-/-	10	9	6	2		
Inversion frequency:						
2Rb+	0.39	0.41	0.34	0.42		
2Rb-	0.61	0.59	0.66	0.58	0.43	0.93
2Rd+/+	20	12	11	5		
2Rd+/-	1	3	1	1		
2Rd-/-	2	2	0	0		
Inversion frequency:						
2Rd+	0.89	0.79	0.95	0.91		
2Rd-	0.11	0.21	0.05	0.09	3.96	0.26
2La+/+	1	4	1	3		
2La+/-	5	7	1	0		
2La-/-	9	6	9	3		
Inversion frequency:						
2La+	0.23	0.44	0.14	0.50		
2La-	0.77	0.56	0.86	0.50	8.57	0.03

Given that no significant difference was found between the methods of collection in the frequency of the inversions 2Rb and 2Rd, the total numbers of females collected by all methods were combined for the analysis of linkage disequilibrium in the population. Despite the significant difference between methods of collection for the 2La inversion, the numbers were also added because the analysis of linkage disequilibrium discriminating by method of collection would leave very low numbers of homozygotes and heterozygotes. The results represent the overall population. The frequency of the inversions 2Rb, 2Rd and 2La of *An. gambiae s.s.* and F values are shown in Table 6.7. The F value was significant for the three inversions, meaning a significant deviation from the expected numbers if the population were in Hardy-Weinberg equilibrium. A deficiency of heterozygotes was observed for the three inversions and also high frequency of inverted homozygotes for 2Rb and 2La and of the standard homozygote for the inversion 2Rd. This arrangement corresponds to the so-called 'Savanna' form (Coluzzi, 1985).

Table 6.7. Frequency of chromosome-2 inversions of *An. gambiae s.s.* collected in the village of Balingho in all methods of collection. (+/+:standard homozygote, +/-:heterozygote, -/-:inverted homozygote). The F value is a measure of the deviation from Hardy-Weinberg deviation. (*): critical value P=0.05.

Inversion		St +/+	Het. +/-	Inv. -/-	Frequency +	-	χ^2	1.96/√n	F
2Rb	o	14	17	27	0.388	0.612			
	e	8.7	27.5	21.7			8.498	0.26	0.382 *
2Rd	o	48	6	4	0.879	0.120			
	e	44.8	12.3	0.8			15.24	0.26	0.512 *
2La	o	9	13	27	0.316	0.684			
	e	4.9	21.1	22.9			7.324	0.28	0.386 *

The number of observed and expected associations between the three inversions is shown in Table 6.8. Analysis using a chi-square is not valid due to expected values lower than 5 for most of the cells. However, a slight tendency can be observed for 2Rb inverted homozygotes and 2Rd standard homozygotes to be present in a higher than expected frequency and the two standard homozygotes to be present in lower frequency than expected. Associations for the inversions 2La/2Rb and 2La/2Rd are presented in the same Table, but the number of individuals were lower than the association for 2Rb and 2Rd, due to lack of readings of the 2La region for some individuals.

6.3.4. Sporozoite rates

Only three out of 1086 mosquitoes were positive by ELISA for the circumsporozoite protein: two *An. gambiae s.s.* collected in exit traps and one *An. melas* collected resting in a bednet. The cytotypes of the two *An. gambiae s.s.* were similar, being both homozygote standard (+/+) for the 2Rd inversion, and homozygote inverted (-/-) for the 2La. One was heterozygote (+/-) and the other inverted homozygote (-/-) for the 2Rb inversion. Both mosquitoes therefore corresponded to the Savanna form.

6.3.5. Human blood index

Results of the females positive for human blood by species and method of collection are presented in Table 6.9. The number of *An. arabiensis* tested was too low to allow any comparison with the other species. Comparing the proportion positive of *An. gambiae s.s.* with *An. melas*, no significant differences were found between the species (using the totals χ^2=1.32, P=0.25). Separating the methods of collection Chi-square could validly be used only for PSC (χ^2=1.7, P=0.19). Fisher's exact tests were made for ETC and PIT. In both cases no significant differences were found between the species. However, the sample size was too low to be conclusive.

No significant differences were found in the overall proportion of *An. gambiae s.s.* with human blood between the different karyotypes (Table 6.10) (using the totals, 2Rb: χ^2=0.607, P=0.738; 2Rd: χ^2=1.393, P=0.498; 2La: χ^2=4.904, P=0.086), although only 27 *An. gambiae s.s.* were analysed.

Table 6.8. Numbers of observed (o) associations between 2Rb, 2Rd and 2La inversion karyotypes in the sample of *An. gambiae s.s.* collected in the village of Balingho. (+/+:standard homozygote, +/-:heterozygote, -/-:inverted homozygote). Number expected assuming linkage equilibrium indicated by e and in small print.

			Karyotypes			
	2Rb		+/+	+/-	-/-	Totals
2Rd	+/+	o	6	16	26	48
		e	11.6	14.1	22.3	
	+/-	o	4	1	1	6
		e	1.4	1.8	2.8	
	-/-	o	4	0	0	4
		e	1.0	1.2	1.9	
Totals			14	17	27	58
	2Rb		+/+	+/-	-/-	Totals
2La	+/+	o	6	2	1	9
		e	2.1	3.0	3.9	
	+/-	o	4	4	5	13
		e	3.0	4.3	5.7	
	-/-	o	1	10	15	26
		e	6.0	8.7	11.4	
Totals			11	16	21	48
	2Rd		+/+	+/-	-/-	Totals
2La	+/+	o	4	3	2	9
		e	7.5	1.1	0.4	
	+/-	o	11	2	0	13
		e	10.8	1.6	0.5	
	-/-	o	25	1	0	26
		e	21.7	3.3	1.1	
Totals			40	6	2	48

Table 6.9. Proportion positive for human blood of each species of the *Anopheles gambiae* complex identified by cytogenetics. Data are classified by method of collection: PSC:spray catches, ETC: exit traps (only feds found in the traps), PIT:pit traps. Number tested in parentheses.

	Species		
Method	*An. gambiae s.s.*	*An.arabiensis*	*An. melas*
PSC	61.1% (18)	87.5% (8)	30.8% (13)
ETC	13.3% (15)	0 (3)	0 (4)
PIT	33.3% (6)	0 (2)	31.4% (35)
ANC	-	-	0 (7)

Table 6.10. Number positive for human blood in *Anopheles gambiae s.s.* carriers of different chromosome 2 karyotypes, by method of collection. PSC:spray catches, ETC: exit traps (only feds found in the traps), PIT:pit traps. Number tested in parentheses.

	Methods of collection				
Karyotypes	PSC	ETC	PIT	Total	Overall percent
2Rb+/+	3 (6)	0 (0)	0 (1)	3 (7)	42.8%
2Rb+/-	1 (2)	2 (3)	2 (3)	5 (8)	62.5%
2Rb-/-	6 (9)	0 (1)	0 (2)	6(12)	50.0%
Total	10(17)	2 (4)	2 (6)	14(27)	51.8%
2Rd+/+	8(12)	2 (4)	2 (5)	12(21)	57.1%
2Rd+/-	1 (3)	0 (0)	0 (1)	1 (4)	25.0%
2Rd-/-	1 (2)	0 (0)	0 (0)	1 (2)	50.0%
Total	10(17)	2 (4)	2 (6)	14(27)	51.8%
2La+/+	1 (4)	0 (0)	1 (3)	2 (7)	28.6%
2La+/-	6 (7)	0 (0)	0 (0)	6 (7)	85.7%
2La-/-	3 (6)	2 (4)	1 (3)	6(13)	46.2%
Total	10(17)	2 (4)	2 (6)	14(27)	51.8%

6.4. DISCUSSION

6.4.1. Species identification

In evaluation of control of malaria with insecticides, the identification of the species involved in transmission is of relevance because differences in behaviour between species could lead to a differential exposure to the insecticide, hence to a differential impact of the control measure. Species identification is also important to monitor specific responses, for example in the development of resistance to the insecticide used. Non-radioactive DNA probes (Hill *et al.*, 1991, 1992, 1994a) show potential for use in the field .

Comparing the DNA results with cytogenetics, a good correspondence was found between the two methods for the identification of *An. arabiensis*, as well as for *An. gambiae s.s.*. However, the method of identification of *An. arabiensis* was considered by Macdowall (1994) as the main disadvantage of this technique, because it relies on the presence of hybridisation with the probe pAngsl (for *An. gambiae s.l.*) and the absence of hybridization with the pAngss (for *An. gambiae s.s.*). When *An. melas* is present together with *An. gambiae s.s.*, a positive control is necessary to avoid mistaking *An. melas* for *An. gambiae s.s.* since the probe for *An. gambiae s.s.* recognized both species (Hill *et al.*, 1992; Hill and Crampton 1994b). This control is difficult to provide since currently there are no existing colonies of *An. melas*.

One of the major limitations of the non-radioactive DNA probes was found to be the variability between and within batches of probes, particularly with the pAngsl, the nature of which is unknown (Macdowall, 1994).

Vij (1995) has carried out more comprehensive comparisons (including samples from South Africa, Kenya, Tanzania, Uganda, Mali, Ghana, Malawi and Namibia) between the same non-radioactive DNA probes and cytogenetics. She found good concordance, and there was inconsistency in hybridization of only one of the probes (pAnq1 SH 2), for *An. arabiensis* and *An. gambiae s.s.*.

Cytogenetics gives species identification besides other information of relevance (frequency of inversions). But great attention has to be given to the procedure for collecting

and preserving the material before the chromosome preparations are made. Different proportions of readable slides were obtained in the study presented in Chapter 7 (80%) and in the study presented in this Chapter (49%), probably as a result of the difference in the methods of handling the samples. Samples referred to in Chapter 7 could be put at low temperatures immediately after collection, while the samples for the study presented in this Chapter were put in Carnoy's only after each individual mosquito had been killed and dissected for other assays (heads for DNA probes, thoraces for ELISA for sporozoites and blood for ELISA for HBI).

In any evaluation of vector control to distinguish the species is necessary. Routine identification of the species of the *An. gambiae* complex is difficult because the available methods are not only time consuming but imperfect. The only method that can reliably distinguish each species from the others is cytogenetics and this is very exigent technique with a limitation of that only semigravid females can be used. DNA probes might help to solve this problem but improvements are necessary, specially in their specificity. Further developments in the specificity of the probes would be ideal to avoid relaying on a combination of results with two different probes.

6.4.2. Species composition, resting behaviour, infectivity and human blood index

Chromosomal identifications were broadly in agreement with previous reports for the area (Bryan *et al.*, 1982): *An. gambiae s.s.* and *An. melas* were the most frequent species collected, and *An. arabiensis* was found in low frequency.

The detection of associations between species and behaviour related to transmission was hampered by the misidentification problems with the DNA probes. This meant that Human bloodmeal and sporozoite ELISA results could be linked to individual identification only for the sample cytogenetically identified. However, the tendencies observed were in agreement with previous reports from the area. *An. gambiae s.s.* was more abundant resting indoors (BNC and PSC) than outdoors while *An. melas* was more abundant resting outdoors in the pit traps than indoors (Table 6.4), which is similar to observations by Bryan *et al.*

(1987). *An. melas* had a lower HBI than *An. gambiae s.s.*, and although this difference was not significant in the few samples tested, it is consistent with the observations of Bryan (1987). She found *An. melas* with a lower HBI (13%) than *An. gambiae s.s.* (45%) among samples collected resting in houses. She also found *An. gambiae s.s.* with higher sporozoite rates than *An. melas* (Bryan, 1979 and 1983). *An. gambiae s.s.* is recognized as the main vector during the rainy seasons throughout the country. However, *An. melas* may have a modest but significant role as a vector in the western half of the country, given that it is found for several months after the end of the rainy season, when *An. gambiae s.s.* is no longer abundant (Bryan, 1983).

6.4.3. Cytotypes of *An. gambiae s.s.*, behaviour, infectivity and human blood index

Although the 2 different incipient species described by Bryan *et al.* (1982) were found in the village, the frequency of the 'Savanna' type was higher than the frequency of the so-called 'Bissau' type. The majority of the mosquitoes sampled were carriers of the inverted form of the 2Rb and 2La regions and standard for the 2Rd. As few carriers of the 2Rb inversion were found, the contrast between carriers of the 2Rb and 2Rd inversions in the tendency to rest indoors or outdoors described by Coluzzi *et al.*, (1979) could not be seen.

Cytogenetics gives information about the frequency of inversions that cannot be obtained by other methods such as DNA probes. This information can be relevant to epidemiology and control, since inversions have been found associated with characteristics important for malaria transmission and control. The association found between the inversion 2La in *An. gambiae s.s.* and infectivity with *Plasmodium* in two separate studies (Petrarca and Beier, 1992; Crews-Oyen *et al.*,1993)), suggest the need for more studies of this kind. From a more practical point of view, associations between inversions and resistance to insecticides would be relevant to the interpretation of control measures. An association has been described between DDT resistance and the 2Rb inversion in *An. arabiensis* in Ethiopia (Nigatu *et al.*, 1995). In a recent study in Tanzania the 2Rb inversion in *An. arabiensis* was found to show higher frequency outdoors (in pit traps and exit traps) than resting indoors

(Mnzava *et al.*, 1995), although this difference was found only in one out of three villages. These differences in behaviour could have an influence in the impact of any intradomiciliary insecticide for malaria control and in particular to the use of impregnated bednets.

One of the aims of this study was to look for differences in the sporozoite rates of different sibling species and cytotypes. This was hampered not only by the problems encountered in identification, but also by the surprisingly low sporozoite rate found in Balingho; previous studies in these areas have found rates of around 2% (Thomson *et al*, 1994 and 1995a). The ideal setting for a study of this kind would be one with high rates of sporozoite positivity and with the presence of the two incipient species in similar frequency.

6.5. SUMMARY

The main purpose of the study presented in this chapter was to determine the extent to which differences between species could affect the evaluation of impregnated bednets in The Gambia. DNA probes and cytogenetics were compared for identification of the species.

Females of *An. gambiae s.l.* were collected using different methods (BNC, PSC, ETC, PIT, ANC) in the village of Balingho (200Km from the Atlantic coast). The species were identified using a non-radioactive DNA probe and a sample was identified by cytogenetics. Both methods were compared. For each individual female, information was recorded about the species identification by DNA probes and cytogenetics, cytotype if *An. gambiae s.s.*, positivity for circumsporozoite protein and human blood index.

The identification using DNA probes showed good agreement with cytogenetics for *An. gambiae s.s.* and *An. arabiensis*. However, some limitations were found. Variability between the batches of the probes was observed and some *An. melas* identified by cytogenetics, were identified as *An. gambiae s.s.* by the DNA probes. This makes essential to have a positive *An. melas* control on each of the filters, which is difficult to provide since currently there are no existing colonies of this species. Further developments in the specificity of the probes would be ideal to avoid relying on a combination of results with two different probes.

The frequency of the species of the *An. gambiae* complex as well as the presence of the two incipient species of *An. gambiae s.s.* agreed with previous reports for the area. Differences in the species composition were found by method of collection. *An. melas* was found more frequently resting outdoors in pit traps and animal baited nets, while *An. gambiae s.s.* was found more frequently resting indoors in bednets, rooms and exit traps. No significant differences were observed in the HBI, although the sample size was too small to be conclusive.

The three inversions in chromosome 2, (arms R and L) of *An. gambiae s.s.* were found: 2Rb, 2Rd and 2La. The frequency of the 'Savanna' type (carriers of 2Rb-2La inverted form in high frequency) was higher than the frequency of the 'Bissau' type (carriers of 2Rd

inverted form in high frequency). Differences in resting behaviour, infectivity or sporozoite rates could not be seen but their absence was not conclusive because of the small sample size and the low frequency of the 'Bissau' cytotype.

CHAPTER 7

SOME ENTOMOLOGICAL FACTORS THAT COULD HAVE INFLUENCED THE LACK OF IMPACT OF THE GAMBIAN NATIONAL IMPREGNATED BEDNET PROGRAMME IN ZONE 5

7.1. INTRODUCTION

In The Gambia, sleeping under permethrin impregnated bednets has been shown to reduce malaria morbidity and mortality in children. In a trial involving 73 villages after the impregnation of bednets with permethrin, cases of fever associated with malaria parasitaemia were reduced by 45% and spleen, parasite and high density parasite rates at the end of the transmission season were reduced by 35%, 39% and 48% respectively in children 1 to 4 years old (Alonso *et al.*, 1993). Mortality was reduced by about 60% (Alonso *et al.*, 1991). Because of these encouraging epidemiological results, a large scale impregnation was initiated as a National Impregnated Bednet Programme (NIBP), operating as part of the Primary Health Care Programme (PHC). The NIBP aimed to cover all the villages which have PHC centres (approximately 400 villages) in a period of 3 years, starting in 1992. Half of the PHC villages received free permethrin to impregnate their own bednets in the first year. In the second year the other half of the villages received free insecticide while the villagers who received it free the previous year had to pay for it. In the third year the insecticide was charged for in all the villages. The impact of the programme on child mortality, morbidity and entomological parameters was evaluated together with its cost-effectiveness.

For the epidemiological, entomological and cost-effectiveness evaluation of the programme in the first year of implementation, five study zones were chosen, representing different geographic areas in the country (see Figure 1.4 in Chapter 1). The study areas involved 104 villages with a population of 115895 of which 35% were children 1 to 9 years old. Villages were paired by size within each zone and the intervention was implemented in one randomly chosen village of each pair.

Results of the epidemiological evaluation of mortality and morbidity in children are given by D'Alessandro *et al.* (1995b). All deaths in children in the study villages were recorded, and the most probable cause of the death was determined by three physicians. The mean packed cell volume (PCV), parasitaemia, high parasitaemia and splenomegaly were also evaluated. The results showed that the mortality in children 1-9 years old was reduced by 25% overall including all zones. However, in zone 5, which is located in the eastern part of the country, mortality in treated villages was actually higher than in the untreated villages, particularly in the 1-2 years old group. Excluding zone 5 from the analysis, the reduction in overall mortality was 38%. Regarding morbidity, the results including zone 5 showed non-significant reduction in the percentage of children with parasitaemia, high density parasitaemia or splenomegaly, but again, when zone 5 was removed from the analysis, there was a significant reduction of about 50% in parasite and high density parasite rates. It was concluded that the impregnation of bednets was successful in reducing malaria mortality and morbidity in zones 1 - 4, but not in zone 5.

The next step was to examine what is known to be different in zone 5 that could have an influence in the ineffectiveness of the NIBP in that area. Entomological and social aspects were considered. Some of the possible differences were:

a) Differences in usage of bednets were found between zones. Zone 3 had the highest (78.6%) and zone 5 the lowest (46.4%) percentage of beds with bednets (D'Alessandro *et al.*, 1995b).

b) Differences in the entomological inoculation rate (EIR) were found between zones in the pre-intervention year (Thomson *et al.*, 1994). Villages in zone 5 had the highest EIR (11.15 and 7.75 in the two villages sampled) followed by zone 3 (5.0 and 4.17 in the two villages). The sporozoite rate was highest in the villages in zone 5, while the highest densities of *An.gambiae s.l.* were found in zone 3.

These two differences (a and b) were given as possible explanations of the failure of the intervention in zone 5 by D'Alessandro *et al.*, 1995b.

c) Evidence for a difference in the exophily of fed females was found between zones

(Thomson *et al.*, 1994). In zones 1, 2 and 3 few blood-fed mosquitoes were collected in exit traps while in Zones 4 and 5 a high proportion were blood-fed. It was proposed by Thomson *et al.* (1994) that this difference in exiting behaviour could have been associated with differences in the distribution of species of the *An. gambiae* complex or in intra-specific forms of *An.gambiae s.s.*

d) There are differences in the ethnic composition of the human population between zones (D'Alessandro *et al.*, 1995b). In zone 5 the majority belong to the Sarahuli tribe (73.2%) while in the other zones Mandinka and Wollof are the main tribes. It was noticed that the Sarahuli houses were bigger and with more square rooms than the houses of other tribes, where traditional rounded huts are predominant. Type of rooms could have an influence in the behaviour of the mosquito vector population e.g. characteristics of the rooms could be related to the exophily of females in zone 5.

e) Because of the difference in exiting behaviour of fed females found in zone 5 (Thomson *et al.*, 1994), the estimates of the EIR might have been biased, leading to a relative underestimation in zone 5. The EIR was calculated using the sporozoite rate and an estimate of the human biting rate. This estimate was calculated from the geometric mean number of human fed *An. gambiae s.l.* collected resting indoors from pyrethrum spray catches and exiting the room and caught in exit traps, and divided by the mean number of occupants of the room (Thomson *et al.*, 1994). This way of estimating the human-biting rate assumes that most of the mosquitoes that fed during the night are accounted for, since they are collected either resting indoors or in their attempt to leave the room.

Estimation of the human-biting rate are generally made using human-landing collections. Despite their limitations, human-landing collections have been recognized as the least biased method for estimating human-vector contact (Service, 1993). Since the mosquitoes are collected in their attempt to feed, differences in post-feeding behaviour such as exophily would not affect the estimate of the biting rates. However, human-landing collections present some difficulties. One of the main problems is the ethical aspect since the risk of contact with infective mosquitoes is increased for collectors who normally sleep

under bednets. Also it is tedious and expensive. Alternative methods need to be evaluated and compared with the standard human-landing collections. The use of light traps in combination with a bednet has been found to be as efficient as human-landing collections to estimate human-biting rates in Tanzania (Lines *et al.*, 1991; Davies *et al.*, 1995; Shiff *et al.*, 1995). Also, similar parous rates and gonotrophic stage to the females collected landing in humans has been found in the LTC (Lines *et al.*, 1991). Sporozoite rates of unfed females showed no significant difference between both methods (Davis *et al.*, 1995). These results encourage the possibility to use LTC for estimating human-vector contact, with the advantage of being more convenient and with the possibility of increasing the sampling replications.

Comparison between methods of collection was necessary to validate the use of fed females collected in spray catches and exit traps and also the use of light traps as alternatives to human-landing collections for the estimates of human-biting rates.

The study presented in this chapter was carried out to investigate whether differences in the vector population could have influenced the effectiveness of the programme in zone 5. Observations were made in square and rounded rooms and with a treated and untreated bednet in one village in zone 5 and compared with another village in zone 3. Zone 3 was selected because reduction in mortality and morbidity for malaria was found after the impregnation of bednets there. Basic entomological questions were addressed, looking for differences between the populations of *An. gambiae s.l.* in the two zones:

a) Species composition.

b) Cytotypes within *An. gambiae s.s.* species.

c) Behaviour (biting cycle, exiting, resting).

d) Responses to the treatment in deterrency, exiting, resting indoors and persistence of the insecticide in the bednets.

e) Relative sampling efficiency of different methods of collection in both zones. Human-landing collections were compared with light traps, pyrethrum spray catches

and exit trap collections.

7.2. MATERIALS AND METHODS

7.2.1. Study area

Two villages, Kulari in zone 5 and Jahally in zone 3, were selected to carry out the observations. Both villages had received free insecticide for impregnation of bednets during the previous year, 1992, and in 1993 they were asked to pay for the insecticide, which not everybody in the villages did. Therefore, both villages had rooms with treated bednets and with untreated ones. This situation allowed us to select both types of rooms in the same village in order to check for room-level effects of the treatment on entomological parameters. Also, in the villages there were two distinctive types of rooms which could have influenced the behaviour of the mosquito population: traditional rounded rooms and square rooms .

Initially, 48 rooms were identified in both villages, but, given the low densities found in Kulari, 12 more rooms were included in this village. The rooms were classified following two criteria: type of room (small traditional rounded and big square) and with or without a treated bednet. Characteristics of the rooms were recorded: material of the walls, roof and floor, presence of ceiling and eaves, number of doors, windows and sleepers in the room. Chi-squared was used to compare the proportions and t-test to compared the mean number of sleepers. Rooms were matched in pairs by type and treatment (large-treated (LT) and large-untreated (LU), small treated (ST) and small untreated (SU)) in the same compound when possible. When a nearby room with a treated bednet was not found, particularly in zone 5, a treated bednet was provided during the collections. Four sets of 4 rooms (LT, LU, ST, SU) were sampled every week, using various method of collection in rotation as described below. Each set of rooms was sampled once with the four methods of collection.

7.2.2. Collection of mosquitoes

Mosquitoes were collected using light traps (LTC), human-landing catches (HLC) indoor and outdoor, exit traps (ETC) and pyrethrum spray catches (PSC) in the same room. The schedule followed during the week was:

	Days						
	S	M	T	W	T	F	S
1st four rooms	LTC	HLC	ETC	PSC			
2nd four rooms		LTC	HLC	ETC	PSC		
3rd four rooms			LTC	HLC	ETC	PSC	
4th four rooms				LTC	HLC	ETC	PSC

Because the use of insecticide for the spray catches leaves traces of insecticide in the room, PSC was always the last method used. It was considered unlikely that any of the other methods would affect subsequent catches in this way. The collections were carried out for 3 weeks in zone 3 and 4 weeks in zone 5 during July-August 1993.

