

Spatial variability in the density, distribution and vectorial capacity of anopheline species in Rufiji district, south-eastern Tanzania

EMMANUEL S.P. KIGADYE^{1*}, GAMBA NKWENGULILA², STEPHEN M. MAGESA³, SALUM ABDULLA⁴

¹Faculty of Science, Technology and Environmental Studies, The Open University of Tanzania. P.O. Box 23409, Dar es Salaam, Tanzania

²Department of Zoology and Wildlife Conservation, University of Dar es Salaam, P. O. Box 35064, Dar es Salaam, Tanzania

³National Institute for Medical Research, Amani Research Centre, P.O. Box 81, Muheza, Tanzania

⁴Ifakara Health Institute, P.O. Box 78373, Dar es Salaam, Tanzania

Abstract: Malaria transmission varies from one area to another and there are also local difference in time and space. The objective of the study was to determine the local variability of entomological parameters namely, mosquito abundance, human biting rate (HBR), sporozoite rate for *Plasmodium falciparum* and entomological inoculation rate (EIR). The study was carried out in Rufiji District south eastern Tanzania from October 2001 and September 2004. Adult mosquitoes were collected indoors by CDC light traps. PCR was employed to identify the species within the *Anopheles gambiae* complex. ELISA was used to determine the sporozoite rate. Over a three year sampling period a total of 64,875 female mosquitoes were caught using light-traps, and of these 28% were *Anopheles gambiae* complex, 25% *An. funestus* Giles, 1% *An. pharoensis* Theobald, 46% *Culex* species and the rest were *Mansonia uniformis* Theobald. Mosquito abundance and species composition varied seasonally, spatially and between years. Using PCR, three members of the *Anopheles gambiae* complex namely *An. gambiae* s.s. Giles (69%), *An. arabiensis* Paton (23%) and *An. merus* Dönitz (7%) were confirmed to occur in the study area. *Plasmodium falciparum* circumsporozoite antigen (CSA) rates were 3.5% for *An. gambiae* complex and 2.3% for *An. funestus*. The mean EIR ranged from 28-275 infective bites/person/year. Transmission indices varied over short distances, seasonally and between years. In conclusion, malaria transmission indices in the study area are one of the highest in Tanzania; and there is high variability of entomological parameters over a small geographical area.

Key words: *Anopheles*, mosquitoes, sporozoite rate, malaria, transmission, entomological inoculation rate, Tanzania

Introduction

Currently malaria is by far the most important disease in Africa (Breman *et al.*, 2004). The World Health Organization estimates that 300-500 million people are affected by malaria each year worldwide, with 1-3 million deaths mainly children under five years of age (WHO, 2005). Malaria is endemic in 100 countries inhabited by over 40% of the world population. Countries of tropical Africa bear the brunt of the disease as over 90% of the total incidences and deaths occur in this region. The disease poses the greatest challenge to further improvement in child survival in sub-Saharan Africa (Craig *et al.*, 1999), as well as household and economic development of these countries (Gallup & Sachs, 2001). Malaria still represents a significant threat to human health in Tanzania despite considerable national and international control efforts.

* Correspondence: Emmanuel S.P. Kigadye; E-mail: ekigadye@uccmail.co.tz

The main contributing factors are the emergence of drug-resistant strains of the parasite, the emergence of mosquitoes that are resistant to insecticides, environmental factors and increased population (Bremam *et al.*, 2004). Unplanned urbanization, enforced migration and poor environmental sanitation are some of the major causes of emergence and re-emergence of malaria disease in developing countries (TDR, 2002). In addition, many control activities are undertaken without regard for the presence of the highly heterogeneous vectorial system, e.g. species complexes with different roles in transmission. Given the need for locally adapted, integrated vector control strategies, a better understanding of the ecology, biology and population structure of the vectorial units is necessary (TDR, 2002).

In Tanzania, *Plasmodium falciparum*, the cause of the most severe human malaria accounts for 90% of all malaria infections. Other species found in Tanzania include *P. malariae*, *P. ovale* and *P. vivax* (Mboera, 2000). Malaria is the leading public health problem in Tanzania. Tanzania experiences the highest transmission rates in Africa (over 300 infection bites per person per year) (Mboera, 2000; Drakeley *et al.*, 2003). Most areas of the country have perennial and intense malaria transmission, others have intense but seasonal transmission, but only a few areas experience low or epidemic transmission; these include the semi-arid areas of central Tanzania, mountain ranges in south-western and north-eastern Tanzania (Mboera *et al.*, 2005).

