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IMPACT OF INSECTICIDE-TREATED NETS PROTECTING CATTLE IN ZERO-GRAZING UNITS ON NUISANCE AND BITING INSECTS IN THE FOREST REGION OF KUMASI, GHANA

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Institute for Parasitology and Tropical Veterinary Medicine of the
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at the Free University of Berlin, Germany

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Abbreviations

AAT	African animal trypanosomosis
ADG	Animal daily gain
a.m.	*ante meridiem* – Latin for “before noon”
ANOVA	Analysis of variance
BCRF	Boadi Cattle Research Farm
BG	Biogents
Bti	*Bacillus thuringensis* var. *israelensis*
C	Celsius
ca.	*circa* – Latin for “about”
CSP1	Circumsporozoite protein
DDT	Dichloro-diphenyl-trichloroethane
EEEV	Eastern equine encephalitis virus
EIR	Entomological inoculation rate
ELISA	Enzyme-linked immunosorbent assay
EPN	Entomopathogenic nematodes
et al.	*et alii* – Latin for “and others”
FAO	Food and Agriculture Organization of the United Nations
GPS	Global positioning system
HBR	Human biting rate
HLC	Human landing catch
IBN	Insecticide-treated bed nets
i.e.	*id est* – Latin for “that is”
IGR	Insect growth regulators
IRS	Indoor residual spraying
ITC	Insecticide-treated cattle
ITN	Insecticide-treated nets
ITT	Insecticide-treated targets
KCCR	Kumasi Centre for Collaborative Research
KNUST	Kwame Nkrumah University of Science and Technology
LLIN	Long lasting insecticide-treated nets
LSD	Least significant difference
MBR	Monthly biting rate
ND	Not done / not dissected / not determined
NP	Nulliparous

OIE	Office International des Epizooties
P	Parous
p	P-Value
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PCV	Packed cell volume
p.m.	*post meridiem* – Latin for " after noon"
POCA	Propylphenol, octenol, p-cresol and acetone
PR	Parity rate
SIT	Sterile insect technique
s.l.	*sensu lato* – Latin for "in the wide sense"
s.s.	*sensu stricto* – Latin for "in the strict sense"
spp.	Species
SR	Sporozoite rate
VC	Vectorial capacity
VEEV	Venezuelan equine encephalitis virus
WEEV	Western equine encephalitis virus
WHO	World Health Organization

1 Introduction and objective

Insects can directly affect animals and man's well being through disease transmission or disturbing nuisance. A large variety of insects, in particular dipterids (Diptera), are vectors of diseases in the Afro-tropical region. A vector is an organism that does not cause disease itself but which spreads infection by passing on pathogens from one host to another. Vector-borne diseases are responsible for a significant fraction of the global disease burden and have substantial effects not only on health but also on the socioeconomic development of affected nations (SACHS and MALANEY, 2002). Livestock productivity in Africa is low due to several factors some of which are its extensive and self sustainable nature following the regional traditions, infrastructure and logistic limitations, scarce farmer training, and overburdened veterinary services. Cattle are commonly attacked by arthropod parasites which besides deteriorating their body condition and creating nuisance can frequently transmit diseases, such as nagana, dermatophilosis, bluetongue disease, and arboviral infections. Diseases increase morbidity and mortality of cattle whereas nuisance leads to animal disturbance and reduction in feed-intake. The incomes of the farmer are reduced and the animal performance potential is never fully achieved (BAUER *et al.*, 1999; ROWLANDS *et al.*, 1999; KAMUANGA *et al.*, 2001). African communities live in a very close relationship with their animals which are frequently the main source of family income. Often animals are kept in stables directly outside households luring haematophagous insects in search of blood meals, increasing the amount of vectors susceptible for disease transmission in these homesteads. Insecticide-treated nets are commonly used as bed nets to protect humans from malaria infections (WHO, 2003a) or as material for traps and targets to control tsetse flies (GOUTEUX *et al.*, 1982; LAVEISSIÈRE and GREBAUT, 1990). This project was inspired by a trial conducted in Kenya where zero-grazed dairy cattle were protected from tsetse flies with a 75 denier net impregnated with lambda-cyhalothrin attached to the enclosures (BAUER *et al.*, 2006). The net was set at 150 cm height as it was not expected for tsetse flies to fly higher (GOUTEUX and JARRY, 1998). In this trial the effect of the treated net was evaluated through the direct observations of the farmers, the measurement of the animals mean packed cell volume (PCV) and the mean hazard rate of trypanosome infections. However insect densities could not be measured, hindering to prove a reductive effect of the net on the nuisance and vector arthropods densities. Observations of the participating farmers hinted to a reduction in animal disturbance

caused by nuisance flies, decrease in attacking tsetse flies, better feed intake, and improvement in milk production. Farmers also reported fewer mosquitoes in their homes and associated this with the near-by presence of the treated net around the enclosures.

The objective of this study was to assess the effect of an insecticide-treated net on the density of insects of veterinary and medical importance (Dipterids) inside and in the surroundings of zero-grazing cattle enclosures by measuring fly and mosquito densities through three different trapping methods. The effect of the insecticide-treated net fence on anophelines was also investigated, due to their role as malaria vectors. Integrative and well managed vector control programs could have an enormous benefit in the afro-tropical region by decreasing morbidity and mortality of human and animal individuals through prevention, reduction and elimination of vector-borne diseases.

2 Literature review

2.1 Insects – Order Diptera

2.1.1 Suborder Nematocera

2.1.1.1 Family Culicidae

Mosquitoes, also known as culicids, are insects belonging to the family Culicidae, Order Diptera (Insecta: Arthropoda). Mosquitoes have a pair of scaled wings, a pair of halteres, a slender body, and long legs. The family Culicidae contains three subfamilies, Anophelinae, Culicinae and Toxorhynchitinae. Female mosquitoes belonging to Anophelinae and Culicinae subfamilies are haematophagous in contrast to Toxorhynchitinae which never suck blood.

Mosquitoes are known vectors of a wide range of diseases afflicting animals and humans in the tropics. Some of these diseases are zoonoses, the etiological agents are maintained in nature in cycles involving arthropod transmission among a variety of susceptible reservoir animal hosts, and many times humans are the definitive hosts.

Anophelines

Anopheles species transmit four different species of parasites of the genus *Plasmodium* which cause malaria affecting humans in endemic areas. *Anopheles gambiae* s.l. is one of the best known, because of its predominant role in the transmission of *Plasmodium falciparum* (TAYLOR *et al.*, 1990; FONTENILLE *et al.*, 1991) and due to the strong anthropophily of some species of this complex (COSTANTINI *et al.*, 1999; PATES *et al.*, 2001; DEKKER *et al.*, 2001). Most *Anopheles* mosquitoes are not exclusively anthropophilic or zoophilic (GITHEKO *et al.*, 1996; KONATE *et al.*, 1999; DUCHEMIN *et al.*, 2001). However, the primary malaria vectors in Africa, *A. gambiae* s.s and *A. funestus*, are strongly anthropophilic (MBOGO *et al.*, 1993) and consequently, the two most efficient malaria vectors (SHILILU *et al.*, 1998; MWANGANGI *et al.*, 2003; BALDET *et al.*, 2003). Some species of *Anopheles* can also serve as the vectors for *Dirofilaria immitis* (TODARO *et al.*, 1977; BUXTON and

MULLEN, 1980; TOLBERT and JOHNSON, 1982; PARKER, 1993; ROSSI *et al.*, 1999), *Wuchereria bancrofti* (KUHLOW and ZIELKE, 1978; UDONSI, 1988; MWANDAWIRO *et al.*, 1997; BOAKYE *et al.*, 2004; RWEGOSHORA *et al.*, 2005), *Brugia malayi* (SUZUKI *et al.*, 1981; VYTHILINGAM *et al.*, 1996), and arboviruses (RICKENBACH *et al.*, 1976; MITCHELL *et al.*, 1987; CUPP *et al.*, 2004).

Culicines

The subfamily Culicinae includes various genera which contain 80% of all mosquito species. They are distinguishable by their short palpae in females, in comparison to the long palpae which characterize female anophelines. Culicine species are known vectors of many arboviral diseases such as yellow fever, dengue fever, Rift Valley fever, Venezuelan equine encephalitis virus (VEEV), Western equine encephalitis virus (WEEV), Eastern equine encephalitis virus (EEEV), Chikungunya, Japanese encephalitis, West Nile virus and many other arboviruses (HAYES *et al.*, 1976; DOHERTY *et al.*, 1979; RUSSELL *et al.*, 1991; MITCHELL *et al.*, 1996; NASCI *et al.*, 2001). Mosquitoes of the genus *Aedes* are also capable of mechanically transmitting the lumpy skin disease virus to cattle (CHIHOTA *et al.*, 2001) which is part of List A Diseases of OIE. Some mosquitoes can transmit filariasis parasites which cause a disfiguring condition (often referred to as elephantiasis) characterized by a distinct swelling of several parts of the body in humans. Besides the dangers that mosquitoes place to public health as vectors of diseases their role as nuisance agents should not be neglected. Areas of great mosquito prevalence suffer losses in tourism due to the unpleasant nuisance and risk of disease transmission created by these insects (THAVARA *et al.*, 1996; SCHILTHUIS and OVERBOSCH, 2000).

2.1.1.2 Family Simuliidae

Black flies (sometimes called buffalo gnats or turkey gnats) are members of the family Simuliidae, the majority of species belong to the genus *Simulium* which medically is by far the most important genus as it contains important vector species such as *Simulium damnosum* s.l. and *S. neavei* (Africa)*, S. ochraceum, S. metallicum,* and *S. callidum* (South and Central Americas). They are usually small, black or grey, with short legs and antennae. Most black flies live on vertebrate blood being of common nuisance for

humans and animals inducing quite painful bites. Animals may suffer loss of condition, decrease in reproduction rate and even occasional death due to anaphylactic shock (JORDAAN and VAN ARK, 1990). Black flies transmit parasitic nematodes of the genus *Onchocerca*. It is known to transmit to cattle *Onchocerca ochengi* and *Onchocerca gutturosa* which provoke nodular dermatitis resulting from the intradermal infestation with microfilarias (BWANGAMOI, 1969). Also *S. erythrocephalum* is capable of causing dermatitis to humans (BEAUCOURNU-SAGUEZ *et al.*, 1993). Black flies are the only vectors of onchocercosis, also known as river blindness, which is caused by *Onchocerca volvulus.* The diseases morbidity is linked to the host's immune response to the microfilarias which may cause blindness and most commonly dermatological changes such as "lizard skin" and "leopard skin" (MURDOCH, 1992; MURDOCH *et al.*, 2002). Onchocercosis is highly prevalent in West, East, and Central Africa and some localized areas of South America (WHO, 2000).

2.1.1.3 Family Ceratopogonidae

Ceratopogonidae, or biting midges, are a family of small mosquitoes (1-4 mm long) in the suborder Nematocera. They are distributed world wide and some of the species are haematophagous pests. Biting midges feed on most vertebrates, including humans, but preferentially ruminants (BLACKWELL *et al.*, 1994). Biting midges breed in moist conditions in a variety of habitats, particularly damp, muddy areas and in faecal and plant matter (MEISWINKEL, 1987; KLINE and WOOD, 1988b; DYCE and MARSHALL, 1989; LARDEUX and OTTENWAELDER, 1997; USLU and DIK, 2006). They have mostly nocturnal feeding habits (SERVICE, 1980), preferring still, warm and dry conditions (FASSOTE *et al.*, 2008). *Culicoides* may be vectors of disease-causing viruses, protozoa, and filarial infections. The list of diseases which *Culicoides* are incriminated include bluetongue disease, equine summer eczema, oropouche fever, vesicular stomatitis, epizootic haemorrhagic disease, EEEV, african horse sickness, *Haemoproteus belopolskyi*, *Leucocytozoon caullereryi*, etc. Areas with high *Culicoides* densities can suffer tourism losses and even property value loss (RATNAYAKE *et al.*, 2006).

2.1.1.4 Family Psychodidae

Sand flies belong to the subfamily Phlebotominae of the family Psychodidae, they are tiny mosquitoes measuring 1-3 mm in length. Sand flies are vectors of leishmaniosis, which is a disease caused by protozoa of the genus *Leishmania*. In the Old World leishmaniosis is transmitted by sand flies of the genus *Phlebotomus* (KILLICK-KENDRICK, 1990) and in the New World by sand flies of the genus *Lutzomyia*. Cutaneous leismaniosis is an anthroponosis caused by *Leishmania tropica*, yet most other *Leishmania* species infecting humans are zoonotic. Its reservoirs are found in small mammals like rodents, hyraxes and canids (ABRANCHES *et al.*, 1983; SIXL *et al.*, 1987; YAGHOOBI-ERSHADI *et al.*, 1996; GITHURE *et al.*, 1996; ROSYPAL *et al.*, 2003). Sand flies of the genus *Phlebotomus* also transmit the sand fly fever virus, which is prevalent in the Mediterranean region (TESH *et al.*, 1977; VERANI *et al.*, 1995). In South America *Lutzomyia verrucarum* is the vector of Carrions Disease caused by *Bartonella bacilliformis* (CACERES, 1993; TOWNSEND, 1914).

2.1.2 Suborder Brachycera

2.1.2.1 Family Tabanidae

Horseflies are members of the family Tabanidae, suborder Brachycera. They are easily recognisable by their remarkable size and large colourful eyes. Horseflies are considered nuisance pests which attack mammals but preferentially livestock. Tabanids induce extremely painful bites; if the number of flies is abundant a commonly occurring problem is blood loss. The average blood meal size of *Tabanus fuscicostatus* is 110% of its unfed weight (49.7 mg) (LEPRINCE and FOIL, 1993), which can severely weaken or even kill livestock. The potential of tabanids to transmit diseases mechanically is quite prominent (FOIL *et al.*, 1987), such as bovine leukaemia (MANET *et al.*, 1989), anaplasmosis (HAWKINS *et al.*, 1982) or trypanosomosis through African species *Atylotus agrestis* (DESQUESNES and DIA, 2003a; DESQUESNES and DIA, 2003b) and *Atylotus fuscipis* (DESQUESNES and DIA, 2004). Species of the genus *Chrysops* are the biological vectors of loiasis, filarial infection caused by the worm *Loa loa*. The host preference of these tabanids for human and simian blood (GOUTEUX *et al.*, 1989) is adjuvant to the maintenance of the transmission of this anthroponosis.

2.1.2.2 Family Glossinidae

Tsetse flies are mostly middle-sized biting flies prevailing only in sub-Saharan Africa. All species and subspecies of tsetse flies belong to the genus *Glossina* and family Glossinidae. Both male and female tsetse flies are bloodsucking insects. Tsetse flies feed on a large variety of domestic and wild animals including birds and reptiles (STAAK *et al.*, 1986; KÜPPER *et al.*, 1990; GOUTEUX *et al.*, 1994; SASAKI *et al.*, 1995; CLAUSEN *et al.*, 1998; SPÄTH, 2000). They can be distinguished through simple anatomical characteristics, their wings fold completely when resting so that one wing rests directly on top of the other over the abdomen in a scissor format. The medial cell of the tsetse flies wings has a characteristic *hatchet* shape resembling a meat chopper. Tsetse flies have a long proboscis which extends directly forward and is attached by a distinct bulb to the bottom of their head. The *Glossina* genus is generally separated into three different groups of species based on distributional, behavioural, and morphological characteristics which are the savannah species, forest species and riverine species. Tsetse flies are the main vectors of human and animal African trypanosomosis, respectively known as sleeping sickness and nagana, caused by protozoa parasites of the genus *Trypanosoma*. Human and animal trypanosomosis constitute a major constraint to the development in sub-Saharan Africa. Tsetse flies also have the potential to transmit viral diseases mechanically, for example Rift Valley fever (HOCH *et al.*, 1985).

2.1.2.3 Family Muscidae

Muscids are insects belonging to the family Muscidae, suborder Brachycera, order Diptera. House flies and stable flies are part of this family; these insects are synanthropic species, i.e. often considered pests, which are not domesticated but live near and benefit from humans.

House flies

House flies belong to the subfamily Muscinae of the family Muscidae. The most common fly occurring in homes is *Musca domestica*, definitely one of the most widely distributed insects and the most familiar of all flies; it is a pest that can transmit serious

diseases (MAYR, 1983; NAZNI *et al.*, 2005; BARRO *et al.*, 2006). House flies are considered mechanical vectors, disseminating diseases such as typhoid fever, cholera, salmonellosis, bacillary dysentery, tuberculosis, anthrax, helminths and many other pathogens of importance to public health (SULAIMAN *et al.*, 2000; GRACZYK *et al.*, 2001; RAHUMA *et al.*, 2005; BOULESTEIX *et al.*, 2005). In third world countries hygiene conditions are often poor, leading to an increase in disease transmission by these insects, as well as an increase in breeding places by accumulation of debris and deficient drainage systems (KHALIL *et al.*, 1994; CHAVASSE *et al.*, 1996; BOADI and KUITUNEN, 2005)

Stable Flies

Stable flies belong to the subfamily Stomoxyinae of the family Muscidae. Stable flies can easily be mistaken for house flies, however a closer observation of the fly shows that while the house fly has a sponging mouth part (for ingesting liquids) the stable fly has a proboscis for sucking blood. Stable flies feed on blood from practically any mammalian including horses, cattle, camels, dogs and even humans. They can breed in several manure sources. These insects are responsible for serious nuisance to animals and also for the mechanical transmission of trypanosomosis (D'AMICO *et al.*, 1996) and dermatophilosis (RICHARD and PIER, 1966; SAMUI and HUGH-JONES, 1990). The average blood meal of a single specimen is ca. 13.7+/-0.6 mg (MIHOK *et al.*, 1995a) which in conditions of heavy nuisance may lead to significant blood losses.

2.2 Vector control

Vector control has a proven record in the prevention and control of vector-borne diseases. The distribution and incidence of vector-borne diseases are strongly determined by the ecological conditions that favour different species of disease vectors. Knowledge and understanding of these characteristics is important to prevent and control such diseases, by reducing vector human contact and vector population density and survival. For many vector-borne diseases there are no vaccines, and drug resistance – or the threat of resistance – is an increasing problem (CLAUSEN *et al.*, 1992; GEERTS *et al.*, 2001; ANENE *et al.*, 2001; GOGTAY *et al.*, 2006; KSHIRSAGAR, 2006). In such circumstances vector control often plays a vital role. In some cases, effective vector control is the primary or even sole measure for preventing

disease outbreaks, for example the chikungunya (Swahili for stooped walk) fever outbreak on Reunion Island in 2006 (BOUTIN, 2006).

There are several types of vector control measures, success depends on a good integration of measures according to each situation and field conditions (Fig. 2.1). Vector control is feasible through five general approaches (BRUCE-CHWATT, 1985):

- Reduction of human/animal-vector contact;
- Destruction of adult vectors;
- Destruction of the vectors in early developing stages;
- Reduction of vector breeding places;
- Social participation of the communities.

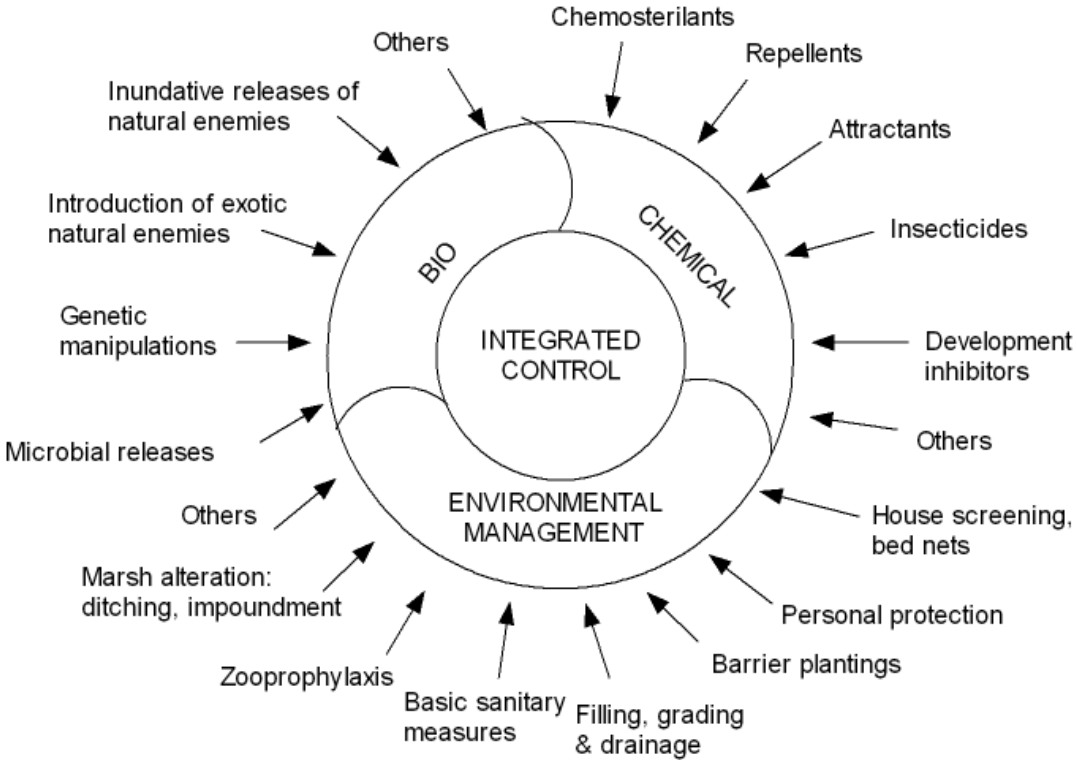

Fig. 2.1 Diagram of the components (environmental management, chemical, biological) and their potential constituent methods to be considered in an "integrated control" approach to mosquito control (source: WHO, 1982).