7.2.2.1. Light trap collections

A CDC light trap was set in each of the four rooms in the evening and collected the following morning. They were set between the bed and the door of the room, at the height of the bed and at the end where the head of the sleeper would lie. The sleeper was under a bednet.

7.2.2.2. Human-landing collections

A team of 12 collectors were recruited from each village, each team having two MRC staff supervisors. The collectors worked individually, one collecting indoors in each of the four rooms, and two other collecting outdoors at locations roughly equidistant between pairs

of similarly-sized rooms. When the treated and untreated rooms in a pair were in different compounds, the outdoor collection took place in the compound of the room with the treated bednet. The collectors worked for alternating periods of 3 hours collection and 3 hours rest, from 19:00h to 07:00h, exchanging collection locations every hour. After 3 hours they were relieved by the other 6 collectors who worked in the same way. All mosquitoes landing on them were collected and placed in paper cups with pads of sugar solution on top.

7.2.2.3. Exit trap collections

One exit trap was set in a window of each of the four rooms. When a window was not available, the trap was set in a doorway covered by a large piece of dark cloth material. The traps were emptied and removed the following morning.

7.2.2.4. Pyrethrum spray catches

After removing the exit trap, the room was sprayed with an aerosol containing tetramethrin 0.10% w/w, d-Allethrin 0.10% w/w, Dichlorvos 0.50% w/w and Permethrin 0.02% w/w (BOP^R) and all the resting indoor mosquitoes were collected.

7.2.3. Sporozoite rates

Heads and thoraces of *An. gambiae s.l.* mosquitoes were assayed by ELISA (Wirtz *et al.*, 1987) to determine the presence of *P. falciparum* circumsporozoite protein.

7.2.4. Species determination

Heads of samples of the mosquitoes collected by each method and in each village were preserved in isopropanol for further analysis using DNA probes technique (Hill *et al*, 1992, 1994a). DNA dot blots were prepared in the month following collection and the papers were preserved to continue with the procedure. Species identification using the probes PnAg1 and PnArab for the identification of *An. gambiae s.s.* and *An. arabiensis* respectivelly was

carried out at the London School of Hygiene and Tropical Medicine by W. Macdowall (1994).

Semigravid *An. gambiae s.l.* mosquitoes were collected for cytological species determination using pyrethrum spray catches in the afternoon, about 5 pm, from August to November 1993. The reason for this was that from the other methods of collection the number of semigravid mosquitoes were few and also because the period of time between collection, sorting and preservation in Carnoy's was too long to ensure good quality preparations. Females collected resting indoors in the afternoon were almost all semigravids. They were immersed immediately in Carnoy's solution (3 parts of absolute ethanol + 1 of glacial acetic acid) and transported in a cool box to the laboratory, where they were placed in a freezer until the chromosome preparation. The chromosome slides were prepared following the method described by Hunt (1973). The species were identified by the banding pattern of the X chromosome together with the pattern of inversions in arms 2R and 2L. The different karyotypes were scored according to the nomenclature of Coluzzi *et al.* (1979).

7.2.5. Persistence of the insecticide

In November 1993, 4 months after the impregnation of bednets, bioassays were carried out to test the persistence of the insecticide on the bednets in the two zones. Sixteen villages were sampled in each zone, 8 in which the insecticide was provided free and 8 in which people had to pay. Samples of different materials of bednet were chosen in each village when available. Whether or not the bednet had been marked as impregnated was recorded. Two pieces of 40cm X 40cm were cut from each bednet, wrapped in aluminium foil and transferred to the laboratory. Fed *An. gambiae s.l.* females were collected resting in bednets in the morning in a village near Farafenni (about 200 km from the coast) for the bioassays. Batches of 20 females were exposed for 3 minutes to each piece of bednet using World Health Organization plastic cones and also to a piece of cloth (nylon netting) without insecticide as control (Figure 7.1). Mosquitoes were transferred to paper cups and a piece of cotton with a sugar solution was placed on top. Mortality was recorded after 24 hours.

Abbot's correction was used when the mortality in the controls was between 5% and 20%: Corrected mortality=((mortality in exposed - mortality in controls)/(100 - mortality in controls)) X 100

Collection of the pieces of bednets was done by M. Jawara and bioassays were carried out by T. Kuyateh and N. Toure.

Figure 7.1. Bioassays with the pieces of bednets from the villages of Jahally and Kulari.

7.3. RESULTS

7.3.1. Species determination

7.3.1.1. DNA probes

The proportion of each species by village and method of collection is presented in Table 7.1. In Jahally (zone 3), *An. gambiae s.s* was the predominant species, comprising 81.4% of the total collected by the different methods. In Kulari (zone 5), both species *An. gambiae s.s* and *An. arabiensis* were found in proportions of around 50%.

Considering the proportions by method of collection, in Jahally *An. arabiensis* were collected in a slightly but significantly higher proportion outdoors (in the HLC outdoors and ETC collections) than indoors (LTC, HLC indoors and PSC) (Chi-square testing outdoors vs. indoors collections: χ^2= 12.73, P<0.01). In Kulari both species were found in similar proportion for all methods of collection.

7.3.1.2. Cytogenetics

A sample of 119 and 140 females were determined by cytogenetics from Jahally and Kulari respectively. The proportion of readable slides was 80%. *An.gambiae s.s.* and *An. arabiensis* were present in both villages (Table 7.2). As found with the DNA probes, *An. arabiensis*was more abundant in Kulari (39.2%) than in Jahally (15.3%) (χ^2=12.56, P<0.01).

The most frequent chromosomal inversions found in *An. gambiae s.s.* were 2R*b* and 2L*a* in both villages (Table 7.2). The number of individuals homozygous for both inversions simultaneously were 54.5% and 47.2% for Jahally and Kulari respectively; no significant difference was found between the villages in the degree of association of both inversions (χ^2=0.41, P=0.52) (Table 7.3). Other inversions found were *j*, *c*, *d* and *u* in the 2R arm. The frequency of these inversions was less than 0.2 in both villages (Table 7.2). Significantly higher frequency of the inversions 2R*j* and 2R*d* in *An. gambiae s.s.* was found in Kulari (Fisher exact test P<0.001) than in Jahally. The cytotypes found in both villages correspond to the 'Savanna' form described by Coluzzi *et al.* (1985), which is typical of savanna areas.

Table 7.1. Species determination by DNA probes of the *Anopheles gambiae* species complex by method of collection: light traps (LTC), human landing indoors (ILC) and outdoors (OLC), exit traps (ETC) and pyrethrum spray catches indoors (PSC) from Jahally in Zone 3 and Kulari in Zone 5.

Village	Method	An.gambiae s.s. %	An.arabiensis %	Total identified	Undetermined (n)
Jahally	LTC	84.7	15.3	85	24
	ILC	84.6	15.4	156	1
	OLC	73.3	26.7	161	14
	ETC	76.3	23.7	101	6
	PSC	86.8	13.2	190	5
Overall		**81.4**	**18.6**	**693**	**50**
Kulari	LTC	41.3	58.7	63	22
	ILC	55.0	44.9	129	0
	OLC	42.7	57.3	89	7
	ETC	59.5	40.5	74	11
	PSC	50.0	50.0	84	0
Overall		**50.3**	**49.7**	**439**	**40**

Table 7.2. Species identification by cytogenetics of members of the *Anopheles gambiae* species complex and polymorphisms of *Anopheles gambiae s.s.* and *An. arabiensis* from Jahally in Zone 3 and Kulari in Zone 5. Samples were from PSC in the afternoon. "+": standard form; "-": inverted form.

	Jahally % (n)				**Kulari % (n)**			
Species:								
An. gambiae s.s.	84.7 (85)				60.8 (120)			
An. arabiensis	15.3 (85)				39.2 (120)			
Polymorphism in	No. individuals			Inversion	No.individuals			Inversion
An. gambiae s.s:	+/+	+/-	-/-	frequency	+/+	+/-	-/-	frequency
Inversions in 2R								
j	56	0	0	0	58	10	4	0.13
b	3	19	34	0.78	3	25	44	0.78
c	54	2	0	0.02	70	2	0	0.01
d	51	5	0	0.04	51	14	7	0.19
u	56	0	0	0	68	5	0	0.03
Inversion in 2L								
a	1	9	47	0.90	1	15	56	0.88
An.arabiesnsis:								
Inversions in 2R								
j	5	3	0	0.19	16	21	4	0.35
b	0	1	7	0.94	0	8	33	0.90
c	0	4	0	0.50	30	10	1	0.15
d	0	1	0	0.50	32	1	0	0.02
e	8	0	0	0	41	0	0	0
f	7	0	0	0	39	0	0	0
Inversion in 2L								
a	5	2	0	0.14	18	16	5	0.33
Inversion in 3R								
a	1	0	0	0	3	0	0	0

Table 7.3. Numbers of observed and associations between the inversions 2R*b* and 2L*a* in *An. gambiae s.s.* from Jahally-Zone 3 and Kulari-Zone 5. Shown in small print are numbers expected assuming random distribution.

Jahally	**2Rb**		**+/+ or +/-**	**-/-**	**Total**	χ^2	**P**
2La	+/+ or +/-	o	6	4	10		
		e	3.8	6.2			
						1.46	0.16
	-/-	o	15	30	45		
		e	17.2	27.8			
Total			21	34	55		

Kulari	2Rb		+/+ or +/-	-/-	Total	χ^2	P
2La	+/+ or +/-	o	6	10	16		
		e	6.2	9.8			
						0.03	0.87
	-/-	o	22	34	56		
		e	21.8	34.2			
Total			27	44	72		

For *An. arabiensis* inversions *a*, *b*, *c*, *d*, *e* and *f* in the 2R chromosome, inversion *a* in the 2L and inversion *a* in the 3R were scored. Although no statistical analysis was made for this species because of the small numbers, no obvious differences were observed between the two villages in the frequency of inversions present (Table 7.2).

7.3.2. Biting behaviour

The mean biting time was calculated for the two villages, indoors and outdoors and by room type, and they are shown in Table 7.4. In both villages the mean biting time was around 2 am and taking into account the standard errors, no significant difference was found between types of room or indoor or outdoor collections.

Table 7.4. Mean biting time and standard error of *An. gambiae s.l.* collected by human-landing from 19:00 to 07:00 hours, indoors and outdoors by type of room (LT: large-treated, LU: large-untreated, ST: small-treated, SU: small-untreated) in Jahally-Zone 3 and Kulari-Zone 5.

	Jahally	Kulari
Indoors		
LT	2:17' ± 14'	1:54' ± 18'
LU	1:51' ± 14'	1:55' ± 19'
ST	2:22' ± 14'	2:07' ± 20'
SU	2:22' ± 14'	2:12' ± 17'
Total	2:13' ± 07'	2:02' ± 09'
Outdoors		
L	2:32' ± 15'	2:40' ± 17'
S	2:05' ± 15'	2:10' ± 19'
Total	2:18' ± 10'	2:58' ± 12'

The indoor:outdoor ratios are presented in Table 7.5. Although the overall ratio was higher in Jahally than in Kulari, the difference is not significant. Neither did the ratio differ significantly between types of room, for all cases the 95% C.I. overlap.

Table 7.5. Ratio of numbers Indoor:Outdoor of *An. gambiae s.l.* collected by human-landing from 18:00 to 07:00 hours, by type of room (LT: large-treated, LU: large-untreated, ST: small-treated, SU: small-untreated) in Jahally-Zone 3 and Kulari-Zone 5.

	Jahally	**Kulari**
	Ratio (95% C.I)	**Ratio (95% C.I.)**
LT	1.72 (1.14-2.30)	0.86 (0.49-1.23)
LU	1.46 (0.88-2.05)	0.77 (0.48-1.08)
ST	1.20 (0.78-1.64)	0.89 (0.48-1.31)
SU	1.08 (0.72-1.45)	1.27 (0.85-1.70)
Overall	**1.36 (1.11-1.63)**	**0.94 (0.75-1.14)**

7.3.3. Density

The geometric means and 95% confidence limits of the number of *An. gambiae s.l.* females collected by each method are shown in Table 7.6. Fed females collected in ETC and PSC (FEP) are also shown. For all the methods of collection the number of females was higher in Jahally than in Kulari (Figure 7.2).

Table 7.6. Geometric mean number and 95% confidence limits of *Anopheles gambiae s.l.* female mosquitoes collected by night in each method of collection: light traps (LTC), indoor and outdoor human-landing collections (ILC and OLC), exit traps (ETC), pyrethrum spray collections (PSC) and fed females in ETC and PSC (FEP) by type of room (LT: large-treated, LU: large-untreated, ST: small-treated, SU: small-untreated) in Jahally-Zone 3 and Kulari-Zone 5.

	LTC	ILC	OLC	ETC	PSC	FEP
Jahally						
LT	11.06 (6.1-19.6)	40.73 (33.0-50.2)	29.43 (19.9-43.1)	5.78 (2.7-11.3)	9.19 (3.4-22.6)	5.83 (2.7-11.5)
LU	21.41 (7.2-60.4)	33.94 (21.7-52.7)		20.22 (10.8-37.1)	16.15 (6.3-39.3)	8.57 (3.2-21.0)
ST	16.10 (5.7-42.8)	36.18 (24.9-52.2)	36.23 (29.4-44.6)	6.07 (3.1-11.3)	11.22 (4.9-24.0)	7.05 (3.9-12.1)
SU	9.65 (3.5-24.4)	30.71 (21.0-44.7)		6.88 (2.7-15.8)	46.14 (23.3-90.6)	10.02 (5.2-18.7)
TOTAL	13.89 (8.6-22.0)	35.21 (29.3-42.3)	32.66 (26.2-40.9)	8.46 (5.7-12.4)	16.81 (10.7-26.2)	7.73 (5.3-11.1)
Kulari						
LT	1.92 (0.9-3.4)	11.93 (7.1-19.8)	21.79 (14.7-32.1)	4.80 (2.8-7.8)	2.13 (1.1-3.8)	2.26 (1.3-3.7)
LU	4.34 (1.9-8.6)	11.22 (6.1-19.9)		3.64 (1.9-6.4)	2.78 (1.3-5.1)	2.23 (1.1-3.9)
ST	5.52 (2.7-10.5)	10.27 (5.9-17.4)	14.55 (8.8-23.5)	0.88 (0.3-1.6)	4.37 (2.1-8.2)	1.49 (0.5-3.1)
SU	4.71 (2.8-7.6)	15.46 (9.4-24.9)		1.22 (0.5-2.2)	11.23 (7.3-17.1)	4.96 (3.2-7.4)
TOTAL	3.91 (2.7-5.4)	12.09 (9.2-15.7)	17.83 (13.0-24.5)	2.25 (1.6-3.1)	4.28 (3.0-5.8)	2.53 (1.8-3.4)

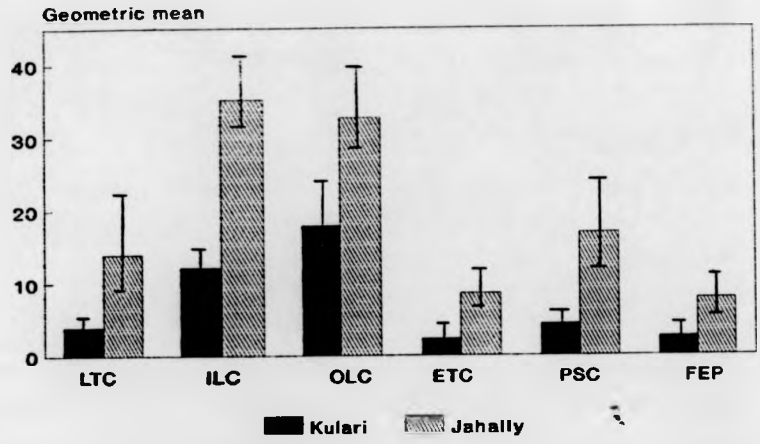

Figure 7.2. Geometric mean numbers of *An. gambiae s.l.* collected by light traps (LTC), human-landing indoors (ILC) and outdoors (OLC), exit traps (ETC), pyrethrum spray catches (PSC) and fed females collected in ETC and PSC in Jahally (zone 3 and Kulari (zone 5).

7.3.4. Characteristics of the rooms

The characteristics of the rooms are shown in Table 7.7. The large-square rooms showed significant differences between the two villages. In Kulari a higher proportion of these rooms were found with ceilings and only one door, and a lower proportion with eaves, compared with the large-square rooms in Jahally. Between the traditional round rooms in both villages significant differences were found in the materials of walls, roof and floor between villages. In Kulari almost all of the round rooms had mud walls, thatched roofs and earth floors. In Jahally, although these materials were predominant for this type of room , other materials were also quite common. No significant differences were found between villages in the number of windows in each type of room or in the number of sleepers per room.

Table 7.7. Characteristics of the study rooms.

	Large-square rooms		Traditional round rooms	
	Jahally (n=24)	Kulari (n=30)	Jahally (n=24)	Kulari (n=30)
Material of walls				
Cement	21 (87.5%)	30 (100%)	7 (29.17%)	0 (0%) **
Mud	3 (12.5%)	0 (0%)	17 (70.8%)	30 (100%)
Material of roof				
Thatched	0 (0%)	0 (0%)	16 (66.7%)	30 (100%) **
Corrugated iron	24 (100%)	30 (100%)	8 (33.3%)	0 (0%)
Material floor				
Cement	22 (91.7%)	30 (100%)	8 (33.3%)	1 (3.3%) **
Earth	2 (8.3%)	0 (0%)	16 (66.6%)	29 (96.7%)
With ceiling	10 (41.%)	26 (86.7%) **	2 (8.3%)	0 (0%)
With eaves	15 (62.5%)	3 (10.0%) **	19 (79.2%)	28 (93.3%)
Number of doors				
1	0 (0%)	26 (86.7%) **	2 (8.3%)	10 (33.3%)
2	24 (100%)	4 (13.3%)	22 (91.7%)	20 (66.7%)
Number of windows				
0	9 (37.5%)	6 (20%)	21 (87.5%)	30 (100%)
1	13 (54.2%)	24 (80%)	3 (12.5%)	0 (0%)
2	2 (8.3%)	0 (0%)	0 (0%)	0 (0%)
Sleepers (mean)	3.0	2.73	2.45	2.43
(95% C.I.)	(2.3-3.7)	(2.1-3.4)	(1.7-3.2)	(1.8-3.0)

** Significant difference between villages.

7.3.5. Size of rooms and treatment effects on the number of females collected

After log-transformation of the total number of *An. gambiae s.l.* collected each night, analysis of variance was carried out separately for each method of collection in each village. The factors analysed were: set of room, size and treatment. In Tables 7.8 and 7.9 the results of the analysis are shown for zone 3 and 5 respectively.

7.3.5.1. Treatment effect

In Jahally a significant difference was found between rooms with a treated bednet and rooms with an untreated bednet in the PSC -fewer indoor resting females being found in treated rooms. Also, significantly greater numbers of mosquitoes were found exiting untreated rooms. This difference was entirely due to an excess in large-untreated rooms, as can be seen from the geometric means in Table 7.6.

In Kulari the effect of the treatment was significant in LTC only. Lower mean number of mosquitoes were seen in treated rooms.

7.3.5.2. Size effect

No difference was found in Jahally for any of the methods of collection related to size of the rooms.

In Kulari, a significant difference was found in ETC and PSC related to size of the room. In ETC, densities were higher in large-square rooms than in small-round ones and the opposite was found in PSC. No significant difference was found in LTC and indoor HLC related to room size in either zone.

A combined analysis was carried out including village (Jahally vs. Kulari) as another factor. For all the methods of collection a significant difference was found between the villages. The number of females collected was higher in Jahally than in Kulari. Other significant differences were found related to size and treatment, but they were influenced by differences within each village, detailed above (data not shown).

Table 7.8. Analysis of variance with the number of mosquitoes collected (transformed to log(n+1)) by each method of collection (LTC:light trap, ILC:indoor landing, OLC:outdoor landing, ETC:exit traps, PSC:pyrethrum spray, FEP: fed females collected in ETC and PSC) in relation to the variables set of rooms (the four rooms: large-treated, large-untreated, small-treated and small-untreated, sampled the same night), size (large and small) and treatment (yes or not) in Jahally-Zone 3.

Dependent Variable: LOG-LTC

Source	DF	Sum of Squares	Mean Square	F	P
Model	14	60.7898080	4.34212914	2.50	0.0152
Error	33	57.3319237	1.73733102		
Corrected Total	47	118.1217317			

Source	DF	Sum of Squares	Mean Square	F	P
SET	11	55.83402495	5.07582045	2.92	0.0084
SIZE	1	0.20273571	0.20273571	0.12	0.7348
TREAT	1	0.23630404	0.23630404	0.14	0.7146
SIZE*TREAT	1	4.51674331	4.51674331	2.60	0.1164

Dependent Variable: LOG-IBC

Source	DF	Sum of Squares	Mean Square	F	P
Model	14	8.23384907	0.58813208	1.59	0.1347
Error	33	12.21428709	0.37012991		
Corrected Total	47	20.44813617			

Source	DF	Sum of Squares	Mean Square	F	P
SET	11	7.38751192	0.67159199	1.81	0.0915
SIZE	1	0.23865452	0.23865452	0.64	0.4277
TREAT	1	0.49470990	0.49470990	1.34	0.2559
SIZE*TREAT	1	0.11297272	0.11297272	0.31	0.5843

Dependent Variable: LOG-OBC

Source	DF	Sum of Squares	Mean Square	F	P
Model	12	4.66950633	0.38912553	1.73	0.1871
Error	11	2.47799896	0.22527263		
Corrected Total	23	7.14750528			

Source	DF	Sum of Squares	Mean Square	F	P
SET	11	4.38069944	0.39824540	1.77	0.1794
SIZE	1	0.28880688	0.28880688	1.28	0.2816

Table 7.8. Continued

Dependent Variable: LOGETC

Source	DF	Sum of Squares	Mean Square	F	P
Model	14	33.22010544	2.37286467	2.40	0.0192
Error	33	32.59765708	0.98780779		
Corrected Total	47	65.81776252			

Source	DF	Sum of Squares	Mean Square	F	P
SET	11	23.61627915	2.14693447	2.17	0.0420
SIZE	1	1.77306234	1.77306234	1.79	0.1895
TREAT	1	4.21180922	4.21180922	4.26	0.0469
SIZE*TREAT	1	3.61895473	3.61895473	3.66	0.0643

Dependent Variable: LOGPSC

Source	DF	Sum of Squares	Mean Square	F	P
Model	14	41.97107844	2.99793417	1.71	0.1024
Error	33	58.01445772	1.75801387		
Corrected Total	47	99.98553616			

Source	DF	Sum of Squares	Mean Square	F	P
SET	11	28.02875461	2.54806860	1.45	0.1980
SIZE	1	3.44457209	3.44457209	1.96	0.1709
TREAT	1	9.17691950	9.17691950	5.22	0.0289
SIZE*TREAT	1	1.32083225	1.32083225	0.75	0.3923

Dependent Variable: LOGFEP

Source	DF	Sum of Squares	Mean Square	F	P
Model	14	19.14026503	1.36716179	1.03	0.4499
Error	33	43.80711977	1.32748848		
Corrected Total	47	62.94738480			

Source	DF	Sum of Squares	Mean Square	F	P
SET	11	17.58113244	1.59828477	1.20	0.3226
SIZE	1	0.27984976	0.27984976	0.21	0.6491
TREAT	1	1.27771438	1.27771438	0.96	0.3337
SIZE*TREAT	1	0.00156845	0.00156845	0.00	0.9728

Table 7.9. Analysis of variance with the number of mosquitoes collected (transformed to log(n+1)) by each method of collection (LTC:light trap, ILC:indoor landing, OLC:outdoor landing, ETC:exit traps, PSC:pyrethrum spray, FEP: fed females collected in ETC and PSC) in relation to the variables set of rooms (the four rooms: large-treated, large-untreated, small-treated and small-untreated, sampled the same night), size (big and small) and treatment (yes or not) in Kulari-Zone 5.