There have been a few studies on malaria and its vectors in the Lower Rufiji River Basin of south-eastern Tanzania (Matola, 1982; Kigadye, 2006). However, epidemiological studies in Kilombero District, which is in the upper Rufiji River basin, have shown that malaria is the major health problem with intense and perennial transmission (Tanner *et al.*, 1992; Abdulla *et al.*, 2001). In the Upper Rufiji River basin, *An. gambiae* s.s. and *An. funestus* have been reported to be the main vectors of malaria with an estimated Entomological Inoculation Rate (EIR) of 200-300 infective bites per person per year (Smith *et al.*, 1993). The objective of the present study was therefore to investigate the entomological factors contributing to malaria transmission in Rufiji district in the Lower Rufiji River basin. To achieve this, a longitudinal survey was carried out to monitor the diversity, spatial and seasonal abundance of *Anopheles* and other human biting mosquitoes. In addition the study also estimated the intensity of transmission and the vectorial capacity of anophelines for malaria transmission.

Materials and Methods

Study area

The study area forms part of the Rufiji River valley in the Rufiji district, in south-eastern Tanzania. The district (7.47° 8'S, 38 39°.17'E) covers an area of approximately 14,500km², located about 150km south of Dar es Salaam. The climate in Rufiji district is characterized by a hot-humid season (December to May) followed by a cool-dry period (June-July) and a hot dry season (August to November). The rainy season consists of three parts, with an average rainfall of 800-1000 mm per year (Richmond *et al.*, 2002). There is usually a short rainy period in October to December, followed by a less rainy and hot period during January-February and long rains (March-May).

Mosquito collection methods

Mosquitoes were collected from six wards in the district. The human population from the six representative wards (Ikwiriri, Kibiti, Mgomba, Umwe, Bungu and Mchukwi) was classified into geographical clusters of 100 people (i.e. people living in the same area), using data from the Rufiji Demographic Surveillance System (RDSS). In each month, a minimum of 10 index people were selected by simple random sampling in each cluster (from the data base). Mosquito catches were carried out in each of the selected households for two consecutive nights' monthly. CDC light traps (John Hock Ltd.) were operated between 18.00-0600hr in two houses every night. Each light trap was hung with its base approximately 1.5 metres above the legs of a person sleeping inside an untreated bed net (Mboera *et al.*, 1998). The traps were set and disconnected by investigators.

Processing and identifying the mosquitoes

Mosquitoes collected each morning were killed in the field with diethyl ether. Female and male *Anopheles gambiae* s.l. and *An. funestus* were counted and identified morphologically using the keys by Gillies & De Meillon (1968) and Gillies & Coetzee (1987). Only females were stored in labelled Eppendorf tubes with desiccant (silica gel) for later laboratory processing at the Ifakara Health Institute in Ifakara. Other anopheline and culicine mosquitoes were scored and discarded.

The PCR method of Scott *et al.* (1993) was used with minor modifications by halving all quantities of DNA primers in order to reduce non specific bands and high rates of PCR amplification failure. One leg from each mosquito was placed in a separate 1.0ml Eppendorf tube to which 12.5µl of PCR master mix was added. This was centrifuged for one minute at 1600 rev/minute in a micro-centrifuge. The PCR procedure included an initial cycle of denaturation at 94°C for 2 minutes followed by 30 cycles of denaturation at 94°C for 30 sec., and a final extra extension step at 72°C for 8min using a Hybaid™ thermal cycler. The resulting amplified DNA was run on an ethidium bromide stained 2.5% agarose gel and photographed under ultraviolet light illumination as described by Scott *et al.* (1993).

Determination of the sporozoite rate

ELISA was used to determine the sporozoite rate in anopheline mosquitoes (Burkot *et al.*, 1984; Wirtz *et al.*, 1987a,b). Absorbance at 405 nm (optical density, OD) was recorded against a blank control with an ELISA plate reader, 30min after addition of substrate. A sample was considered positive if it gave a visually detectable green colour with an OD value at least twice the mean OD of seven negative control wells on that plate (Beier *et al.*, 1988).

Entomological Inoculation Rate

The entomological inoculation rate estimate was based on light trap catches (Lines *et al.*, 1991), without an allowance for the number of occupants per room. In the present study the calculated conversion factor was 1.7, obtained from light trap catches and human landing catches calibration. The formula used to estimate EIR was therefore: $1.7 \times (\text{no. of sporozoite positive ELISA}/\text{no. of mosquitoes tested}) \times (\text{no. of mosquitoes collected}/\text{no. of catches}) \times 365$ days.