Since the invention of DDT (dichloro-diphenyl-trichloroethane) and detection of its residual effect against adult vectors, pesticides have been used on a large scale in the Afro-tropical region. Massive amounts of organochlorines and organophosphorus pesticides were used in aerial sprayings and outdoor spraying as well as for agricultural

purposes. Reductions in vector densities were often achieved and in certain locations vector-borne diseases were eradicated (BRUCE-CHWATT, 1977; WEBBER, 1979; MABASO *et al.*, 2004). However, due to development of pesticide resistance (DAVIDSON and ZAHAR, 1973; HARGREAVES *et al.*, 2003) as well as the ecological impact of these pesticides more vector-targeted and environment-friendly ideas had to be deliberated.

2.2.1 Chemical control - destruction of adult vectors

Control measures directed towards the adult vectors have a more broad applicability than measures directed towards the vector's early stages of development which require solid knowledge of the vector's behaviour and choice of breeding place according to the location (WHO, 2006a). If the vectors breeding places are sufficiently limited in extent and definable, larval control can make a significant contribution to vector control (NASCI *et al.*, 1994; PALMER *et al.*, 1996; FILLINGER and LINDSAY, 2006). However, it should be recognized that it only works by reducing the density of local vector populations and does not prevent immigration from outside the area of control. In the case of *Anopheles* spp. it also does not reduce the chances of survival to the dangerous age at which they can carry sporozoites. Therefore vector control directed towards adult vectors brings better results in broad geographical intervention areas as well as in malaria vector control (WHO, 1982).

2.2.1.1 Insecticide-treated cattle (ITC)

Insecticide-treated cattle (ITC) through pyrethroid pour-on solutions were used in the prospect of a parallel reduction in transmission of trypanosomosis and tsetse fly density (BAUER *et al.*, 1988; BAUER *et al.*, 1989; BAUER *et al.*, 1992; VALE *et al.*, 1999). However, in areas of high vector density its effects on vector populations and disease prevalence can be mediocre and ephemeral (GOUTEUX *et al.*, 1996; HARGROVE *et al.*, 2000; OKIRIA *et al.*, 2002). Usage of pour-on solutions had nevertheless a positive effect on animal production being consistently cost-effective (BAUER *et al.*, 1992; BAUER *et al.*, 1995). Yet, the continuous treatments are sometimes neglected by the African farmers and substituted by alternative control methods, like old motor oil and household disinfectants jeopardizing successful vector control (HLATSHWAYO and

MBATI, 2005). The use of ITC is a promising method to help reduce the burden of trypanosomosis; however, the uncontrolled use of this tool might lead to pyrethroid resistance of tick populations and possible exacerbation of tick-borne diseases (EISLER *et al.*, 2003).

2.2.1.2 Insecticide-treated targets (ITT)

Insecticide-treated targets (ITT) are used for tsetse control, they consist in a simple and cheap device which involves a suspended screen of blue and black cloth (often known as a tsetse target) impregnated with a pyrethroid insecticide such as deltamethrin. Flies are attracted by the blue and black segments and land on the target, quickly succumbing to the insecticide. The effectiveness of insecticide-treated traps and ITT's can be greatly enhanced by addition of appropriate odour bait such as short-chain aromatic compounds like acetone or octenol (VALE, 1982).

2.2.1.3 Insecticide-treated nets (ITN)

Insecticide-treated nets (ITN's) are most commonly used as bed-nets (IBN's). IBN's have proven to protect people from mosquito bites and mainly malaria transmission (BRADLEY *et al.*, 1986). Its use has proven to be cost effective (WISEMAN *et al.*, 2003) and of great significance in terms or reducing malaria prevalence, especially amongst infants (SNOW *et al.*, 1988; ALONSO *et al.*, 1991; TER KUILE *et al.*, 2003; LEENSTRA *et al.*, 2003) and pregnant women (GAMBLE *et al.*, 2006). However, IBN's are dependant of a series of factors such as the vectors behaviour (PATES and CURTIS, 2005), the users sleeping hours, correct maintenance of the net, and self-interest to buy and use an IBN (ALAII *et al.*, 2003; OSERO *et al.*, 2005). Yet, IBN's continue to be one of the most promising malaria control strategies and are part of the WHO's Roll Back Malaria program which envisages to halve the malaria burden by 2010. Insecticide-treated bed nets (IBN's) are currently the main malaria prevention tool, yet only 2% of African children are currently sleeping under an IBN, and only 15% are sleeping under any kind of net (WHO, 2003a). One of the problems is the low re-treatment rate of the nets; however, with the creation of long lasting insecticide-treated nets (LLIN's) the problematic of regular re-treatments can be solved. LLIN's consist, for instance, of polyester net impregnated with deltamethrin mixed in a resin that coats the netting fibres and releases the insecticide progressively; when used indoors they are

expected to last about three years with around twenty washings (KROEGER *et al.*, 2004).

2.2.1.4 Indoor residual spraying (IRS)

Indoor residual spraying (IRS) consists in spraying the indoor surfaces with insecticidal solutions in order to control vectors by killing mosquitoes entering houses and resting on sprayed surfaces. IRS was extensively done in rural African regions in the pursuit of malaria vector control (MABASO *et al.*, 2004). However, IRS is not useful for controlling exophilic vectors (TRUNG *et al.*, 2005; PATES and CURTIS, 2005), although it might be effective against exophagic/endophilic insects which enter dwellings to rest after feeding (GUNASEKARAN *et al.*, 2005). Studies which compared the efficiency of IBN's and IRS have concluded that IRS provides better results in malaria vector control (CURTIS and MNZAVA, 2000). Indoor residual spraying has also been found to be more cost-effective than IBN's in communities with low seasonal risks of malaria infection (GUYATT *et al.*, 2002a; GUYATT *et al.*, 2002b).

2.2.2 Chemical control - destruction of vectors in early developing stages

2.2.2.1 Insect growth regulators (IGR) and larvicides

Insect growth regulators (IGR), also known as growth inhibitors, are substances which interrupt or inhibit the life cycle of an insect by disrupting the normal activity of the hormone system, affecting the development, reproduction, or metamorphosis of the target insect. The target insect's growth is inhibited and kept from reaching the critical adult stage, therefore preventing it from reproducing. Of the many used IGR's the most widely applied are methoprene and pyriproxyfen which are commonly used in combination with adulticides in spot-on solutions to inhibit the growth of immature fleas. IGR's are also used in arthropod vector control programs by inhibiting the development of mosquito larvae. However, success depends on an effective localization of suitable breeding places (YAPABANDARA *et al.*, 2001; YAPABANDARA and CURTIS, 2004).

Other substances which are used to control arthropod vectors in developing stages include biological insecticides, such as the microbial larvicides *Bacillus sphaericus* and

*Bacillus thuringiensis israelensi*s (Bti) (WHO, 2006b). Bti is a larvicide made of a bacteria found naturally in the soil called *Bacillus thuringensis* var. *israelensis* (Bti). It is mixed with water and sprayed onto surfaces where the larvae are found. Bti is ingested by the larvae, bacterial spore is formed in the midgut and death occurs shortly after. The spores germinate in the dead larvae and complete a growth cycle (KHAWALED *et al.*, 1990). It has proven effective against mosquitoes and black flies larvae in the laboratory as well as in the field (GUILLET and DE BARJAC, 1979; LACEY *et al.*, 1982; RABINOVITCH *et al.*, 1999; SHARMA *et al.*, 2003; LEE and ZAIRI 2005; LEE and ZAIRI, 2006). The benefit of this product is that it does not affect other organisms (LARGET and CHARLES, 1982; GHARIB and HILSENHOFF, 1988; MITTAL *et al.*, 1994; DICKMAN, 2000).

2.2.2.2 Insect chemosterilants

An insect chemosterilant is any chemical compound used to control insect pests by causing temporary or permanent sterility of one or both sexes or preventing maturation of the young to a sexually functional adult stage. Laboratory-reared insects are sterilized by placing them in contact with treated materials and afterwards released. The mating of sterilized insects with fertile insects produces no offspring, resulting in reduction of the pest population (BORKOVEC, 1976). Chemosterilants can be categorized into two types of compounds, depending on their action: antimetabolites, such as amethopterin and aminopterin; which cause sterility in female insects by preventing egg formation; and alkylating agents such as tepa, metepa, and apholate which cause changes in genetic material and chromosomal damage in both male and female reproductive cells. Studies proved that it is not advisable to use chemosterilants directly on natural populations, because most chemosterilants cause genetic changes and are thus dangerous both to most animals and man (LABRECQUE and FYE, 1978; L'VOVA, 1981).

2.2.3 Non-chemical control

2.2.3.1 Biological control

Biological control is to control undesired pests through the introduction of man-chosen living organism into ecosystems which might act as predators, parasites, or disease

inflictors attacking the harmful insect. It is a form of manipulating nature to increase a desired effect. However, introducing a new living organism or favouring a pre-existing one in any ecosystem entails taking risks of disturbing the intended equilibrium through unexpected consequences (LACEY and ORR, 1994). Biological control of arthropod pests has been mainly used in agriculture, but also for the control of arthropod disease vectors (RAWLINS, 1989). Two examples are the control of *Aedes aegypti*, vector of dengue and yellow fever through the introduction of *Toxorynchitinae* mosquitoes whose carnivore larvae feed on *Aedes* spp. larvae (RAWLINS *et al.*, 1991; TIKASINGH and EUSTACE, 1992; MIYAGI *et al.*, 1992) and the introduction of larvivorous fish which feed on diverse mosquito larvae (VELIMIROVIC and CLARKE, 1975; SABATINELLI *et al.*, 1991; RUSSELL *et al.*, 1996). The use of entomopathogenic nematodes (EPN) such as Steinernematidae and Heterorhabditidae to control insects pests are a promising tool for biological control (LIU *et al.*, 2000; HOMINICK, 1990), especially concerning ticks (KOCAN *et al.*, 1998). EPN are highly mobile soil-dwelling round-worms, the infective juvenile form seeks suitable hosts such as arthropod larvae in the soil and infects them via natural opening. Once inside the host the infective juveniles release symbiotic bacteria which are toxic to arthropods, killing the host within 48 hours. The bacteria produce antibiotics which prevent the invasion of contaminating soil microbes avoiding the putrefaction of the cadaver. The infective nematodes then complete one to several generations inside the host. When all the host tissues have been consumed new generations of infective juveniles emerge, all carrying the symbiotic bacteria with them in search of new hosts (HOMINICK, 1990). The low production costs through simple technology make it suitable for developed and developing countries (HOMINICK, 1990).

2.2.3.2 Nuclear techniques (SIT)

Insect pests can be controlled or eradicated through a nuclear technique also known as sterile insect technique (SIT). The concept consists in colonizing and mass rearing males of the target insect pest and sterilizing them by ionizing radiation. After initial reduction of the target population by conventional means, the reared insects are aerially released into the field on a sustained basis and in sufficient numbers to achieve appropriate sterile to wild insect over-flooding ratios. The wild females will have no offspring following mating with a released sterile male, leading to a reduction in the natural pest population. Unlike insecticidal techniques SIT has no effect on non-target organisms and becomes more efficient with decreasing density of the target population.

SIT has been successfully used to combat tsetse populations, eradication of *Glossina austeni* (Diptera: Glossinidae) was achieved for instance on the island of Unguja, Zanzibar using the SIT as last phase of the campaign after tsetse population suppression with ITC and ITT (VREYSEN *et al.*, 2000).This technique fits well within the concept of integrated pest management; its complementary use in a phased approach with other suppression techniques can result in maximum efficiency (RAWLINS, 1989; VREYSEN, 2001). However, comparative studies indicate that insecticidal techniques such as ITC may be more cost effective than SIT (VALE and TORR, 2005).

2.2.3.3 Sanitation

Destruction of the vectors in early developing stages such as larvae or pupae for individual and family protection is viable through peri-domestic sanitation (WHO, 1982). However, community protection implies a strong entomological knowledge of vector behaviour as well as ecological circumstances. Measures incorporate identification, elimination or reduction of man-made breeding places through small scale drainage, environmental sanitation, urban-agriculture and water management as well as implementation of intermittent irrigation programs (WHO, 1982). Reduction of breeding places through source reduction involves continuous programs and community involvement, being therefore only viable in smaller communities.

2.2.3.4 Reduced host-vector interface

Reduction of the contact between humans or animals and vectors has been mainly obtained with screening of houses or stables with nets, bed-nets, protective clothing, zooprophylaxis and repellents. Zooprophylaxis consists in the reduction of human-vector contact by diverting the blood-sucking vectors to domestic animals which act as dead-end or decoy hosts (WHO, 1982), for example malaria. It has been described that by increasing the number of cattle in mixed dwellings the chances of people being bitten by anophelines may decrease, since a considerable proportion of bites are inflicted on animals (ROSS, 1910). However, recently, authors have come to the conclusion that the presence of cattle in mixed dwellings tends to increase the man biting rate of vectors (SOTA and MOGI, 1989; HEWITT *et al.*, 1994; BOUMA and ROWLAND, 1995). On the other hand, cattle kept in separate cattle sheds outside of

the human dwellings tend to reduce the man biting rate if the vector is preferentially zoophilic (SEYOUM *et al.*, 2002; SAUL, 2003). It is also considered that zooprophylaxis if not combined with an insecticidal approach may have very little effect on malaria vector control (KAWAGUCHI *et al.*, 2003).

2.3 Entomological techniques

2.3.1 Sampling methods - mosquitoes

Adult mosquitoes are collected during their active period or resting period according to the purpose of the entomological survey. Most haematophagous mosquitoes have crepuscular and nocturnal activities.

2.3.1.1 Hand catch

Hand collection of indoor-resting mosquitoes is usually done early in the morning from walls and surfaces using a simple aspirator tube. Aspirator tubes can easily be made with rubber tubing, small plastic container and a piece of nylon stocking, for instance. Mosquitoes are afterwards transferred into paper cups with covered netting or some other suitable container for transportation.

Mosquitoes can also be caught outdoors using the aspirator on vegetation, on solid surfaces in sheltered places, such as the banks of streams and ditches, holes in rocks, cracks in walls, caves, animals borrows, on the trunks or stems of larger vegetation such as banana trees, in old termite mounds or in shelters specially constructed for the purpose (SERVICE, 1980).

2.3.1.2 Spray sheet collection

Spray sheet collection of mosquitoes is done by spraying pyrethrin in a closed space to knock down mosquitoes resting inside a house and collecting them on white sheets spread on all flat surfaces. This technique reflects more adequately the density of mosquitoes in a house and seasonal changes in indoor resting density than hand collection of indoor resting mosquitoes because many mosquitoes may be hidden from

sight as well as dependant on the workers perseverance and time spent searching for mosquitoes (SERVICE, 1980).

With the sheet collection method it may be possible to collect all mosquitoes from a well-closed room sprayed with pyrethrin solution.

2.3.1.3 Exit traps

Mosquitoes may also be collected using simple exit traps which are fitted into well sealed rooms with only few exit points for mosquitoes. These traps are used to observe the daily movements of mosquitoes into and out of houses allowing a distinction between the mosquito species which bite and rest indoors (endophagic and endophilic) and those that only bite indoors but rest outdoors (endophagic and exophilic) (WHO, 2003b).

2.3.1.4 Human landing catch (HLC)

Mosquitoes can also be caught in their active period when females are in search of a blood meal and are attracted to men and animals. The number of vectors attempting to bite humans and/or animals is important information and can be measured by using men or animals as bait; mosquitoes which land on the skin are caught before they bite (WHO, 1975). The catcher should sit quietly in a selected place with clothing adjusted so that the legs are exposed to the knees. Each catcher holds a torch lamp which is turned on every couple of seconds and without brusque movements inspects his legs in search of mosquitoes. When a mosquito is spotted it is dazzled with the torch light at close range and before it has inserted its mouthparts a test tube is carefully placed over the mosquito which responds by flying into it. Mosquitoes may also be aspirated into individual plastic test tubes. The test tubes are transferred into labelled bags according to the hour of catch. Each catcher is in possession of one mosquito bag for each hour of the night. Catches should be done from dusk to dawn but may also be done only during certain hours if the peak biting time is known. In the first case, two shifts of mosquito catchers are needed. Catches can be done indoors, outdoors or both, according to the purpose of the entomological survey. Animals can also be used as bait to catch mosquitoes which try to take a blood meal, the animal should be alone or stationed at a short distance from other animals, mosquitoes are spotted with a torch

light, caught using aspirators and kept in paper cups according to hour of catch (WHO, 2003b).

Ethical considerations about routinely exposing workers to the risk of contracting malaria are to be taken into consideration (WHO, 2003b); therefore workers should take an appropriate drug for prophylaxis of malaria. In the case of the workers having native resistance to malaria prompt treatment should be offered free of charge in case of illness. Although the human landing catch might be ethically controversial the mechanical traps may not always be reliable for studies which require calculations of mosquito densities and EIR's (entomological inoculation rate). Therefore the human landing catch remains as selected method for most studies (LAGANIER *et al.*, 2003; MBOERA, 2005; MATHENGE *et al.*, 2005). However, mechanical traps beneficiate the advantage of not having a human disturbance factor. Mechanical traps allow a better comparison of results because all traps, a priori, function and attract insects equally whereas different catchers attract mosquitoes differently and may use different catch techniques.

2.3.1.5 Baited mosquito traps

Mosquitoes may also be collected using human/animal baited traps. Mosquitoes are drawn into the trap in seek of a blood meal but never reach the host being captured in a trap compartment. This method is suitable for catching a large amount of mosquitoes but does not give accurate information about the times at which biting occurred (WHO, 2003b). This information is best obtained by making hourly collections during the night. Some mosquitoes are attracted to carbon dioxide, consequently traps baited with CO_2, can be used to catch mosquitoes. Carbon dioxide can be obtained from gas cylinders, sublimation of solid carbon dioxide (dried ice), or even generated from yeast (SAITOH *et al.*, 2004; OLI *et al.*, 2005). Traps baited with carbon dioxide can be used during the day or night.

Odour-baited traps have been developed with the aim to attract mosquitoes using blends of odorous substances present on animal or human skin such as lactic acid, ammonia, and fatty acids (MCCALL *et al.*, 1996; COSTANTINI *et al.*, 1998; KLINE, 1998a; DUCHEMIN *et al.*, 2001; SILVA *et al.*, 2005). Odour-baited traps have been particularly successful catching the main dengue fever vector *Aedes aegypti* (KROCKEL *et al.*, 2006; MACIEL-DE-FREITAS *et al.*, 2006; WILLIAMS *et al.*, 2006).

Such a trap may imitate a human host and attract mainly anthropophilic mosquitoes. Some traps may even be baited with pieces of human clothing which incorporate still human odours, for instances foot odour from used cotton or nylon socks (NJIRU *et al.*, 2006).

2.3.1.6 Light traps

Light traps can attract mosquitoes using several types of light sources such as a normal tungsten light bulb, a fluorescent tube, black-light bulb or even a mercury vapour discharge lamp. The number and type of mosquito species caught by a light trap is greatly influenced by different light intensity and spectral emission (NOVAK, 1967; WILTON and FAY, 1972). Some mosquito species are attracted to UV light, whereas other species are more attracted to small incandescent lights. Biting midges, black flies and phlebotomines may be caught using special black light traps (SERVICE, 1979; ANDERSON and LINHARES, 1989; GALATI *et al.*, 2001). Some mosquito species even have greater activity in moonlit nights (CHARLWOOD *et al.*, 1986). Light traps are only used at night. Both carbon dioxide baited traps and light traps do not give information on biting rates or host preferences but are considered useful due to the absence of a human disturbance factor which is present by human baited traps and human bait catch. These traps are nevertheless very useful in entomological surveys whose primary interest is to know which mosquito species are present in a particular area.

2.3.2 Sampling methods: nuisance flies and tsetse flies

Tsetse flies of both sexes bite mostly during the day. They are attracted to dark colours, generally blue or black, and attack preferentially moving hosts. Traps use blue and black cloth in a shape that attracts flies and then funnels them upwards into a trap cage – monoconical or biconical shape. Traps may also be odour-baited increasing its attractiveness and number of caught insects (VALE, 1974). Some tsetse flies are highly attracted to carbon dioxide or acetone, but also ruminant's urine (SPÄTH, 1995). The effectiveness of traps can be enhanced by addition of carbon dioxide (dried ice) or an odour-bait, such as acetone, octenol or POCA (odour blend of propylphenol, octenol, p-cresol and acetone) (VALE, 1982; JAENSON *et al.*, 1991). The deployment of traps has been used to reduce tsetse flies density and therefore trypanosomosis

transmission (KUZOE AND SCHOFIELD, 2004). Attempts to eradicate tsetse flies through trapping began in the early 1900's (BRUTO DA COSTA *et al*, 1916). Trapping and integrated tsetse control in Príncipe Island resulted in eradication of *Glossina palpalis* for sixty years after its reinvasion in 1956 (LAPEYSSONIE, 1988) More sophisticated traps were designed such as the Harris trap, similar to the Malaise trap, based on the principle that tsetse are attracted to a dark vertical screen. The biconical trap was developed in the seventies (CHALLIER and LAVEISSIÈRE, 1973) which exploited the idea of contrast between the trap and the background vegetation based exclusively on visual attractiveness. The Vavoua Trap, developed in Ivory Coast, also known as monoconical trap, came in the line of the biconical trap having as major advantage its lower price and high catching effectiveness (LAVEISSIÈRE and GREBAUT, 1990). Besides tsetse flies also stable flies and horseflies can be effectively caught with the Vavoua trap or biconical trap (JAENSON *et al.*, 1991; MIHOK *et al.*, 1995b). Recently a new multipurpose trap model called "Nzi" (Swahili for fly) has been developed to catch tsetse flies, stable flies and horseflies (MIHOK, 2002; MIHOK *et al.*, 2006). Stable flies can also be caught with adhesive traps (KAUFMAN *et al.*, 2005; TAYLOR and BERKEBILE, 2006).