Dependent Variable: LOG-LTC

Source	DF	Sum of Squares	Mean Square	F	P
Model	17	36.54927016	2.14995707	3.23	0.0010
Error	42	27.94107611	0.66526372		
Corrected Total	59	64.49034626			

Source	DF	Sum of Squares	Mean Square	F	P
SET	14	29.81903354	2.12993097	3.20	0.0018
SIZE	1	1.65829663	1.65829663	2.49	0.1219
TREAT	1	2.98436162	2.98436162	4.49	0.0401
SIZE*TREAT	1	2.08757838	2.08757838	3.14	0.0837

Dependent Variable: LOG-IBC

Source	DF	Sum of Squares	Mean Square	F	P
Model	17	35.49682966	2.08804880	3.82	0.0002
Error	42	22.93079274	0.54597126		
Corrected Total	59	58.42762240			

Source	DF	Sum of Squares	Mean Square	F	P
SET	14	34.29418133	2.44958438	4.49	0.0001
SIZE	1	0.13132110	0.13132110	0.24	0.6264
TREAT	1	0.36253440	0.36253440	0.66	0.4197
SIZE*TREAT	1	0.70879282	0.70879282	1.30	0.2610

Dependent Variable: LOG-OBC

Source	DF	Sum of Squares	Mean Square	F	P
Model	15	18.28210039	1.21880669	5.69	0.0012
Error	14	2.99882290	0.21420164		
Corrected Total	29	21.28092329			

Source	DF	Sum of Squares	Mean Square	F	P
SET	14	17.18834579	1.22773899	5.73	0.0012
SIZE	1	1.09375460	1.09375460	5.11	0.0403

Table 7.9. Continued

Dependent Variable: LOG-ETC

Source	DF	Sum of Squares	Mean Square	F	P
Model	17	25.01190907	1.47128877	2.37	0.0119
Error	42	26.12466957	0.62201594		
Corrected Total	59	51.13657864			

Source	DF	Sum of Squares	Mean Square	F	P
SET	14	11.80017288	0.84286949	1.36	0.2180
SIZE	1	12.69261632	12.69261632	20.41	0.0001
TREAT	1	0.02554730	0.02554730	0.04	0.8404
SIZE*TREAT	1	0.49357257	0.49357257	0.79	0.3781

Dependent Variable: LOG-PSC

Source	DF	Sum of Squares	Mean Square	F	P
Model	17	22.14583118	1.30269595	1.27	0.2566
Error	42	43.00886704	1.02402064		
Corrected Total	59	65.15469822			

Source	DF	Sum of Squares	Mean Square	F	P
SET	14	6.23612051	0.44543718	0.43	0.9533
SIZE	1	10.76744144	10.76744144	10.51	0.0023
TREAT	1	3.70581218	3.70581218	3.62	0.0640
SIZE*TREAT	1	1.43645705	1.43645705	1.40	0.2429

Dependent Variable: LOG-FEP

Source	DF	Sum of Squares	Mean Square	F	P
Model	17	13.59833099	0.79990182	1.07	0.4121
Error	42	31.41285608	0.74792514		
Corrected Total	59	45.01118707			

Source	DF	Sum of Squares	Mean Square	F	P
SET	14	7.31404611	0.52243187	0.70	0.7629
SIZE	1	0.40052340	0.40052340	0.54	0.4684
TREAT	1	2.83505203	2.83505203	3.79	0.0583
SIZE*TREAT	1	3.04870945	3.04870945	4.08	0.0499

7.3.6. Exiting behaviour

Differences between the two villages were found in exiting behaviour. Related to the type of rooms, in large-square rooms in Kulari females showed a tendency to exit more and rest less indoors than in the traditional small round rooms. This tendency was not observed in Jahally (geometric means in Table 7.6 and results of the analysis of variance in Tables 7.8 and 7.9).

Comparing the number of unfed, fed and gravid *An. gambiae s.l.* as a proportion of the total collected in ETC, in Jahally the majority of the exiting females were unfed (73.39%) and only 4.6% were fed. In Kulari unfed, fed and gravid females were collected in approximately equal numbers (Table 7.10).

The proportion of females exiting rooms was calculated as the number collected in ETC as a proportion of the total collected in ETC plus PSC, as both collections were carried out the same morning. The majority of exiting mosquitoes were unfed in both villages and in both treated and untreated rooms (Table 7.11). A significantly higher proportion of fed females were collected in the ETC in Kulari than in Jahally in both treated and untreated rooms.

Table 7.10. Proportion of unfed (UF), fed (FF), semi-gravid (SG) and gravid (GR) females collected in exit traps in rooms with (T) or without (U) permethrin-treated bednets Jahally-Zone 3 and Kulari-Zone 5.

	JAHALLY			KULARI		
	T	U	Overall	T	U	Overall
UF	71.7%	74.1%	73.4%	57.1%	16.9%	38.8%
FF	4.4%	4.6%	4.6%	19.6%	38.4%	28.2%
SG	19.9%	8.8%	12.1%	8.3%	0.9%	4.9%
GR	4.0%	12.5%	9.9%	15.0%	43.8%	28.2%
No. collected	251	602	853	133	112	245

Table 7.11. Number of unfed (UF), fed (FF), semigravids (SG) and gravid (GR) *Anopheles gambiae s.l.* as a proportion of the total females exiting or resting indoors (collected in exit traps and in spray catches) in both villages.

	Rooms with a treated bednet		Rooms with an untreated bednet	
	Jahally	**Kulari**	**Jahally**	**Kulari**
UF	97.3 % (185)	98.7 % (77)	99.5 % (448)	86.4 % (22)
FF	4.6 % (239)	26.6 % (99)	6.0 % (464)	29.3 % (147)
SG	23.9 % (209)	42.3 % (26)	12.6 % (419)	7.69 % (13)
GR	7.8 % (128)	19.1 % (105)	18.4 % (407)	22.5 % (218)
Total	32.9 % (761)	43.3 % (307)	34.6 % (1738)	28.0 % (400)

7.3.7. Sporozoite rates

The sporozoite rates by method of collection and the overall means in the two villages are shown in Table 7.12. Differences were found in the rates by method of collection within villages. In Jahally, mosquitoes from ETC showed a significantly higher sporozoite rate than mosquitoes collected by the other methods (χ^2=12.09, P=0.0015). In Kulari, higher sporozoite rates were found in mosquitoes from PSC and LTC than from HLC or ETC (χ^2=23.98, P<0.001 and χ^2=22.13, P<0.001 for PSC and LTC respectively compared with HLT plus ETC rates). Overall, sporozoite rates in Kulari were higher than in Jahally (M-H χ^2=118.27, P<0.001).

Table 7.12. Sporozoite rates in Jahally-Zone 3 and Kulari-Zone 5 by method of collection: human-landing (HLC), pyrethrum spray catches (PSC), light traps (LTC), exit traps (ETC). Both villages contained treated and untreated bednets.

Method	Jahally % (n)		Kulari % (n)	
IHLC	*		1.38	(1232)
OHLC			1.52	(527)
I+OHLC	0.26	(3032)	1.42	(1759)
PSC	0.32	(2216)	5.09	(452)
LTC	0.33	(1803)	4.73	(528)
ETC	1.19	(752)	0.83	(242)
Overall	0.38	(7803)	2.52	(2981)

(*): Separated data for indoor and outdoor landing collections are not available.

7.3.8. Infective biting rates (IBR)

Estimates of the IBR for both villages are presented in Table 7.13. Geometric mean number of *An. gambiae s.l.* collected in LTC, indoor HLC and fed females collected in ETC and PSC (FEP) were used to compare the estimates of IBR. Separate estimates of IBR are given according to treatment of the room, as collections were carried out in the presence of treated bednets and deterrence or excito-repellency are expected to influence the numbers collected. There were differences in estimated IBR according to the sampling method used. For example, the IBR was higher when estimated using indoor HLC than when using FEP, mainly because of the differences in the mean number of females per night collected by both methods. Differences in sporozoite rates between methods of collection also influenced the estimated IBR, e.g. females from ETC and PSC were found with significantly higher sporozoite rates in Jahally and Kulari respectively than females from HLC.

Table 7.13. Infective biting rates (IBR) estimated from collections in light traps (LTC), indoor human-landing (IHLC) and from collection of fed females in exit traps and pyrethrum-spray catches (FEP) in Jahally-zone 3 and Kulari-zone 5

Method of collection	Treatment of bednet	Village	Geom. mean	Sporozoite rate (%)[1]	IBR
LTC	Treated	Jahally	13.36	0.33	4.41
		Kulari	3.36	4.73	15.89
	Untreated	Jahally	14.45	0.33	4.77
		Kulari	4.52	4.73	21.38
	Overall	Jahally	13.89	0.33	4.58
		Kulari	3.91	4.73	18.49
IHLC	Treated	Jahally	38.4	0.26[2]	10.13
		Kulari	11.1	1.38	15.32
	Untreated	Jahally	32.3	0.26	8.52
		Kulari	13.2	1.38	18.21
	Overall	Jahally	35.2	0.26	9.15
		Kulari	12.1	1.38	16.68
FEP	Treated	Jahally	6.4	0.54	3.45
		Kulari	1.8	3.60	6.63
	Untreated	Jahally	9.3	0.54	5.01
		Kulari	3.4	3.60	12.25
	Overall	Jahally	7.7	0.54	4.17
		Kulari	2.5	3.60	9.11

(1) No. positive mosquitoes by method of collection and village pooling data for treated or untreated rooms.
(2) Sporozoite rate from Indoor+Outdoor landing collections in Jahally.

Despite the differences in magnitude of the estimated IBR between method of collection, the estimated IBR for Kulari was higher by each method than for the corresponding method in Jahally; the difference was around 2-fold when using IHLC and FEP and around 4-fold when using LTC.

7.3.9. Relative sampling efficiency between methods of collection

The numbers of *An. gambiae s.l.* caught by each method in each room are shown in Table 7.14. The method of analysis followed that used by Lines *et al.* (1991); after log-transforming the counts by room to stabilize the variance, indoor human landing catches were plotted against the other methods of collection. Correlation coefficients were calculated and analysis of the difference between methods related to the average of the two methods was carried out where there weresignificant correlations.

The plots of indoor HLC and the other methods are shown in Figures 7.2 and 7.3. Pearson correlation coefficients between all methods are shown in Table 7.15. A significant correlation was obtained between LTC and indoor HLC in zone 5 only, and between indoor and outdoor HLC in the same zone. The total of blood-fed females collected in the ETC and PSC (FEP) were also included in the correlations in order to compare them with the number of *An. gambiae s.l.* collected in human-landing collections. No correlation was found between them in either of the zones.

Although the same rooms were used for each method of collection, the variability between days could not be taken into account in the correlations, since a different trap method was used every day in each set of rooms. An attempt was made to account, at least partially, for the day effect by doing an analysis of variance including the variables set of rooms, trap, and the interactions week*day and trap*week*day, and then using the residuals for the correlations. By carrying out the analysis in this way, the day effect was included in the model and removed from the residuals. Therefore, a clearer picture of the degree of association between traps might have been expected. However, the degree of association

between trapping methods did not improve (Table 7.16). Significant correlations were obtained only in zone 5 between spray catches and indoor-landing collections, between indoor and outdoor landing catches and between outdoor and light traps. None of the correlations were significant in zone 3.

The subsequent analysis plotting the differences between mosquito numbers collected by the different methods against the average between the methods was not applicable since no significant correlation was seen between the methods, particularly between LTC and FEP with indoor HLC.

7.3.10. Persistence of the insecticide

A summary of the results of the bioassays in each village is shown in Table 7.17. A complete list of the percent of mortality by bednet, showing the material of each bednet and whether or not they were marked is presented in Appendix 3. Table 7.18. shows the results of the analysis of variance and the mean percent of mortality by zone, payment, material of the bednet and whether or not they were found marked. The results showed no significant difference in the bioassay results between the two zones and also between the variables analysed (Paid versus free impregnation, material of the bednet and marked or unmarked bednet). The average percent mortalities were 49.2% and 44.7% for zones 3 and 5 respectively. The interaction zone*payment was significant, because, as it can be seen in the means, the percent mortality in bioassays from zone 3 with free treatment were higher than with payment and the contrary was found in zone 5.

Table 7.14. Number of females *An. gambiae s.l.* caught by light traps (LTC), indoor and outdoor landing catches (ILC and OLC), exit traps (ETC) and pyrethrum spray catches (PSC) in large (L) and small (S) rooms with (T) and without (U) treated bednets in Jahally-Zone 3 and in Kulari-Zone 5.

Village	Size-Treatment	Group	LTC	ILC	OLC	ETC	PSC	No.FEDS IN ETC	No.FEDS IN PSC
Jahally:									
	LT	1	16	43	11	0	48	0	27
	LU	1	408	40		7	65	0	25
	ST	1	68	60	24	4	105	1	31
	SU	1	71	36		1	53	1	10
	LT	2	5	30	31	2	31	0	12
	LU	2	200	104		6	0	0	0
	ST	2	38	45	75	12	30	0	11
	SU	2	96	47		0	63	0	35
	LT	3	8	49	69	7	6	0	3
	LU	3	27	61		9	2	1	0
	ST	3	0	60	21	34	1	3	0
	SU	3	5	41		14	48	2	1
	LT	4	1	43	20	19	0	3	0
	LU	4	3	43		17	47	0	12
	ST	4	1	45	36	1	13	0	11
	SU	4	2	18		0	32	0	5
	LT	5	1	102	93	8	9	0	5
	LU	5	0	75		9	6	0	3
	ST	5	8	70	64	11	26	0	11
	SU	5	19	49		25	12	0	7
	LT	6	2	23	62	10	9	0	6
	LU	6	3	20		37	17	0	9
	ST	6	3	43	35	2	9	0	5
	SU	6	0	68		16	72	1	47
	LT	7	20	80	30	6	32	0	13
	LU	7	5	30		4	8	0	1
	ST	7	253	48	33	3	9	2	4
	SU	7	3	51		7	109	0	11
	LT	8	29	33	53	0	23	0	8
	LU	8	31	94		16	123	0	50
	ST	8	115	85	56	24	42	0	14
	SU	8	4	9		6	57	0	9
	LT	9	7	28	11	41	64	2	34
	LU	9	285	11		49	48	1	48
	ST	9	16	16	39	5	0	0	0
	SU	9	0	78		7	44	0	14

Table 7.14. Continued

Village	Size-Treatment	Group	LTC	ILC	OLC	ETC	PSC	No.FEDS IN ETC	PSC
Jahally:									
	LT	10	49	56	23	13	0	0	0
	LU	10	23	34		150	121	17	84
	ST	10	91	17	41	22	3	0	3
	SU	10	32	58		51	1	0	0
	LT	11	16	57	22	5	16	0	8
	LU	11	9	12		39	26	5	15
	ST	11	13	19	23	1	25	0	15
	SU	11	55	14		40	125	0	39
	LT	12	39	61	16	7	0	0	0
	LU	12	32	10		84	2	0	1
	ST	12	4	10	30	14	9	0	7
	SU	12	22	10		8	55	0	10
Kulari:									
	LT	1	3	11	15	12	4	4	1
	LU	1	6	12		6	10	4	0
	ST	1	3	3	11	0	0	0	0
	SU	1	7	12		5	12	4	5
	LT	2	0	7	8	0	2	0	2
	LU	2	4	4		1	0	0	0
	ST	2	6	0	0	1	5	0	5
	SU	2	4	0		0	22	0	19
	LT	3	0	12	29	4	1	0	0
	LU	3	8	19		8	4	6	0
	ST	3	5	24	17	0	3	0	0
	SU	3	20	34		2	6	2	3
	LT	4	7	68	42	8	2	0	2
	LU	4	120	49		1	2	1	2
	ST	4	10	12	23	1	1	0	0
	SU	4	9	16		7	44	1	17
	LT	5	0	2	34	2	5	0	3
	LU	5	0	0		17	5	0	1
	ST	5	0	4	20	2	1	0	0
	SU	5	4	15		1	37	0	6
	LT	6	0	2	36	5	2	0	2
	LU	6	3	35		9	16	4	3
	ST	6	7	23	32	0	1	0	0
	SU	6	6	15		0	17	0	1

Table 7.14. Continued

Village	Size-Treatment	Group	LTC	ILC	OLC	ETC	PSC	No.FEDS IN ETC	PSC
Kulari:									
	LT	7	2	3	35	3	0	2	0
	LU	7	2	3		6	4	0	2
	ST	7	2	28	39	1	23	0	5
	SU	7	6	21		0	9	0	3
	LT	8	0	7	22	7	0	0	0
	LU	8	2	6		9	0	7	0
	ST	8	1	16	19	1	27	0	16
	SU	8	0	24		0	7	0	3
	LT	9	8	47	44	38	4	0	1
	LU	9	10	31		0	4	0	1
	ST	9	12	45	17	5	6	2	2
	SU	9	1	39		2	1	2	1
	LT	10	4	36	54	11	1	2	1
	LU	10	4	60		10	6	4	3
	ST	10	25	14	22	10	12	1	7
	SU	10	1	48		4	19	3	7
	LT	11	0	10	8	1	0	1	0
	LU	11	7	17		3	12	2	6
	ST	11	2	2	5	0	0	0	0
	SU	11	9	5		2	5	2	3
	LT	12	2	11	10	4	6	2	3
	LU	12	2	4		1	1	0	0
	ST	12	0	6	16	2	16	0	2
	SU	12	2	13		2	9	0	7
	LT	13	6	9	3	3	2	0	2
	LU	13	1	6		0	0	0	0
	ST	13	0	17	6	1	10	0	0
	SU	13	4	20		0	12	0	2
	LT	14	2	33	32	4	2	3	0
	LU	14	5	17		2	0	0	0
	ST	14	21	24	23	0	3	0	1
	SU	14	13	21		3	13	0	5
	LT	15	6	25	35	8	26	6	15
	LU	15	18	13		11	5	1	3
	ST	15	37	15	36	0	9	0	3
	SU	15	12	25		0	6	0	1

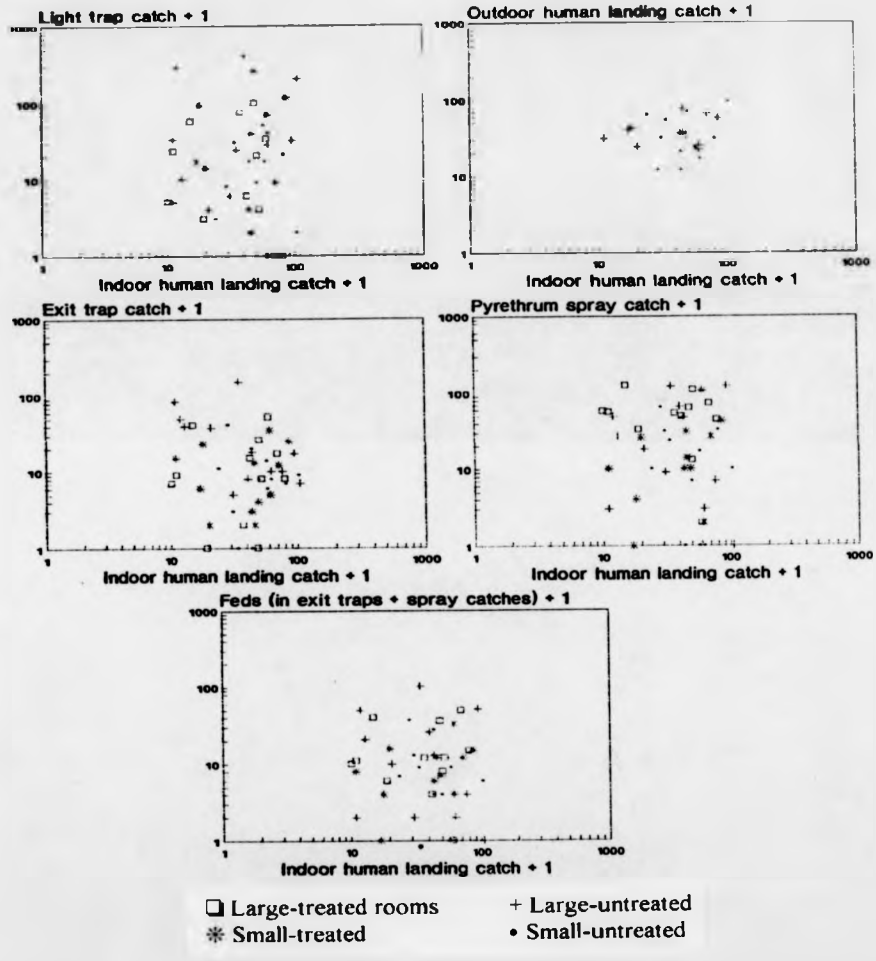

Figure 7.3. Correlation of the log-transformed (n+1) number of females of *An. gambiae s.l.* collected by the different methods, compared with human-landing collections in Jahally-Zone 3

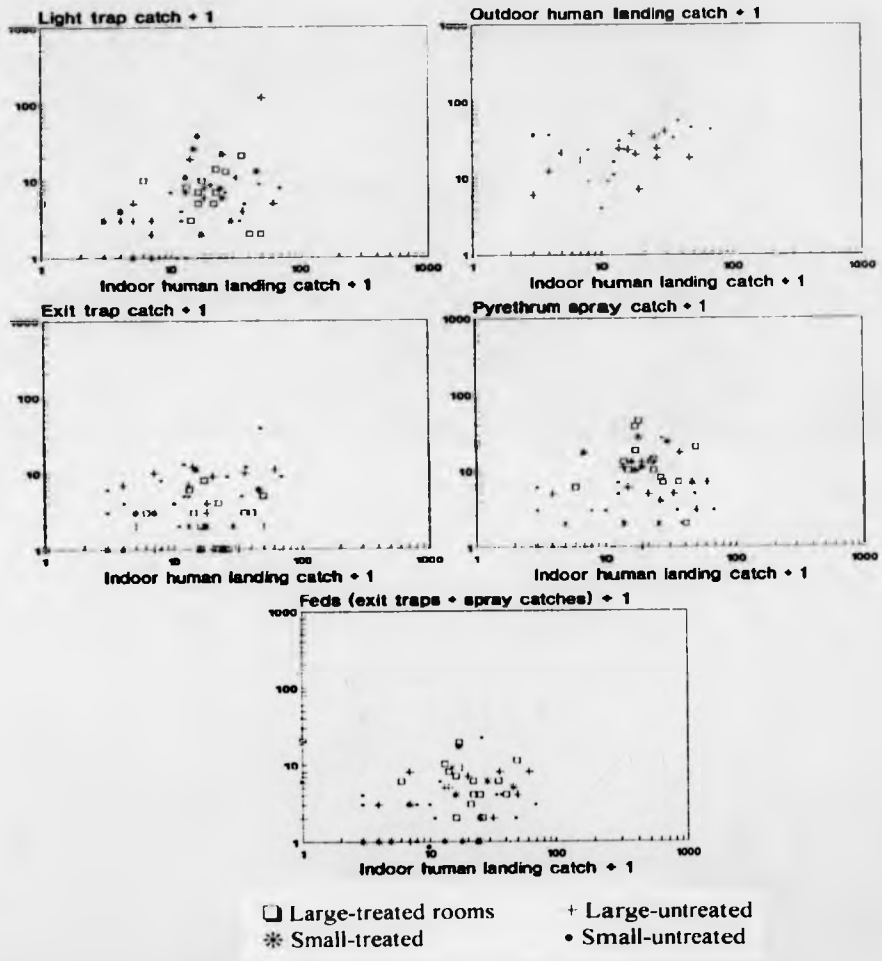

Figure 7.4. Correlation of the log-transformed (n+1) number of females of *An. gambiae s.l.* collected by the different methods, compared with human-landing collections in Kulari-Zone 5

Table 7.15. Pearson correlation coefficients of the number of female *An. gambiae s.l.* transformed to log(n+1) collected by the different methods of collection in Jahally-Zone 3 and Kulari-Zone 5. LTC= light trap, ILC= indoor landing, OLC= outdoor landing, ETC= exit trap, PSC= pyrethrum spray.

Village	Method	ILC	OLC	ETC	PSC	FEP
Jahally	LTC	-0.055	-0.012	0.019	0.035	0.051
	P	0.708	0.953	0.894	0.809	0.727
	n	48	24	48	48	48
	ILC		0.131	-0.141	-0.102	-0.088
	P	-	0.542	0.338	0.487	0.551
	n		24	48	48	48
	OCL			0.038	0.059	-0.100
	P	-	-	0.858	0.781	0.641
	n			24	24	24
	ETC				-0.086	0.042
	P	-	-	-	0.560	0.773
	n				48	48
	PSC					**0.887**
	P	-	-	-	-	**0.0001**
	n					**48**
Kulari	LTC	**0.388**	0.103	-0.014	0.192	0.231
	P	**0.002**	0.586	0.912	0.140	0.076
	n	**60**	30	60	60	60
	ILC		**0.546**	0.127	0.218	0.155
	P	-	**0.001**	0.333	0.093	0.236
	n		**30**	60	60	60
	OLC			0.354	0.068	0.066
	P	-	-	0.055	0.717	0.727
	n			30	30	30
	ETC				0.068	**0.337**
	P	-	-	-	0.601	**0.008**
	n				60	**60**
	PSC					**0.706**
	P	-	-	-	-	**0.0001**
	n					**60**

Table 7.16. Pearson correlation coefficients with the residuals after the analysis of variance accounting for the day effect, between methods of collection in Jahally-Zone 3 and Kulari-Zone 5. LTC= light trap, ILC= indoor landing, OLC= outdoor landing, ETC= exit trap, PSC= pyrethrum spray.