Data analysis

Analysis was performed using the SPSS® software version 10 (SPSS, Inc., Chicago, IL). Mosquito counts were transformed to $[\ln(x + 1)]$ to stabilize the variance before analysis. Two-way ANOVA was carried out to test variation within species between sites and seasons. Regression analysis was carried out to assess the effect of rainfall on the abundance of mosquitoes. Mann-Whitney test was used to test for significant difference between the sporozoite rates of *An. gambiae* s.l. and *An. funestus*. Z-test was used to test for significant difference between EIRs of *An. gambiae* s.l. and *An. funestus*.

Ethical clearance and informed consent

The study received ethical approval from the Medical Research Coordination Committee of the National Institute for Medical Research, Tanzania (Certificate No. NIMR/HQ/R.Sa/Vol. IX/192). Informed consent was obtained from local health authorities and village committees as well as individual house occupants.

Results

Mosquito abundance, spatial and temporal variation

A total of 64,875 female mosquitoes were collected between October 2001 and September 2004. The most abundant mosquitoes were *Culex* species which accounted for 46% followed by *An. gambiae* complex (28%) and *An. funestus* (25%). Other mosquitoes included a few *An. pharoensis* and *Mansonia uniformis* all accounting for 1% of the total mosquito collection.

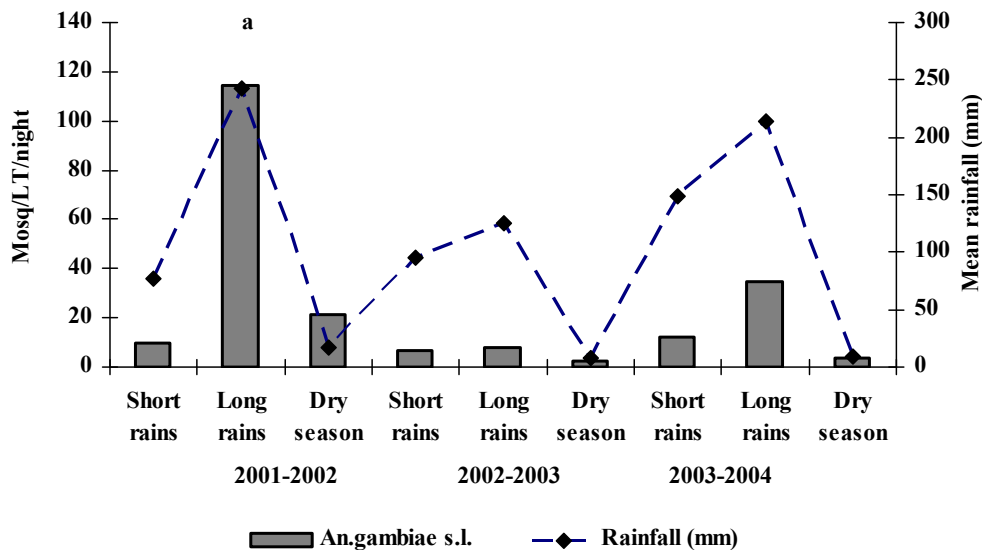


Figure 1: Seasonal variation of female *An. gambiae* s.l. in relation to rainfall

Species composition varied among the wards. There was a significant variation in the abundance of mosquitoes between years: *An. gambiae* complex (ANOVA, $F = 3.084$, $P < 0.05$), *An. funestus* (ANOVA, $df = 5$, $F = 6.637$, $P < 0.001$), *Culex* sp. (ANOVA, $df = 5$, $F = 3.669$, $P < 0.001$). Density of *An. gambiae* s.l. correlated positively to rainfall ($r = 0.9997$, $P < 0.05$) (Figure

1). The density of *An. funestus* was slightly higher during the dry than rainy season. However, the difference was not statistically significant (Figure 2). The abundance of *Culex* sp. correlated significantly with rainfall ($r = 0.4.066$, $P < 0.05$). The abundance of other mosquitoes was very low and was recorded only during the long rains.