2.3.3 Dissection techniques (Culicidae)

Dissection of mosquitoes is routinely done in order to determine ovarian age or infectivity relative to particular vector-borne diseases like filariasis or malaria. Dissecting material include finely pointed forceps, dissecting needles, slides, PBS solution, staining material, physiological solution, and water. Dissections are performed under a binocular. The female insect may be killed using chloroform, some other anaesthetic or it may be knocked down by refrigeration, this way maintaining all the important structures fresh for dissection (SERVICE, 1980).

2.3.3.1 Determination of species, age and infection incrimination

Determination of mosquito genus and species is commonly done using specific morphological keys. However there are cases when different species are not morphologically differentiable. In these situations it is required to run a PCR in order to determine the correct mosquito species. The body of the female insect is placed on a

microscopic slide and the wings and legs are pulled out, these structures can be macerated and used for PCR.

Age determination of female mosquitoes can be done through ovary dissection (DETINOVA, 1962). The age of the mosquito is determined in relative terms considering as older mosquitoes those which have already laid an egg batch (parous) and young mosquitoes as those which have not yet reproduced (nulliparous). Differentiation between parous and nulliparous females is possible through examination of the condition of the ovarian tracheal system. Nulliparous females display orderly rolled-up tracheoles (skeins) and parous females have distended tracheoles forming a tracheal net (Fig. 2.2).

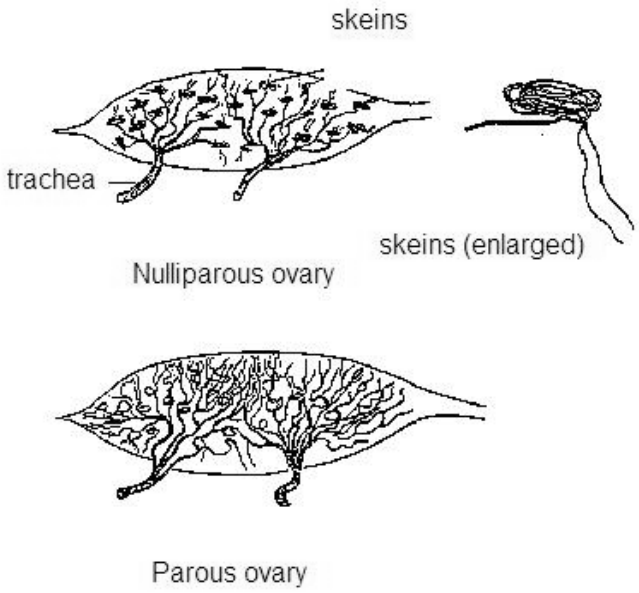

Fig. 2.2 Appearance of nulliparous and parous mosquito ovaries (source: WHO, 2003b).

Parity determination is necessary in order to calculate the parity rate as well as to identify the number of mosquitoes necessary for the determination of the sporozoite rate. Anopheles mosquitoes can only become infective after a blood meal, therefore only parous females are possible sporozoite carriers.

Female anophelines are dissected for malaria parasites. Vectors may be identified through dissection of salivary glands or through immunological methods such as ELISA (BURKOT, 1984a; BURKOT, 1984b; WHO, 2003b). The dissection of the salivary glands of individual mosquitoes looking for sporozoites has two major disadvantages.

In areas where vectors have low sporozoite rates it is necessary to dissect many mosquitoes to establish a sporozoite rate, and this method does not allow the identification of the species of malaria parasite involved. The immunological method (ELISA) is routinely used to detect sporozoites in a mosquito. This method allows not only a quantification of the number of sporozoites present, but it also allows a precise species identification (BEIER *et al.*, 1988a; MA *et al.*, 1990). The limitation of the immunological method is that it does not distinguish between infected and infectious mosquitoes (BEIER *et al.*, 1987; ROBERT *et al.*, 1988). Since the specific surface antigen detected (CSP1) is also present in sporozoites inside oocysts, which have not yet made their way to the salivary glands, the sporozoite rate determined in this way can be an over-estimation by up to 50% (BEIER *et al.*, 1988b; BEIER *et al.*, 1990; SOKHNA *et al.*, 1998). PCR is recently used as an alternative test for detecting malarial sporozoites in mosquitoes presenting high specificity and sensitivity (TASSANAKAJON *et al.*, 1993; STOFFELS *et al.*, 1995; VYTHILINGAM *et al.*, 1999).

2.3.4 Entomological parameters in malaria vector control

Parity rate (PR) corresponds to the percentage of parous mosquitoes. Under stressful conditions caused by vector control measures female mosquito populations tend to be mainly composed of nulliparous specimens which correspond to the young females rising from their breeding places (DETINOVA, 1962).

Human biting rate (HBR) is the average number of bites per person per period of time; this parameter can be presented as a daily biting rate, weekly biting rate or monthly biting rate. Human-biting rates are best estimated by all-night collections of mosquitoes that come to feed on human baits (WHO, 2003b).

The sporozoite rate (SR) corresponds to the percentage of mosquitoes with sporozoites in their salivary glands and usually varies between 1 and 20% (MACDONALD, 1957).

The entomological inoculation rate (EIR) is a measure of the number of infective bites each person receives per period of time, and is a direct measure of the risk of human exposure to the bites of infective mosquitoes (WHO, 2003b). The EIR can be calculated based on mosquito density, multiplying the HBR by the SR. This parameter is a useful indicator of the intensity of malaria transmission and provides information on

seasonal variations in transmission (WHO, 2003b). EIR's can vary greatly between 1 and 1000 infective bites per person per year (BEIER *et al.*, 1999).

Vectorial capacity (VC) measures the potential for malaria transmission, based on several key parameters of vector populations. It is defined as the daily rate at which future inoculations arise from a currently infective case and it is directly related to the number of bites per person per day (or man-biting rate), feeding habits (anthropophilic vs zoophilic) and life expectancy of the mosquito. The equation used to calculate VC is C = ma2pn/–logcp, where C = vectorial capacity, m = density of vectors in relation to humans, a = number of blood meals taken on humans per vector per day, p = daily survival probability of vectors (measured in days), and n = incubation period in the vector (measured in days) (MACDONALD, 1957). The formula expresses the capacity of a vector population to transmit malaria based on the potential number of secondary inoculations originating per day from an infected person. The formula is specific for a given species of vector, because different species vary with respect to m, a, and p. If several vector species coexist, the VC is the sum of the vectorial capacities of each of the individual vector species. Vectorial capacity is an essential component of mathematical models of malaria transmission.

3 Materials and methods

3.1 Study site

The field studies took place within the premises of the Boadi Cattle Research Farm of the Kwame Nkrumah University for Science and Technology (KNUST), Kumasi, Ghana, at 6º41' latitude and 1º32' longitude. The climate is characterized as semi-equatorial with an annual rainfall of around 1400 mm with two distinct rainy seasons. The annual mean temperature is from 26 to 29ºC with humidity ranging from 65 to nearly 100% (ENYCLOPEDIA BRITANNICA, www.britannica.com/EBchecked/topic/232376/Ghana). The site was characterized as Ghanaian forest region, having areas of dense vegetation and high grass fields. Through the site ran a slow water course of about 20 meters width surrounded by a gallery, being wider during rainy season and sometimes inexistent during dry season. The trial was conducted during the months of October and November of 2005, corresponding to the transition of the rainy season to the dry season. Four similar sites at a distance of 20-40 meters from the water course were chosen on which four identical stables were built (Fig. 3.1).

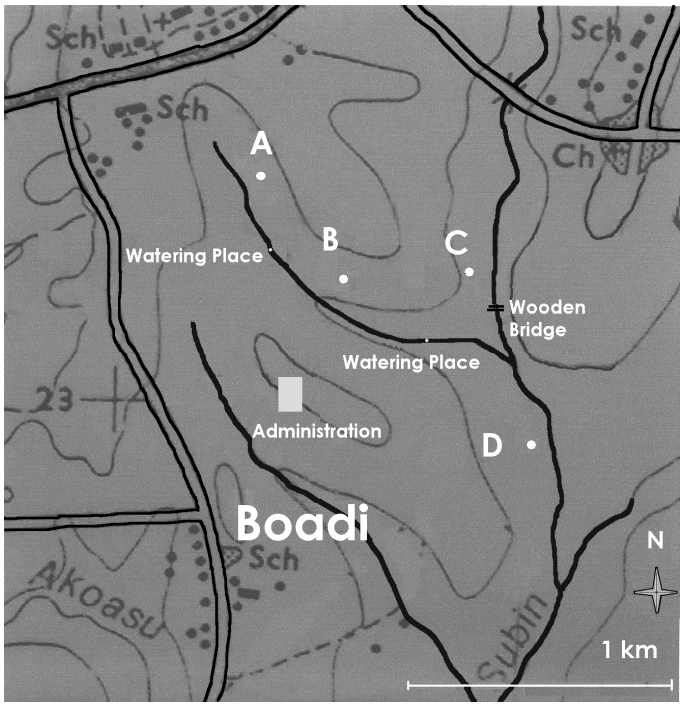

Fig. 3.1 Map of the trial site at the Boadi Cattle Research Farm, outside Kumasi, Ghana, 2005. Points A, B, C, and D represent the locations where the stables were built in the vicinity of a small water course. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

3.2 Experimental stables

The pen dimensions were six by seven meters (area), half the pen was roofed with corrugated iron sheets and all pens were surrounded by a one meter high chicken wire fence (Fig.3.2). The treated and the control net were attached in two stables against the chicken wire. The stable ground was concrete and had a small slope in order to facilitate removal of the dung.

The pens were all 500 meters apart from each other. Geographical coordinates of the pens were measured using a portable GPS reader with average 10 m accuracy: A (N 6º 41' 23,9''; W 1º 32' 30,7''); B (N 6º 41' 12,1''; W 1º 32' 17,9''); C (N 6º 41' 18,8 ''; W 1º 32' 8,3''); D (N 6º 41' 6,1''; W 1º 32' 3,3'').

Fig. 3.2 Photo of one of the four identical trial pens built on the Boadi Cattle Research Farm and experimental animals. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

3.3 Experimental animals

A total of six zebus were chosen from the herd of the Boadi Cattle Research Farm. All animals were black male zebus of approximately 80-120 kg weight and with ages between 1 and 3 years. All animals were kept on a zero-grazing system and were weekly rotated between pens B, C, and D during the trial period.

3.4 Experimental nets

The treated net consisted of a black 150 denier polyester fibre with 2 x 2mm mesh width, and impregnated with 80-120 mg of deltamethrin per square meter. The untreated net consisted of the same but untreated material.

Samples from the treated net in pen D were regularly taken for a period of eight months and submitted to bioassays using *Musca domestica* and *Aedes aegypti.* Persistence of insecticide activity in the treated net exposed to outdoor conditions was tested. Bioassays were done by covering the inside of a small cylindrical container (5 cm diameter x 10 cm height) with experimental net, the control container was coated using the non-impregnated net. Each net sample was tested twice by releasing the insects into the container through a small hole submitting them to 10 seconds contact with the net. The insects were then transferred into a large cage through a wide opening at the end of the tube and the number of paralyzed insects was counted after 5 minutes, 10 minutes, 15 minutes, 6 hours and 24 hours.

3.5 Experimental design

The trial was performed for a period of six weeks during which the effectiveness of the treated net protecting cattle against nuisance and biting insects was evaluated. The stables were randomly selected as Pens A, B, C and D. Pen A served as a negative control, with no animals and no netting attached to the chicken wire; Pen B had a mechanical protection with the untreated net and two zebus; Pen C served as unprotected control with two zebus; and Pen D served as the experimental stable with two zebus and an impregnated net attached to the surrounding chicken wire fence.

3.6 Sampling and recording methods

3.6.1 Sampling methods

3.6.1.1 Mono-conical traps (Vavoua traps)

The mono-conical traps were first designed in Vavoua, Côte d'Ivoire (LAVEISSIÈRE and GREBAUT, 1990). They are suitable in catching tsetse flies (*Glossina*) and other Diptera such as Muscinae and Stomoxyinae. It consists of a mosquito netting cone attached to a circular piece of galvanized metal wire and placed above three screens joined together at angles of 120° (Fig. 3.3). Each screen is two-thirds blue and one-third black, the black joining together in the middle. The flies land on the screens, fly upwards towards the light, pass through the upper cone and finally enter the cage where they are collected.

Fig. 3.3 Mono-conical trap. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

The sites selected for trap deployment were in the vicinity of the stable locations. The traps were set in pairs at all locations, one trap at a distance of 20 m from the stable and the other trap directly outside the fringing gallery vegetation of the water course. During the six weeks they were deployed at weekly intervals for 24h.

3.6.1.2 Human landing catch (HLC)

Human landing catch is a standard method used for collecting mosquitoes in entomological surveys (WHO, 1975). The human catcher serves as bait for blood sucking mosquitoes. The catch is performed from dusk until dawn; during which catchers sit on small stools with bare legs and a flashlight, waiting for mosquitoes in search of a blood meal (Fig. 3.4). The mosquito catcher examines his legs with a flashlight every 10 seconds avoiding any sudden movement. Once the mosquito lands on the catcher's bare skin it is entrapped in a small test tube.

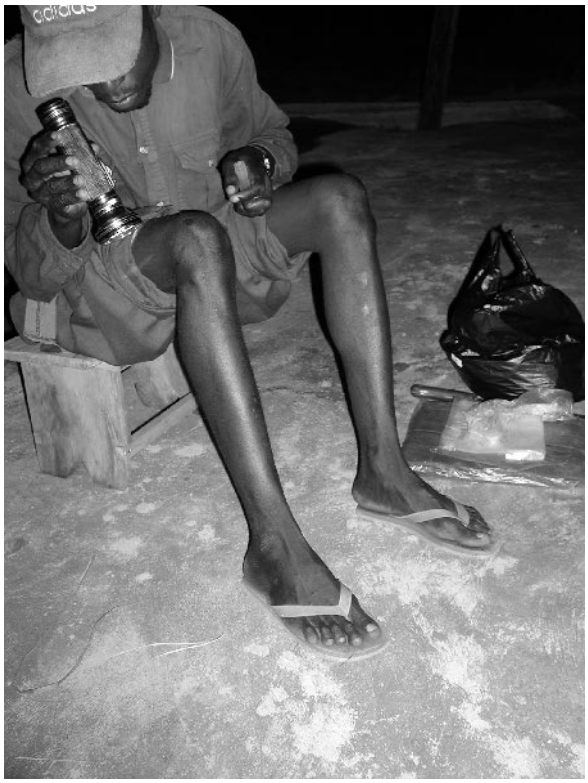

Fig. 3.4 Mosquito catcher during a human landing catch. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

Catchers were grouped in two shifts, from 18:00 to 24:00 and from 00:00 to 06:00. During each shift catchers were distributed in pairs for all pens, one mosquito catcher remained within the stable and the other sat approximately 20 m away. Each mosquito catcher was given a set of twelve plastic bags, labelled with date, hour and location. The mosquitoes were separated according to their hour of catch and site. All workers were offered treatment free of charge in case of contracting malaria. Temperature and precipitation were hourly recorded.

3.6.1.3 Odour-baited trap (BG-Sentinel™ trap)

The BG-Sentinel™ mosquito trap (BioGents, Regensburg) was originally designed to catch *Aedes aegypti* (GEIER *et al.*, 1996; PAPPENBERGER *et al.*, 1996; BOSCH *et al.*, 2000; DEKKER *et al.*, 2005). This trap is an odour baited mechanical trap powered by a car battery (Fig. 3.5), with a built-in fan which disperses the odour into the surrounding environment and simultaneously draws air into the catch bag (Fig. 3.6). The odour bait consists of a combination of non-toxic substances that are specifically found on human skin: ammonia, lactic acid, and fatty acids (the exact composition is not provided by the manufacturer).

Fig. 3.5 Mechanical odour baited mosquito trap powered by a car battery – BG-Sentinel™ trap. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

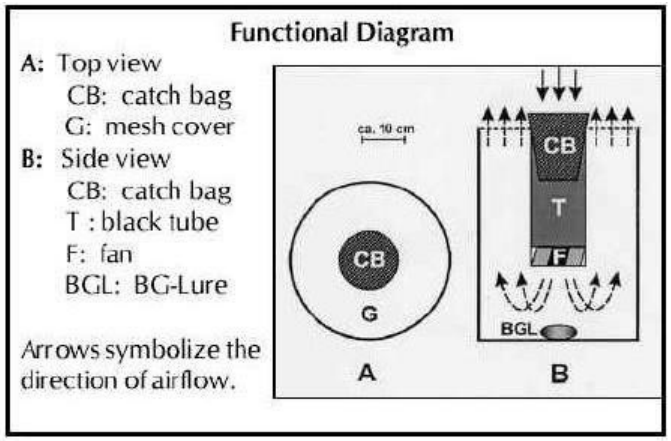

Fig. 3.6 BG-Sentinel™ trap, transversal and longitudinal sections; Functional Diagram illustrating the air currents produced by the in-built fan and all other traps components.

The BG-Sentinel™ traps were weekly deployed inside each pen. The traps were set at dusk and retrieved in the morning.

3.6.2 Recording methods

3.6.2.1 Digital photos of selected animal body regions

In order to quantify the infestation with house flies and stable flies seven representative body regions were selected: head, fore and hind leg, lower neck, flank, lateral thorax and abdomen. Photos were taken with a digital camera from close range twice weekly of both animals present in each pen (B, C, and D). Body regions of both animals were twice photographed from both sides. The mean number of nuisance flies per animal on all selected body regions for each pen was calculated as shown in Fig. 3.7.

$$\frac{\Sigma ai + \Sigma bi + \Sigma aii + \Sigma bii}{4}$$

Figure 3.7 Mathematical formula used to calculate mean number of flies per animal (animals - a, b) on all selected body regions during photo sessions taken twice weekly (experimental days- i, ii) each week.

3.6.2.2 Video recordings of animal defensive movements

Video recordings of about 30 seconds were taken twice weekly from the animals inside pens B, C, and D. The following defensive movements were counted: head throws, front and hind leg stamps, skin twitches (panniculus reflex) and tail flicks. An average number of defensive movements during 30 seconds regarding each pen was calculated as shown in Fig 3.8.

$$\frac{\Sigma ai + \Sigma bi + \Sigma aii + \Sigma bii}{4}$$

Figure 3.8 Mathematical formula used to calculate average number of animal defensive movements done by two animals (animals - a, b) during two 30 second long video recording taken twice weekly (experimental days - i, ii) each week.

3.7 Handling of collected insects

Insects were killed at -20 ºC permitting their handling during morphological examination. Anopheline mosquitoes were immobilized at 4ºC to prevent deterioration of the ovaries.

3.7.1 Flies

Flies were morphologically examined, counted and separated into Muscinae, Stomoxyinae, and Tabanidae; all other insects were discarded after recording.

3.7.2 Mosquitoes

Mosquitoes (Nematocera: Culicidae) were morphologically identified and grouped into Culicinae and Anophelinae.

Anophelines were identified up to species level using a morphological key (GILLIES and COETZEE, 1968). Parity determination was done through ovary dissection (DETINOVA, 1962). Differentiation between parous and nulliparous females was achieved through examination of the condition of the tracheal system of their ovaries. Dissection was performed by separating the abdomen from the mosquito thorax and placement on microscopic slide over a drop of PBS solution. The integument of the abdomen was cut with dissecting needles on both sides of about the fourth segment. One needle was hold flat across the proximal segment to hold the abdomen in place while the other needle was used to gently pull the caudal segment apart; in doing so the gut and ovaries were withdrawn on the slide. The ovaries were removed and transferred to the edge of a drop of pure water placed separately on the slide and let to dry for approximately 24 hours. During the drying process air enters the trachea, tracheal branches and tracheoles making the ovaries completely visible. Microscopic examination of the dried-out ovaries permitted distinction between nulliparous females which displayed orderly rolled-up tracheoles (skeins) and parous females which had distended tracheoles forming a tracheal net.

Sporozoites rates for *Plasmodium falciparum* were determined by KCCR staff using head and thorax of parous mosquitoes through ELISA method. The antibody used was anti *Plasmodium falciparum* circumsporozoite protein antibody, the microtiter plates were coated with mab-peroxidase ie. monoclonal antibody peroxidase enzyme conjugate (BURKOT *et al.*, 1984a; BURKOT *et al.*, 1984b).

3.7.3 Other haematophagous mosquitoes

Other haematophagous insects, such as biting midges and sand flies, were counted. Half of the collected insects were stored in small test tubes in alcohol 70%; the other half was stored dry in Eppendorfs as reference samples.