Village	Method	ILC	OLC	ETC	PSC
Jahally	LTC	-0.029	0.297	0.149	-0.131
	P	0.845	0.157	0.309	0.374
	n	48	24	48	48
	ILC		-0.290	-0.033	-0.180
	P	-	0.168	0.819	0.220
	n		24	48	48
	OCL			-0.105	0.011
	P	-	-	0.636	0.956
	n			24	24
	ETC				0.175
	P	-	-	-	0.234
	n				48
Kulari	LTC	0.127	**-0.581**	-0.135	0.233
	P	0.332	**0.0008**	0.303	0.072
	n	60	**30**	60	60
	ILC		**0.470**	-0.149	**0.338**
	P	-	**0.008**	0.253	**0.008**
	n		30	60	60
	OLC			0.177	-0.084
	P	-	-	0.349	0.657
	n			30	30
	ETC				0.016
	P	-	-	-	0.901
	n				60

Table 7.17. Average percent of mortality from bioassays in villages that paid (Y) or received the insecticide free (N) in zones 3 and 5.

Zone	Village	Paid	No. exposed	Percent mortality	Average
ZONE 3					
	Jafai	Y	201	70.07	
	Jahally	Y	193	78.19	
	Kudang	Y	196	63.41	
	PatheSame	Y	201	22.33	
	FasAbdou	Y	200	38.09	
	MbienMaka	Y	200	43.17	
	Mbian	Y	205	27.17	
	Patchar	Y	193	34.52	
	Sarengai	Y	209	22.80	44.42%
	Sotokoy	N	198	67.53	
	FulaBantang	N	186	67.64	
	Saruja	N	191	63.08	
	Bati	N	201	57.99	
	GaleManda	N	197	54.78	
	TabaNani	N	200	23.54	
	MamuFana	N	201	50.23	54.97%
Overall					49.22
ZONE 5					
	Dingiri	Y	199	21.52	
	Kudang	Y	198	31.18	
	Sudowol	Y	199	21.14	
	Sarebojo	Y	199	58.26	
	Badari	Y	199	75.95	
	Sotumasire	Y	194	65.05	
	Samunding	Y	187	87.46	
	Numuyel	Y	153	80.34	55.11%
	BanitoKekoro	N	199	17.55	
	Julangel	N	202	31.79	
	Misira	N	193	31.94	
	Koina	N	226	29.22	
	Bakadagi	N	209	23.83	
	DembakKuta	N	199	52.47	
	Gambissara	N	201	54.76	
	Garowai	N	199	36.27	
	Bantakore	N	76	71.88	38.86%
Overall					44.77

Table 7.18. (a) Analysis of variance of the arc-sin transformed percent of mortality in bioassays in zones 3 and 5. (b) Mean mortality before and after back transformation.

(a) Dependent Variable: ARCSIN

Source	DF	Sum of Squares	Mean Square	F	P
Model	12	2.12810328	0.17734194	2.46	0.0058
Error	147	10.57648109	0.07194885		
Corrected Total	159	12.70458438			

Source	DF	Sum of Squares	Mean Square	F	P
ZONE	1	0.07965563	0.07965563	1.11	0.2944
PAY	1	0.06892452	0.06892452	0.96	0.3293
MATERIAL	4	0.56090538	0.14022634	1.95	0.1054
MARK	1	0.21376780	0.21376780	2.97	0.0869
ZONE*PAY	1	0.88645010	0.88645010	12.32	0.0006
ZONE*MATERIAL	4	0.31839985	0.07959996	1.11	0.3558

(b) Variable	**N**	**Arcsin Mean**	**SD**	**Back-transformed Mean**
Zone 3	80	0.777	0.268	49.22%
Zone 5	80	0.733	0.296	44.77%
Pay	83	0.777	0.307	49.17%
Free	77	0.731	0.252	44.65%
Cotton netting	7	0.781	0.201	49.60%
Cotton sheeting	42	0.708	0.294	42.32%
Nylon jersey	18	0.877	0.328	59.19%
Nylon netting	72	0.778	0.273	49.35%
Nylon sheeting	21	0.654	0.238	37.08%
Marked	60	0.794	0.307	50.86%
Unmarked	100	0.732	0.265	44.68%

7.4. DISCUSSION

7.4.1. Relative sampling efficiency of different methods of collection

Light traps (LTC) and human-fed females resting in rooms and in exit traps (FEP) have been used as alternative methods to human-landing catches to estimate the human-vector contact. When human-vector contact is estimated using human fed females in rooms and in exit traps (Lindsay *et al.*, 1989b, Thomson *et al.*, 1995a) bias might occur when comparisons are made between more and less exophilic populations as was the case with the population of *An. gambiae s.l.* in zones 3 and 5; or when the indoor resting behaviour is altered, as is the case when sampling in the presence of permethrin-impregnated bednets. Human-landing collections or light traps would be of more use for those cases of differences in exophily or indoor resting behaviour. However, it is important to standardize the different methods under local conditions as it has been noted by Lines *et al.* (1991).

The design of the collections presented in this chapter were carried out with this kind of method comparison in mind, but has the problem of not having taken into account the day to day variation. A different design in which every method of collection were used every day, would have avoided this problem. However, such a design was not possible since pyrethrum spray catches had to be the last method of the series used in each room. This was because traces of insecticide could affect the collection of mosquitoes for the next few days.

As Altman and Bland (1983) have pointed out, when comparing methods, the degree of correlation is proportional to the between measurements variability. The variability in the numbers of mosquitoes collected was in most cases not significantly different between types of rooms or treatment condition as can be seen in the geometric means (Table 7.6). It seems that within a village, night to night and house to house variations can be great and this may have caused the lack of correlation between the methods observed. In Tanzania, Lines *et al.* (1991) found a significant correlation between LTC and HLC sampling different villages in different seasons, covering a wide range of densities. Similarly, the work of Davis *et al.* (1995) covered different seasons. A more extensive sampling in both time and space would be more appropriate to compare the relative sampling efficiency of the different

methods.

Another factor to consider is the possible differences in attractiveness of the different species to light traps. In Kenya (Githeko *et al.*, 1994) a significant correlation was found between human-landing and light traps for *An. arabiensis* but not for *An. funestus*. In the study presented in this chapter, determination of species was not linked with the information used for the correlations between the methods of collections though in both villages there was a mixture of *An. gambiae s.s.* and *An. arabiensis*.

The difference in sporozoite rates between females collected in the different methods of collection cannot be attributed to a difference in species composition between methods, since the species composition was rather similar in the different methods of collection in both villages.

Using the different methods of collection to estimate the IBR, this was in general higher in Kulari than in Jahally (Table 7.13). The ratio between villages of the estimated IBR using IHLC or FEP was similar (2-fold) for both methods, which give similar differences between areas to previous estimates (Thomson *et al.*, 1994 and 1995a). However, this similarity in ratios may have arisen by chance, given the differences found in the estimated human-mosquito bites and sporozoite rates between methods of collection Therefore, the validation of the use of FEP or LTC as a substitute for IHLC was not conclusive. A more extensive study in time and space and allowing for the day to day variation would be required.

7.4.2. Why did the NIBP not work in zone 5?

The fact that the NIBP reduced mortality and morbidity for malaria in 4 of the evaluation zones but not in zone 5 raised the question of whether entomological factors in this zone were part of the reasons that caused the programme not to be so effective in this zone.

7.4.2.1. *Anopheles gambiae* species and cytotypes

Differences between the zones in the proportions of *An. gambiae s.s.* and *An. arabiensis* were found, *An. arabiensis* being found in a higher proportion in zone 5 than in zone 3. As a vector, *An. gambiae s.s.* is widely recognized as more efficient than *An. arabiensis* (White, 1974), however, the participation of *An. arabiensis* in transmission in this zone has not been studied.

According to the descriptions by Coluzzi *et al.* (1985), the cytotypes of *An. gambiae s.s.* found in both zones (Jahally in zone 3 and Kulari in zone 5) from indoor resting females, indicate that the mosquitoes belong to the 'Savanna' form, which is characterized by high frequency of the inversions 2R*b*-2L*a*. Heterogeneities in behaviour of anophelines associated with chromosomal inversions have been described. For example, *An. gambiae s.s* carriers which were of the 2R*b*-2L*a* inversions were found more frequently indoors while carriers of the inversion 2R*d* were found more frequently outdoors in Garki, Nigeria (Coluzzi *et al.*, 1979). In some cases such intraspecific polymorphism may affect vector potential. *An. gambiae s.s* carriers of the standard form of the inversion 2La were associated with at least a two times higher sporozoite rate in Kenya (Petrarca and Beier, 1992). Determination of whether there is an association between the inversions *j* and *d* and sporozoite rate or exophilic behaviour of *An. gambiae s.s.* in zone 5 would be of interest. As the sample analysed by cytogenetics was only from indoor-resting females, the frequency of inversions can be referred only to this fraction of the population. A more extensive sampling including other methods of collection would be necessary to be conclusive about the differences found between the two zones. Sampling by other methods requires one to feed unfed females (most frequently found in HLC and ETC) and to maintain them until the semi-gravid stage is reached.

7.4.2.2. Biting and exiting behaviour

Higher exposure of the people to mosquito bites could be an important element for

the difference in impact of the NIBP in zone 5. However, differences in the *An. gambiae s.l.* biting behaviour between zones 5 and 3 were not found. The biting cycle was similar in both villages as were the indoor:outdoor ratios.

The presence of a treated bednet affected the indoor resting density in both zones as expected due to the excito-repellent effect of the insecticide. Although the difference was on the border line of significance in Kulari (Table 7.9), in both villages the mean numbers of females found were lower resting indoors in rooms with treated bednets.

Type of rooms seemed to have an influence in the tendency to exit of *An. gambiae s.l.* females in Kulari. Exit traps in large-square rooms collected significantly higher numbers of females than in the traditional small round rooms. Also, the number of females resting indoors collected by PSC was less in large-square rooms than in rounded ones. In Jahally this tendency was not found. Characteristics of the large-square rooms in Kulari may have influenced this finding. For instance, more than 85% of the square rooms in Kulari had a ceiling and no eaves while those percentages were around 40% in Jahally (Table 7.7).

Fed females were found in higher frequency in exit traps in zone 5 than in zone 3 (Table 7.10. and Thomson *et al.*, 1995a). This finding could have been due to the differences in bednet usage between the two zones. Mosquitoes would have more opportunity to blood-feed in villages with low bednet usage such in zone 5. However, calculating the proportion of fed females in exit traps related to the total fed females found in the rooms (resting indoors and in exit traps) the proportion of feds exiting was still higher in zone 5 compared with zone 3 (Table 7.11), which indicates a more exophilic behaviour of *An. gambiae s.l.* in zone 5.

This exophilic behaviour cannot be attributed to differences in species composition between the zones, as was suggested by Thomson *et al.*, (1994) because, although the proportion of *An. arabiensis* in zone 5 was higher than in zone 3, mosquitoes collected outdoors (in exit traps and outdoor biting) had similar species composition as those collected indoors (spray catches, light traps and human-landing) in zone 5 (Table 7.1). *An. arabiensis* is considered to be a more exophilic species than *An. gambiae s.s* (White, 1972), however in the present study this behaviour was found only in the village in zone 3.

7.4.2.3. Persistence of the insecticide

The persistence of the insecticide after 4 months of impregnation in both zones was similar to a previous report from The Gambia (Lindsay *et al.*, 1993). As no significant difference was found between zones (Table 7.17 and 7.18) and no records of resistance of any of the *An. gambiae* species complex to permethrin has been documented in The Gambia (M. Jawara, personal communication) we can conclude that the impregnation was carried out in a similar way in each zone and that poor insecticide treatment did not explain the failure of the NIBP in zone 5.

7.4.2.4. EIR and bednet usage

The density of *An. gambiae s.l.*, measured by the different methods of collection, was found to be lower in zone 5 compared with zone 3, and sporozoite rates were higher in zone 5. The entomological inoculation rate was higher in zone 5 than in zone 3, which is in agreement with the previous study by Thomson *et al.* (1994). The higher EIR has been suggested as one of the reasons for the lack of impact of the programme in this particular area (D'Alessandro *et al.*, 1995b) given that, to reduce transmission in areas with high endemicity, the impact on the vectorial capacity of the mosquito population needs to be reduced more drastically than in areas of low endemicity (Rozendaal and Curtis, 1989; Chapter 1).

However, in the entomological evaluation of the NIBP, EIR on unprotected humans did not decline in zone 5 or in any other zone, after the impregnation of bednets (Thomson *et al.*, 1995a). Personal rather than community protection is therefore provided by the impregnated bednets in The Gambia (Thomson *et al.*, 1995a; Chapter 2).

If no community protection ('mass-killing effect) is achieved by the impregnation of bednets, the rate of individual bednet usage becomes a crucial factor. The percentage of beds with bednets in the study zones were surveyed by D'Alessandro *et al.* (1995b), zone 3 had the highest proportion of beds with bednets (78.6%) and zone 5 the lowest (46.4%). Recently M. Aikins (personal communication) compared the bednet usage among children

1 to 4 years old in 8 villages in zone 5 and 3. Differences between the zones were found: on average in zone 3, 7.5% of the children slept without a bednet while in zone 5 the percentage reached 25%.

Considering the parasitaemia results (% *P. falciparum* positive) in relation to bednet usage in PHC villages that received free insecticide in zone 5, a significant difference was found (χ^2= 8.83, P=0.003) between children sleeping under a treated bednet (56.6% positive n=83) and the ones sleeping without bednet (80.3% positive n=61) (U. D'Alessandro, personal communication). In zone 3, only 3 out of 173 children were sleeping without a bednet. This supports the hypothesis of the low bednet usage as one of the major reasons for the failure of the NIBP in zone 5.

Disease (clinical malaria) or mortality rates have been found to be relatively more sensitive parameters to changes in exposure than prevalence of parasitaemia (see section 1.6 in Chapter 1). Therefore a greater difference would have been expected if disease parameters had been measured and analysed in relation to bednet usage.

Other possibilities include differences between groups (treated and control villages) in the level of malaria and an "unlucky" randomization which left the most malarious villages in the treated group. Also, there is a possibility that some insecticide passed from villages assigned to the treated group to the control villages. These possibilities however, may be only part of the explanation for the difference between zone 5 and the other zones.

In conclusion, the explanation for the lack of impact of the NIBP on mortality and morbidity in zone 5 appeared to be more related to the low bednet usage than any of the entomological parameters considered in this study.

7.5. SUMMARY

During the first year of the National Impregnated Bednet Programme (NIBP) in The Gambia, bednets in half of the PHC villages (with a primary health centre) were impregnated with permethrin. The impact of the NIBP on mortality and morbidity in children was evaluated in 5 zones from west to east across the country, comparing pairs of treated and untreated PHC villages. A reduction in mortality and morbidity was seen in children (1-9 years old) in all zones, except in zone 5 where mortality in children (particularly in 1-2 years old), and parasitaemia and high-density parasitaemia were higher in treated villages in this zone.

A study was undertaken to see if entomological factors were part of the reason that the NIBP was not as effective in zone 5 as in the other zones. Two villages were selected, Jahally and Kulari in zones 3 and 5 respectively to carry out the observations. The aspects studied were: species composition, cytotypes of *An. gambiae s.s.*, biting cycle, exiting and resting behaviour, responses to the treatment with regard to deterrency, exiting, indoor resting behaviour and persistence of the insecticide. The collection of mosquitoes were carried out in sets of small round traditional rooms and large square rooms in both villages to see if the type of room could make a difference in the behaviour of the mosquito populations.

Some entomological differences were found between Jahally (zone 3) and Kulari (zone 5):

a) *An. gambiae s.s.* was found in higher proportion in zone 3 (80%) than in zone 5 (50%-60%); *An. arabiensis* was found in higher proportion in zone 5 (40%-50%) than in zone 3 (15%).

b) Some chromosomal inversions in *An. gambiae s.s.* (2R*j* and 2R*d*) showed higher frequency in zone 5

c) Fed females of *An. gambiae s.l.* showed more exophilic behaviour in zone 5 than in zone 3.

Other parameters showed no significant differences:

a) The cytotype of *An. gambiae s.s.* found in both villages was 'Savanna' form.

b) The mean biting time and indoor:outdoor biting ratio *An. gambiae s.l.* did not differ between the villages.

c) Persistence of the insecticide on bednets did not differ.

d) No evidence was found that the treatment had a differential effect related to type of rooms.

Bednet usage may play a more significant role in the differential effect of the NIBP in zones 3 and 5 than the entomological parameters measured in this study.

The relative sampling efficiency of human-landing collections was compared with light traps, exit traps and pyrethrum-spray catches. In general there were few significant correlations between methods, due probably to night to night and house to house variation and a limited sampling in both duration and geographical extension.

CHAPTER 8

GENERAL DISCUSSION, CONCLUSIONS AND FUTURE WORK

8.1. The 'mass-killing effect'

The results from a survey in 10 pairs of treated and untreated villages and a cross-over study, together with previous studies (Lindsay *et al.*, 1993; Thomson *et al.*, 1995a), confirm the absence of a 'mass-killing effect' of permethrin-treated bednets in The Gambia. Females of the *An. gambiae* species complex were found to be as abundant, long lived and infective in villages with treated bednets as in villages with untreated bednets (Chapter 2). Also, no evidence was found for a repellency at village level due to the impregnated bednets (Chapter 3)

Therefore, the fact that treated bednets reduce malaria mortality and morbidity in children cannot be explained by reduction of density, survival rates or sporozoite rates of the vector population at a village level.

The absence of the 'mass-killing effect' raises two kind of questions. Firstly, why is this effect not seen in The Gambia while in other African countries (e.g.Tanzania, Zaire) a clear 'mass-effect' has been found after the introduction of treated bednets? Secondly, what are the consequences and implications of the lack of mass-killing effect?

8.1.1. Possible reasons for the difference between other African countries and The Gambia concerning the "mass-killing effect".

One possibility is that the "mass-killing effect" was masked by movements of mosquitoes between treated and untreated villages, as proposed by Lindsay *et al.* (1993). Results of two mark-release recapture experiments regarding this question showed that an estimated 17.2% (95% C.I. 11%-26.7%) mosquitoes in one of the villages involved in the National Impregnated Bednet Programme (NIBP) had originated in another such village about 2km away (Chapter 3). These results were similar to previous estimates of movements between smaller villages (Thomson *et al.*, 1995b). It would be desirable to go beyond the

above estimate to interpret these results in terms of how much of interchange of mosquitoes between villages would be necessary to mask a 'mass-killing effect'. A mathematical model could help to resolve this question, but this was beyond the scope of this thesis.

No evidence was found for an effect on movements from and to villages with permethrin-treated bednets i.e. repellency at village level (Chapter 3). However, mark-release recapture experiments and comparison of the estimation of movements would not be the most appropriate way to assess this effect, because of the wide confidence limits in the movement indices. However, density was not reduced and this would be expected to be found if repellency at a village level is occurring.

However, if the 'mass-killing effect' in treated villages were being masked by the immigrants from neighbouring untreated villages, we would have expected to observe an indication of this in the recapture rates in the mark-release recapture experiments. In the absence of repellency at a village level, recapture rates would have been lower in treated, than in the untreated villages because of the lower survival rates of the vector population in treated villages. However, this was not consistently found. In fact, one of the treated villages actually had the highest recapture rate in one of the experiments (Chapter 3).

At the same time, significant differences have been reported in the proportion of immigrant mosquitoes in untreated villages between Tanzania and The Gambia in similar mark-release recapture experiments (Chapter 3).

Another possible explanation is that mosquitoes are not generally killed by treated bednets in The Gambia, perhaps because the material of the bednets in The Gambia (mostly heavy cotton) is different from the ones used both in previous experimental hut studies where killing of mosquitoes has been measured (Lindsay *et al.*, 1991; Miller *et al.*, 1991) and in countries where a mass-killing effect following insecticide-impregnation of bednets at a community level has been seen (Magesa *et al.*, 1991; Karch *et al.*, 1993), which is light nylon material. In a recent experimental hut trial, low mortality was found when Gambian bednets were used (2 nylon netting, 2 synthetic sheeting and 1 synthetic muslin netting) compared with previous results using the same experimental huts with imported nylon bednets (Nagle,

1994).

Although the results of resistance tests confirm the susceptibility of the different populations of *An. gambiae s.l.* (M. Jawara, personal communication), the possibility that the vector in The Gambia may be slightly more tolerant to permethrin than populations in other countries cannot be completely ruled out.

In conclusion, the reasons for the absence of the "mass-killing effect" in The Gambia are not very clear. Movements of mosquitoes between villages continues to be the most likely possible explanation of the absence of a "mass-killing effect" in The Gambia in contrast with other African countries.

8.1.2. Consequences of the lack of mass-killing effect

8.1.2.1. How therefore is protection against malaria acquired by children?

Impregnated bednets could have had other effects on the population of *An. gambiae s.l.* apart from reduction in density, longevity and sporozoite rates, that would help to explain the way in which protection against malaria is acquired by children.

Changes in biting location

The rate of infective biting on children might be reduced through diversion of mosquitoes from biting children indoors to other hosts outdoors. No significant differences were found in the indoor landing densities in the presence or absence of treated bednets and no significant differences were found in the Indoor:Outdoor ratio (Chapter 4).

Changes in biting time

A shift in biting time might also lead to a change in the rate of biting on children rather than other hosts. No indication was found of a shift in the biting time in treated villages (Chapter 4).

Changes in biting frequency

Instead of being diverted to the hosts, the mosquitoes that would normally (in the absence of treated bednets) feed on children might fail to feed at all, or be delayed in

obtaining a meal. Pulses of recapture of fed females were found at days 2 and 4 after release, suggesting a 2 day gonotrophic cycle in untreated villages (Chapter 4). No evidence of a change in the gonotrophic cycle length was seen. However, the number recaptured in the presence of treated bednets was too low to rule out the possibility that females failed to feed, or were delayed in feeding, in the presence of treated bednets.

Changes in host choice

No significant reduction in the Human Blood Index of indoor resting females was found in treated villages (Chapter 4). Despite the small number of females tested, this result together with previous similar results (Lindsay *et al.*, 1989; Thomson *et al.*, 1995a) fails to support the hypothesis that the mosquitoes that would otherwise bite children feed on bovids instead. However, the HBI of indoor resting females is not an adequate index of the behaviour of the whole population. The HBI in outdoor resting females is also necessary for an overall estimate of host choice in the mosquito population at a village level (Garrett-Jones, 1964). The study presented in Chapter 5 tried to address this question. No tendency was found for the presence of treated bednets to cause a significant reduction in the HBI of outdoor resting females. But, doubts regarding the methodology used to identify host species remains.

Evidence for a significant reduction of mosquito bloodmeals from children was found associated with the presence of treated bednets in the study villages, as shown in Chapter 5. However, it is not clear whether these results are reliable. Assuming that they are, they clearly imply that in this case there was diversion of bites from children to adults as an effect of permethrin-treated bednets. This effect would help to explain why no differences in the human blood index between treated and untreated villages have been found.

Further work is needed for firmer conclusions. The priorities are, firstly, to assess changes in the HBI in the overall vector population and secondly to assess children/adult biting frequency in the presence and absence of treated bednets. Both would need sampling outdoor as well as indoor resting females. The second may require the use of PCR to individually identify the source of bloodmeals (Gookol *et al.*, 1993) or aminopropeptide of

human procollagen type I (Bodker *et al.*, 1995) to distinguish blood from people with growing bones (i.e. mostly children).

8.1.2.2. Implications of the absence of a "mass-killing effect"

Implications for epidemiological evaluation

Personal rather than community protection against malaria is therefore predicted by the entomological results in The Gambia. Evaluation of the epidemiological impact of treated bednets could be carried out at individual level taking into account personal bednet usage rather than that at village level. In the epidemiological evaluation in The Gambia the results were checked by mass surveys which recorded individual use of treated bednets and these confirmed that the protective effect was associated with personal protection (D'Alessandro *et al.*, 1995a) rather than a community effect. In addition, in Zone 5, in which the NIBP seemed to have failed, a significant reduction in parasitaemia was noticed in users compared with non-users of treated bednets (U. D'Alessandro, unpublished data).

Operational implications

Knowledge of whether impregnated bednets can be expected to produce a 'mass-killing effect' at the village level under local conditions is important for the planning of implementation programmes of malaria control. If a 'mass-killing effect' occurs then, as with DDT house spraying, programs encouraging high coverage are likely to be most effective. On the other hand, if only individual protection is given by the use of impregnated bednets, different strategies could be employed, for example encouraging target groups especially at risk for malaria to acquire and impregnate their bednets.

The occurrence of individual protection of permethrin-treated bednets and the absence of a 'mass-killing effect' at a community level makes it justifiable to direct intervention programmes to high risk target groups like children and pregnant women rather than whole communities. Coordinated programmes with other organizations working with the same

target groups could facilitate the use of impregnated bednets.

If mosquitoes are diverted to bite animals instead of people sleeping under treated bednets, there is no reason not to promote individual acquisition of a treated bednet as the strategy for implementation of this control measure. However, if diversion to unprotected people is occurring, ethical considerations are necessary in the development of such strategies.

8.2. Entomological differences in Zone 5 and 3 of The Gambia and failure of the NIBP in zone 5.

The lack of impact of the NIBP on mortality and morbidity in Zone 5 (in the extreme east of The Gambia), appeared to be more related to low bednet usage than any of the entomological parameters considered in this study (Chapter 7). Some differences were found between Zones 5 and 3 in respect of species composition, frequency of different cytotypes within *An. gambiae s.s.*, and in the infectivity and density of *An. gambiae s.l.*. However, how these differences may have affected the impact of treated bednets is not clear. More studies are needed to relate, for example, the *An. gambiae s.s.* inversions that were found in zone 5 in higher frequency (2Rj and 2Rd) with differences that could lead to a difference in the effect of impregnated bednets. It is already known (Thomson *et al*, 1994; Thomson *et al.*, 1995a) that the entomological inoculation rate (EIR) in Zone 5 was higher than in any other zone, and this has been considered as an important factor to explain the difference in impact on mortality and morbidity in this zone, in which the analysis was done at village level (D'Alessandro *et al.*, 1995b). However, the analysis of parasitaemia by individual bednet usage showed a significant reduction in parasitaemia in children that slept under treated bednets even in zone 5 (U. D'Alessandro, unpublished data), despite the high EIR there. This is in agreement with conclusions reached in the studies reported here on the way impregnated bednets appear to work in The Gambia, which is by providing personal protection, reducing mosquito bites on protected people, and not by a "mass-killing effect" at a village level.

8.3. Differences in behaviour between species and incipient species of the *An. gambiae* complex and treated bednets

Differences in behaviour between the members of the *An. gambiae* species complex and between cytotypes of *An. gambiae s.s.* might influence the effect of the impregnated bednets. This question was addressed in a study carried out in the central part of the country (Chapter 6). The behaviour of the species of the *An. gambiae* complex was in agreement with previous studies: *An. gambiae s.s.* showed the most anthropophilic and endophilic tendencies, while *An. melas* showed a higher degree of exophily and zoophily. Few *An. arabiensis* were found. These behavioural differences between species may have an influence on the impact of the NIBP. One could anticipate that in the presence of treated bednets, areas in which *An. melas* is frequent would be less likely to show a reduction in density or longevity of this species in particular since they would be less exposed to the insecticide. However, in the entomological evaluation of the NIBP, evidence for a reduction in the indoor resting density (evaluated in rooms with an untreated bednet) was found in the pair of villages in zone II, where *An. melas* is present in relatively high frequency (Thomson *et al.*, 1995a). It would be of interest to determine whether diversion to animals of *An. melas*, one of the more zoophilic species of the *An. gambiae* complex, could have accounted for these results.

Two incipient species within *An. gambiae s.s.* have been described in The Gambia and both of these were found in Balingho (Chapter 6). The 'Savanna' form (characterized by predominance of the 2Rb-2La inversions) showed much higher frequency than the 'Bissau' form (predominance of the 2Rd inversion). Because of the small numbres of the later, it was impossible to assess potential contrast in behaviour between the two incipient species.

8.4. Comparison of methods

8.4.1. Method of collection comparison

Comparisons of the sampling efficiency of human-landing collections with other methods is an important question for entomology in general and for the evaluation of

impregnated bednets in The Gambia in particular. Light traps (Lines *et al.*, 1991; Shiff *et al.*, 1995) and counting of human-fed females in rooms (Lindsay *et al.*, 1993; Thomson *et al.*, 1995a) have been used as alternative methods to assess human-biting rates. The results of the comparison between these methods (Chapter 7) was not conclusive, probably because the amount of the sampling in both duration and geographical extension was limited (4 weeks, 2 villages). In general there were few significant correlations between the methods. It seems that within a village, night to night and house to house variations (which could not be adequately separated in this study) can be great and this may have caused the lack of correlation between the methods observed. In any case, the method comparison results reported here contrast with those of Lines *et al.* (1989) in which sampling was carried out over a number of villages and in different seasons. A balanced design and more extensive coverage is recommended for further studies of this kind.

8.4.2. Comparison of cytogenetics and DNA probes for species identification of members of the *An. gambiae* species complex in The Gambia

The use of DNA probes for the identification of the species was reliable in comparison with cytogenetics, for identification of *An. gambiae s.s.* and *An. arabiensis*, and this method has the advantage of allowing identification of large numbers of specimens relatively rapidly and with minimal training. However, some limitations were found. Variability between batches of the probes was observed, (Macdowall, 1994) and some *An. melas*, identified by cytogenetics, were identified as *An. gambiae s.s.* by the DNA probes. This stressed the necessity, with the existing probes, for strict quality control in the production of the probes, and for a positive control for *An. melas*. Currently there are no existing colonies of *An. melas*, and so such control material is difficult to provide. The alternative would be to produce more specific probes so that each species can be positively identified by a fully specific test (Chapter 6).

REFERENCES

Aikins, M.K., H. Pickering, P.L. Alonso, U. D'Alessandro, S.W. Lindsay, J. Todd and B.M. Greenwood. 1993. A malaria control trial using insecticide-treated bed nets and targeted chemoprophylaxis in a rural area of The Gambia, West Africa. 4. Perceptions of the causes of malaria and of its treatment and prevention in the study area. **Transactions of the Royal Society of Tropical Medicine and Hygiene**, 87(supplement 2):20-24.

Alonso, P.L., S.W. Lindsay, J.R.M. Armstrong, M. Conteh, A.G. Hill, P.H. David, G. Fegan, A. de Francisco, A.J. Hall, F.C. Shenton, K. Cham, and B.M. Greenwood. 1991. The effect of insecticide-treated bed nets on mortality of Gambian children. **The Lancet**, 337:1499-1502.

Alonso, P.L., S.W. Lindsay, J.R.M. Armstrong Schellenberg, K. Keita, P. Gomez, F.C. Shenton, A.G. Hill, P.H. David, G.Fegan, K. Cham and B.M. Greenwood. 1993. A malaria control trial using insecticide-treated bed nets and targeted chemoprophylaxis in a rural area of The Gambia, West Africa. 6. The impact of the intervention on mortality and morbidity from malaria. **Transactions of the Royal Society of Tropical Medicine and Hygiene**, 87(supplement 2):37-44.

Altman, D.J. and J.M. Bland. 1983. Measurement in Medicine: the analysis of method comparison studies. **The Statistician**, 32(307-317.

Bauer, H.M., Y. Ting, C.E. Greer, J.C. Chambers, C.J. Tashiro, J. Chimera, A. Reingold, M. Manos. 1991. Genital human papillomavirus infection in female university students as determined by a PCR-based method. **Journal of the American Medical Association**, 265(4):473-477.

Beach, R.F., T.K. Ruebush II, J.D. Sexton, P.L. Bright, A.W. Hightower, J.G. Breman, D.L. Mount and A.J. Oloo. 1993. Effectiveness of permethrin-impregnated bed nets and curtains for malaria control in a holoendemic area of Western Kenya. **American Journal of Tropical Medicine and Hygiene**, 49(3):290-300.

Bermejo, A. and H. Veeken. 1992. Insecticide-impregnated bed nets for malaria control: A review of the field trials. **Bulletin of the World Health Organization**, 70(3):293-296.

Bo, P., L. Zu-Zi, H. Qi-Lin. 1995. Studies and application of pyrethroid treated bednet for control of malaria vectors in P.R. China. **Chinese Journal for Parasitic Disease Control**, 8(1):54-58.

Bodker, R., O. Orum, B. Teisner, E.M. Pedersen, R. Thompson and B. Hogh. 1995. Aminopropeptide of human procollagen type I: a marker for the identification of blood from children in the mosquito blood meal. **Transactions of the Royal Society of Tropical Medicine and Hygiene**, 89:449-453.

Boreham, P.F.L., J.A. Chandler and J. Jolly. 1978. The incidence of mosquitoes feeding on mothers and babies at Kisumu, Kenya. **Journal of Tropical Medicine and Hygiene**, 81:63-67.

Boreham, P.F.L. and G.R. Port. 1982. The distribution and movement of engorged females of *Anopheles gambiae* Giles (Diptera:Culicidae) in a Gambian village. **Bulletin of Entomological Research**, 72:489-495,

Bradley, A.K., A.M. Greenwood, P. Byass, B.M. Greenwood, K. Marsh, S. Tulloch and R. Hayes. 1986. Bed-nets (Mosquito-nets) and morbidity from malaria. **The Lancet**, 2-1:204-207.

Bryan, J.H. and M.E. Smaley. 1978. The use of ABO blood groups as markers for mosquito biting studies. **Transactions of the Royal Society of Tropical Medicine and Hygiene**, 72:357-360.

Bryan, J. H. 1979. Observations on the member species of the *Anopheles gambiae* complex in The Gambia, West Africa. **Transactions of the Royal Society of Tropical Medicine and Hygiene**, 73:463-466.

Bryan, J.H. 1980. Use of the palpal ratio and the number of pale bands on the palps in separating *Anopheles gambiae* Giles s.s. and *An. melas* Theobald (Diptera: Culicidae). **Mosquito Systematics**, 12: 153-163.

Bryan, J.H., M.A. Di Deco, V. Petrarca and M. Coluzzi. 1982. Inversion polymorphism and incipient speciation in *Anopheles gambiae s.str.* in The Gambia, West Africa. **Genetica**, 59:167-176.

Bryan, J.H. 1983. *Anopheles gambiae* and *A. melas* at Brefet, The Gambia, and their role in malaria transmission. **Annals of Tropical Medicine and Parasitology**, 77(1):1-12.

Bryan, J.H., V. Petrarca, M.A. Di Deco, M. Coluzzi. 1987. Adult behaviour of members of the *Anopheles gambiae* complex in The Gambia with special reference to *An. melas* and its chromosomal variants. **Parassitologia**, 29:221-249

Brown, J.H. 1970. The estimation of Wright's fixation index from genotypic frequencies. **Genetica**, 41:399-406.

Burkot, T.R., W.G. Goodman and G.R. DeFoliart. 1981. Identification of mosquito bloodmeals by enzyme linked immunosorbent assay. **American Journal of Tropical Medicine and Hygiene**, 30:1336-1341.

Busvine, J.R. 1993. **Disease transmission by insects**. Springer-Verlag Editors. 361pp.

Campbell, H., P. Byass and B.M. Greenwood. 1987. Bed nets and malaria supression. **The Lancet**, 1-2:859-860.

Carnevale, P., V. Robert, C. Boudin, J.M. Halna, L. Pazart, P. Gazin, A. Richard and J. Mouchet. 1988. La lutte contre le paludisme par des moustiquaires impregnees de pyrethrinoides au Burkina Faso. **Bulletin de la Societe de Pathologie Exotique et de ses Filiales**, 81:832-846.

Carnevale, P., P. Bitsindou, L. Diomande and V. Robert. 1992. Insecticide impregnation can restore the efficiency of torn bed nets and reduce man-vector contact in malaria endemic areas. **Transactions of the Royal Society of Tropical Medicine and Hygiene**, 86:362-364.

Charlwood, J.D. 1986. A differential response to mosquito nets by *Anopheles* and *Culex* mosquitoes from Papua New Guinea. **Transactions of the Royal Society of Tropical Medicine and Hygiene**, 80:958-960.

Charlwood, J.D. and Dagoro, H. 1987. Impregnated bed nets for the control of filariasis transmitted by *Anopheles punctulatus* in rural Papua New Guinea. **Papua New Guinea Medical Journal**, 30:199-202.

Charlwood J.D. and P.M. Graves. 1987. The effect of permethrin-impregnated bednets on a population of *Anopheles farauti* in coastal Papua New Guinea. **Medical and Veterinary Entomology**, 1:319-327.

Charlwood, J.D., P.M. Graves and T.F. de C. Marshal. 1988. Evidence for a 'memorized' home range in *Anopheles farauti* females from Papua New Guinea. **Medical and Veterinary Entomology**, 2:101-108.

Cheng, H., Y. Wen, K. Wuanmin and L. Chongyi. 1995. Large-scale spraying of bednets to control mosquito vectors and malaria in Sichuan, China. **Bulletin of the World Health Organization**, 73(3):321-328.

Choi, H.W., J.G. Breman, S.M. Teutsch, S. Liu, A.W. Hightower and J.D. Sexton. 1995. The effectiveness of insecticide-impregnated bed nets in reducing clases of malaria infection: A meta analysis of published results. **American Journal of Tropical Medicine and Hygiene**, 52(5):377-382.

Cleaveland, S. 1996. The epidemiology of rabies and canine distemper in the Serengeti, Tanzania. PhD Thesis. Department of Medical Parasitology, London School of Hygiene and Tropical Medicine. University of London.

Coluzzi, M. 1964. Morphological divergences in the *Anopheles gambiae* complex. **Rivista di Malariologia**, 43:197-232.

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

Coluzzi M., Sabatini A.,Petrarca V. and Di Deco M.A. 1979. Chromosomal differentiation and adaptation to human environments in the *Anopheles gambiae* complex. **Transactions of the Royal Society of Tropical Medicine and Hygiene**, 73: 483-497.

Coluzzi M. 1984. Heterogeneities of the malaria vectorial system in Tropical Africa and their significance in malaria epidemiology and control. **Bulletin of the World Health Organization**, 62 (suppl.):107-113

Coluzzi, M., V. Petrarca, M. A. Di Deco. 1985. Chromosomal inversion intergradation and incipient speciation in *Anopheles gambiae*. **Bulletin of Zoology**, 52:45-63.

Coulson, R.M.R., C.F. Curtis, P.D. Ready, N. Hill and D.F. Smith. 1990. Amplification and analysis of human DNA present in mosquito bloodmeals. **Medical and Veterinary Entomology**, 4:357-366.

Curtis, C.F. and P. Rawlings. 1980. A preliminary study of dispersal and survival of *Anopheles culicifacies* in relation to the possibility of inhibiting the spread of insecticide resistance. **Ecological Entomology**, 5:11-17.

Curtis, C.F., J.D. Lines, P. Carnevale, V. Robert, C. Boudin, J.M. Halna, L. Pazart, P.Gazin, A. Richard, J. Mouchet, J.D. Charlwood, P.M. Graves, M.I. Hossain, T. Kurihara, K. Ichimori, L. Zuzi, L. Baolin, G. Majori, G. Sabatinelli, M. Coluzzi, K.J. Njunwa, T.J. Wilkes, R.W. Snow, and S.W. Lindsay. 1991. Impregnated bed nets and curtains against malaria mosquitoes. In: **Control of Disease Vectors in the Community**, Ed. Curtis, C.F., Wolfe Ltd. pp233.

Curtis, C.F., J. Myamba and T.J. Wilkes. 1992. Various pyrethroids on bednets and curtains. **Memorias do Instituto Oswaldo Cruz**, 87 (Supplemento 3):363-370.

Curtis, C.F. 1992. Personal protection methods agaist vectors of disease. **Review of Medical and Veterinary Entomology**, 80(10):544-553.

Curtis,C.F., J. Myamba and T.J. Wilkes. 1996. Comparison of different insecticides and fabrics for anti-mosquito bednets and curtains. **Medical and Veterinary Entomology**, 10:1-11.

Crews-Oyen A.E., V. Kumar and F.H. Collins. 1993. Association of two esterase genes, a chromosomal inversion, and susceptibility to *Plasmodium cynomolgi* in the African malaria vector *Anopheles gambiae*. **American Journal of Tropical Medicine and Hygiene**, 49(3):341-347.

D'Alessandro, U., M.K. Aikins, P. Langerock, S. Bennett and B.M. Greenwood. 1994. Nationwide survey of bednet use in rural Gambia. **Bulletin of the World Health Organization**, 72(3):391-394.

D'Alessandro, U., B.O. Olaleye, W. McGuire, M.C. Thomson, P. Langerock, S. Bennett and B.M. Greenwood. 1995a. A comparison of the efficacy of insecticide-treated and untreated bed nets in preventing malaria in Gambian children. **Transactions of the Royal Society of Tropical Medicine and Hygiene**, 89:596-598.

D'Alessandro, U., B.O. Olaleye, W. McGuire, P. Langerock, S. Bennett, M.K. Aikins, M.C. Thomson, M.K. Cham, B.A. Cham and B.M. Greenwood. 1995b. Mortality and morbidity from malaria in Gambian children after introduction of an impregnated bednet programme. **The Lancet**, 345:479-483.

Darriet, F., V. Robert, N. Tho Vien and P. Carnevale. 1984. Evaluation of the efficacy of permethrin-impregnated intact and perforated mosquito nets against vectors of malaria. WHO mimeographed document WHO/VBC/84.899.

Davis, J.R., T. Hall, E.M. Chee, A. Majala, J. Minjas and C.J. Shiff. 1995. Comparison of sampling anopheline mosquitoes by light-trap and human-bait collections indoors at Bagamoyo, Tanzania. **Medical and Veterinary Entomology**, 9:249-255.

Dawson-Saunders, B. and R.G. Trapp. 1994. **Basic and Clinical Biostatistics**. Prentice-Hall International.

De Zulueta, J. and J.R. Cullen. 1963. Deterrent effect of insecticides on malaria vectors. **Nature**, 200:860-861

Dye, C. 1986. Vectorial Capacity: Must we meassure all its components? **Parasitology Today**, 2(8):203-209.

Emerson, P.M. 1992. An investigation into whether *Anopheles gambiae sensu latu* exhibit a preference for a restricted home range in an area of The Gambia. MRC report, 25pp.

Garrett-Jones, C. 1964. The human blood index of malaria vectors in relation to epidemiological assessment. **Bulletin of the World Health Organization**, 30:241-261.

Garrett-Jones, C. and B. Grab. 1964. The assessment of insecticidal impact on the malaria mosquito's vectorial capacity, from data on the proportion of parous females. **Bulletin of the World Health Organization**, 31:71-86.

Genton, B., J. Hii, F. Al-Yaman, R. Paru, H-P. Beck, M. Ginny, H. Dagoro, D. Lewis and M.P. Alpers. 1994. The use of untreated bednets and malaria infection, morbidity and immunity. **Annals of Tropical Medicine and Parasitology**, 88(3):263-270.

Gillies, M.T. 1961. Studies on the dispersion and survival of *Anopheles gambiae* Giles in East Africa, by means of marking and release experiments. **Bulletin of Entomological Research**, 52:99-127.

Gillies, M.T. and B. De Meillon. 1968. **The *Anophelinae* of Africa South of the Sahara (Ethiopian Zoogeographical Region).** Second Edition. The South African Institute for Medical Research. Johannesburg. 343p

Gillies, M.T. and M. Coetzee. 1987. **A supplement to the *Anophelinae* of Africa South of Sahara (Afrotropical Region).** The South African Institute for Medical Research. Johannesburg. 143p.

Githeko, A.K., M.W. Service, C.M. Mbogo, F.A. Atieli and F.O. Juma. 1994. Sampling *Anopheles arabiensis*, *A. gambiae sensu lato* and *A. funestus* (Diptera:Culicidae) with CDC light-traps near a rice irrigation area and a sugarcane belt in western Kenya. **Bulletin of Entomological Research**, 84:319-324.

Githeko, A.K., C.N.M. Mbogo, C.F. Curtis, J. Lines and C. Lengeler. 1996. Entomological monitoring of large-scale vector-control interventions. **Parasitology Today**, 12(4):127-128.

Glynn, J.R., J. Lines and D.J. Bradley. 1994. Impregnated bednets and the dose-severity relationship in malaria. **Parasitology Today**, 10(7):279-281.

Glynn, J.R., W.E. Collins, G.M. Jeffery and D.J. Bradley. 1995. Infecting dose and severity of falciparum malaria. **Transactions of the Royal Society of Tropical Medicine and Hygiene**, 89:281-283.

Golkool, S., C.F. Curtis and D.F. Smith. 1993. Analysis of mosquito bloodmeals by DNA profiling. **Medical and Veterinary Entomology**, 7:208-215.

Graves, P.M., B.J. Bradin, J.D. Charlwood, T.R. Burkot, J.A. Cattani, M. Ginny, J. Paino, F.D. Gibson and M.P. Alpers. 1987. Reduction in incidence and prevalence of *Plasmodium falciparum* in under-5-year-old children by permethrin impregnation of mosquito nets. **Bulletin of the World Health Organization**, 65(6):869-877.

Greenwood, B.M., A.K. Bradley, A.M. Greenwood, P. Byass, K. Jammeh, K. Marsh, S. Tulloch, F.S.J. Oldfield and R. Hayes. 1987. Mortality and morbidity from malaria among children in a rural area of The Gambia, West Africa. **Transactions of the Royal Society of Tropical Medicine and Hygiene**, 81:478-486.

Greenwood, B.M., A.M. Greenwood, A.K. Bradley, R.J. Snow, P. Byass, R.W. Hayes and A.B.H. N'Jie. 1988. Comparison of two strategies for control of malaria within a primary health care programme in The Gambia. **The Lancet**, 1-2:1121-1127.

Greenwood, B.M. 1991. Malaria chemoprophylaxis in endemic regions. In: **Malaria waiting for the vaccine**. G.A.T. Targett. John Wiley & Sons Ltd.

Greenwood, B.M., K. Marsh and R. Snow. 1991. Why do some African children develop severe malaria? **Parasitology Today**, 7:277-281.

Haynes, K.F. 1988. Sublethal effects of neurotoxic insecticides on insect behaviour. **Annual Review of Entomology**, 33:149-168.

Hemingway J., S.W. Lindsay, G.J. Small, M. Jawara and F.H. Collins. 1995. Insecticide susceptibility status in individual species of the *Anopheles gambiae* complex (Diptera:Culicidae) in an area of The Gambia where pyrethroid impregnated bednets are used extensively for malaria control. **Bulletin of Entomological Research**, 85:229-234.

Henderson, R.H. 1991. Malaria and the World Health Organization. In: **Malaria waiting for the vaccine**. G.A.T. Targett. John Wiley & Sons Ltd.

Hii, J.L.K., Y.S. Vun, K.F. Chin, R. Chua, S. Tambakau, E.S. Binisol, E. Fernandez, N. Singh and M.K.C. Chan. 1987. The influence of permethrin-impregnated bednets and mass drug administration on the incidence of *Plasmodium falciparum* malaria in children in Sabah, Malaysia. **Medical and Veterinary Entomology**, 1:397-407.

Hii, J.L.K., L. Kanai, A. Foligela, S.K.P. Kan, T.R. Burkot and R.A. Wirtz. 1993. Impact of permethrin-impregnated mosquito nets compaired with DDT house-spraying against malaria transmission by *Anopheles farauti* and *An. punctulatus* in the Solomon Island. **Medical and Veterinary Entomology**, 7(4):333-338.

Hii, J.L.K., M.H. Birley, L. Kanai, A. Foligeli and J. Wagner. 1995. Comparative effects of permethrin-impregnated bednets and DDT house spraying on survival rates and oviposition interval of *Anopheles farauti* No. 1 (Diptera:Culicidae) in Solomon Islands. **Annals of Tropical Medicine and Parasitology**, 89(5):521-529.

Hill, N, N. Rogers and M. Looker. 1989. The effects of sublethal contact with permethrin on development of *Plasmodium* oocysts in the vector *Anopheles stephensi.* **Transactions of the Royal Society of Tropical Medicine and Hygiene**, 83(3):425

Hill, N. and L. Ranford-Cartwright. 1993. *Plasmodium falciparum* and *P. yoelii* oocyst development in *Anopheles* mosquitoes exposed to sublethal doses of insecticides. **Transactions of the Royal Society of Tropical Medicine and Hygiene**, 87:379.

Hill, S.M., R.Urwin, T.F. Knapp and J.M. Crampton. 1991. Synthetic DNA probes for the identification of sibling species in the *Anopheles gambiae* complex. **Medical and Veterinary Entomology**, 5:455-463.