3.8 Entomological data analysis

Mosquitoes were dissected for parity. Parity rates (PR) were calculated for each experimental pen and relative to both anophelines specimens:

PR = (Nr. parous mosquitoes / Total nr. mosquitoes) * 100

Monthly biting rates (MBR) were calculated by multiplying the average number of mosquitoes caught per man night per site by 30 (days for a month).

Sporozoite rates (SR) were calculated in each study site and relative to both anophelines specimens:

SR= (Nr. infected mosquitoes/ Total nr. mosquitoes) * 100

The entomological inoculation rate (EIR) was calculated by multiplying the sporozoite rates by the monthly biting rate.

3.9 Statistical analysis

The statistical analysis was done using SPSS Version 13.0 for Windows. ANOVA tests were used in each set of data to determine if there was a significant difference between the pens, in order to determine where the difference was located LSD (least significance difference) tests were used. Results were considered significant when $p<0.05$. Box plots were drawn to describe each data set (Fig. 3.7).

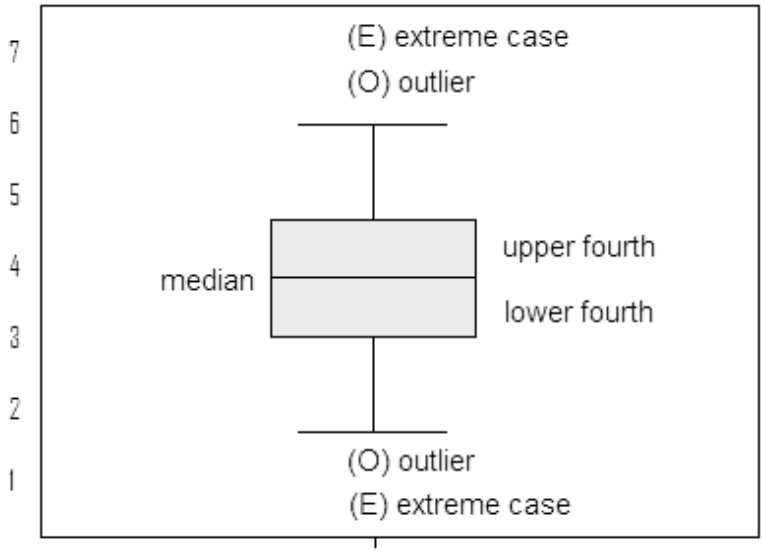

Fig. 3.7 Description of the elements of a box plot.

The top of the box is called the upper fourth**.** It is at the 75th percentile of the observations. The bottom of the box is called the lower fourth. It is at the 25th percentile of the observations. Therefore 50 % of the observations fall within the box. The interquartile range is the distance between the upper fourth and the lower fourth. The horizontal line through the box represents the median. The ends of the whiskers represent the largest and smallest values that are not outliers. An outlier (O) is defined as a value which is smaller (or larger) than 1.5 box-lengths from the lower fourth (upper fourth). The box-length is defined as the interquartile range. An extreme case (E), also called extreme outlier, is defined as a value that is smaller (or larger) than 3 box-lengths from the lower fourth (upper fourth). Outliers and extreme cases are observations which are numerically distant from the rest of the data. Statistics derived from data sets which include outliers and extreme cases may be misleading.

4 Results

4.1 Nuisance and biting flies

4.1.1 Mono-conical traps

Results of the mono-conical traps give an estimate of fly density in the vicinity of each pen. The majority of insects caught with the mono-conical traps were stable flies (52.6%) and house flies (45.4%). Occasionally, other insects such as Tabanidae (1.7%), Calliphoridae (0.2%) and Sarcophagidae (0.1%) were caught in the traps (Table 4.1). Insects of non-medical importance were discarded. An increase in the fly catches was observed during the trial. This was particularly pronounced after the third week where the total fly catch tripled.

Table 4.1 Total number of weekly catches with the mono-conical traps during the trial period. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

Total number of weekly catches with the mono-conical traps							
	Week						
Insect	1	2	3	4	5	6	Total
Musca	9	18	27	158	501	277	990
Stomoxys	10	26	43	252	535	363	1229
Tabanidae	0	1	6	6	4	4	21
Sarcophagidae	0	0	0	1	2	0	3
Calliphora	0	0	0	1	1	1	3
Total	19	45	76	418	1043	645	2246

There were clear differences between the total catches near pens B and C opposed to A and D (Table 4.2). Total catch percentiles of the trial show that B (untreated net) and C (no net) had similar results, with 44 and 49%; contrasting with A (negative control) and D (treated net) where the percentiles amounted to 4 and 3%, respectively. However, statistically the catches in the vicinity of all pens did not significantly differ (p=0,141).

Table 4.2 Total numbers and percentages of all insects caught with mono-conical traps (Others, representative of Sarcophagidae and Calliphoridae) during the trial period in the vicinity of each pen. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

Total numbers and percentages of all insects caught with mono-conical traps						
Location	Insects				Total	%
	Muscinae	Stomoxyinae	Tabanidae	Others		
A - Negative control	66	16	7	2	91	4
B - Untreated net	389	599	6	3	997	44
C - No net	498	587	3	1	1089	49
D - Treated net	37	27	5	0	69	3
Total	990	1229	21	6	2246	100

The catches around pen D (treated net) were constantly lower throughout the trial when compared with the catches around other pens (Fig. 4.1); they were even lower than the catches around pen A (negative control) which had no animals.

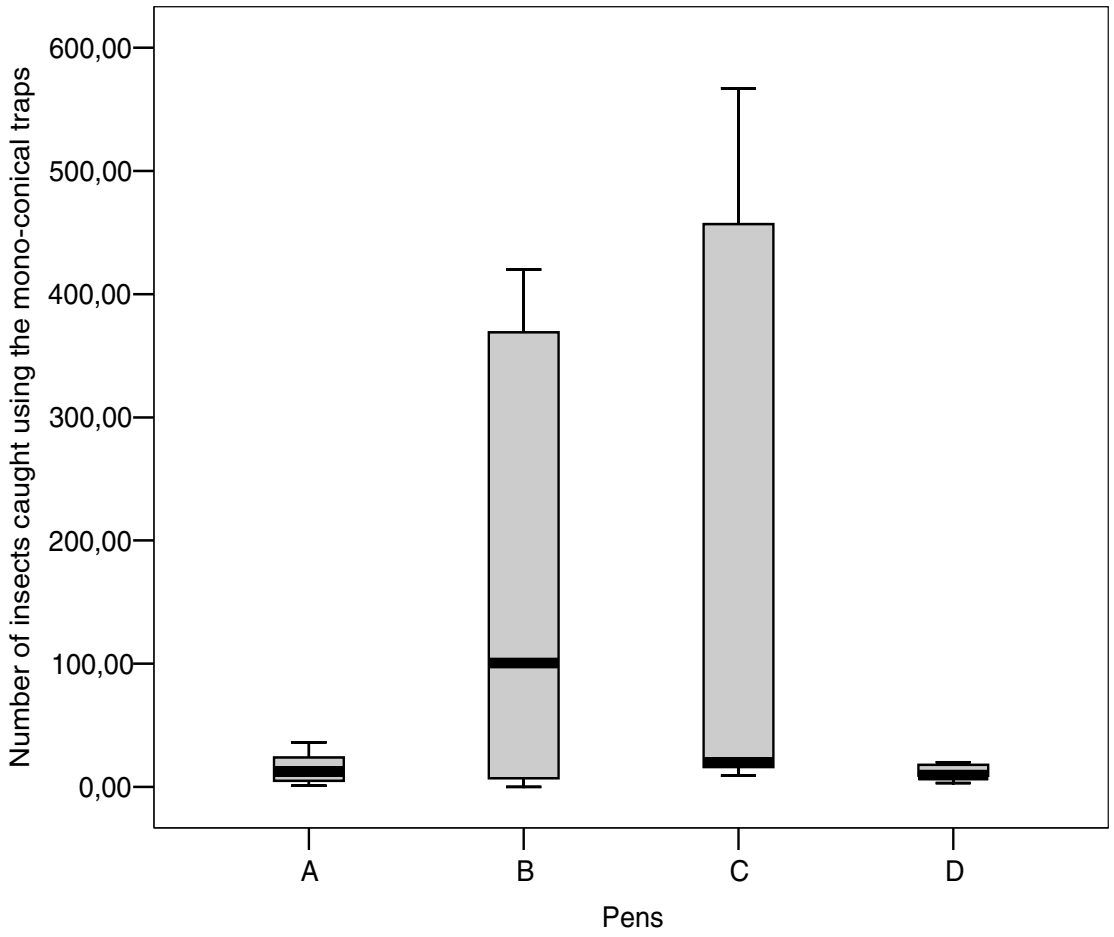

Fig. 4.1 Boxplot of the number of insects caught with the mono-conical traps around the pens showing the median, quartiles, and extreme values registered in each pen. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

Pen C had the highest catch with 49%, closely followed by B with 44%, comprising 92% of the total fly catch. In comparison to B and C the catches around D were 93% lower. The catches of A (negative control) and D were comparable.

4.1.2 Digital photo observations

Digital photos of both animals were taken twice weekly in pens B, C and D. The mean number of attacking flies on all selected body regions per animal was calculated.

The number of attacking flies was consistently higher in pens B and C than in pen D (Fig. 4.2).

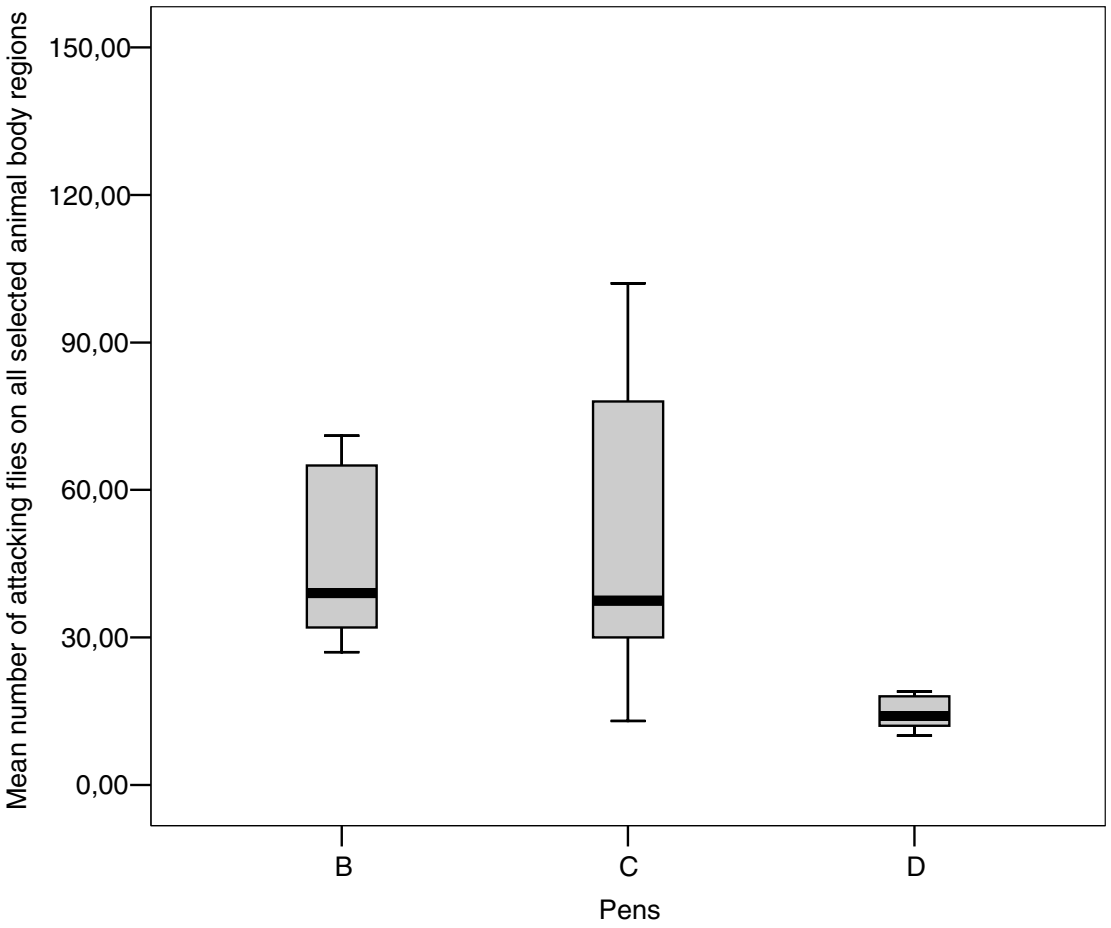

Fig. 4.2 Boxplot of the mean number of insects recorded on digital photos attacking selected animal body regions of the animals inside the pens showing the median, quartiles, and extreme values registered in each pen. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

Table 4.3 Mean number of attacking flies on all selected animal body regions in pens B, C and D. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

Mean numbers of counted flies on all selected body regions			
Week	B – Untreated net	C – No net	D – Treated net
1	27	13	12
2	32	33	19
3	38	30	10
4	71	42	18
5	40	102	12
6	65	78	16
Total	272	298	85

The mean number of flies on all selected animal body regions in each pen amounted to 45.5, 49.7 and 14.5 for B, C and D respectively. During the first three trial weeks pens B (untreated net) and C (no net) had mean fly counts that were 2.4 and 1.9 times higher than in pen D (treated net) . After the third trial week the fly counts in pens B and C increased distinctly, being about 4-5 times higher than in D. Pen D (treated net) had a relatively stable mean fly count throughout the trial, never going beyond 20 flies (Table 4.3).

There was a significant difference in the number of counted flies among all pens ($p=0.029$). Pen D had a significantly lower number of counted flies relative to pen B ($p=0.028$) and C ($p=0.015$).

Pens B and C had similar total fly count percentiles, with, respectively, 41.5 and 45.3%, comprising 86.8% of all flies counted on animal body regions. Pen D (treated net) had an inferior percentile with only 13.2% of the total fly count.

4.1.3 Video recording observations

Monitoring and counting of the defensive movements such as head throws, front and hind leg stamps, skin twitches (panniculus reflex) and tail flicks, caused by fly attacks was assessed by recording 30 second-long video sequences of both animals inside all pens twice weekly. The mean number of defensive movements by one animal registered in a 30 seconds sequence relative to each pen was calculated weekly.

Table 4.4 Weekly mean numbers of defensive movements per 30 seconds per animal. No video could be taken during the first trial week. ND – Not determined. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

Mean number of animal defensive movements				
	Location			
Week	B - Untreated net	C - No net	D - Treated net	Total
1	ND	ND	ND	-
2	34.25	41.75	2.5	78.5
3	10.5	13	1	24.5
4	59.25	25	17.5	101.75
5	14.75	65	6	85.75
6	19.5	19	2.5	41

Pens B and C had consistently higher weekly means of defensive movements per animal (Fig. 4.4). The defensive movements in D were below 10 with the exception of week 4 where the fly challenge was very high in all pens (Table 4.4). However, statistically the number of defensive movements recorded in each pen was not significantly different (p=0.064).

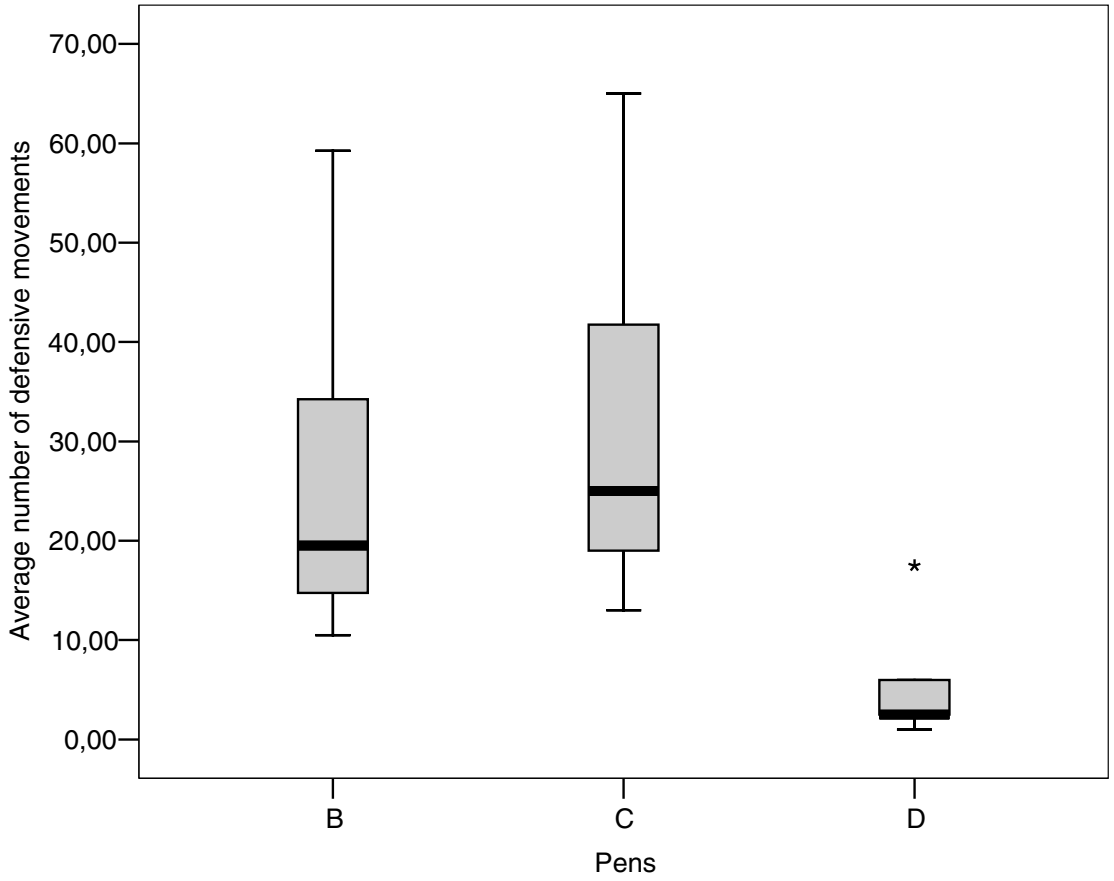

Fig. 4.3 Boxplot of the average number of defensive movements performed by the animals inside the pens showing the median, quartiles, extreme values, and an extreme case (⋆) registered in each pen. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

The animals of pens B and C had comparable defensive movements percentiles with respectively, 42 and 49 %, comprising 91% of all defensive movements observed in the recorded videos. Pen D had an inferior percentile with only 9% of the total defensive movements counted. The animals in pen D (treated net) showed 80% fewer defensive movements in comparison with B (untreated net) and C (no net) (Fig. 4.3).

4.2 Mosquitoes

4.2.1 Human landing catch (HLC)

Most of the mosquitoes caught with this technique were culicines comprising 76% of the total catch; the remaining 24% were anophelines. The majority of mosquitoes were caught at site B (34%), followed by A (29%), C (23%) and D (14%).The highest catch was during the fifth week, and the lowest catch was recorded during the second week. Weather conditions and moon phases during the catch nights were registered (Table 4.5).

Table 4.5 Climatic conditions and moon phase recorded during weekly human landing catch. Due to technical problems it was not possible to record temperature readings during the fifth human landing catch. ND – Not determined; Boadi Cattle Research Farm, Kumasi, Ghana 2005.

Climatic Conditions during HLC's					
Week	Average temperature	Maximal temperature	Minimal temperature	Precipitation (mm^3)	Moon phase
1	24,4	26,7	23,4	0	○
2	23,6	26,2	20,9	0	☾
3	23	25,1	21,9	0	●
4	23,2	27,3	22,2	0	☽
5	ND	ND	ND	0	○
6	24,9	28,2	23,1	0	☾

During the trial period the average temperature measured in Kumasi Ghana was 26ºC, average minimal daily temperature was 21.6ºC and average maximal daily temperature was 31ºC (Ghana Meteorological Agency, 2005). There was a total rainfall of 100, 4 mm^3 and 13 days of rain (KCCR). Nevertheless, during the nights of the human landing catches no precipitation was measured.

4.2.1.1 Culicines

Culicines represented the majority of mosquitoes (76%). Mosquitoes of the genus *Mansonia* and *Culex* were most common among the catches. However, due to the difficulty of the culicine genera and species identification culicine mosquitoes were only quantified by site. The peak in culicine biting activity was observed between 01:00 and 02:00 a.m. (Fig. 4.4).