Hill, S.M., R. Urwin and J.M. Crampton. 1992. A simplified, non-radioactive DNA probe protocol for the field identification of insect vector specimens. **Transactions of the Royal Society of Tropical Medicine and Hygiene**, 86:213-215.

Hill, S.M. and J.M. Crampton. 1994a. Synthetic DNA probes to identify members of the *Anopheles gambiae* complex and to distinguish the two major vectors of malaria within the complex, *An. gambiae s.s.* and *An. arabiensis*. **American Journal of Tropical Medicine and Hygiene**, 50(3):312-321.

Hill, S.M. and J.M. Crampton. 1994b. **The use of synthetic DNA probes for the field identification of members of the *Anopheles gambiae* complex: A Methods Book.** Liverpool School of Tropical Medicine.

Holmes, P.R. and M.H. Birley. 1987. An improved method for survival rate analysis from time series of haematophagous dipteran populations. **Journal of Animal Ecology**, 56:427-440.

Hossain, M.I. and C.F. Curtis. 1989. Permethrin-impregnated bednets: behavioural and killing effects on mosquitoes. **Medical and Veterinary Entomology**, 3:367-376.

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

Irwin, D.M., T.D. Kocher, A.C. Wilson. 1991. Evolution of the cytochrome b gene of mammals. **Journal of Medical Evolution**, 32:128-144.

Jaenson, T.G.T., M.J. Gomes, R.C. Barreto dos Santos, V. Petrarca, D. Fortini, J. Evora and J. Crato. 1994. Control of endophagic *Anopheles* mosquitoes and human malaria in Guinea Bissau, West Africa by permethrin-treated bed nets. **Transactions of the Royal Society of Tropical Medicine and Hygiene**, 88:620-624.

Jambulingam, P., K. Gunasekharan, S.S. Sahu, P.K. Hota, B.K. Tyagi and M. Kalyanasundaram. 1989. Effect of permethrin impregnated bednets in reducing population of malaria vector *Anopheles culicifacies* in a tribal village of Orissa state (India). **Indian Journal of Medical Research**, 89:48-51.

Jana-Kara, B.R., W.A. Jihullah, B. Shahi, V. Dev, C.F. Curtis and V.P. Sharma. 1995. Deltamethrin impregnated bednets against *Anopheles minimus* transmitted malaria in Assam, India. **Journal of Tropical Medicine and Hygiene**, 98:73-83.

Karch, S., B. Garin, N. Asidi, Z. Manzambi, J.J. Salaun and J. Moudhet. 1993. Moustiquaires impregnees contre le paludisme au Zaire. **Annales de la Societe Belge de Medecine Tropicale**, 73: 37-53.

Kere, N.K. 1992. Permethrin impregnated bednets and DDT residual spraying, multicentre comparative trial in Solomon Islands. Ph.D. Thesis, University of London.

Kere, N.K. 1993. The effect of permethrin impregnated bednets on the incidence of *Plasmodium falciparum*, in children of north Guadalcanal, Solomon Islands. **Southeast Asean Journal of Tropical Medicine and Public Health**, 24(1):130-137

Le Goff G.L., V. Robert, E. Fondjo and P. Carnevale. 1992. Efficacy of insecticide impregnated bed-nets to control malaria in a rural forested area in southern Cameroon. **Memorias do Instituto Oswaldo Cruz**, 87 (Supplemento 3):355-359.

Li Zuzi, X. Jinjiang, Li Bangquan, Z. Taihua and L. Mingxin. 1987. Mosquito nets impregnated with deltamethrin against malaria vectors in China. WHO mimeographed document WHO/VBC/87.939.

Li Zuzi, Z. Mancheng, W. Yuguang, Z. Binglin, L. Guangyu and H. Hui. 1989. Trial of deltamethrin impregnated bed nets for the control of malaria transmitted by *Anopheles sinensis* and *Anopheles anthropophagus*. **American Journal of Tropical Medicine and Hygiene**, 40(4):356-359.

Lindsay, S.W., F.C. Shenton, R.W. Snow and B.M. Greenwood. 1989a. Responses of *Anopheles gambiae* complex mosquitoes to the use of untreated bednets in The Gambia. **Medical and Veterinary Entomology**, 3:253-262.

Lindsay, S.W., R.W. Snow, G.L. Broomfield, M. Semega Janneh, R.A. Wirtz and B.M. Greenwood. 1989b. Impact of permethrin-treated bednets on malaria transmission by the *Anopheles gambiae* complex in The Gambia. **Medical and Veterinary Entomology**, 3:263-271

Lindsay, S.W., J.H. Adiamah, J.E. Miller and J.R.M. Armstrong. 1991. Pyrethroid-treated bednet effects on mosquitoes of the *Anopheles gambiae* complex in The Gambia. **Medical and Veterinary Entomology**, 5:477-483.

Lindsay, S.W., J.H. Adiamah and J.R.M. Armstrong. 1992. The effect of permethrin-impregnated bednets on house entry by mosquitoes (Diptera:Culicidae) in The Gambia. **Bulletin of Entomological Research**, 82:49-55.

Lindsay, S.W., P.L. Alonso, J.R.M. Armstrong Schellenberg, K. Keita, P. Gomez, F.C. Shenton, M. Jawara and B.M. Greenwood. 1993. Impact of permethrin-impregnated bed nets on malaria vectors. A malaria control trial using insecticide-treated bed nets and targeted chemoprophylaxis in a rural area of The Gambia, West Africa. 7. Impact of permethrin-impregnated bed nets on malaria vectors. **Transactions of the Royal Society of Tropical Medicine and Hygiene**, 87(supplement 2):45-52.

Lines, J.D., J. Myamba and C.F. Curtis. 1987. Experimental hut trials of permethrin-impregnated mosquito nets and eave curtains against malaria vectors in Tanzania. **Medical and Veterinary Entomology**, 1:37-51.

Lines, J.D., C.F. Curtis, T.J. Wilkes and K.J. Njunwa. 1991. Monitoring human-biting mosquitoes (Diptera:Culicidae) in Tanzania with light-traps hung beside mosquito nets. **Bulletin of Entomological Research**, 81:77-84.

Lines, J. and J.R.M. Armstrong. 1992. For a few parasites more: Inoculum size, vector control and strain-specific immunity to malaria. **Parasitology Today**, 8(11):381-383.

Lines, J. 1996. Technological issues. Chapter 2 **Net gain:operational aspects of a new health intervention for preventing malaria**. Eds. C. Langeler, J. Cattani and D. De Savigny. IDR Publications, Canada.

Lyimo, E.O., F.H.M. Msuya, R.T. Rwegoshora, E.A. Nicholson, A.E.P. Mnzava, J.D. Lines and C.F. Curtis. 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.

Nagle, L. C. S. 1994. Experimental hut studies on the ability of bednets impregnated with permethrin to kill mosquitoes in The Gambia. M.Sc. project report. Department of Medical Parasitology, London School of Hygiene and Tropical Medicine. 54pp.

Nigatu W., C.F. Curtis and M. Lulu. 1995. Test for association of DDT resistance with inversion polymorphism in *Anopheles arabiensis* from Ethiopia. **Journal of the Mosquito Control Association**, 11(2):238-240.

Njau, R.J.A., F.W. Mosha and J.F.M. Nguma. 1993. Field trials of pyrethroid impregnated bednets in northern Tanzania. 1. Effect on malaria transmission. **Insecticide Science Applicated**, 14(5/6):575-584.

Njunwa, K. J. 1993. Studies on the productivity of *Anopheles* breeding sites in relation to adult mosquito density. PhD Thesis. Department of Medical Parasitology, London School of Hygiene and Tropical Medicine. University of London.

MacCormack, C.P. and R.W. Snow. 1986. Gambian cultural preferences in the use of insecticide-impregnated bednets. **Journal of Tropical Medicine and Hygiene**, 89: 295-302.

MacCormack, C.P. 1991. Appropriate Vector Control in Primary Health Care. In **Control of Disease Vectors in the Community**, Ed. C.F.Curtis, Wolfe Ltd. pp. 233.

Macdowall, W.G. 1994. The identification of members of the *Anopheles gambiae* complex, from The Gambia, using DNA probes. M.Sc. project report. Department of Medical Parasitology, London School of Hygiene and Tropical Medicine. 42pp.

Magesa, S.M., T.J. Wilkes, A.E.P. Mnzava, K.J. Njunwa, J. Myamba, M.D.P. Kivuyo, N. Hill, J.D. Lines and C.F. Curtis. 1991. Trial of pyrethroid impregnated bednets in an area in Tanzania holoendemic for malaria. Part II. Effects on the malaria vector population. **Acta Tropica**, 49:97-108.

Marsh, K. 1992. Malaria- a negleted disease? **Parasitology**, 104:S53-S69.

Menon, A., R.W. Snow, P. Byass, B.M.Greenwood, R.J. Hayes and A.B. N'Jie. 1990. Sustained protection against mortality and morbidity from malaria in rural Gambian children by chemoprophylaxis given by village health workers. **Transactions of the Royal Society of Tropical Medicine and Hygiene**, 84(6):768-772.

Miller, J.E. 1990. Laboratory and field studies of insecticide impregnated fibres for mosquito control. Ph.D. Thesis, University of London 336pp.

Miller, J.E., S.W. Lindsay and J.R.M. Armstrong. 1991. Experimental hut trials of bednets impregnated with synthetic pyrethroid or organophosphate insecticide for mosquito control in The Gambia. **Medical and Veterinary Entomology**, 5:465-476.

*Mnzava, A.E.P., R.T. Rwegoshora, T.J. Wilkes, M. Tanner and C.F. Curtis. 1995. *Anopheles arabiensis* and *An. gambiae* chromosomal inversion polymorphism, feeding and resting behaviour in relation to insecticide house-spraying in Tanzania. **Medical and Veterinary Entomology**, 9:316-324.

Molineux, L. and G. Gramiccia. 1980. **The Garki Project**, World Health Organization, Geneva.

Muirhead Thomson, R.C. 1947. The effects of house spraying with pyrethrum and with DDT on *Anopheles gambiae* and *A. melas* in West Africa. **Bulletin of Entomological Research**, 38:449-464

Msuya, F.H.M. and C.F. Curtis. 1991. Trial of pyrethroid impregnated bednets in an area of Tanzania holoendemic for malaria. Part 4. Effects on incidence of malaria infection. **Acta Tropica**, 49:165-171.

Onori, E., P.F. Beales and H.M. Gilles. 1993. Rationale and technique of malaria control. Chapter 10. In: **Bruce-Chwatt's Essential Malariology**. Third Edition. H.M. Gilles and D.A. Warrell Editors.

Petrarca, V. and J.C. Beier. 1992. Intraspecific chromosomal polymorphism in the *Anopheles gambiae* complex as a factor affecting malaria transmission in the Kisumu area of Kenya. **American Journal of Tropical Medicine and Hygiene**, 46(2):229-237.

Pleass, R.J., J.R.M. Armstrong, C.F. Curtis, M. Jawara and S.W. Lindsay. 1993. Comparison of permethrin treatments for bednets in The Gambia. **Bulletin of Entomological Research**, 83:133-140.

Port, G.R., P.F.L. Boreham and J.H. Bryan. 1980. The relationship of host size to feeding by mosquitoes of the *Anopheles gambiae* Giles complex (Diptera:Culicidae). **Bulletin of Entomological Research**, 70:133-144.

Port, G.R. and P.F.L. Boreham. 1982. The effect of bed nets on feeding by *Anopheles gambiae* Giles (Diptera: Culicidae). **Bulletin of Entomological Research**, 72:483-488.

Rawlings, P., C.F. Curtis, M.B. Wickramasinghe and J. Lines. 1981. The influence of age and season on dispersal and recapture of *Anopheles culicifacies* in Sri Lanka. **Ecological Entomology**, 6:307-319.

Ree, H.I. 1988. Experimental hut studies on the effect of permethrin-treated mosquito-nets against *Anopheles farauti* in The Solomon Islands. WHO mimeographed document WHO/VBC/88.963.

Renshaw, M. M.W. Service and M.H. Birley. 1994. Host finding, feeding patterns and evidence for a memorized home range of the mosquito *Aedes cantans*. **Medical and Veterinary Entomology**, 8:187-193.

Richards Jr, F.O., R.E. Klein, R. Zea Flores, S. Weller, M. Gatica, R. Zeissig and J. Sexton. 1993. Permethrin-impregnated bed nets for malaria control in northern Guatemala: Epidemiological impact and community acceptance. **American Journal of Tropical Medicine and Hygiene**, 49(4):410-418.

Robert, V. and P. Carnevale. 1991. Influence of Deltamethrin treatment of bed nets on malaria transmission in the Kou valley, Burkina Faso. **Bulletin of the World Health Organization**, 69(6):735-740.

Rozendaal, J.A. 1989. Impregnated mosquito nets and curtains for self-protection and vector control. **Tropical Diseases Bulletin**, (supplement) 86:1-41.

Rozendaal, J.A., J. Voorham, J.P.M. Van Hoof and B.F.J. Oostburg. 1989. Efficacy of mosquito nets treated with permethrin in Suriname. **Medical and Veterinary Entomology**, 3:353-365.

Rozendaal, J.A. and C.F. Curtis. 1989. Recent research on impregnated mosquito nets. **Journal of the American Mosquito Control Association**, 5(4):500-507.

Saiki, R.K., S. Scharf, F. Faloona, K.B. Mullis, G.T. Horn, H.A. Erlich, N. Arnheim. 1985. Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. **Science**, 230:1350-1354.

Samarawickrema, W.A., A.D. Parkinson, N. Kere and O. Galo. 1992. Seasonal abundance and biting behaviour of *Anopheles punctulatus* and *An.koliensis* in Malaita Province, Solomon Island, and a trial of permethrin impregnated bednets against malaria transmission. **Medical and Veterinary Entomology**, 6:371-378.

Service, M.W. 1993. **Mosquito Ecology; Field Sampling Methods**. Elsevier Applied Science. London, 583pp.

Sexton J.D. 1994. Impregnated bed nets for malaria control: Biological success and social responsability. **American Journal of Tropical Medicine and Hygiene**, 50(6)Supplement:72-81.

Sexton, J.D., T.K. Ruebush II, A.D. Brandling-Bennett, J.G. Breman, J.M. Roberts, J.S. Odera and J.B.O. Were. 1990. Permethrin-impregnated curtains and bed-nets prevent malaria in Western Kenya. **American Journal of Tropical Medicine and Hygiene**, 43(1):11-18.

Shiff, C.J., J.N. Minjas, T. Hall, R.H. Hunt, S. Lyimo and J.R. Davis. 1995. Malaria infection potential of anopheline mosquitoes sampled by light trapping indoors in coastal Tanzanian villages. **Medical and Veterinary Entomology**, 9:256-262.

Smith, A. 1965. A verandah-trap hut for studying the house-frequenting habits of mosquitoes and for assessing insecticides. I.-A description of the verandah-trap hut and of studies on the egress of *Anopheles gambiae* Giles and *Mansonia uniformis* (Theo.) from an untreated hut. **Bulletin of Entomological Research**, 56:161-165.

Smith, A. and D.J. Webley. 1968. A verandah-trap hut for studying the house-frequenting habits of mosquitoes and for assesing insecticides. III. The effect of DDT on behaviour and mortality. **Bulletin of Entomological Research**, 59:33-46.

Snow, R.W., K.M. Rowan and B.M. Greenwood. 1987a. A trial of permethrin-treated bed nets in the prevention of malaria in Gambian children. **Transactions of the Royal Society of Tropical Medicine and Hygiene**, 81:563-567.

Snow, R.W., Jawara M. and C.F. Curtis. 1987b. Observations on *Anopheles gambiae s.l.* (Diptera:Culicidae) during a trial of permethrin-treated bed nets in The Gambia. **Bulletin of Entomological Research**, 77:279-286.

Snow, R.W., K.M. Rowan, S.W. Lindsay and B.M. Greenwood. 1988a. A trial of bed nets (mosquito nets) as a malaria control strategy in a rural area of The Gambia, West Africa. **Transactions of the Royal Society of Tropical Medicine and Hygiene**, 82:212-215.

Snow, R.W., S.W. Lindsay, R.J. Hayes and B.M. Greenwood. 1988b. Permethrin-treated bed nets (mosquito nets) prevent malaria in Gambian children. **Transactions of the Royal Society of Tropical Medicine and Hygiene**, 82:838-842.

Somboon, P. 1993. Forest malaria vectors in northwest Thailand and a trial of control with pyrethroid-treated bednets. Ph.D. Thesis, University of London 249pp.

Somboon, P., J. Lines, A. Aramrattana, U. Chitprarop, S. Prajakwong and C. Khamboonruang. 1995. Entomological evaluation of community-wide use of lambdacyhalothrin-impregnated bed nets against malaria in a border area of north-west Thailand. **Transactions of the Royal Society of Tropical Medicine and Hygiene**, 89:248-254.

Stich, A.H.R., C.A. Maxwell, A.A. Haji, D.M. Haji, A.Y. Machano, J.K. Mussa, A. Matteelli, H. Haji and C.F. Curtis. 1994. Insecticide-impregnated bed nets reduce malaria transmission in rural Zanzibar. **Transaction of The Royal Society of Tropical Medicine and Hygiene**, 88:150-154.

Tami-Hirsch, A. 1994. Use of PCR to identify the individual host on whom mosquitoes in Gambian experimental huts had fed. M.Sc. project report. Department of Medical Parasitology, London School of Hygiene and Tropical Medicine. 40pp.

Taylor, B. 1975. Changes in the feeding behaviour of a malaria vector *Anopheles farauti Lav.*, following use of DDT as a residual spray in houses in The British Solomon Islands Protectorate. **Transactions of the Royal Entomological Society of London**, 127:277-292.

Thomson, M.C., U. D'Alessandro, U. Bennett, S. Connor, P. Langerock, M.Jawara, J. Todd and B.M. Greenwood. 1994. Malaria prevalence is inversely related to vector density in The Gambia, West Africa. **Transactions of the Royal Society of Tropical Medicine and Hygiene**, 88:609-720.

Thomson, M.C., J.H. Adiamah, S.J. Connor, M. Jawara, S. Bennett, U. D'Alessandro, M. Quinones, P. Langerock and B.M. Greenwood. 1995a. Entomological evaluation of the Gambia's National Impregnated Bednet Programme. **Annals of Tropical Medicine and Parasitology**, 89(3):229-242.

Thomson, M.C., S. Connor, M. L. Quinones, M. Jawara, J. Todd and B. M. Greenwood. 1995b. Measuring movement of the malaria vector *Anopheles gambiae s.l.* between villages in The Gambia. **Medical and Veterinary Entomology**, 9(4):413-419.

Vij, R. 1995. DNA probes and cytogenetics for the identification of *Anopheles gambiae* sibling species. PhD Thesis, Liverpool School of Tropical Medicine.

Vulule, J.M., R.F. Beach, F.K. Atieli, J.M. Roberts, D.L. Mount and R.W. Mwangi. 1994. Reduced susceptibility of *Anopheles gambiae* to permethrin associated with the use of permethrin-impregnated bed nets and curtains in Kenya. **Medical and Veterinary Entomology**, 8:71-75.

Vulule, J.M., R.F. Beach, F.K. Atieli, D.L. Mount, J.M. Roberts and R.W. Mwangi. 1996. Long-term use of permethrin-impregnated nets does not increase *Anopheles gambiae* permethrin tolerance. **Medical and Veterinary Entomology**, 10:71-79.

Vythilingam, I., L.C. Foo, G.L. Chiang, S.T. Chan, K.L. Eng, S. Mahadevan, J.W. Mak and K. Inder Singh. 1995. The impact of permethrin impregnated bednets on the malaria vector *Anopheles maculatus* (Diptera:Culicidae) in aboriginal villages of Pos Betau Pahang, Malaysia. **Southeast Asian Journal of Tropical Medicine and Public Health**, 26(2):354-358.

White, G.B. 1974. *Anopheles gambiae* complex and disease transmission in Africa. **Transactions of the Royal Society of Tropical Medicine and Hygiene**, 68(4):278-301.

WHO Technical Report Series 857. 1995. Vector control for malaria and other mosquito-borne diseases. World Health Organization. Geneva. 94pp.

Wirtz, R.A., Burkot, T.R., Graves, P.M. and R.G. Andre. 1987. Field evaluation of enzyme-linked immunosorbent assays for *Plasmodium falciparum* and *Plasmodium vivax* sporozoites in mosquitoes, (Diptera:Culicidae) from Papua New Guinea. **Journal of Medical Entomology,** 24:433-437.

Zebra, E. 1988. Insecticidal activity of pyrethroids on insects of medical importance. **Parasitology Today,** 4:53-57.

Zoulani, A., P. Carnevale and L. Penchenier. 1994. Influence des moustiquaires impregnees de deltamethrine sur le cycle d'agressivite d'*Anopheles gambiae* a Djoumouna, Congo. **Annales de la Societe Belge de Medecine Tropicale**, 74:83-91.

APPENDIX 1. List of recaptures in the Mark-release recapture experiment in 1992. C:Compound number; R:Room number; Gon.Rec.: Gonothrophic stage when recaptured; Gon.Rel.: Gonothrophic stage when released.

No.	Recapture Village	Date of Recapture	Meth.	C	R	Gon. Rec.	Colour	Release Village	Gon. Rel.	Most probable date of release
1	Saruja	07/08/92	PSC	25	2	FF	Green	Saruja	UF	06/08/92
2	Saruja	07/08/92	PSC	25	2	FF	Green	Saruja	UF	06/08/92
3	Saruja	07/08/92	PSC	25	2	FF	Green	Saruja	UF	06/08/92
4	Saruja	07/08/92	PSC	25	2	FF	Green	Saruja	UF	06/08/92
5	Saruja	07/08/92	PSC	25	2	FF	Green	Saruja	UF	06/08/92
6	Saruja	07/08/92	PSC	25	2	FF	Green	Saruja	UF	06/08/92
7	Saruja	07/08/92	PSC	25	2	GR	Green	Saruja	UF	06/08/92
8	Saruja	07/08/92	PSC	25	2	GR	Green	Saruja	UF	06/08/92
9	Wellingara	09/08/92	PIT			UF	Orange	Wellingar	UF	
10	Brikamaba	12/08/92	ETC	11	3	PF	Blue	Brikamab	UF	
11	Wellingara	12/08/92	ETC	8	1	PF	Red	Wellingar	UF	
12	Wellingara	13/08/92	ETC	8		UF	Orange	Wellingar	UF	10/08/92
13	Brikamaba	13/08/92	ETC	118	2	SG	Blue	Brikamab	UF	10/08/92
14	Wellingara	20/08/92	PSC	2	2	FF	Orange	Wellingar	UF	
15	Brikamaba	21/08/92	ETC	251	1	FF	Magenta	Brikamab	UF	18/08/92
16	Brikamaba	21/08/92	ETC	236	4	FF	Magenta	Brikamab	UF	18/08/92
17	Brikamaba	21/08/92	ETC	251	1	FF	Magenta	Brikamab	UF	18/08/92
18	Brikamaba	21/08/92	ETC	213	1	FF	Magenta	Brikamab	UF	18/08/92
19	Saruja	22/08/92	ETC	17	5	UF	Green	Saruja	UF	
20	Saruja	23/08/92	PSC	21	2	FF	Green	Saruja	UF	
21	Wellingara	26/08/92	BNC			FF	Orange	Wellingar	UF	
22	Brikamaba	26/08/92	PSC	248	2	FF	Blue	Brikamab	UF	
23	Brikamaba	26/08/92	PSC	248	2	FF	Blue	Brikamab	UF	
24	Wellingara	27/08/92	BNC	1		PF	Orange	Wellingar	UF	
25	Brikamaba	28/08/92	PSC	113	1	GR	Blue	Brikamab	UF	
26	Saruja	23/08/92	PSC	21	2	FF	Green	Saruja	UF	
27	Brikamaba	04/09/92	ETC	239	3	FF	Blue	Brikamab	UF	
28	Brikamaba	04/09/92	ETC	239	3	FF	Blue	Brikamab	UF	
29	Brikamaba	04/09/92	ETC	239	3	FF	Blue	Brikamab	UF	
30	Brikamaba	04/09/92	ETC	216	1	GR	Blue	Brikamab	UF	
31	Brikamaba	04/09/92	ETC	216	1	PF	Blue	Brikamab	UF	
32	Wellingara	04/09/92	BNC	13		FF	Orange	Wellingar	UF	
33	Wellingara	05/09/92	ETC	27	1	UF	Orange	Wellingar	UF	
34	Wellingara	05/09/92	BNC	13		SG	Orange	Wellingar	UF	
35	Saruja	05/09/92	BNC	10		FF	Purple	Saruja	FF	01/09/92
36	Wellingara	06/09/92	ETC	7	1	UF	Orange	Wellingar	UF	
37	Wellingara	06/09/92	ETC	27	1	UF	Orange	Wellingar	UF	
38	Wellingara	06/09/92	PSC	30	2	GR	Magenta	Wellingar	FF	05/09/92
39	Saruja	06/09/92	PSC	70	2	GR	Turquoi	Saruja(W)	FF	05/09/92
40	Brikamaba	06/09/92	ETC	224	1	FF	Blue	Brikamab	UF	
41	Brikamaba	06/09/92	ETC	224	1	FF	Blue	Brikamab	UF	
42	Brikamaba	06/09/92	ETC	239	3	FF	Blue	Brikamab	UF	
43	Brikamaba	06/09/92	ETC	239	3	FF	Blue	Brikamab	UF	

APPENDIX 1. Continued.

No.	Recapture Village	Date of Recapture	Meth.	C	R	Gon. Rec.	Colour	Release Village	Gon. Rel.	Most probable date of release
44	Brikamaba	06/09/92	ETC	239	3	FF	Blue	Brikamab	UF	
45	B.nding	07/09/92	BNC			GR	Orange	Wellingar	UF	
46	Saruja	07/09/92	ETC	76	2	UF	Orange	Wellingar	UF	
47	Wellingara	07/09/92	ETC	27	1	UF	Orange	Wellingar	UF	
48	Wellingara	07/09/92	BNC	28		FF	Magenta	Wellin(S)	FF	05/09/92
49	Wellingara	08/09/92	BNC	12		FF	Orange	Wellingar	UF	
50	Wellingara	10/09/92	ETC	27	1	UF	Purple	Saruja	FF	07/09/92
51	Wellingara	10/09/92	BNC	14	1	FF	Orange	Wellingar	UF	
52	Wellingara	10/09/92	BNC	28	1	FF	Orange	Wellingar	UF	
53	Wellingara	10/09/92	ETC	32	1	UF	Orange	Wellingar	UF	
54	Wellingara	10/09/92	PSC	8	1	FF	Orange	Wellingar	UF	
55	Wellingara	11/09/92	BNC	29		FF	Orange	Wellingar	UF	
56	Wellingara	11/09/92	ETC	32	1	UF	Orange	Wellingar	UF	
57	Wellingara	11/09/92	ETC	27	1	UF	Orange	Wellingar	UF	
58	Saruja	12/09/92	PSC	92	1	UF	Red	Wellingar	FF	10/09/92
59	Saruja	12/09/92	ETC	70	1	GR	Turquoi	Saruja(W)	FF	11/09/92
60	Saruja	12/09/92	PSC	67	1	GR	Turquoi	Saruja(W)	FF	11/09/92
61	Saruja	12/09/92	PSC	67	1	FF	Turquoi	Saruja(W)	FF	08/09/92 or 110992
62	Saruja	12/09/92	BNC	21	1	FF	Purple	Saruja	FF	10/09/92
63	Wellingara	12/09/92	ETC	24	3	UF	Red	Wellingar	FF	10/09/92
64	Wellingara	12/09/92	PSC	5	1	GR	Magenta	Wellin(S)	FF	11/09/92
65	Wellingara	12/09/92	BNC	17	1	FF	Orange	Wellingar	UF	
66	Wellingara	12/09/92	PSC	30	1	FF	Orange	Wellingar	UF	
67	Wellingara	12/09/92	BNC	27	1	FF	Orange	Wellingar	UF	
68	Wellingara	12/09/92	ETC	27		UF	Orange	Wellingar	UF	
69	Wellingara	12/09/92	ETC	27		UF	Orange	Wellingar	UF	
70	Wellingara	12/09/92	ETC	27		UF	Orange	Wellingar	UF	
71	Wellingara	12/09/92	ETC	7		UF	Orange	Wellingar	UF	
72	Wellingara	12/09/92	PSC	5		SG	Orange	Wellingar	UF	
73	Wellingara	12/09/92	ETC	24	3	UF	Orange	Wellingar	UF	
74	Wellingara	12/09/92	BNC	12		FF	Orange	Wellingar	UF	
75	Brikamaba	12/09/92	ETC	224	1	FF	Blue	Brikamab	UF	
76	Brikamaba	12/09/92	ETC	224	1	FF	Blue	Brikamab	UF	
77	Brikamaba	12/09/92	ETC	224	1	FF	Blue	Brikamab	UF	
78	Brikamaba	12/09/92	ETC	224	1	FF	Blue	Brikamab	UF	
79	Wellingara	13/09/92	ETC	7	1	GR	Red	Wellingar	FF	10/09/92
80	Wellingara	13/09/92	ETC	30	1	UF	Magenta	Wellin(S)	FF	11/09/92
81	Wellingara	13/09/92	BNC	13	1	FF	Magenta	Wellin(S)	FF	11/09/92
82	Wellingara	13/09/92	BNC	17	1	FF	Orange	Wellingar	UF	
83	Saruja	13/09/92	ETC	92	1	FF	Orange	Wellingar	UF	
84	Saruja	13/09/92	PIT	1		UF	Magenta	Wellin(S)	FF	11/09/92
85	Saruja	13/09/92	BNC	10		FF	Turquoi	Saruja(W)	FF	11/09/92
86	Saruja	13/09/92	PSC	69	3	FF	Magenta	Wellin(S)	FF	11/09/92
87	Brikamaba	13/09/92	ETC	118	2	GR	Blue	Brikamab	UF	
88	Brikamaba	13/09/92	ETC	118	2	FF	Blue	Brikamab	UF	
89	Wellingara	14/09/92	ETC	32	1	UF	Magenta	Wellin(S)	FF	11/09/92
90	Wellingara	14/09/92	ETC	30	1	UF	Orange	Wellingar	UF	

APPENDIX 1. Continued.

No.	Recapture Village	Date of Recapture	Meth.	C	R	Gon. Rec.	Colour	Release Village	Gon. Rel.	Most probable date of release
91	Wellingara	14/09/92	PSC	10	1	FF	Orange	Wellingar	UF	
92	Wellingara	15/09/92	PSC	36	1	FF	Orange	Wellingar	UF	
93	Wellingara	15/09/92	BNC	18		FF	Orange	Wellingar	UF	
94	Wellingara	15/09/92	BNC	19		FF	Lime	Wellingar	FF	12/09/92
95	Wellingara	15/09/92	BNC	18		FF	Magenta	Wellin(S)	FF	11/09/92
96	Wellingara	15/09/92	BNC	23		FF	Red	Wellingar	FF	13/09/92
97	Saruja	15/09/92	ETC	54	1	UF	Purple	Saruja	FF	13/09/92
98	Saruja	15/09/92	BNC	14		FF	Red	Wellingar	FF	13/09/92
99	Saruja	16/09/92	ETC	92	1	UF	Turquoi	Saruja(W)	FF	14/09/92
100	Wellingara	16/09/92	ETC	36	1	SG	Orange	Wellingar	UF	
101	Wellingara	16/09/92	ETC	32	1	GR	Orange	Wellingar	UF	
102	Wellingara	16/09/92	ETC	27	1	SG	Green	Saruja	UF	
103	Wellingara	16/09/92	ETC	7	1	UF	Green	Saruja	UF	
104	Wellingara	16/09/92	BNC	13		FF	Turquoi	Saruja(W)	FF	14/09/92
105	Wellingara	16/09/92	BNC	17		FF	Turquoi	Saruja(W)	FF	14/09/92
106	Wellingara	16/09/92	BNC	17		FF	Red	Wellingar	FF	13/09/92
107	Saruja	16/09/92	PIT	3		FF	Red	Wellingar	FF	13/09/92
108	Brikamaba	16/09/92	ETC	118	2	FF	Blue	Brikamab	UF	
109	Brikamaba	16/09/92	ETC	118	2	FF	Blue	Brikamab	UF	
110	Brikamaba	16/09/92	ETC	118	2	FF	Blue	Brikamab	UF	
111	Brikamaba	16/09/92	ETC	118	2	FF	Blue	Brikamab	UF	
112	Brikamaba	16/09/92	ETC	118	2	GR	Blue	Brikamab	UF	
113	Brikamaba	16/09/92	ETC	118	2	FF	Turquoi	Saruja(W)	FF	14/09/92
114	Wellingara	17/09/92	ETC	27	1	UF	Magenta	Wellin(S)	FF	14/09/92
115	Saruja	17/09/92	BNC	57		SG	Purple	Saruja	FF	13/09/92
116	Wellingara	17/09/92	BNC	14		SG	Orange	Wellingar	UF	15/09/92
117	Wellingara	18/09/92	BNC	32		FF	Turquoi	Saruja(W)	FF	14/09/92
118	Wellingara	18/09/92	ETC	7	1	UF	Red	Wellingar	FF	13/09/92
119	Brikamaba	18/09/92	ETC	118	2	GR	Blue	Brikamab	UF	15/09/92
120	Brikamaba	18/09/92	ETC	118	2	FF	Blue	Brikamab	UF	15/09/92
121	Brikamaba	18/09/92	ETC	224	1	FF	Blue	Brikamab	UF	15/09/92
122	Brikamaba	18/09/92	ETC	224	1	FF	Blue	Brikamab	UF	15/09/92
123	Brikamaba	18/09/92	ETC	118	2	GR	Blue	Brikamab	UF	15/09/92
124	Brikamaba	18/09/92	ETC	224	1	FF	Blue	Brikamab	UF	15/09/92
125	Brikamaba	18/09/92	ETC	224	1	FF	Blue	Brikamab	UF	15/09/92
126	Brikamaba	18/09/92	ETC	224	1	FF	Blue	Brikamab	UF	15/09/92
127	Brikamaba	18/09/92	ETC	224	1	FF	Blue	Brikamab	UF	15/09/92
128	Saruja	18/09/92	PSC	6	3	FF	Turquoi	Saruja(W)	FF	14/09/92
129	Saruja	30/09/92	ETC	25	1	SG	Turquoi	Saruja(W)	FF	29/09/92
130	Saruja	01/10/92	ETC	70	3	UF	Green	Saruja	UF	28/09/92
131	Brikamaba	01/10/92	ETC	118	2	FF	Blue	Brikamab	UF	28/09/92
132	Brikamaba	01/10/92	ETC	118	2	SG	Blue	Brikamab	UF	28/09/92
133	Brikamaba	01/10/92	ETC	118	2	FF	Blue	Brikamab	UF	28/09/92
134	Saruja	02/10/92	ETC	12	1	UF	Peach	Saruja	FF	30/09/92
135	Saruja	02/10/92	BNC	7		FF	Turquoi	Saruja(W)	FF	29/09/92
136	Brikamaba	03/10/92	ETC	46	9	FF	Turquoi	Saruja(W)	FF	29/09/92

APPENDIX 1. Continued.

No.	Recapture Village	Date of Recapture	Meth.	C	R	Gon. Rec.	Colour	Release Village	Gon. Rel.	Most probable date of release
137	Wellingara	03/10/92	BNC	18		FF	Red	Wellingar	FF	01/10/92
138	Wellingara	03/10/92	BNC	12		FF	Magenta	Wellingar	FF	29/09/92
139	Wellingara	04/10/92	ETC	30	1	UF	Darkgre	Saruja	UF	03/10/92 or30/09/92
140	Saruja	04/10/92	BNC	10		FF	Turquoi	Saruja(W)	FF	02/10/92
141	Saruja	04/10/92	BNC	11		FF	Turquoi	Saruja(W)	FF	02/10/92
142	Brikamaba	05/10/92	PIT	125		GR	Darkblu	Brikamab	UF	02/10/92
143	Brikamaba	05/10/92	ETC	118	2	GR	Darkblu	Brikamab	UF	02/10/92
144	Brikamaba	05/10/92	ETC	118	2	UF	Darkblu	Brikamab	UF	02/10/92
145	Brikamaba	07/10/92	ETC	14	3	UF	Lightblu	Brikamab	UF	06/10/92 or03/10/92
146	Brikamaba	07/10/92	ETC	14	3	UF	Lightblu	Brikamab	UF	06/10/92 or03/10/92
147	Brikamaba	07/10/92	PIT	125		FF	Blue	Brikamab	UF	04/10/92 or01/10/92
148	Brikamaba	07/10/92	ETC	216	1	FF	Lightblu	Brikamab	UF	06/10/92
149	Brikamaba	08/10/92	ETC	10	1	FF	Lightblu	Brikamab	UF	06/10/92
150	Saruja	08/10/92	ETC	72	1	FF	Purple	Saruja	FF	04/10/92
151	Wellingara	08/10/92	ETC	27	1	UF	Orange	Wellingar	UF	07/10/92
152	Wellingara	08/10/92	ETC	12	3	UF	Orange	Wellingar	UF	07/10/92
153	Wellingara	09/10/92	ETC	37	1	UF	Darkgre	Saruja	UF	06/10/92
154	Wellingara	09/10/92	BNC	12	3	SG	Yellowi	Wellingar	UF	06/10/92
155	Wellingara	10/10/92	BNC	12		FF	Darkblu	Brikamab	UF	05/10/92 or08/10/92
156	Brikamaba	10/10/92	ETC	216	1	SG	Darkblu	Brikamab	UF	05/10/92 or08/10/92
157	Brikamaba	10/10/92	ETC	216	1	GR	Darkblu	Brikamab	UF	05/10/92 or08/10/92
158	Brikamaba	10/10/92	ETC	216	1	GR	Darkblu	Brikamab	UF	05/10/92 or08/10/92
159	Brikamaba	10/10/92	ETC	224	1	GR	Blue	Brikamab	UF	07/10/92
160	Brikamaba	10/10/92	ETC	224	1	FF	Blue	Brikamab	UF	04/10/92
161	Wellingara	11/10/92	ETC	14	3	PF	Orange	Wellingar	UF	10/10/92
162	Wellingara	11/10/92	ETC	13	1	UF	Orange	Wellingar	UF	07/10/92 or10/10/92
163	Saruja	12/10/92	BNC	27		FF	Darkgre	Saruja	UF	09/10/92
164	Saruja	12/10/92	PSC	39	4	PF	Turquoi	Saruja	FF	08/10/92
165	Saruja	13/10/92	ETC	76	2	GR	Orange	Wellingar	UF	10/10/92
166	Brikamaba	14/10/92	ETC	46	9	FF	Turquoi	Saruja	FF	08/10/92 or11/10/92
167	Wellingara	14/10/92	ETC	30	1	SG	Orange	Wellingar	UF	10/10/92
168	Saruja	14/10/92	ETC	92	1	SG	Peach	Saruja	FF	09/10/92
169	Brikamaba	15/10/92	ETC	231	2	PF	Blue	Brikamab	UF	13/10/92
170	Brikamaba	15/10/92	ETC	239	2	FF	Darkblu	Brikamab	UF	14/10/92
171	Brikamaba	15/10/92	ETC	239	2	FF	Darkblu	Brikamab	UF	14/10/92
172	Wellingara	15/10/92	BNC	23		FF	Lime	Wellingar	FF	12/10/92
173	Wellingara	15/10/92	ETC	27		UF	Red	Wellingar	FF	13/10/92
174	Wellingara	15/10/92	BNC	12		SG	Yellowis	Wellingar	UF	12/10/92
175	Wellingara	15/10/92	ETC	7		UF	Yellowis	Wellingar	UF	12/10/92
176	Wellingara	16/10/92	ETC	27		UF	Magenta	Wellin(S)	FF	14/10/92
177	Saruja	15/10/92	PSC	59	7	FF	Magenta	Wellin(S)	FF	11/10/92
178	Saruja	15/10/92	PSC	50	2	GR	Turquoi	Saruja(W)	FF	14/10/92
179	Brikamaba	16/10/92	ETC	10	1	UF	Blue	Brikamab	UF	13/10/92
180	Brikamaba	16/10/92	ETC	82	2	FF	Darkblu	Brikamab	UF	14/10/92
181	Brikamaba	16/10/92	ETC	82	2	FF	Darkblu	Brikamab	UF	14/10/92
182	Brikamaba	16/10/92	ETC	118	2	FF	Lightblu	Brikamab	UF	12/10/92
183	Wellingara	16/10/92	BNC	23		SG	Magenta	Wellin(S)	FF	11/10/92

APPENDIX 2. List of recaptures in the Mark-release recapture experiment in 1993. T:Treatment (T:Treated, U:Untreated); C:Compound number; R:Room number; LE:Considered as having layed eggs (Yes or No); DA:Days after release.

No.	Recapt. Village	T	Date of Recapture	Method	C	R	Gon.	Colour	Release Village	T	Date of Release	LE	DA
1	Madina	T	07/10/93	PSC	18	3	GR	Red	Madina	T	06/10/93	N	1
2	Jakoto	U	08/10/93	ETC2			UF	Blue	Madina	T	07/10/93	N	1
3	Jakoto	U	08/10/93	ETC2			UF	Blue	Madina	T	07/10/93	N	1
4	Jakoto	U	08/10/93	PSC	9	2	FF	Green	Jakoto	U	06/10/93	Y	2
5	Madina	T	09/10/93	ETC	3		UF	Green	Jakoto	U	06/10/93	Y	3
6	Jakoto	U	09/10/93	BNC	12		FF	Magenta	Jakoto	U	05/10/93	Y	4
7	Modikay	U	09/10/93	BNC			FF	Lightblue	Jakoto	U	07/10/93	Y	2
8	Jakoto	U	09/10/93	PSC	10	1	GR	Green	Jakoto	U	06/10/93	Y	3
9	Jakoto	U	09/10/93	PSC	9		SG	Yellowish	Jakoto	U	08/10/93	N	1
10	Jakoto	U	09/10/93	PSC	10	2	GR	Yellowish	Jakoto	U	08/10/93	N	1
11	Madina	T	10/10/93	ETC2	5	5	UF	Magenta	Jakoto	U	05/10/93	Y	5
12	Jakoto	U	10/10/93	PSC	2	4	FF	Yellowish	Jakoto	U	08/10/93	Y	2
13	Jakoto	U	10/10/93	PSC	2	4	GR	Yellowish	Jakoto	U	08/10/93	Y	2
14	Jakoto	U	10/10/93	PSC	11	1	FF	Yellowish	Jakoto	U	08/10/93	Y	2
15	Jakoto	U	10/10/93	PSC	2	4	GR	Green	Jakoto	U	06/10/93	Y	4
16	Jakoto	U	10/10/93	PSC	4	2	GR	Lightblue	Jakoto	U	07/10/93	Y	3
17	Jakoto	U	10/10/93	PSC	4	2	GR	Lightblue	Jakoto	U	07/10/93	Y	3
18	Jakoto	U	10/10/93	PSC	14	5	GR	Darkblue	Jakoto	U	09/10/93	N	1
19	Jakoto	U	11/10/93	PSC	9	2	GR	Green	Jakoto	U	06/10/93	Y	5
20	Jakoto	U	11/10/93	PSC	2	2	FF	Green	Jakoto	U	06/10/93	Y	5
21	Jakoto	U	11/10/93	PSC	16	2	SG	Darkgr	Jakoto	U	10/10/93	N	1
22	Jakoto	U	11/10/93	PSC	10	2	FF	Lightblue	Jakoto	U	07/10/93	Y	4
23	BKFula	U	11/10/93	BNC			FF	Lightblue	Jakoto	U	07/10/93	Y	4
24	BKFula	U	11/10/93	BNC			FF	Lightblue	Jakoto	U	07/10/93	Y	4
25	Madina	T	11/10/93	PSC	17	6	GR	Turquoise	Madina	T	10/10/93	N	1
26	Madina	T	11/10/93	PSC	3	1	FF	Purple	Madina	T	09/10/93	Y	2
27	Jakoto	U	12/10/93	BNC	11		UF	Lightblue	Jakoto	U	07/10/93	Y	5
28	Jakoto	U	12/10/93	BNC	9		FF	Darkgr	Jakoto	U	10/10/93	Y	2
29	Jakoto	U	12/10/93	PSC	8	2	GR	Yellowish	Jakoto	U	08/10/93	Y	4
30	BKFula	U	12/10/93	BNC			FF	Darkgr	Jakoto	U	10/10/93	Y	2
31	BKFula	U	12/10/93	BNC			FF	Turquoise	Madina	T	10/10/93	Y	2
32	Barokun	U	13/10/93	BNC			FF	Purple	Madina	T	09/10/93	Y	4
33	Jakoto	U	13/10/93	PSC	4	2	FF	Purple	Madina	T	09/10/93	Y	4
34	Jakoto	U	13/10/93	ETC2			UF	Darkgr	Jakoto	U	10/10/93	Y	3
35	Jakoto	U	13/10/93	ETC2			UF	Yellowish	Jakoto	U	08/10/93	Y	5
36	Jakoto	U	13/10/93	BNC	2		GR	Darkgr	Jakoto	U	10/10/93	Y	3
37	BKFula	U	14/10/93	BNC			FF	Darkgr	Jakoto	U	10/10/93	Y	4
38	Jakoto	U	14/10/93	PSC	17	1	FF	Darkgr	Jakoto	U	10/10/93	Y	4
39	Ebria	U	15/10/93	BNC			FF	Darkblue	Jakoto	U	09/10/93	Y	6
40	Jakoto	T	21/10/93	PSC	17	2	GR	Magenta	Jakoto	T	20/10/93	N	1
41	Madina	U	21/10/93	PSC	7	1	FF	Brightgr	Madina	U	19/10/93	Y	2
42	Madina	U	21/10/93	PSC	6	1	GR	Red	Madina	U	20/10/93	N	1
43	Madina	U	21/10/93	BNC	7		FF	Green	Jakoto	T	19/10/93	Y	2

APPENDIX 2. Continued.

No.	Recapt. Village	T	Date of Recapture	Method	C	R	Gon.	Colour	Release Village	T	Date of Release	LE	DA
44	Madina	U	21/10/93	BNC	10		FF	Green	Jakoto	T	19/10/93	Y	2
45	Madina	U	22/10/93	BNC	7		FF	Red	Madina	U	20/10/93	Y	2
46	Madina	U	22/10/93	BNC	18		FF	Red	Madina	U	20/10/93	Y	2
47	Madina	U	22/10/93	BNC	18		SG	Blue	Madina	U	21/10/93	N	1
48	Madina	U	22/10/93	PSC	25	1	FF	Darkgr	Jakoto	U	10/10/93	Y	12
49	Madina	U	22/10/93	PSC	5	2	FF	Red	Madina	U	20/10/93	Y	2
50	Madina	U	22/10/93	PSC	23	7	GR	Blue	Madina	U	21/10/93	N	1
51	Madina	U	22/10/93	PSC	22	3	GR	Blue	Madina	U	21/10/93	N	1
52	Madina	U	22/10/93	PSC	28	1	GR	Brightgr	Madina	U	19/10/93	Y	3
53	Madina	U	22/10/93	PSC	5	2	FF	Green	Jakoto	T	19/10/93	Y	3
54	Madina	U	22/10/93	PSC	20	1	FF	Magenta	Jakoto	T	20/10/93	Y	2
55	Madina	U	22/10/93	PSC	5	2	GR	Blue	Madina	U	21/10/93	N	1
56	Madina	U	22/10/93	PSC	24	6	GR	Red	Madina	U	20/10/93	N	2
57	Madina	U	22/10/93	PSC	5	3	FF	Red	Madina	U	20/10/93	Y	2
58	Jakoto	T	23/10/93	PSC	9	4	FF	Lightblue	Jakoto	T	21/10/93	Y	2
59	Madina	U	23/10/93	ETC	6	3	UF	Brightgr	Madina	U	19/10/93	Y	4
60	Madina	U	23/10/93	PSC	18	3	FF	Red	Madina	U	20/10/93	Y	3
61	Madina	U	23/10/93	PSC	17	1	GR	Magenta	Jakoto	T	20/10/93	Y	3
62	Madina	U	23/10/93	PSC	7	3	GR	Orange	Madina	U	22/10/93	N	1
63	Madina	U	23/10/93	PSC	17	1	FF	Blue	Madina	U	21/10/93	Y	2
64	Madina	U	23/10/93	PSC	18	3	FF	Red	Madina	U	20/10/93	Y	3
65	Madina	U	23/10/93	BNC	18		FF	Blue	Madina	U	21/10/93	Y	2
66	Madina	U	23/10/93	BNC	18		FF	Brightgr	Madina	U	19/10/93	Y	4
67	Jakoto	T	24/10/93	PSC	9	9	FF	Red	Madina	U	20/10/92	Y	4
68	Jakoto	T	24/10/93	PSC	1		FF	Lightblue	Jakoto	T	21/10/93	Y	3
69	Jakoto	T	24/10/93	PSC	2		FF	Yellowish	Jakoto	T	22/10/93	Y	2
70	Madina	U	24/10/93	PSC	10	2	GR	Blue	Madina	U	21/10/93	Y	3
71	Madina	U	24/10/93	PSC	21	3	FF	Orange	Madina	U	22/10/93	Y	2
72	Madina	U	24/10/93	PSC	15	2	GR	Blue	Madina	U	21/10/93	Y	3
73	Madina	U	24/10/93	PSC	5	4	FF	Orange	Madina	U	22/10/93	Y	2
74	Madina	U	24/10/93	PSC	5	4	FF	Magenta	Jakoto	T	20/10/93	Y	4
75	Madina	U	24/10/93	PSC	11	2	GR	Red	Madina	U	20/10/93	Y	4
76	Madina	U	24/10/93	PSC	26	2	GR	Orange	Madina	U	22/10/93	Y	2
77	Madina	U	24/10/93	PSC	21	3	GR	Magenta	Jakoto	T	20/10/93	Y	4
78	Madina	U	24/10/93	BNC	9		FF	Lightblue	Jakoto	T	21/10/93	Y	3
79	Madina	U	24/10/93	BNC	22		FF	Green	Jakoto	T	19/10/93	Y	5
80	Madina	U	24/10/93	BNC	5		FF	Yellowish	Jakoto	T	22/10/93	Y	2
81	Madina	U	24/10/93	BNC	3		FF	Orange	Madina	U	22/10/93	Y	2
82	Madina	U	24/10/93	BNC	7		GR	Lightblue	Jakoto	T	21/10/93	Y	3
83	Jakoto	T	25/10/93	PIT 3			GR	Darkgr	Jakoto	T	24/10/93	N	1
84	Jakoto	T	25/10/93	PSC	M		GR	Red	Madina	U	20/10/93	Y	5
85	Jakoto	T	25/10/93	PSC	M		FF	Darkblue	Jakoto	T	23/10/93	Y	2
86	Jakoto	T	25/10/93	PSC	M		FF	Darkgr	Jakoto	T	24/10/93	N	1
87	Jakoto	T	25/10/93	PSC	M		GR	Darkgr	Jakoto	T	24/10/93	N	1
88	Jakoto	T	25/10/93	PSC	5	4	GR	Darkgr	Jakoto	T	24/10/93	N	1
89	Jakoto	T	25/10/93	PSC	7	3	GR	Yellowish	Jakoto	T	22/10/93	Y	3

APPENDIX 2. Continued.