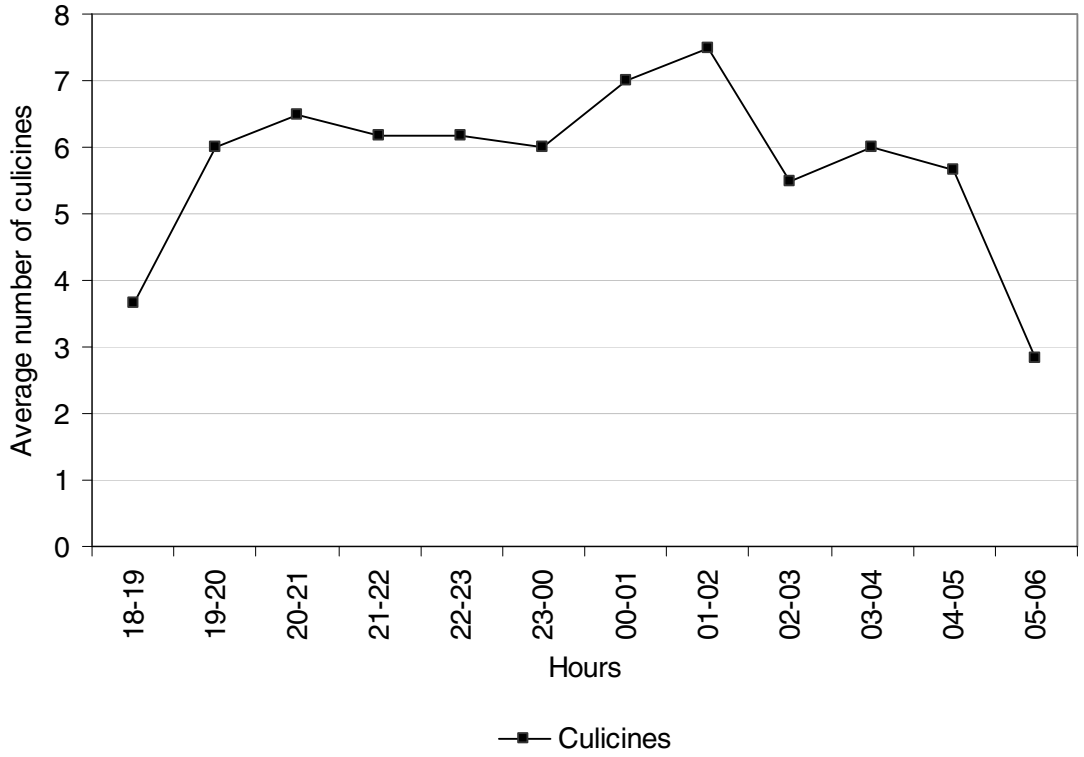

Fig. 4.4 Average number of culicines caught per hour during human landing catches. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

During each monitoring one catcher sat inside the pen and a second 20 meters outside the pen. The objective was to assess a possibly larger effect of the treated net on mosquitoes in the vicinity of the protected pen as had been observed by the participating farmers in Kenya (BAUER *et al.*, 2006).

Table 4.6 Total numbers of culicines caught in- and outside each stable during the weekly HLC's Boadi Cattle Research Farm, Kumasi, Ghana 2005.

Total numbers of culicines caught inside and outside the pens										
	Location									
	A – Negative control		B – Untreated net		C – No net		D – Treated net		Total	
Week	in	out	in	out	in	out	in	out	in	out
1	57	79	73	118	71	95	19	52	220	344
2	37	70	48	47	13	40	12	35	110	192
3	63	55	148	73	59	30	32	56	302	214
4	70	68	77	127	36	82	10	62	193	339
5	90	150	90	168	119	85	25	45	324	448
6	62	98	78	123	50	73	30	71	220	365
Total	379	520	514	656	348	405	128	321	1369	1902

The culicine catch outside the pens was greater than inside. Pen B which was protected with an untreated net had the highest culicine catches out- and inside the pen. The lowest catches were registered out- and inside the pen D which was protected with a treated net (Table 4.6).

The numbers of culicines caught inside all pens were significantly different (p=0.004). The culicine catch in pen D (treated net) was significantly different to the other pens; differences were significant between pens A and D (p=0.013), B and D (p=0.000) and C and D (p=0.026) (Fig. 4.5).

The number of culicines caught outside all pens was also significantly different (p=0.030). Pen B (untreated net) had significantly higher catches than other pens with animals; differences were significant between B and C (p=0.03) and B and D (p=0.005) (Fig. 4.6).

In pen A 27% fewer culicines were caught inside than outside, in B the difference amounted to 22% and in C to 14% fewer culicines. Pen D had the greatest difference between the number of mosquitoes caught in- and outside: on average the inner catches comprised only 28.5% of the total catch at this site. At the other pens inner catches comprised about 44% of the total catches for each location (A-42.2%; B-43.9%; C-46.2%).

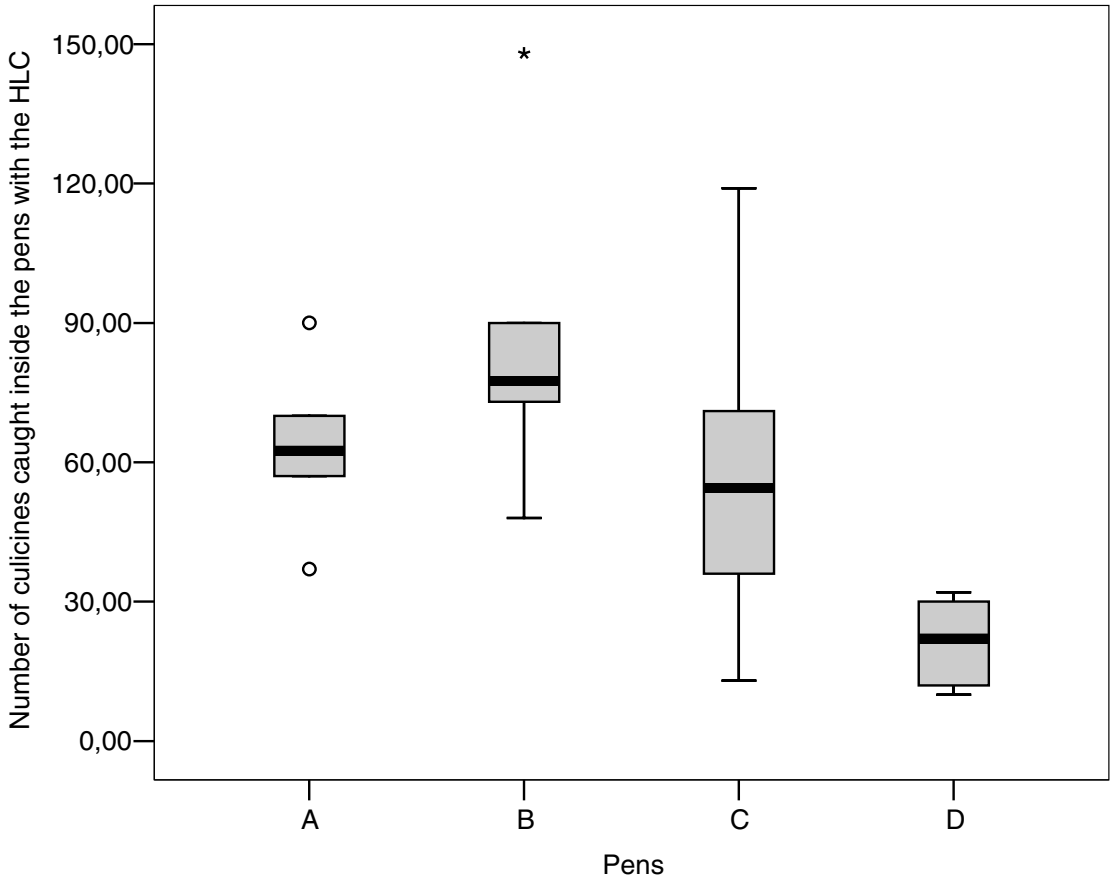

Fig. 4.5 Boxplot of the total number of culicines caught inside the pens using the HLC showing the median, quartiles, extreme values, an extreme case (⋆) and two outliers (○) registered in each pen. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

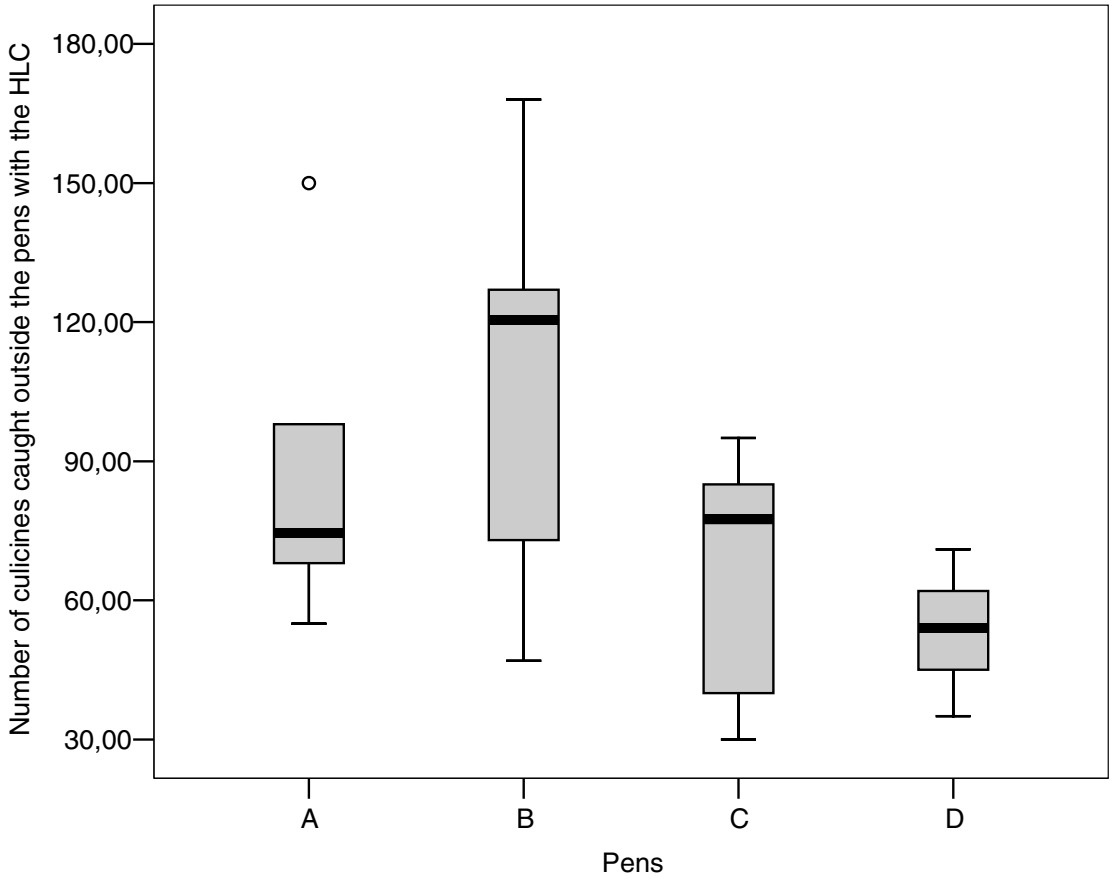

Fig. 4.6 Boxplot of the total number of culicines caught outside the pens using the HLC showing the median, quartiles, extreme values and one outlier (○) registered in each pen. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

4.2.1.2 Anophelines

Anophelines represented 22% of the total human landing catch. The site at which most anophelines were caught was A (34%), followed by B (26%), C (23%) and D (17%). Highest anopheline catches were observed during the first and third week, the lowest catch was recorded during the sixth week.

The caught anophelines were identified as *Anopheles gambiae* s.l. (46.1%)*, Anopheles ziemanni* (53.5%), and *Anopheles funestus* (0.4%) (Table 4.7).

Table 4.7 Number of different anopheline species caught during the weekly HLC's. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

Total numbers of *A. gambiae* s.l., *A. ziemanni*, and *A. funestus* caught with the HLC's				
Week	*A.gambiae* s.l.	*A.ziemanni*	*A.funestus*	Total
1	108	157	1	266
2	62	33	2	97
3	94	178	0	272
4	61	103	1	165
5	47	83	0	130
6	16	75	0	91
Total	388	629	4	1021

Both *Anopheles gambiae* s.l. and *Anopheles ziemanni* showed activity peeks between 02:00 and 03:00 (Fig. 4.7).

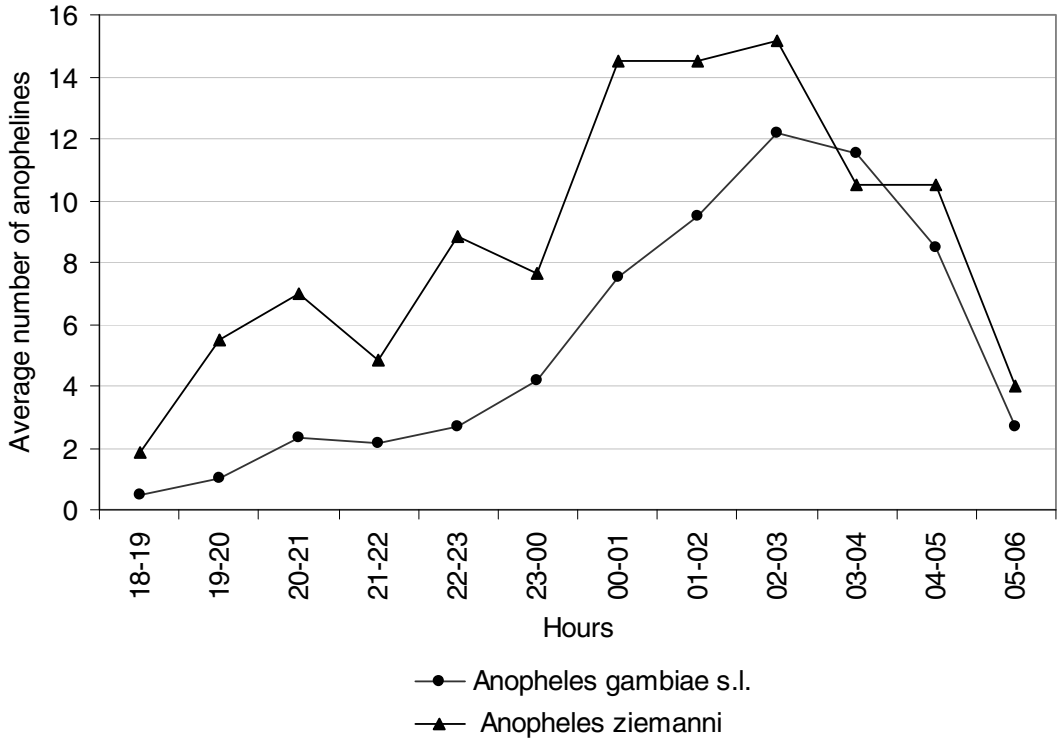

Fig. 4.7 Average number of *Anopheles gambiae* s.l. and *Anopheles ziemanni* caught per hour during human landing catches. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

On average the catchers caught 40% more *Anopheles ziemanni* than *Anopheles gambiae* s.l. at each site. The highest catch for *Anopheles gambiae* s.l. was observed in the first week and tended to decrease during the trial period (Table 4.8).

Table 4.8 Total numbers of *Anopheles gambiae* s.l. caught in- and outside each stable during the weekly HLC's Boadi Cattle Research Farm, Kumasi, Ghana 2005.

Total number of *Anopheles gambiae* s.l. inside and outside the pens										
	Location									
	A – Negative control		B – Untreated net		C – No net		D – Treated net		Total	
Week	in	out	in	out	in	out	in	out	in	out
1	38	5	10	10	11	18	5	11	64	44
2	18	10	4	14	1	10	3	2	26	36
3	23	10	20	3	7	8	12	11	62	32
4	15	0	12	6	9	5	7	7	43	18
5	21	3	4	4	11	2	1	1	37	10
6	6	3	1	0	3	2	0	1	10	6
Total	121	31	51	37	42	45	28	33	242	146

Catches between outer and inner positions were comparable with exception of pen A. Catches of *Anopheles gambiae* s.l. inside pen A were highest, comprising almost 80% of all catches recorded inside the pens. The catches of B, C and D differed only slightly between the respective positions.

The number of *Anopheles gambiae* s.l. inside all pens was significantly different ($p=0.005$). Pen A (negative control) registered a significantly higher catch compared to inside catches of other pens; catches were significantly different between pens A and D ($p=0.001$), A and C ($p=0.004$) and A and B ($p=0.009$) (Fig. 4.8).

The number of *Anopheles gambiae* s.l. caught outside the pens was not significantly different ($p=0.860$) (Fig. 4.9)

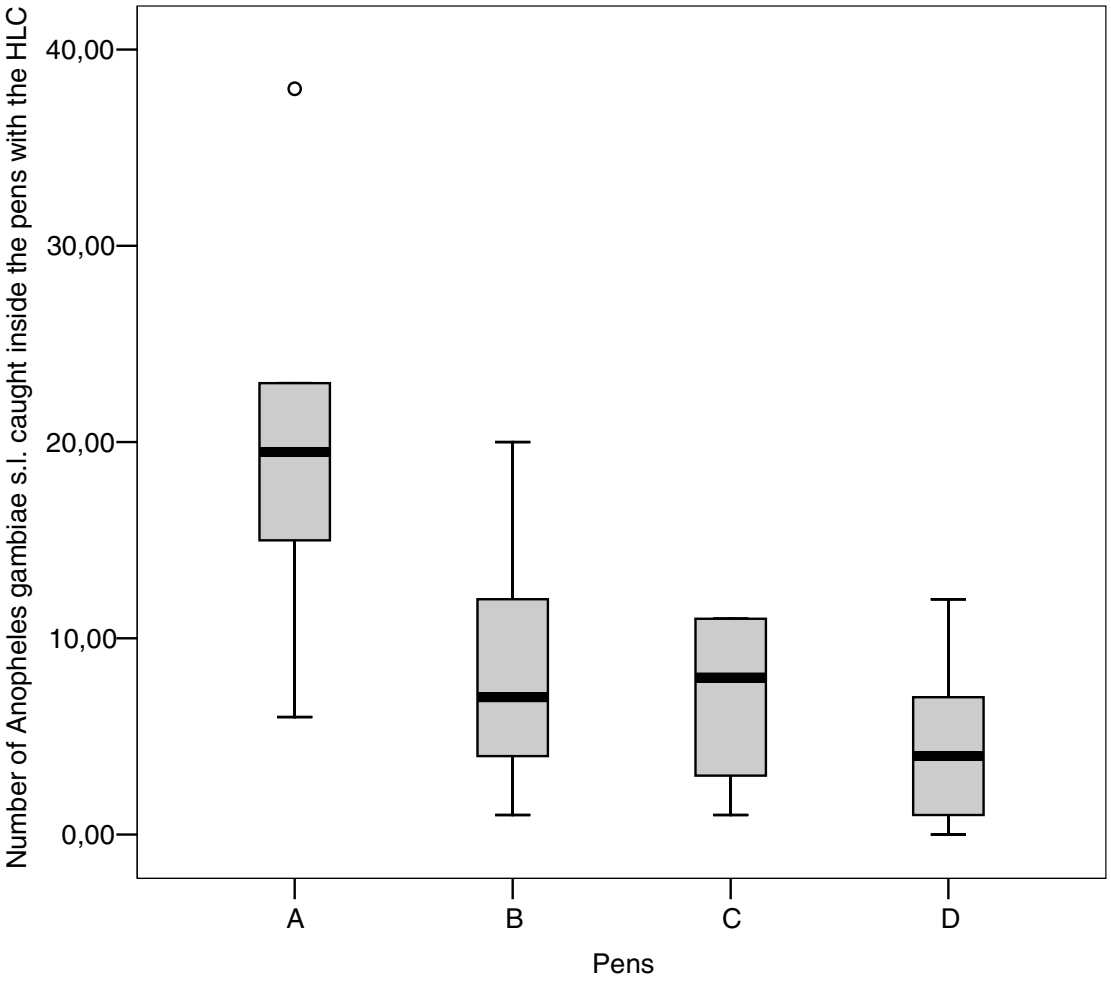

Fig. 4.8 Boxplot of the total number of *Anopheles gambiae* s.l. caught inside the pens using the HLC showing the median, quartiles and extreme values registered in each pen. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

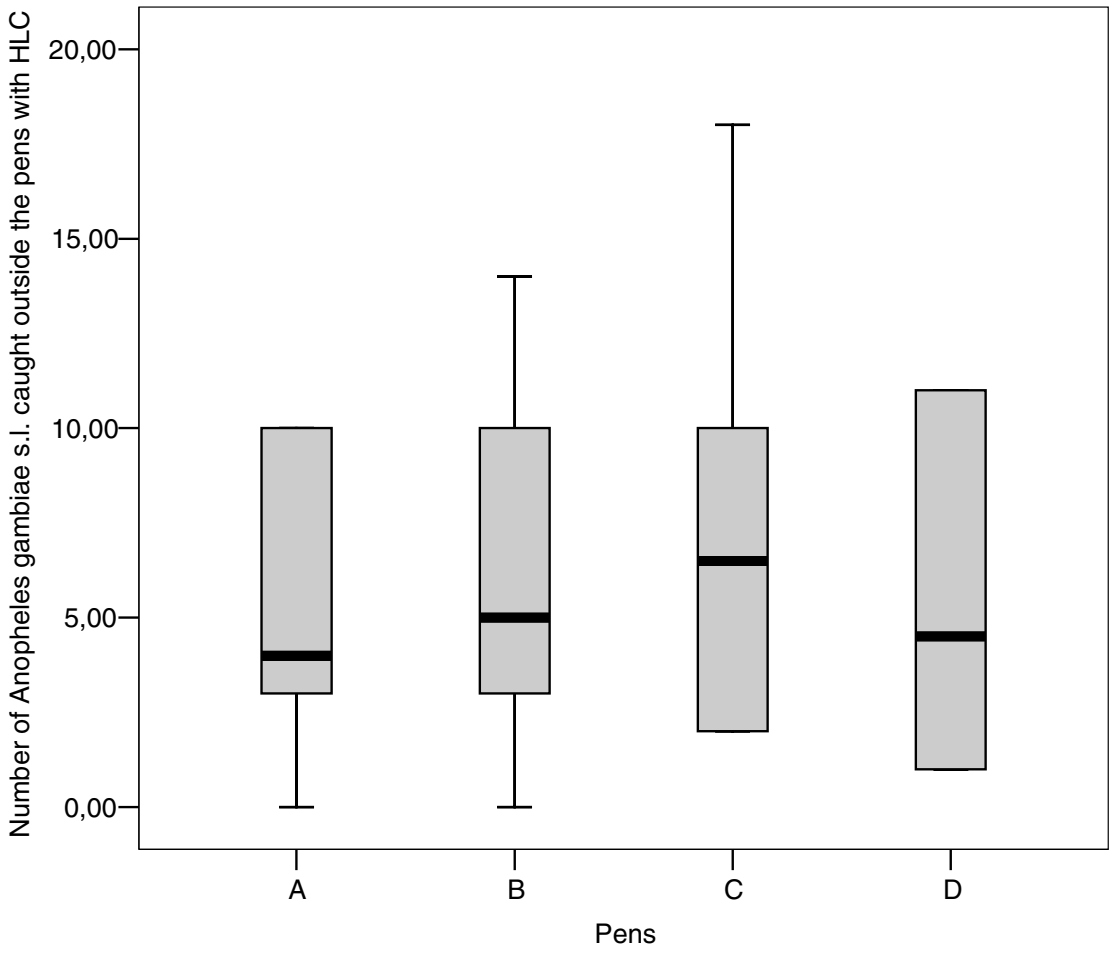

Fig. 4.9 Boxplot of the total number of *Anopheles gambiae* s.l. caught outside the pens using the HLC showing the median, quartiles and extreme values registered in each pen. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

The number of weekly catches of *Anopheles ziemanni* remained at a level with little variation (Table 4.9). Most of the *Anopheles ziemanni* (80%) were caught outside the pens. The highest catch was observed outside A and lowest inside pen C. At pen A 90% more *A. ziemanni* were caught out- than inside. The figures for the other pens were respectively B (54%), C (76%) and D (58%).