No.	Recapt. Village	T	Date of Recapture	Method	C	R	Gon.	Colour	Release Village	T	Date of Release	LE	DA
90	Madina	U	25/10/93	BNC	28		FF	Lightblue	Jakoto	T	21/10/93	Y	4
91	Madina	U	25/10/93	BNC	28		FF	Magenta	Jakoto	T	20/10/93	Y	5
92	Madina	U	25/10/93	BNC	16		FF	Magenta	Jakoto	T	20/10/93	Y	5
93	Madina	U	25/10/93	BNC	18		SG	Turquoise	Madina	U	24/10/93	N	1
94	Madina	U	25/10/93	BNC	28		SG	Turquoise	Madina	U	24/10/93	N	1
95	Madina	U	25/10/95	PSC	6	1	SG	Turquoise	Madina	U	24/10/93	N	1
96	Madina	U	25/10/93	PSC	19	2	SG	Turquoise	Madina	U	24/10/93	N	1
97	Madina	U	25/10/93	PSC	3	1	GR	Turquoise	Madina	U	24/10/93	N	1
98	Madina	U	25/10/93	PSC	28	2	GR	Magenta	Jakoto	T	20/10/93	Y	5
99	Madina	U	25/10/93	PSC	6	2	GR	Turquoise	Madina	U	24/10/93	N	1
100	Madina	U	25/10/93	PSC	18	5	GR	Turquoise	Madina	U	24/10/93	N	1
101	Madina	U	25/10/93	ETC	2		UF	Darkblue	Jakoto	T	23/10/93	Y	2
102	Ebria	U	25/10/93	BNC			FF	Blue	Madina	U	21/10/93	Y	4
103	Ebria	U	25/10/93	BNC			FF	Darkblue	Jakoto	T	23/10/93	Y	2
104	Ebria	U	25/10/93	BNC			FF	Blue	Madina	U	21/10/93	Y	4
105	BKFula	U	26/10/93	BNC			FF	Turquoise	Madina	U	24/10/93	Y	2
106	BKFula	U	26/10/93	BNC			FF	Turquoise	Madina	U	24/10/93	Y	2
107	BKMand	U	26/10/93	BNC			FF	Darkgr	Jakoto	T	24/10/93	Y	2
108	Barokun	U	26/10/93	BNC			FF	Darkgr	Jakoto	T	24/10/93	Y	2
109	Barokun	U	26/10/93	BNC			FF	Darkgr	Jakoto	T	24/10/93	Y	2
110	Jakoto	T	26/10/93	PIT 1	11		FF	Darkgr	Jakoto	T	24/10/93	Y	2
111	Jakoto	T	26/10/93	ETC	6	1	UF	Yellowish	Jakoto	T	22/10/93	Y	4
112	Madina	U	26/10/93	ETC	6	3	UF	Turquoise	Madina	U	24/10/93	Y	2
113	Madina	U	26/10/93	BNC	2		FF	Turquoise	Madina	U	24/10/93	Y	2
114	Madina	U	26/10/93	BNC	26		FF	Darkgr	Jakoto	T	24/10/93	Y	2
115	Madina	U	26/10/93	BNC	5		FF	Yellowish	Jakoto	T	22/10/93	Y	4
116	Madina	U	26/10/93	BNC	26		FF	Turquoise	Madina	U	24/10/93	Y	2
117	Madina	U	26/10/93	BNC	5		FF	Turquoise	Madina	U	24/10/93	Y	2
118	Madina	U	26/10/93	PSC	3	4	FF	Yellowish	Jakoto	T	22/10/93	Y	4
119	Madina	U	26/10/93	BNC	28		GR	Purple	Madina	U	23/10/93	Y	3
120	Madina	U	26/10/93	PSC	3	3	FF	Turquoise	Madina	U	24/10/93	Y	2
121	Madina	U	26/10/93	PSC	8	2	FF	Turquoise	Madina	U	24/10/93	Y	2
122	Madina	U	26/10/93	PSC	3	2	FF	Turquoise	Madina	U	24/10/93	Y	2
123	Madina	U	26/10/93	PSC	11	1	FF	Turquoise	Madina	U	24/10/93	Y	2
124	Madina	U	26/10/93	PSC	17	1	GR	Purple	Madina	U	23/10/93	Y	3
125	Madina	U	26/10/93	PSC	8	2	FF	Turquoise	Madina	U	24/10/93	Y	2
126	Madina	U	26/10/93	PSC	17	1	FF	Magenta	Jakoto	T	20/10/93	Y	6
127	Madina	U	26/10/93	PSC	25	2	GR	Blue	Madina	U	21/10/93	Y	5
128	Jakoto	T	27/10/93	PIT 1	9		FF	Magenta	Jakoto	T	20/10/93	Y	7
129	Jakoto	T	27/10/93	PIT 3	6		FF	Turquoise	Madina	U	24/10/93	Y	3
130	Madina	U	27/10/93	PSC	26	1	GR	Turquoise	Madina	U	24/10/93	Y	3
131	Madina	U	27/10/93	PSC	26	1	GR	Turquoise	Madina	U	24/10/93	Y	3
132	Madina	U	27/10/93	PSC	5	2	FF	Blue	Madina	U	21/10/93	Y	6
133	Madina	U	27/10/93	PSC	22	1	GR	Yellowish	Jakoto	T	22/10/93	Y	5
134	Madina	U	27/10/93	PSC	28	5	GR	Turquoise	Madina	U	24/10/93	Y	3
135	Barokun	U	27/10/93	BNC			FF	Magenta	Jakoto	T	20/10/93	Y	7

APPENDIX 2. Continued.

No.	Recapt. Village	T	Date of Recapture	Method	C	R	Gen.	Colour	Release Village	T	Date of Release	LE	DA
136	Barokun	U	27/10/93	BNC			FF	Magenta	Jakoto	T	20/10/93	Y	7
137	BKFula	U	27/10/93	BNC			FF	Blue	Madina	U	21/10/93	Y	6
138	Madina	U	27/10/93	BNC	18		FF	Turquoise	Madina	U	24/10/93	Y	3
139	Madina	U	27/10/93	BNC	5		GR	Darkgr	Jakoto	T	24/10/93	Y	3
140	Madina	U	27/10/93	BNC	28		FF	Red	Madina	U	20/10/93	Y	7
141	Madina	U	27/10/93	BNC	17		FF	Magenta	Jakoto	T	20/10/93	Y	7
142	Madina	U	27/10/93	BNC	28		FF	Turquoise	Madina	U	24/10/93	Y	3
143	BKFula	U	28/10/93	BNC			FF	Magenta	Jakoto	T	20/10/93	Y	8
144	BKFula	U	28/10/93	BNC			FF	Yellowish	Jakoto	T	22/10/93	Y	6
145	BKMand	U	28/10/93	BNC			GR	Yellowish	Jakoto	T	22/10/93	Y	6
146	Madina	U	28/10/93	BNC	24		FF	Turquoise	Madina	U	24/10/93	Y	4
147	Jakoto	T	29/10/93	PIT 2	11		FF	Blue	Madina	U	21/10/93	Y	8
148	Madina	U	29/10/93	PSC	19		FF	Turquoise	Madina	U	24/10/93	Y	5

APPENDIX 3. Bioassays results by individual bednet and sides of the bednet (A:alive mosquitoes; D:dead mosquitoes).

Village	Zone	Pay	Date	Comp	Mark	Type	Side 1		Side 2		Control		%Mort Test	%Mort Control	%corr. ABBOT	%Mor Village
							A	D	A	D	A	D				
JAFAI	3	Y	10/11/93	29	Y	NS	5	15	7	13	19	1	70.00	5.00	70.00	
				7	Y	NN	5	16	3	16	19	1	80.00	5.00	80.00	
				29	Y	CS	5	15	10	11	19	1	63.41	5.00	63.41	
				10	Y	NN	5	19	7	12	19	1	72.09	5.00	72.09	
				14	Y	CS	0	18	13	6	19	1	64.86	5.00	64.86	70.07
SOTOKOI	3	N	10/11/93	18	N	CS	9	11	2	17	40	0	71.79	0	71.79	
				22	N	NN	11	8	11	9	40	0	43.59	0	43.59	
				14	N	CS	6	13	3	17	40	0	76.92	0	76.92	
				8	N	NN	7	13	9	11	40	0	60.00	0	60.00	
				11	N	CS	6	14	0	21	40	0	85.37	0	85.37	67.53
F/BANTA	3	N	11/11/93	56	Y	NN	0	17	0	19	38	2	100.0	5.00	100.0	
				69	Y	CS	9	11	2	14	38	2	69.44	5.00	69.44	
				22	Y	NN	8	10	5	13	38	2	63.89	5.00	63.89	
				26	Y	NS	8	11	8	6	38	2	51.52	5.00	51.52	
				74	Y	NN	13	10	8	14	38	2	53.33	5.00	53.33	67.64
JAHALLY	3	Y	11/11/93	30	N	CS	6	14	5	12	34	1	70.27	2.86	70.27	
				49	N	NN	1	18	2	16	34	1	91.89	2.86	91.89	
				42	N	NS	2	18	1	21	34	1	92.86	2.86	92.86	
				63	Y	NN	2	17	2	19	34	1	90.00	2.86	90.00	
				49	N	NN	11	9	9	8	34	1	45.95	2.86	45.95	78.19
SARUJA	3	N	11/11/93	45	N	NJ	4	15	8	9	31	1	66.67	3.13	66.67	
				38	N	NS	16	3	10	5	31	1	23.53	3.13	23.53	
				5	Y	NJ	5	16	1	18	31	1	85.00	3.13	85.00	
				66	N	CS	7	11	6	14	31	1	65.79	3.13	65.79	
				2	Y	NJ	5	17	6	15	31	1	74.42	3.13	74.42	63.08
KUDANG	3	Y	12/11/93	44	N	NN	7	12	13	4	42	0	44.44	0	44.44	
				28	N	NN	6	15	3	15	42	0	76.92	0	76.92	
				28	N	CS	3	18	5	15	42	0	80.49	0	80.49	
				37	N	NN	0	20	15	4	42	0	61.54	0	61.54	
				37	N	NJ	13	10	6	12	42	0	53.66	0	53.66	63.41
PATEH SA	3	Y	15/11/93	39	N	NN	16	4	19	1	41	0	12.50	0	12.50	
				30	N	NN	8	12	13	8	41	0	48.78	0	48.78	
				30	N	NJ	14	6	18	2	41	0	20.00	0	20.00	
				29	N	NJ	19	1	19	2	41	0	7.32	0	7.32	
				38	N	NS	16	4	14	5	41	0	23.08	0	23.08	22.33
BATI	3	N	15/11/93	29	Y	CN	7	13	3	16	41	0	74.36	0	74.36	
				25	Y	CS	17	6	14	5	41	0	26.19	0	26.19	
				14	Y	NN	7	12	8	13	41	0	62.50	0	62.50	
				27	Y	NS	9	10	10	10	41	0	51.28	0	51.28	
				18	Y	NN	7	13	3	18	41	0	75.61	0	75.61	57.99
F/ABDOU	3	Y	16/11/93	24	N	NN	12	7	13	8	40	1	37.50	2.44	37.50	
				19	N	CN	11	9	14	5	40	1	35.90	2.44	35.90	
				18	N	CS	16	4	13	6	40	1	25.64	2.44	25.64	
				16	N	NN	17	4	16	5	40	1	21.43	2.44	21.43	
				8	N	NJ	7	13	5	15	40	1	70.00	2.44	70.00	38.09
G/MANDA	3	N	16/11/93	12	Y	CS	11	9	8	10	40	1	50.00	2.44	50.00	
				29	Y	NN	13	8	14	7	40	1	35.71	2.44	35.71	
				44	N	CN	14	6	12	8	40	1	35.00	2.44	35.00	
				28	Y	NN	5	14	3	17	40	1	79.49	2.44	79.49	
				30	Y	NN	4	15	6	13	40	1	73.68	2.44	73.68	54.78
T/BANANI	3	N	17/11/93	6	N	CS	15	5	17	3	40	0	20.00	0	20.00	
				13	N	NN	19	1	16	4	40	0	12.50	0	12.50	
				12	N	NS	14	6	12	7	40	0	33.33	0	33.33	
				18	N	NN	17	3	14	7	40	0	24.39	0	24.39	
				10	N	NS	12	8	17	3	40	0	27.50	0	27.50	23.54

APPENDIX 3. Continued.

Village	Zone	Pay	Date	Comp	Mark	Type	Side 1 A	Side 1 D	Side 2 A	Side 2 D	Control A	Control D	%Mort Test	%Mort Control	%corr. ABBOT	%Mort Village
M/FANA	3	N	17/11/93	44	N	CS	9	11	5	15	40	0	65.00	0	65.00	
				18	N	NN	11	8	7	14	40	0	55.00	0	55.00	
				51	N	NN	6	14	13	8	40	0	53.66	0	53.66	
				46	N	NS	12	7	15	6	40	0	32.50	0	32.50	
				19	N	NN	13	7	9	11	40	0	45.00	0	45.00	50.23
M/MAKA	3	Y	18/11/93	5	N	CN	16	4	12	9	39	1	31.71	2.50	31.71	
				11	N	NJ	16	4	7	14	39	1	43.90	2.50	43.90	
				18	N	NJ	9	11	2	17	39	1	71.79	2.50	71.79	
				15	N	NN	15	5	13	7	39	1	30.00	2.50	30.00	
				14	N	CS	13	7	11	8	39	1	38.46	2.50	38.46	43.17
MBIAN	3	Y	18/11/93	3	N	NJ	16	5	17	3	39	1	19.51	2.50	19.51	
				2	N	CS	17	3	18	2	39	1	12.50	2.50	12.50	
				17	N	NN	15	5	17	4	39	1	21.95	2.50	21.95	
				1	N	NN	16	4	9	12	39	1	39.02	2.50	39.02	
				10	N	CN	15	5	9	13	39	1	42.86	2.50	42.86	27.17
PATCHAR	3	Y	24/11/93	55	N	NN	8	12	11	9	40	0	52.50	0	52.50	
				58	N	NN	17	3	13	7	40	0	25.00	0	25.00	
				70	N	NN	15	6	9	10	40	0	40.00	0	40.00	
				37	N	NN	10	7	14	4	40	0	31.43	0	31.43	
				53	N	NS	12	7	17	2	40	0	23.68	0	23.68	34.52
SARENGA	3	Y	24/11/93	151	N	NN	13	7	15	5	40	0	30.00	0	30.00	
				152	N	CS	17	3	18	2	40	0	12.50	0	12.50	
				153	N	NS	17	4	17	2	40	0	15.00	0	15.00	
				154	N	NS	12	8	13	16	40	0	48.98	0	48.98	
				18	N	NN	19	1	18	2	40	0	7.50	0	7.50	22.80
BANITO	5	N	22/11/93	10	Y	CS	18	2	17	3	40	1	12.50	2.44	12.50	
				9	N	NS	12	8	13	7	40	1	37.50	2.44	37.50	
				3	N	NN	19	1	17	3	40	1	10.00	2.44	10.00	
				13	Y	NN	19	1	16	3	40	1	10.26	2.44	10.26	
				32	N	CS	15	5	18	2	40	1	17.50	2.44	17.50	17.55
JULANG	5	N	22/11/93	29	Y	NS	14	6	17	3	40	1	22.50	2.44	22.50	
				11	Y	CS	11	9	16	4	40	1	32.50	2.44	32.50	
				8	N	NN	19	2	15	5	40	1	17.07	2.44	17.07	
				46	Y	NN	12	8	3	17	40	1	62.50	2.44	62.50	
				31	N	NN	17	3	14	7	40	1	24.39	2.44	24.39	31.79
MISIRA	5	N	25/11/93	5	Y	NN	12	7	9	8	40	0	41.67	0	41.67	
				40	N	NN	4	15	11	14	40	0	65.91	0	65.91	
				2	N	CS	20	2	13	5	40	0	17.50	0	17.50	
				7	Y	NS	17	3	14	6	40	0	22.50	0	22.50	
				53	Y	CS	11	3	18	1	40	0	12.12	0	12.12	31.94
KOINA	5	N	25/11/93	1	N	NN	16	7	23	4	40	0	22.00	0	22.00	
				88	N	NN	16	10	16	9	40	0	37.25	0	37.25	
				32	N	CS	13	4	15	6	40	0	26.32	0	26.32	
				70	N	NN	11	8	15	6	40	0	35.00	0	35.00	
				27	Y	NN	14	11	21	1	40	0	25.53	0	25.53	29.22
DINGIRI	5	Y	29/11/93	21	Y	NN	16	5	17	3	39	1	19.51	2.50	19.51	
				18	Y	NN	15	5	10	10	39	1	37.50	2.50	37.50	
				61	Y	NN	14	6	15	5	39	1	27.50	2.50	27.50	
				78	Y	CS	16	3	18	2	39	1	12.82	2.50	12.82	
				59	Y	CS	17	3	18	1	39	1	10.26	2.50	10.26	21.52
BAKADAG	5	N	29/11/93	25	Y	CS	17	2	16	4	39	1	15.38	2.50	15.38	
				83	Y	NN	16	4	14	6	39	1	25.00	2.50	25.00	
				4	Y	NS	18	3	29	1	39	1	7.84	2.50	7.84	
				3	Y	NJ	12	8	13	6	39	1	35.90	2.50	35.90	
				82	Y	NN	11	9	15	5	39	1	35.00	2.50	35.00	23.83

APPENDIX 3. Continued.

Village	Zone	Pay	Date	Comp	Mark	Type	Side 1 A	Side 1 D	Side 2 A	Side 2 D	Control A	Control D	%Mort Test	%Mort Control	%corr. ABBOT	%Mort Village
KUDAM	5	Y	29/11/93	4	N	NN	12	8	9	11	39	1	47.50	2.50	47.50	
				34	N	CS	16	4	18	2	39	1	15.00	2.50	15.00	
				8	Y	NN	16	4	15	3	39	1	18.42	2.50	18.42	
				6	Y	NN	8	12	11	9	39	1	52.50	2.50	52.50	
				34	Y	NS	17	3	14	6	39	1	22.50	2.50	22.50	31.18
DKUN/KU	5	N	30/11/93	1	N	NJ	16	5	13	7	40	0	29.27	0	29.27	
				1	N	NN	7	12	5	15	40	0	69.23	0	69.23	
				1	N	CS	5	14	13	7	40	0	53.85	0	53.85	
				33	N	NN	16	4	6	14	40	0	45.00	0	45.00	
				49	N	CS	8	12	6	14	40	0	65.00	0	65.00	52.47
GAMBISA	5	N	30/11/93	15	N	CS	10	11	11	9	40	0	48.78	0	48.78	
				14	N	CS	6	14	7	13	40	0	67.50	0	67.50	
				8	Y	NN	5	15	3	17	40	0	80.00	0	80.00	
				16	N	NS	13	7	12	8	40	0	37.50	0	37.50	
				12	Y	NN	13	7	11	9	40	0	40.00	0	40.00	54.76
SUDUWOI	5	Y	30/11/93	22	N	CS	17	3	14	6	40	0	22.50	0	22.50	
				32	N	CS	13	7	15	4	40	0	28.21	0	28.21	
				34	Y	CS	18	2	17	3	40	0	12.50	0	12.50	
				39	N	CS	16	3	16	5	40	0	20.00	0	20.00	
				17	Y	CS	14	6	17	3	40	0	22.50	0	22.50	21.14
SAREBOI	5	Y	1/12/93	22	N	NN	15	5	8	12	39	1	42.50	2.50	42.50	
				25	Y	NN	6	14	8	12	39	1	65.00	2.50	65.00	
				22	Y	NS	7	12	12	8	39	1	51.28	2.50	51.28	
				2	N	NN	4	16	7	13	39	1	72.50	2.50	72.50	
				3	N	NN	9	11	7	13	39	1	60.00	2.50	60.00	58.26
GARAWOI	5	N	1/12/93	7	N	NN	11	9	7	12	39	1	53.85	2.50	53.85	
				112	N	NS	14	6	17	3	39	1	22.50	2.50	22.50	
				115	N	CS	16	4	14	6	39	1	25.00	2.50	25.00	
				7	Y	NN	11	9	13	7	39	1	40.00	2.50	40.00	
				2	N	CS	13	7	11	9	39	1	40.00	2.50	40.00	36.27
BADARI	5	Y	2/12/93	23	Y	NJ	3	17	5	15	40	0	80.00	0	80.00	
				11	N	CS	4	16	2	18	40	0	85.00	0	85.00	
				16	Y	NN	3	17	1	18	40	0	89.74	0	89.74	
				5	N	NJ	14	6	11	9	40	0	37.50	0	37.50	
				17	Y	CS	3	17	2	18	40	0	87.50	0	87.50	75.95
SOTUMA	5	Y	2/12/93	2	Y	NN	7	11	9	11	40	0	57.89	0	57.89	
				36	Y	CN	12	8	9	11	40	0	47.50	0	47.50	
				18	Y	CN	6	14	2	16	40	0	78.95	0	78.95	
				34	Y	NN	5	14	7	12	40	0	68.42	0	68.42	
				33	N	NS	7	13	4	16	40	0	72.50	0	72.50	65.05
SAMUNDI	5	Y	3/12/93	18	N	NN	4	12	6	11	24	4	69.70	14.29	64.65	
				43	N	NJ	6	14	0	21	24	4	85.37	14.29	82.93	
				10	N	NN	0	19	0	18	24	4	100.0	14.29	100.0	
				17	N	NJ	3	16	0	15	24	4	91.18	14.29	89.71	
				20	Y	NJ	0	20	0	22	24	4	100.0	14.29	100.0	87.46
BANTANG	5	N	3/12/93	1	N	CS	3	20	6	14	24	4	79.07	14.29	75.58	
				21	Y	NJ	4	9	5	15	24	4	72.73	14.29	68.18	71.88
NUMUYE	5	Y	3/12/93	67	N	CS	0	20	4	18	24	4	90.48	14.29	88.89	
			7/12/93	42A	N	NN	16	15	0	31	31	8	74.19	20.51	67.53	
				42B	N	NN	4	19	2	24	31	8	87.76	20.51	84.60	80.34
DANKUNKU			6/12/93	500MG		CN	0	22	0	22	34	2	100.0	5.56	100.0	
DANKUNKU			13/12/93	500MG		CN	6	13	8	12	39	1	64.10	2.50	64.10	82.05