Table 4.9 Total numbers of *Anopheles ziemanni* in- and outside each stable during the weekly HLC's. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

Total number of *Anopheles ziemanni* inside and outside the pens										
	Location									
	A – Negative control		B – Untreated net		C – No net		D – Treated net		Total	
Week	in	out	in	out	in	out	in	Out	in	out
1	3	40	16	39	9	33	14	3	42	115
2	1	11	3	5	0	6	3	4	7	26
3	5	57	20	29	8	48	3	8	36	142
4	3	19	8	29	0	14	2	28	13	90
5	5	31	5	14	9	9	2	8	21	62
6	1	17	5	9	2	7	8	26	16	59
Total	18	175	57	125	28	117	32	77	135	494

Catches of *Anopheles ziemanni* inside all pens were not significantly different ($p=0.153$), nor the catches outside ($p=0.333$) (Fig 4.10 and Fig. 4.11).

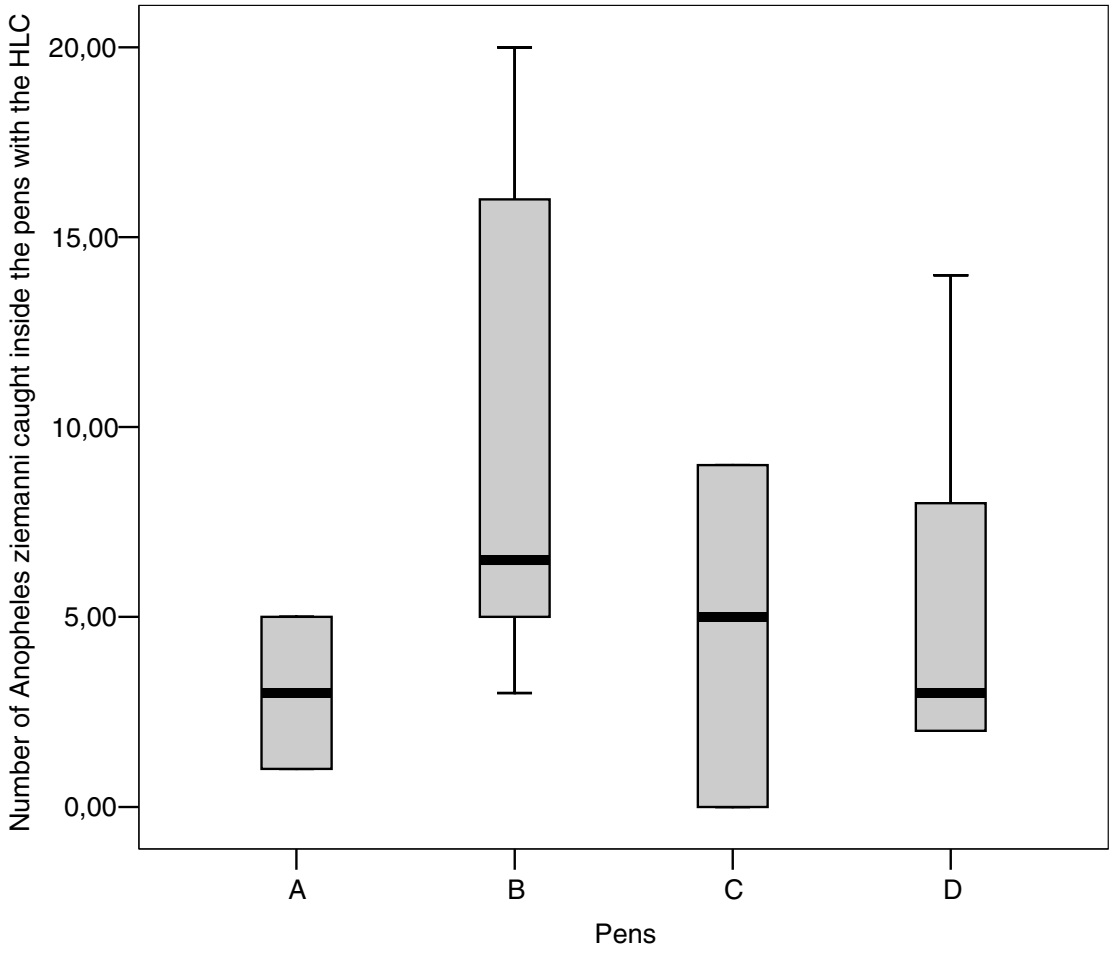

Fig. 4.10 Boxplot of the number of *A. ziemanni* caught inside the pens showing the median, quartiles, and extreme values registered in each pen. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

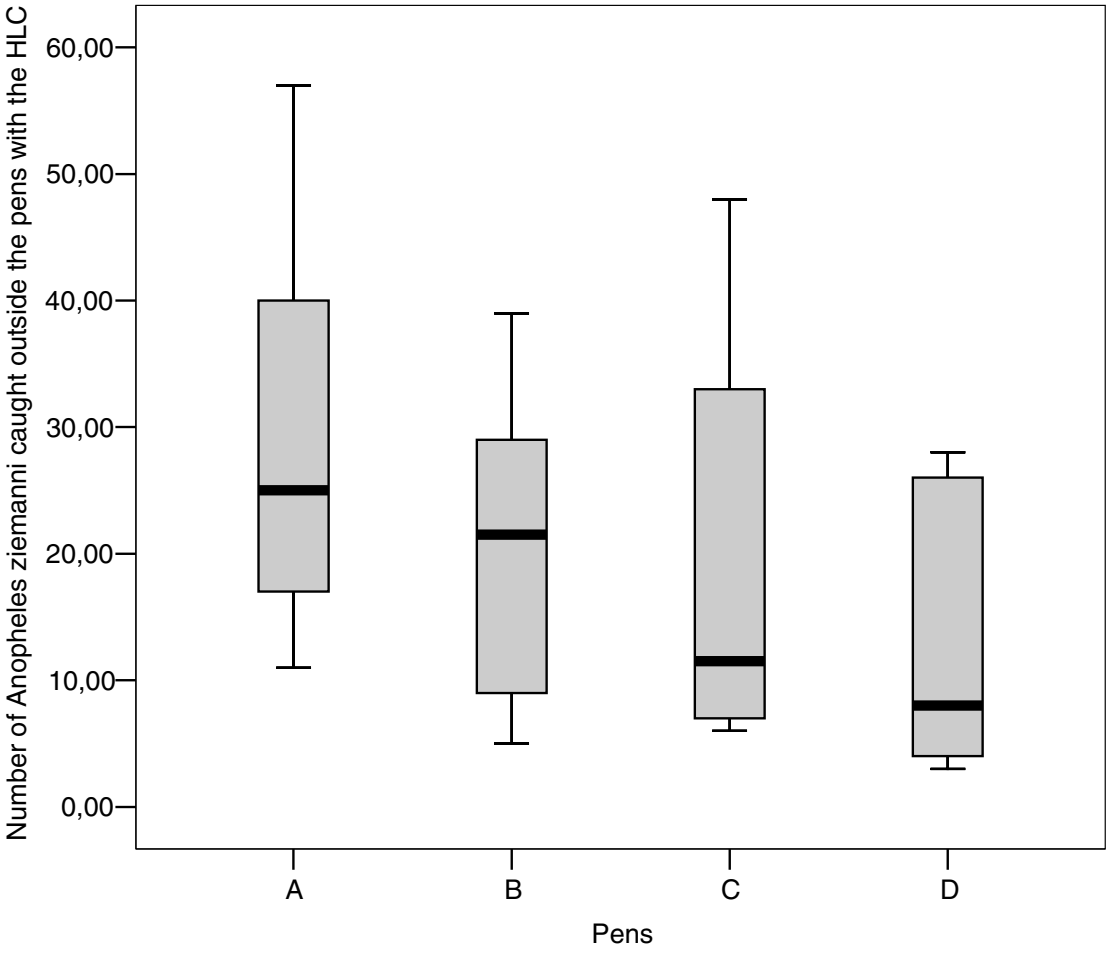

Fig. 4.11 Boxplot of the number of *Anopheles ziemanni* caught outside the pens showing the median, quartiles, and extreme values registered in each pen. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

4.2.1.3 Entomological data analysis

Parity Rates

All caught anophelines (except *Anopheles funestus* which only composed 0.4% of the anopheline catch) using the HLC method were dissected for their parity. Mosquitoes were categorized as parous, older mosquitoes which had already laid eggs, and nulliparous, younger mosquitoes which had not yet laid an egg batch. Damage of the ovary system precluded dissection of some (1%) mosquitoes. Parity rates were calculated relatively to each anopheline species and each pen.

Table 4.10 Dissection results of *Anopheles gambiae* s.l. for parity. P – Parous; NP – Nulliparous; ND – Not dissected; PR – Parity rate. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

Dissection results for *A.gambiae* s.l.												
	A -Negative control			B - Untreated net			C - No net			D - Treated net		
Week	P	NP	ND	P	NP	ND	P	NP	ND	P	NP	ND
1	29	14	0	16	3	1	24	4	1	6	10	0
2	21	7	0	12	6	0	6	5	0	3	2	0
3	22	11	0	15	7	1	10	4	1	18	4	1
4	7	8	0	11	7	0	11	3	0	3	11	0
5	17	7	0	3	5	0	10	3	0	1	1	0
6	5	4	0	1	0	0	1	4	0	1	0	0
Total	101	51	0	58	28	2	62	23	2	32	28	1

Table 4.11 Parity rates for *Anopheles gambiae* s.l.. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

Parity rates for *Anopheles gambiae* s.l.				
Location	A – Negative control	B – Untreated net	C - No net	D – Treated net
Parity rate (%)	66,4	67,4	72,9	53,3

The majority of dissected *Anopheles gambiae* s.l. were parous (66,1%), the remaining were nulliparous and only 1% were not dissected. Most parous *Anopheles gambiae* s.l. were dissected from the catches in pen A (101), followed by pen C (62), pen B (58) and pen D (32) (Table 4.10). Not dissected mosquitoes were excluded for the parity determination. Pen C (no net) had the highest mean *Anopheles gambiae* s.l. parity rate whereas pen D (treated net) had the lowest (Table 4.11) (Fig. 4.12).

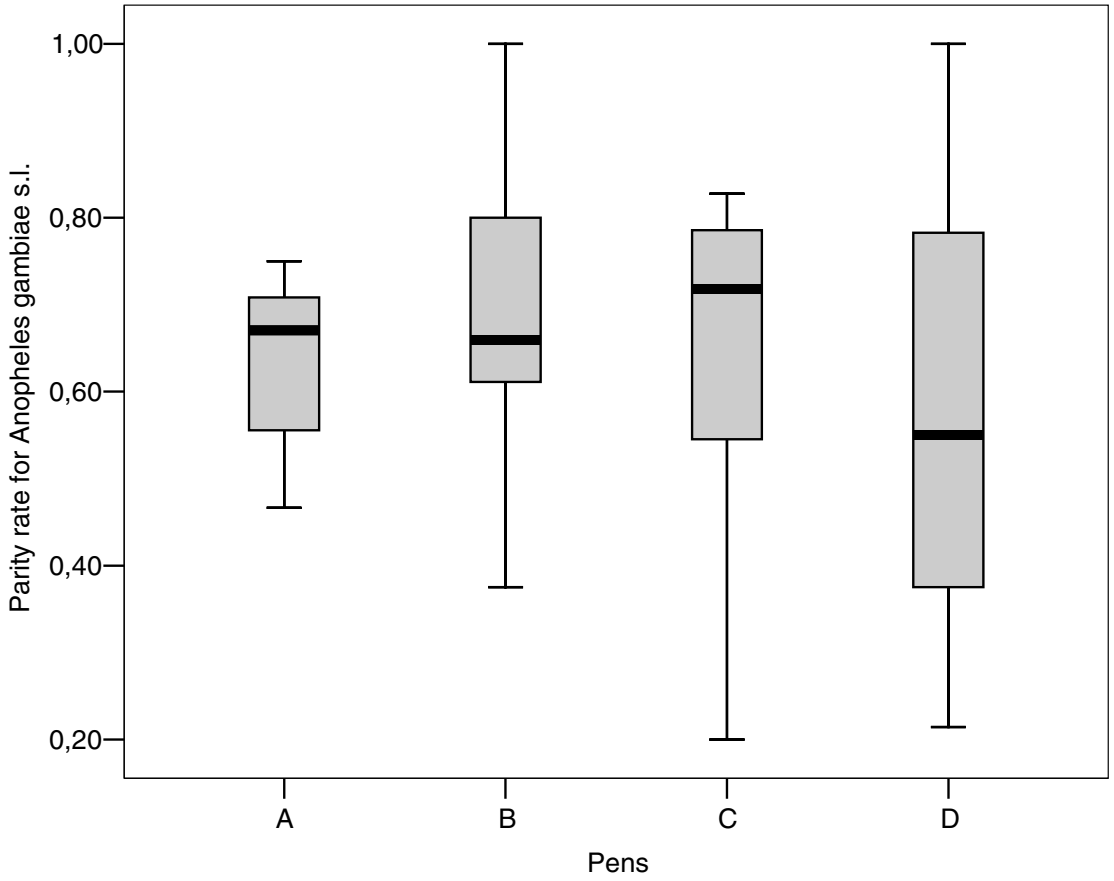

Fig. 4.12 Boxplot of the parity rate for *Anopheles gambiae* s.l. showing the median, quartiles, and extreme values registered in each pen. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

Table 4.12 Dissection results of *Anopheles ziemanni* for parity. P – Parous; NP – Nulliparous; ND – Not dissected. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

Dissection results for parity *Anopheles ziemanni*												
Week	A – Negative control			B – Untreated net			C – No net			D – Treated net		
	P	NP	ND	P	NP	ND	P	NP	ND	P	NP	ND
1	18	25	0	38	16	1	21	21	0	9	7	1
2	7	5	0	6	2	0	1	4	1	7	0	0
3	38	22	2	34	15	0	32	24	0	10	1	0
4	18	4	0	27	10	0	13	1	0	25	5	0
5	20	16	0	13	6	0	10	7	1	4	6	0
6	15	3	0	11	2	1	4	5	0	21	12	1
Total	116	75	2	129	51	2	81	62	2	76	31	2

Table 4.13 Parity rates for *Anopheles ziemanni*. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

Parity rates for *Anopheles ziemanni*				
Location	A – Negative control	B – Untreated net	C - No net	D – Treated net
Parity rate (%)	60,7	71,7	56,6	71,0

Most parous *Anopheles ziemanni* were dissected from the catches done in pen B (129), followed by pen A (116), pen C (81) and pen D (76). The majority of dissected *Anopheles ziemanni* were parous (64,7%), the remaining were nulliparous and only 1% were not dissected (Table 4.12). Not dissected mosquitoes were excluded for the parity determination. Pen B (untreated net) had the highest *Anopheles ziemanni* parity rate (71,7%) whereas pen C (no net) had the lowest (56,6%) (Table 4.13) (Fig. 4.13).

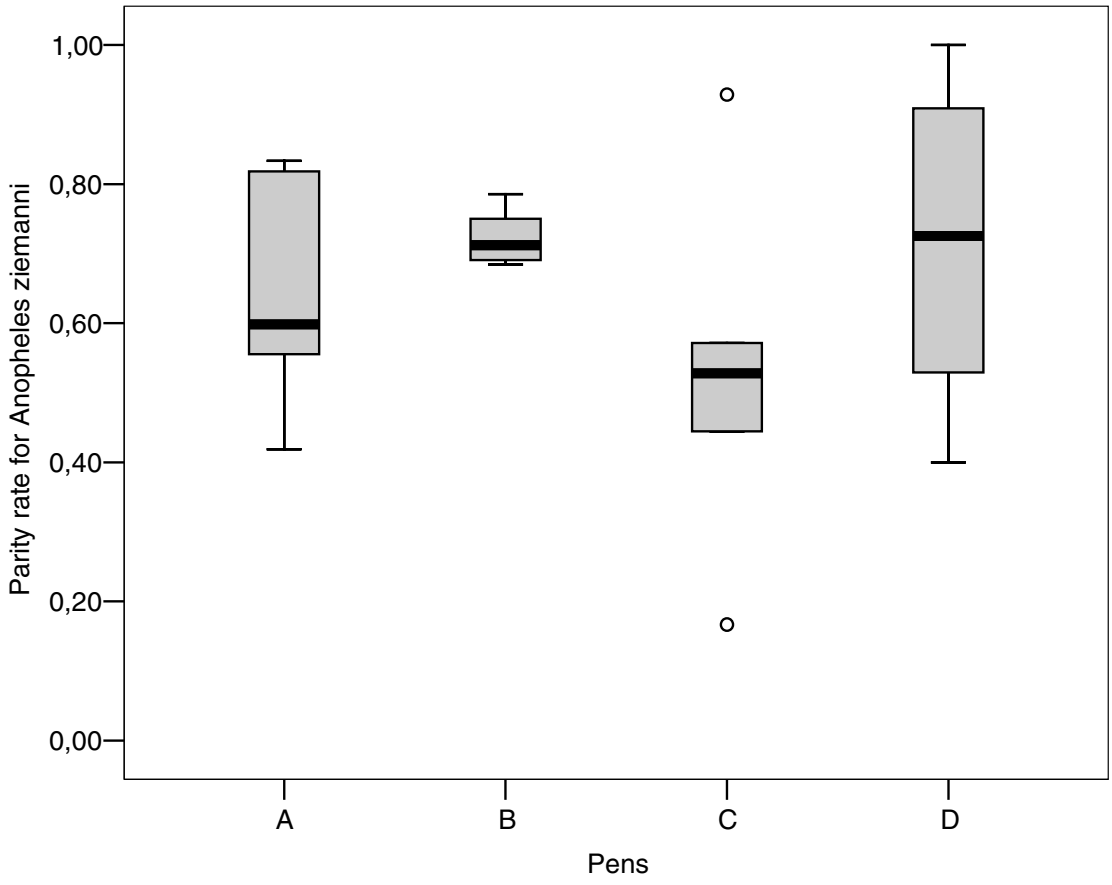

Fig. 4.13 Boxplot of the parity rate for *Anopheles ziemanni* showing the median, quartiles, extreme values and outliers (○) registered in each pen. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

The mean parity rates of the anophelines caught using the HLC method were similar between anopheline specimens in all experimental pens, values ranged between 53,3% and 72.9%. Parity rates concerning *Anopheles gambiae* s.l. registered in all different pens were not significantly different (p=0.857), nor the parity rates concerning *Anopheles ziemanni* (p=0.265). The highest PR registered relative to *Anopheles gambiae* s.l. was in pen C (72.9%) and lowest in pen D (53.3%). The highest PR for *Anopheles ziemanni* was in pen B (71.7%) and lowest in pen C (56.6%) (Table 4.13).

Monthly biting rates

Monthly biting rates (MBR) were calculated for *Anopheles gambiae* s.l., *Anopheles ziemanni* and culicines, inside and outside all study sites (Table 4.14). MBR's were highest among culicines at all study sites. Among the anophelines the highest MBR's belonged to *Anopheles ziemanni*.

Table 4.14 Monthly biting rates registered at each study site for *Anopheles gambiae* s.l., *Anopheles ziemanni* and culicines. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

Monthly biting rates at all sites				
Site		*A. gambiae* s.l.	*A. ziemanni*	culicines
A – Negative control	in	605	90	1895
B - Untreated net	in	255	285	4495
C - No net	in	210	140	3280
D - Treated net	in	140	160	1740
A – Negative control	out	155	875	2600
B - Untreated net	out	185	625	2570
C - No net	out	225	585	5850
D - Treated net	out	165	385	2025

The MBR's for *Anopheles gambiae* s.l. were comparable between in- and outside the pens, contrasting with *Anopheles ziemanni* whose MBR's were distinctly higher outside the pens.

Generally low MBR's were registered at the site of the treated (pen D). The highest MBR for *A. gambiae* s.l. was registered inside the negative control (A -no animals); and the highest MBR for *A. ziemanni* was outside the negative control (A – no animals).

4.3 Catches with the BG-Sentinel™ trap

The BG-Sentinel™ traps, odour-baited mechanical traps were only placed inside each pen. They caught a variety of haematophagous insects. The results of the mechanical traps are indicative of the density of haematophagous insects present within the pens and are not associable with a human disturbing factor.

The majority of caught insects belonged to the family Ceratopogonidae, also known as biting midges, with 58% of the total catch. Culicines represented 22% of the catch followed by phlebotomines with 19%, and anophelines with only 1%.

Table 4.15 Total number of insects caught during each week with the BG-Sentinel™ traps in each stable. ND- Not done due to battery malfunction. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

Total numbers of insects caught during each week with the BG-Sentinel™ Traps					
Week	A – Negative control	B - Untreated net	C - No net	D - Treated net	Total
1	14	112	46	24	196
2	30	37	30	20	117
3	ND	999	912	234	2145
4	71	482	136	133	822
5	128	580	42	249	999
6	ND	104	189	92	385
Total	243	2314	1355	752	4664

Most of the insects were caught in B (50%) which had the majority of midges, culicines and phlebotomines, followed by C with 29%, D with 16% and A with 5%. A (negative control) had the lowest catch. D had the lowest catch in comparison to all other pens with animals (Table 4.15 and Fig. 4.14). However the number of caught insects between all pens was not statistically different ($p=0.268$), even when discarding pen A and comparing only the catches done in pens B, C and D ($p=0.339$).

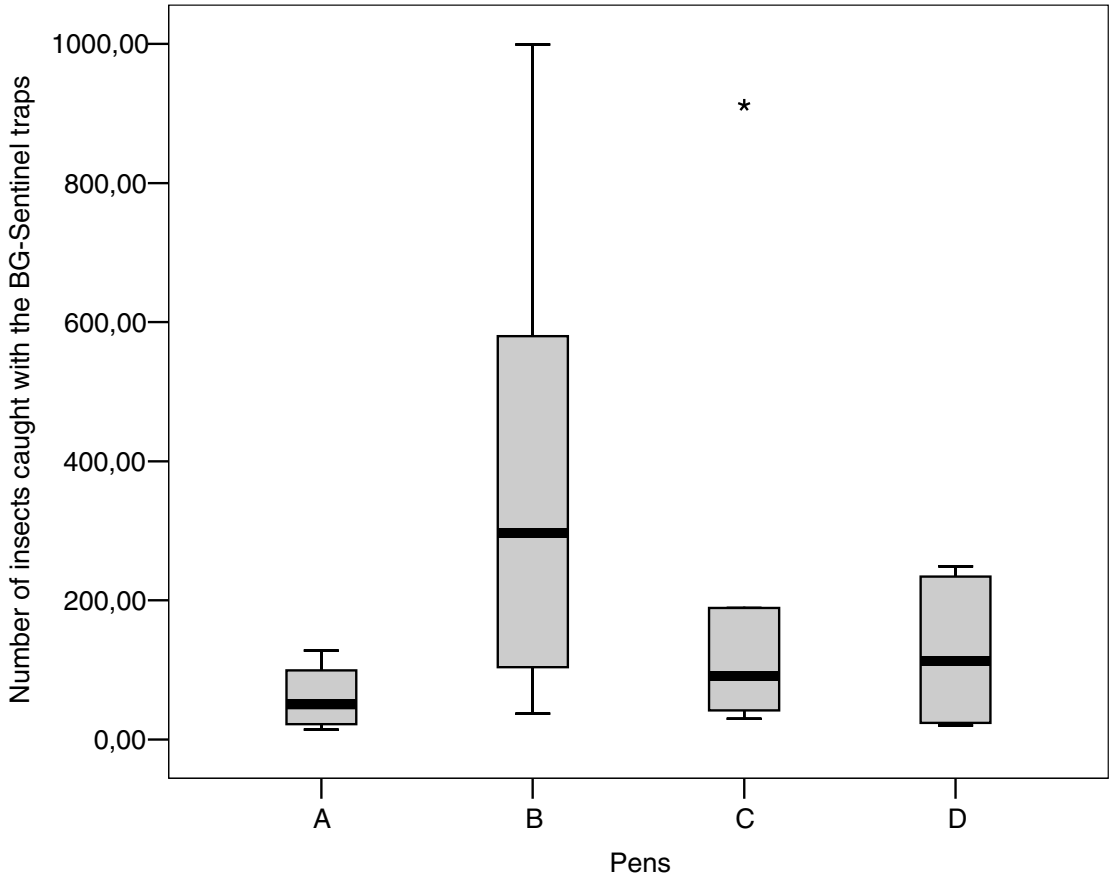

Fig. 4.14 Boxplot of the total number of insects caught using the BG-Sentinel™ traps showing the median, quartiles, extreme values and a extreme case (⋆) registered in each pen. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

Anophelines were caught in very low number, only 1% of the total catch accounts for anopheline mosquitoes. The highest number of anophelines caught was in pen C (11), followed by D (10) and B (8) (Table 4.15).

Biting midges and phlebotomines were only recorded prior to the third week. In A (negative control) no midges were caught (Table 4.16).

Table 4.16 Total number of haematophagous insects caught weekly using the BG-Sentinel™ mosquito traps in each pen. A – Negative control, B – Untreated net, C – No net, D – Treated net. ND – Not done due to battery malfunction. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

Total number of insects caught with BG-Sentinel™ traps																
	Anophelines				Culicines				Phlebotomines				Biting midges			
Pen	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Week 1	1	1	0	0	13	111	46	24	0	0	0	0	0	0	0	0
Week 2	0	1	1	2	30	36	29	18	0	0	0	0	0	0	0	0
Week 3	ND	0	4	3	ND	98	75	31	ND	141	73	28	ND	760	760	171
Week 4	1	4	2	0	39	33	31	7	31	99	82	108	0	346	21	18
Week 5	2	1	1	2	45	88	3	18	81	70	21	8	0	421	17	220
Week 6	ND	1	3	3	ND	22	82	30	ND	79	84	27	ND	2	19	32
Subtotal	4	8	11	10	127	388	266	128	112	389	260	171	0	1529	817	441
Total	33				909				932				2787			

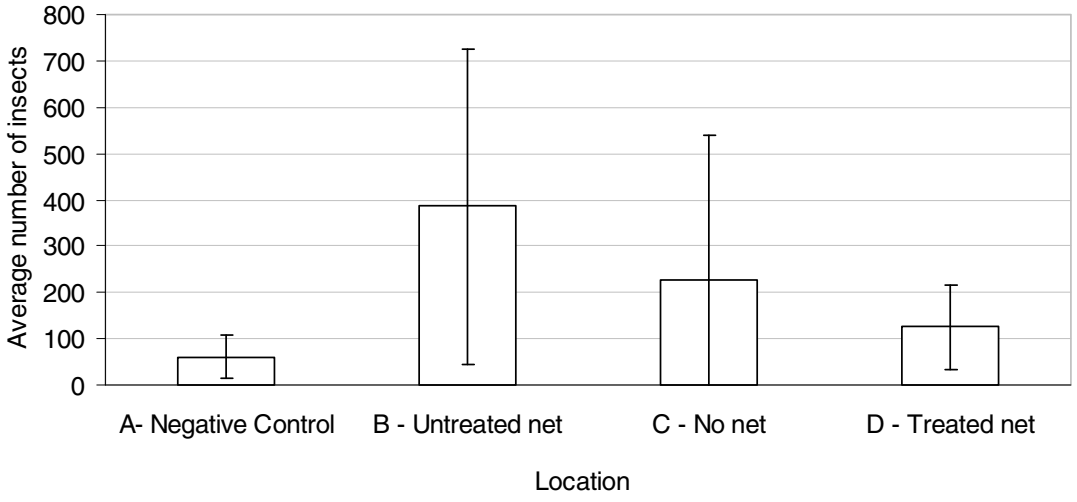

Fig. 4.15 Average number and respective standard deviations of insects caught throughout the trial period using the BG-Sentinel mosquito trap in each stable. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

For a more legitimate comparison between pens the average number of caught insects per catch night was calculated. The highest average number of caught insects during the entire trial was in B (untreated net), followed by C (no net) and D (treated net). The

average catch in pen A (negative control) was the lowest concerning most insects, with the exception culicines where pen D (treated net) had the lowest catch. Pen D had lowest average catches in comparison with other pens with animals (B and C). Average catches in pen B (untreated net) were highest regarding most insects, with exception of anophelines where pen C (no net) had the highest catch (Fig. 4.15).

4.4 Evaluation of the insecticide activity persistence in the treated net

The persistence of insecticide activity in the experimental nets exposed to outdoor conditions in the Afro-tropical region was tested over a period of 9 months subsequent to the trial. Bioassays were conducted using net material regularly collected from the trial site and assessed with laboratory-reared *Musca domestica* and *Aedes aegypti*.

Table 4.17 Percentage of active flies (*Musca domestica*) after contact with tested nets. The number of active flies was counted 5 minutes, 10 minutes, 15 minutes, 6 hours and 24 hours after 10 seconds exposure to the tested net. Tested nets included an untreated control net and six samples of treated net removed from the trial site at different times. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

Percentage of active flies (*Musca domestica*) after contact with tested nets							
Time	Control	Nov 05	Feb 06	Apr 06	May 06	Jun 06	Aug 06
5 min	100	99	97	69	73	99	100
10 min	100	28	8	7	9	95	100
15 min	100	1	1	3	2	59	96
6 h	94	0	0	0	5	23	6
24 h	86	4	11	12	6	86	28

Table 4.18 Percentage of active mosquitoes (*Aedes aegypti*) after contact with tested nets. The number of active mosquitoes was counted 5 minutes, 10 minutes, 15 minutes, 6 hours and 24 hours after 10 seconds exposure to the tested net. The same netting material was used as for the fly tests. The test relative to the netting collected in August could not be done due to logistical problems. Boadi Cattle Research Farm, Kumasi, Ghana 2005.

Percentage of active mosquitoes (*Aedes aegypti*) after contact with tested nets						
Time	Control	Nov 05	Feb 06	Apr 06	May 06	Jun 06
5 min	98	97	94	97	94	99
10 min	98	61	49	17	47	83
15 min	98	6	0	2	19	59
6 h	97	0	0	0	2	16
24 h	93	0	4	0	2	16

Almost 80 % of all flies exposed to the treated nets were paralyzed after 10 minutes. Flies exposed to nets collected in June and August 2006 required longer to be paralyzed. Flies exposed to the samples from June and August 2006 had recovery rates of more than 80% and 20%, respectively after 24 hours (Table 4.17).

More than 80% of the mosquitoes exposed to the treated nets were paralyzed after 15 minutes, with the exception of the net sampled in June 2006 where only 40% were paralyzed. However, more than 80% of all mosquitoes exposed to treated nets were paralyzed 6 hours later. The percentage of recovering mosquitoes after 24 hours remained below 20% (Table 4.18).

5 Discussion

5.1 Entomological monitoring

5.1.1 Impact of the nets on nuisance and biting flies

5.1.1.1 Assessment with mono-conical traps, photos and videos

In order to achieve a reliable comparison between study sites a collection of baseline data concerning the muscid density at each site before the attachment of the nets would have been of great assistance. Photos and videos were usually taken at noon. However studies show oscillations between the amount of attacking flies along the different periods of the day, being highest in early morning and late afternoon and lowest around noon (PATTERSON, 1989). Thus, perhaps the measured fly density was an underestimation.

Catches with the mono-conical traps were distinctly enhanced by cattle presence. Although percentile differences were very large, statistically there were no significant differences in the number of caught insects with the mono-conical traps ($p=0.141$). To obtain statistically significant results the study should have been done over longer time period which would have allowed the collection of more data and lower variance. During the first three weeks muscid densities were very low, subsequently they rose considerably with higher number of caught insects, insects counted on body regions and number of defensive movements. This was likely due to the constant accumulation of animal dung in the premises which permitted the introduction of new suitable breeding places for flies (MENDES and LINHARES, 2002). The animals within the pen protected with a treated net had significantly fewer insects counted on their body regions ($p=0.029$) and performed the least defensive movements ($p=0.064$). Altogether the stabled animals protected by the treated net showed a 70% reduction of attacking nuisance insects, and an 80% reduction of defensive movements. The animals in this pen appeared calmer and more concentrated on feed intake. Therefore the treated net of pen D protected the animals to a considerable extent against nuisance insects, whereas the untreated net did not serve as an effective barrier.

It is assumed the treated net killed most of the muscids trying to enter that particular pen reducing the fly density of the surrounding area. The use of an untreated net in pen B showed no benefits. The high catches in this pen associated with the high number of counted nuisance insects and defensive movements performed by the stabled animals indicate that the untreated net seemed to retain the insects which entered the pen.

Tsetse flies were never caught during this study, although they are highly prevalent in Ghana (MAHAMA *et al.*, 2004a; MAHAMA *et al.*, 2004b). The veterinary officers at the Boadi farm noted that earlier tsetse flies were commonly seen. However, in the last years they have become uncommon (personal communication, Mr. Donkoh, Veterinary Official of the BCRF, 2005), reasons for this are unclear. Most of the insects caught with the mono-conical traps were house flies (Musca) and stable flies (*Stomoxys*), both are widely spread around the world and are considered to be synanthropic species. These insects are common livestock pests responsible for nuisance and mechanical transmission of several disease agents (MAYR, 1983; RICHARD and PIER, 1966; SAMUI and HUGH-JONES, 1990; D'AMICO *et al.*, 1996; NAZNI *et al.*, 2005; BARRO *et al.*, 2006). In Africa little is done to protect animals against these insect pests. Most commonly used are pour-on solutions, also called ITC (insecticide treated cattle). To achieve success with ITC's continuous treatments of most herd animals are necessary (OKIRIA *et al.*, 2002). The mediocre outcome of using ITC's alone result in a suboptimal solution for the problem (GOUTEUX *et al.*, 1996; HARGROVE *et al.*, 2000; OKIRIA *et al.*, 2002). Integrative measures combining ITC's and ITT's (insectide treated targets) have shown to be more beneficial (HARGROVE *et al.*, 2000). Unlike ticks, and similar to tsetse flies *Stomoxys* do not spend much time on their host (FOIL and YOUNGER, 2006); the possibility of controlling stable flies through ITC's is therefore small. In addition, insufficient doses of active compound are usually found on the animals legs (VALE *et al.*, 1999) due to the distance from the application point as well as to weathering. Attempts to control stable flies using ITT's have proven to be useful in stable fly control (FOIL and YOUNGER, 2006). The use of treated fences to control *Stomoxys* flies attacking cattle must be further researched. Reduction of stable flies attacking cattle is likely to result in the decrease of mechanically transmitted diseases such as dermatophilosis. The relief of diseases like animal trypanosomes could mean socio-economic improvement to African communities (SHAW *et al.*, 2006). The impact of the insecticide treated net on animal vector-borne diseases should be thoroughly assessed in the future. Also, measures resulting in stable fly control will bring relief from nuisance, resulting in an improvement of animal condition and production. Animal blood loss caused by *Stomoxys* bites may amount to about 300 ml

per day (PARR, 1959). Studies have attempted to estimate the outcome on milk yields by controlling nuisance caused by stable flies (MULLENS *et al.*, 2006). Protected cattle have shown potential increase of milk yields up to 1.0 L per day per cow (MORGAN and BAILIE, 1980). Cattle exposed to stable flies can also suffer in average a 0.16 Kg/day reduction in ADG (CATANGUI *et al.*, 1993). This weight loss can occur without compensatory recovery after removal of the stable fly stress (CAMPBELL *et al.*, 2001). The improvement of animal condition using treated nets for protection against nuisance flies must still be quantified through production rates measurements such as weight gain and milk yields.

5.1.2 Impact of the nets on mosquitoes

5.1.2.1 Assessment with HLC (human landing catch)

The highest mosquito catch (Culicidae) was observed during the fifth week. This catch night corresponded to a full moon night. Mosquitoes are known to be more active during moonlit nights forming sometimes swarms for mating purposes (CHARLWOOD *et al.*, 1986; DE MEILLON, 1951). Catches were lowest during the second week, probably due to the slight decrease in temperature measured during the night. In order to achieve a reliable comparison between study sites a collection of baseline data concerning the culicid density at each site before the attachment of the nets would have been of great assistance.

Culicines

Most of the mosquitoes caught using the HLC method were culicines. The treated net protected to some extent the animals from culicine mosquitoes as the number of culicines caught at pen D (treated net) was significantly the lowest ($p=0.002$). Catches at all pens were lowest inside; the pen protected with the treated net had distinctly fewer inner catch, which indicates a somewhat protective effect of net. The pen protected by an untreated net presented the highest catch; this confirms the advantage of a treated net over an untreated net for the purpose of animal protection against culicines. Also, the untreated net provided less protection than no net at all. The amount of attacking culicines was high in the pen which had no animals; this was

possibly due to the fact that the catcher was the only available host in the premises whereas at the other sites the animals were also a possible host. The peak in culicine catch was recorded between 1-2 a.m.

Anophelines

Three different anopheline species were caught using the human landing catch method, most of which were identified as *Anopheles ziemanni* (53.5%), thought to be a preferentially exophagic and zoophilic species (CHANDLER *et al.*, 1975). The remaining mosquitoes caught were mainly *Anopheles gambiae* s.l. (46.1%) and *Anopheles funestus* (0.4%).

The percentage of *Anopheles funestus* was extremely low (0.4%). The very endophilic and antropophilic characteristics of this species (DE MEILLON, 1951) are likely to be in some extent accountable for the low catches observed at Boadi. The study site was not an urbanized area, thus with a small human density; it is known that *A. funestus* is preferably present in sections occupied by humans (DE MEILLON, 1951). Another factor which might have influenced the low catch of *A. funestus* is the seasonality of this species. Heavy rains are known to inhibit the breeding of this species (DE MEILLON, 1951). *Anopheles funestus* are therefore mainly present during the dry season. In contrast, *Anopheles gambiae* s.s is most abundant during the rainy season and declines to low population levels during the dry season (DE MEILLON, 1951).

The peak in *Anopheles gambiae* s.l. biting activity was observed between 02:00 and 03:00 a.m.. This finding disagrees with the common behavior of this species complex which biting peak is usually observed earlier in the evening (GEISSBÜHLER *et al.*, 2007; TAYE *et al.*, 2005; WANJI *et al.*, 2003; GILLIES and COETZEE, 1968; GELFAND, 1955).

The *Anopheles gambiae* s.l., were not submitted to PCR analysis, however, according to the region it is most probably *Anopheles gambiae* s.s. (AFRANE *et al.*, 2004; APPAWU *et al.*, 2004; KRISTAN *et al.*, 2003; COETZEE *et al.*, 2000; APPAWU *et al.*, 1994). Both *A. gambiae* s.s. and *A. funestus* are known to be the main malaria vectors in the Afro-tropical region (DE MEILLON 1951). The role of *A. ziemanni* as a possible malaria transmitter is still in evaluation (KAMAU *et al.*, 2006). However, earlier and recent studies proved that *Anopheles ziemanni* could carry malaria sporozoites

(ANTONIO-NKONDJIO *et al.*, 2006; GILLIES, 1964; CARUS, 1902). The malaria transmission potential of *A. ziemanni* as secondary vector should be given more importance given the fact that most malaria control strategies aim at endophagic species (ANTONIO-NKONDJIO *et al.*, 2006; GILLIES, 1964).

The *A. gambiae* s.l. were mostly caught within the pens possibly attributable to the endophagic character of *A. gambiae* s.s.. The number of *Anopheles gambiae* s.l. caught inside pen A was significantly the highest (p= 0.005), this indicates that the animal presence in the other pens significantly deviated possible anopheline bites from the human host. This may suggest a zooprophylaxis against malaria transmission. It has been observed that cattle kept in separate sheds outside of the human dwellings tend to reduce the man biting rate if the vector is preferentially zoophilic (SEYOUM *et al.*, 2002; SAUL, 2003). However, some authors concluded that the presence of cattle in mixed dwellings tends to increase the man biting rate of vectors (SOTA and MOGI, 1989; HEWITT *et al.*, 1994; BOUMA and ROWLAND, 1995). Stll, it has been shown that the combination of zooprophylaxis and insecticidal control by using insecticide treated cattle near dwelling houses results in decrease of malaria vector density (MAHANDE *et al.*, 2007). The combined use of zooprophylaxis and chemical control can also result in effective vector control without the development of undesirable insecticidal resistance (KAWAGUCHI *et al.*, 2003). The role of insecticide treated fences as a malaria protective measure through zooprophylaxis must be further investigated. The treated net protected the catchers inside the pen to some degree from the attack of *A. gambiae* s.l. given that catches were approximately a third lower than in other pens containing animals.

In all locations *Anopheles ziemanni* was the most dominant species being mostly caught outside the pens. This is possibly because of its exophagic characteristics (CHANDLER *et al.*, 1975).

Pen C (no net) also registered low catches which could have been thanks to different ecological characteristics of the site which location seemed to be more affected by wind than others. Pen B (untreated net) registered considerably higher catches among the pens with animals. It appears that the untreated net is inadequate to protect animals against blood-sucking insects. The untreated net in pen B seemed to retain the insects within the pen.

Entomological data analysis

The monthly biting rates (MBR) of *Anopheles gambiae* s.l. were generally highest inside the pens. This was consistent with the endophilic character of *Anopheles gambiae* s.s. (DE MEILLON, 1951). The animals possibly lured *A. gambiae* in search of a blood meal despite its anthropophilic behaviour. The low MBR inside the treated net pen indicates a possible protective effect of the treated net (Table 5.1).

On the other hand, the MBR of *Anopheles ziemanni* were generally highest outside the pens. This was consistent with the exophagic and exophilic character of *Anopheles ziemanni*. Pens with cattle attracted most *A. ziemanni*. However, the highest MBR was registered outside the pen with no animals, indicating a contradictory result. One reason may be that most mosquito catchers regularly worked with cattle; therefore their clothing could contain odours which attracted *A. ziemanni*. Another reason was the aspect that the catcher was the only available host at site A (Table 5.2).

The parity rates (PR) calculated for each pen relative to *Anopheles gambiae* s.l. were not significantly different (p= 0.870) nor were the PR relative to *Anopheles ziemanni* (p=0.279). The lowest parity rate concerning *Anopheles gambiae* s.l. was calculated in pen D (treated net). This could suggest that the introduction of the treated net in the location might have had an effect on the local population of *Anopheles gambiae* s.l. causing a reduction of older females. The PR is expected to decrease in the case of a successful vector control measure since the female mosquito population tends to be mostly composed by freshly emerging females (DETINOVA, 1962). In order to determine if the introduction of the treated net in the location was responsible for this change another study should be projected over a longer time period. Parity rates of *Anopheles ziemanni* were similar in all pens ranging from 56.6% in C and 71.7% in B.

The sporozoite rates (SR) concerning *Anopheles gambiae* s.l. (courtesy of the KCCR, Ghana) were similar among all pens ranging from 0 to maximum value of 3.57% inside pen D. Entomological inoculation rates (EIR) concerning *A. gambiae* s.l. ranged from 0 to 15 infective bites per person per month. These results indicate extremely high malaria transmission intensity (BEIER *et al.*, 1999) and are consistent with the holoendemic character of malaria in Ghana. The highest EIR (*A. gambiae* s.l.) was registered inside the pen with no animals. The lack of animals inside this pen was perhaps responsible for this result; the presence of the animals in other sites may have

acted as zooprophylaxis, protecting to some extent the human catchers. Results are inconclusive concerning the protective effect of the treated net. Studies should be projected to evaluate if zooprophylaxis combined with insecticide-treated nets surrounding cattle enclosures could be of importance to malaria control (Table 5.1).

Anopheles ziemanni has been described as a zoophilic species and is not generally considered a malaria vector, however immunological tests carried out by the KCCR staff have found positive *A. ziemanni* specimens. Most of the positive *A. ziemanni* were found outside the pens and the highest EIR was outside pen A (negative control) with 20 infective bites per person per month. Taking into consideration the high EIR which was registered, the importance and potential of this species as vector of malaria must be further researched. Confirmation of these results should be performed by PCR so that more conclusions may be drawn (Table 5.2).

Table 5.1 *Anopheles gambiae* s.l. densities and malaria transmission parameters at all sites. A (negative control), B (untreated net), C (no net) and D (treated net). [1] Average number of *A. gambiae* s.l. caught per night over six weeks with standard deviation in parenthesis; [2] MBR: monthly human biting rate; [3] PR: parity rate; [4] SR: sporozoite rate; [5] EIR: Entomological inoculation rate (per month); [*] Parity rates were calculated per pen (A;B;C;D) and not per location (in/out), the population of mosquitoes caught in and outside the pens is the same. Boadi Cattle Research Farm, Kumasi Ghana, 2005.

Anopheles gambiae s.l. densities and malaria transmission parameters						
Location		*Anopheles gambiae* s.l. [1]	MBR[2]	PR (%)[3]*	SR (%)[4]	EIR[5]
A	in	20,17 (10,57)	605	66,4	2,48	15,00
B	in	8,50 (6,98)	255	67,4	1,96	5,00
C	in	7,00 (4,20)	210	72,9	0,00	0,00
D	in	4,67 (4,41)	140	53,3	3,57	5,00
A	out	5,17 (4,07)	155	66,4	3,23	5,00
B	out	6,17 (5,08)	185	67,4	2,70	5,00
C	out	7,50 (6,06)	225	72,9	2,22	5,00
D	out	5,50 (4,81)	165	53,3	0,00	0,00

SR: Sporozoite rates were determined by the KCCR.

Table 5.2 *Anopheles ziemanni* densities and malaria transmission parameters at all sites. A (negative control), B (untreated net), C (no net) and D (treated net). [1] Average number of *A. ziemanni* caught per night over six weeks with standard deviation in parenthesis; [2] MBR: monthly human biting rate; [3] PR: parity rate; [4] SR: sporozoite rate; [5] EIR: Entomological inoculation rate (per month); * Parity rates were calculated per pen (A;B;C;D) and not per location (in/out) the population of mosquitoes caught in and outside the pens is the same. Boadi Cattle Research Farm, Kumasi Ghana, 2005.

Anopheles ziemanni densities and malaria transmission parameters						
Location		Anopheles *ziemanni*[1]	MBR[2]	PR (%)[3]*	SR (%)[4]	EIR [5]
A	in	3,00 (1,79)	90	60.7	0,00	0,00
B	in	9,50 (6,89)	285	71.7	0,00	0,00
C	in	4,67 (4,46)	140,1	56.6	0,00	0,00
D	in	5,33 (4,80)	159,9	71.0	3,13	5,00
A	out	29,17 (17,19)	875,1	60.7	2,29	20,00
B	out	20,83 (13,42)	624,9	71.7	1,60	10,00
C	out	19,50 (17,17)	585	56.6	1,71	10,00
D	out	12,83 (11,18)	384,9	71.0	1,30	5,00

SR: Sporozoite rates were determined by the KCCR.

5.1.2.2 Assessment with BG- Sentinel ™ traps

The BG-Sentinel™ trap proved efficient in catching biting midges (Ceratopogonidae) and sand flies (Phlebotominae). However, it was less efficient catching anophelines (only 1% of total catch). Most of the mosquitoes (Culicidae) caught belonged to the genus *Mansonia*, and *Culex*. *Aedes* mosquitoes were seldom caught despite the trap being designed to particularly catch *Aedes aegypti* (GEIER *et al.*, 1996; PAPPENBERGER *et al.*, 1996; BOSCH *et al.*, 2000; DEKKER *et al.*, 2005). A reason for this low catch is that the traps were set from dusk till dawn and *Aedes* mosquitoes are active during the day. The low catch may also be explained by the possible low density of *Aedes* spp. in the region due to the presence of mosquitoes of the genus *Toxorynchitinae* in the area whose carnivorous larvae feed on *Aedes* larvae (ADDY *et al.*, 1996). The BG-Sentinel™ trap used in this study was a model which did not

incorporate a black light; the use of this additional attractant could possibly improve catch results.

The number of biting midges and sand flies caught in the traps was remarkably high, despite only been caught after the trial's third week. The reason for this might be due to high seasonal variations of these insects, together with the transition period from rainy to dry season (DIPEOLU and OGUNRINADE, 1977).

Biting midges were only caught within pens containing animals, in pen A (negative control – no animals) no biting midges were caught. This situation may be explained by the zoophilic behaviour of some species of biting midges which are attracted to animals. Many of the biting midges when observed under a microscopic proved to be engorged which denotes that the insecticide treated net did not prevent them from attacking the stalled animals. Unfortunately, due to battery malfunction, the deployment of the BG-Sentinel trap in pen A (negative control) was not possible during the third and fifth week leaving this pen with only four sampled weeks. These circumstances make it impossible to conclude on the absence of biting midges in pen A. Nonetheless the non-existence of animals in the pen is indicative of a smaller attraction factor for these insects and therefore a low catch. On average, pen A had the lowest catch of most insects, with the exception of culicines where the lowest catch was recorded in pen D. The highest catch was in pen B (untreated net) with 50% of all insects. These results point to an inadequacy of the untreated net as protective against a broad range of insects including biting midges and sand flies. In pen D (treated net) the amount of caught insects was somewhat lower (only 16% of the total catch) indicating a modest protection against blood sucking insects. Nonetheless, the number of insects caught using the BG-Sentinel trap was not significantly different ($p=0.268$).

5.1.3 Comparison between HLC and BG- Sentinel ^{TM} trap

The results obtained in this trial show that the human landing catch was performing better for catching mosquitoes in comparison with the odour-baited trap BG-Sentinel^{TM}. Both culicines and anophelines were caught in much greater number using the human landing method (Fig. 5.1). Numerous insect catches provide more reliable data for calculation of mosquito densities and malaria transmission parameters (WHO, 2006a).

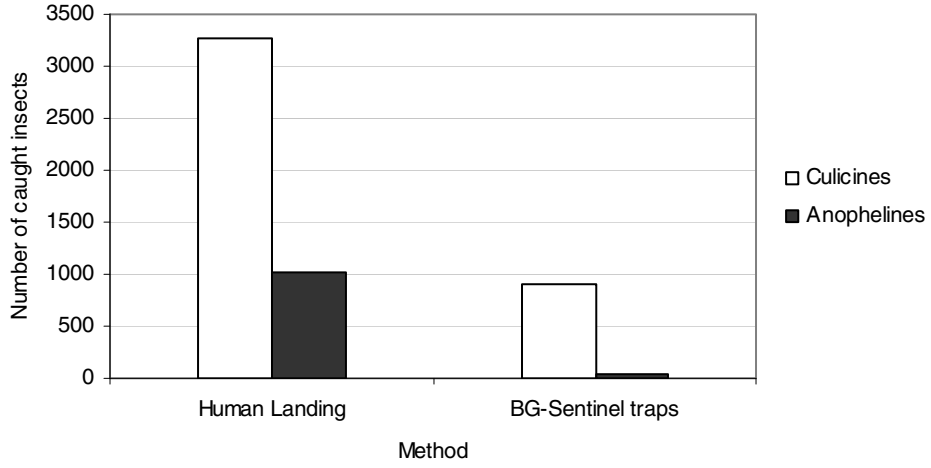

Fig. 5.1 Comparison of the number of culicines and anophelines caught using the human landing method and the BG-Sentinel™ traps. Boadi Cattle Research Farm, Kumasi, Ghana, 2005.

Nevertheless, the BG-Sentinel™ traps showed potential in catching biting midges and sand flies. The small size of these insects makes it very hard to catch them during a landing catch.

5.2 Persistence of insecticide activity in the treated net

The experimental nets demonstrated satisfactory insecticidal effect on *Musca domestica* and *Aedes aegypti* for 8 to 10 months following exposure to African climatic conditions. Mosquitoes showed greater sensibility to the insecticide being quicker paralyzed with fewer recovering individuals after 24 hours. The time necessary to paralyze the insects increased as a consequence of the duration of exposure to the climatic conditions. However, 6 hours after exposure to the nets more than 70% of the flies and 80% of the mosquitoes were paralyzed. Under natural conditions these insects would be predated before recovery. Differences of insecticide availability on the net surface may explain the somewhat contradictory results of June and August during the laboratory bio-assays. The net manufacturers claim that the availability of insecticidal molecule is dependant on the temperature. The experimental net was attached to pen D in the beginning of September 2005 and tests were conducted until the month of August 2006. Results showed that the persistence of insecticide in the net was affected by climatic conditions; however it still remained satisfactory after 10 months of exposure to tropical conditions.

6 Conclusions and recommendations

The insecticide-treated fence distinctly reduced the disturbance by biting flies and nuisance flies. Defensive movements of the protected cattle were reduced by 80% in comparison to an unprotected control or to cattle protected with an untreated net. The impact of this reduction on animal production parameters such as weight gain and milk yields requires further studies. Protection of livestock by reducing biting and nuisance insects may not only increase animal production and farmer's income but also reduce disease transmission. Studies should be projected in order to further evaluate the impact of insecticide-treated fences on the transmission of vector-borne diseases such as trypanosomosis or bluetongue between stalled animals. However, the effect of treated net proved to be unsatisfactory against biting midges, further research concerning the net itself must be done to analyse the necessary adjustments.

A successive study should be designed over a longer period of time including rotation of the treated and untreated nets between the experimental locations in order to better clarify the results.

The treated net proved to remain effective after 8-10 months of exposure to tropical African climatic conditions, studies should be done over longer periods of time to assess the longevity of the treated net. The cost-efficiency relation of using insecticide-treated fences to protect livestock from nuisance and biting insects should be estimated in developing and developed countries.

The role of *Anopheles ziemanni* as a secondary malaria vector should be further assessed with the purpose of promoting efficient malaria control strategies.

The effect of combining zooprophylaxis and insecticide treated fences should be evaluated for the control of anopheline mosquitoes. Studies must be projected over longer time periods and in locations where baseline data exist.

BG-Sentinel^{TM} traps can be useful for catching biting midges and sand flies; in this study they were found to be less performing in catching malaria mosquitoes.

7 Summary

Impact of insecticide-treated nets protecting cattle in zero-grazing units on nuisance and biting insects in the forest region of Kumasi, Ghana

The objective of this study was to assess the effect of an insecticide-treated net on the density of insects of veterinary and medical importance (Dipterids) inside and in the surroundings of zero-grazing cattle enclosures by measuring fly and mosquito densities through three different trapping methods. The effect of the insecticide-treated net fence on anophelines was also investigated because of their role as malaria vectors

As experimental setup, four similar sites were chosen for the construction of four pens. These were built approximately half a kilometer from each other and were all near a small water course surrounded by dense vegetation. Six black zebu bulls of comparable size were chosen as experimental animals and remained within the pens throughout the trial. Pens were denominated as A, B, C and D; pen A served as the negative control pen with no animals and no netting; pen B with two zebu bulls was surrounded by an untreated net (100 cm height); pen C with two animals had no netting, and pen D also with two animals was protected by a deltamethrin impregnated net (100 cm height). The trial was performed for six weeks during the months of October and November 2005. For the entomological monitoring three insect catching methods were applied: mono-conical traps, odor-baited traps (BG-Sentinel^{TM} Trap) and human landing catch (HLC) with volunteer human baits in order to determine the mosquito biting rates. The caught insects were then counted and identified. Anopheline mosquitoes were dissected to assess parity. Serological identification of malaria infected mosquitoes was conducted by the KCCR through ELISA and sporozoite rates were determined. In addition, monthly biting rates (MBR) and entomological inoculation rates (EIR) were calculated. The annoyance caused by biting and nuisance flies was monitored twice weekly by digital photos of selected animal body regions and thirty-second video recordings of each animal. Weekly means of counted flies and recorded defensive movements were calculated per animal.

In and around pen D (treated net) the results showed a consistently low catch of insects with all catching methods, as well as a considerable reduction (70-80%) of

nuisance and animal disturbance. Animals appeared calmer and displayed an undisturbed fodder intake whereas in other pens the nuisance and biting flies created significant annoyance. Most mosquitoes caught with the HLC method were caught in pen B (untreated net) demonstrating that an untreated net provides no protection. Many mosquitoes were caught with the HLC method in pen A (negative control), possibly due to the absence of animals as alternative host to divert the insects from the human catcher. It is acknowledged that animals can be protective against malaria – a circumstance commonly known as zooprophylaxis. The BG Sentinel™ trap (without black light) caught distinctly fewer mosquitoes than the HLC method and was considered unsuitable for catching anophelines. However, it proved to be valuable for catching biting midges (Ceratopogonidae) and sand flies (Phlebotominae). Analysis of malaria transmission parameters (MBR, SR, EIR) revealed slight differences among experimental pens.

Studies should be continued over a longer time period to determine if the use of insecticide-treated nets surrounding animal enclosures influences malaria transmission. Furthermore, the impact on weight gain and milk yield should be evaluated. Cost efficiency of using insecticide-treated net fences by African farmers must be estimated. Considerable benefits for livestock keepers both in intensive as well as in traditional farming systems (nomadic grazing economy) are likely to be obtained.

8 Zusammenfassung

Untersuchung zur Wirksamkeit insektizidbehandelter Netze zum Schutz von Rindern gegen medizinisch und veterinärmedizinisch bedeutsame Insekten im Waldgebiet von Kumasi, Ghana.

Ziel dieser Studie war die Beurteilung der Wirkung eines insektizid-behandelten Netzes auf das Vorkommen medizinisch/veterinärmedizinisch bedeutsamer Insekten (Diptera) innerhalb und in der Umgebung von Rinderstallungen durch Messung des Anflugs von Fliegen und Mücken mit drei verschiedenen Fangmethoden. Die Wirkung des insektizid-behandelten Netzzaunes auf Anophelesmücken wurde wegen ihrer Rolle als Malariaüberträger ebenfalls untersucht.

Als Versuchsanordnung wurden vier Stallungen an ähnlichen Standorten aufgebaut. Die Stallungen wurden etwa einen halben Kilometer entfernt voneinander errichtet und lagen, umgeben von dichter Vegetation, nahe einem kleinen Wasserlauf. Sechs schwarze Zebubullen von vergleichbarer Größe wurden als Versuchstiere ausgewählt, die für die gesamte Versuchdauer innerhalb der Stallungen verblieben, aber wöchentlich zwischen den Stallungen B, C und D rotierten. Die Stallungen wurden als A, B, C und D bezeichnet. Stall A diente als Negativ-Kontrolle, d.h. ohne Tiere und ohne Netz; Stall B mit zwei Zebubullen war umgeben von einem unbehandelten Netz (100 cm Höhe); Stall C mit zwei Tieren hatte keinen Netzzaun und Stall D mit ebenfalls zwei Tieren war von demselben aber mit Deltamethrin imprägnierten Netz (100 cm Höhe) umgeben. Der Versuch erstreckte sich über sechs Wochen und wurde in den Monaten Oktober und November 2005 durchgeführt. Für die entomologischen Folgeuntersuchungen wurden drei Fangmethoden in allen Ställen angewandt: Monokonische Fallen, Ansaugfallen mit Geruchsstoffen (BG-Sentinel^{TM} Trap) und Lebendfallen mit freiwilligen Versuchspersonen (*human landing catch*, HLC) zur Bestimmung der Mückenstechraten. Die Insekten wurden anschließend ausgezählt und bestimmt. Anophelesmücken wurden seziert und auf Parität untersucht. Der Anteil mit Sporozoiten infizierten Mücken wurde serologisch im ELISA durch das *Kumasi Centre for Collaborative Research in Tropical Medicine* bestimmt und die Sporozoitenraten (SR) determiniert. Weiter wurden monatliche Stechraten (MBR) und

entomologische Inokulationsraten (EIR) berechnet. Die Belästigung durch Stechfliegen und andere Lästlingsinsekten wurde zweimal in der Woche durch Aufnahme digitaler Fotos von ausgewählten Körperregionen der betroffenen Rinder und durch Video-Aufnahmen über 30 Sekunden von jedem Tier erfasst. Aus den gezählten Fliegen und Abwehrbewegungen wurde der wöchentliche Durchschnitt pro Tier berechnet.

In und um Stall D (behandeltes Netz) zeigten die Ergebnisse einen gleichbleibend niedrigen Fang von Insekten bei allen Fangmethoden sowie erhebliche Reduktionen (70-80%) der Belästigungen und Störungen der Tiere. Die Tiere erschienen ruhiger und ungestörter bei der Futteraufnahme, wohingegen in den anderen Stallungen Lästlingsinsekten erhebliche Störungen der Tiere verursachten. Die meisten Mücken wurden mit der HLC-Methode im Stall B (unbehandeltes Netz) gefangen. Dies zeigt, dass ein unbehandeltes Netz keinen Schutz bietet. Von den mit der HLC-Methode gefangenen Mücken wurde der größte Teil in Stall A (Negativ-Kontrolle) festgestellt. Als Ursache kann das Fehlen von Tieren vermutet werden, die die Insekten vom Menschen ablenken, ein Umstand, der als Schutz gegen die Malaria dienen könnte - auch allgemein bekannt als *Zooprophylaxis*. Die BG Sentinel™ Falle (ohne Schwarzlicht) fing deutlich weniger Mücken als die HLC-Methode und wurde als ungeeignet für den Fang von Anophelesmücken gewertet. Hingegen erwies sie sich als eine wirksame Falle für den Fang von Gnitzen (Ceratopogonidae) und Sandmücken (Phlebotominae). Die Analyse der Malaria-Übertragungsparameter (MBR, SR, EIR) zeigte nur leichte Unterschiede zwischen den experimentellen Stallungen.

Um festzustellen, ob die Malariaübertragung durch insektizid-behandelte Netze um Tierställe nachhaltig beeinflusst wird, sollten weitergehende Studien über einen längeren Zeitraum geplant werden. Auch sollte hierbei die Auswirkung auf die Gewichtszunahme und Milchleistung der geschützten Tiere ermittelt werden. Die Kosteneffizienz der Verwendung insektizid-behandelter Netzzäunen durch afrikanische Bauern sollte evaluiert werden, um die Vorteile für die Tierhalter sowohl in intensiven als auch in den traditionellen Haltungssystemen (Weidenwirtschaft von Nomaden) bewerten zu können.

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Selbständigkeitserklärung

Hiermit bestätige ich, dass ich die vorliegende Arbeit selbständig und nur unter Zuhilfenahme der angegebenen Literatur erstellt habe.

Berlin, den 23 Juni, 2009

Marta Ferreira Maia