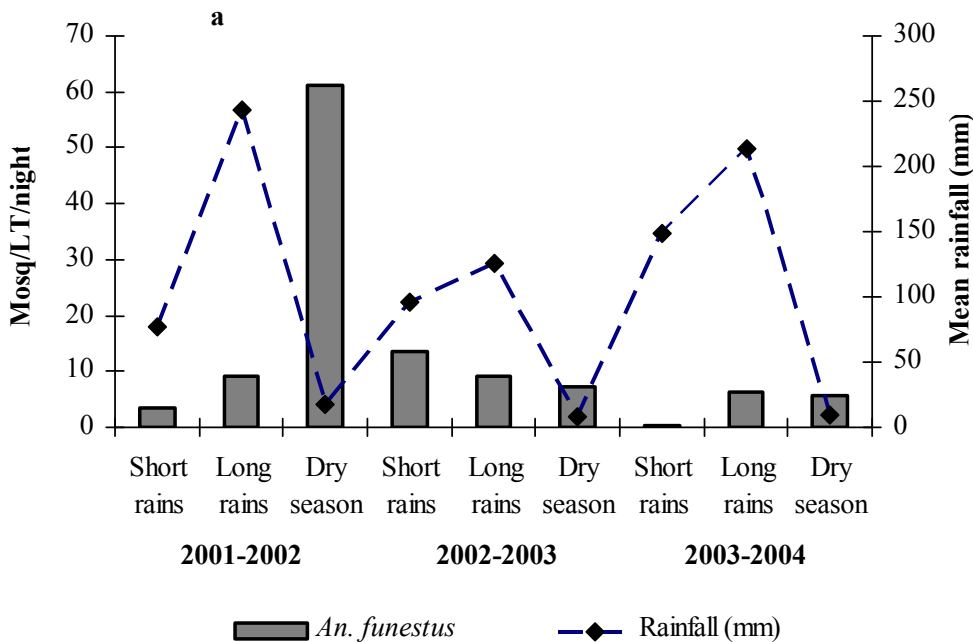


Figure 2: Seasonal variation of female *An. funestus* in relation to rainfall

Speciation of the members of the Anopheles gambiae complex

A total of 600 *An. gambiae* complex females were selected for speciation, these comprised 100 mosquitoes from each ward. Thirty eight (38) specimens did not produce positive results. Therefore, 562 specimens were differentiated into three sibling species of the *An. gambiae* complex and the results were as follows: 69% of the specimens produced fragments equivalent to *An. gambiae* s.s. (390 bp), 23% were identified as *An. arabiensis* (315bp) and 7% produced a fragment equal in size to *An. merus* (464 bp). *An. gambiae* s.s and *An. merus* were more abundant on the plateau than on the flood plain (Fisher's exact test, $P < 0.0001$). The abundance of *An. arabiensis* was similar among the six wards (Fisher's exact test, $P = 1656$).

Sporozoite rate

A total of 33,639 female anophelines were examined by ELISA for *P. falciparum* circumsporozoite antigen (CSA). Overall, the *P. falciparum* CSA rate for both *An. gambiae* s.l. and *An. funestus* was 2.9 %. The *P. falciparum* rate for *An. gambiae* s.l. was 3.4 % and 2.3 % for *An. funestus*; there was no significant difference between the two rates (Mann-Whitney test, $U = 547$, $P = 0.2534$). There was no significant difference in the *P. falciparum* CSA rates of *An. gambiae* s.l between the six wards (ANOVA, $df = 5$, $F = 1.585$, $P = 0.183$). However, the *P. falciparum* CSA rates for *An. funestus* varied significantly between the wards (ANOVA, $df = 5$, $F = 4.266$, $P < 0.05$). The CSA rate of *An. gambiae* s.l. varied significantly between seasons (ANOVA, $df = 2$, $F = 1.381$, $P < 0.05$), and was generally high during the rainy seasons ($r =$

0.7101, $P < 0.05$). However, the CSA rate of *An. funestus* did not show significant seasonal variation (ANOVA, $df = 2$, $F = 0.055$, $P = 0.946$).

The Entomological Inoculation Rate

Estimates of EIR for *An. gambiae* s.l. ranged from 0 to 1085.5 infective bites per person year and for *An. funestus* ranged from 0 to 768 infective bites. The combined EIR for both species ranged from 0 to 1087 infective bites per person per year. The EIR for *An. gambiae* s.l. varied significantly between wards. (ANOVA, $df = 29$, $F = 8.132$, $P = 0.001$). However, the EIR for *An. funestus* did not vary significantly between the wards (ANOVA, $df = 29$, $F = 0.201$, $P = 0.857$). When combined the EIR for both species showed no significant variation between wards (ANOVA, $df = 29$, $F = 0.379$, $P = 0.854$). The EIR for *An. gambiae* s.l. varied significantly between years (ANOVA, $df = 35$, $F = 16.05$, $P < 0.05$), while that of *An. funestus* did not vary between years (ANOVA, $df = 35$, $P = 0.159$). Generally the EIR for *An. gambiae* s.l. correlated significantly to rainfall ($r = 0.872$, $df = 35$, $P = 0.002$) but that of *An. funestus* did not ($r = 0.126$, $df = 35$, $P = 0.748$). The EIR rates for *An. gambiae* s.l. were higher than that of *An. funestus* (T-test, $t = 1.701$, $df = 89$, $P < 0.0001$) (Fig. 4).

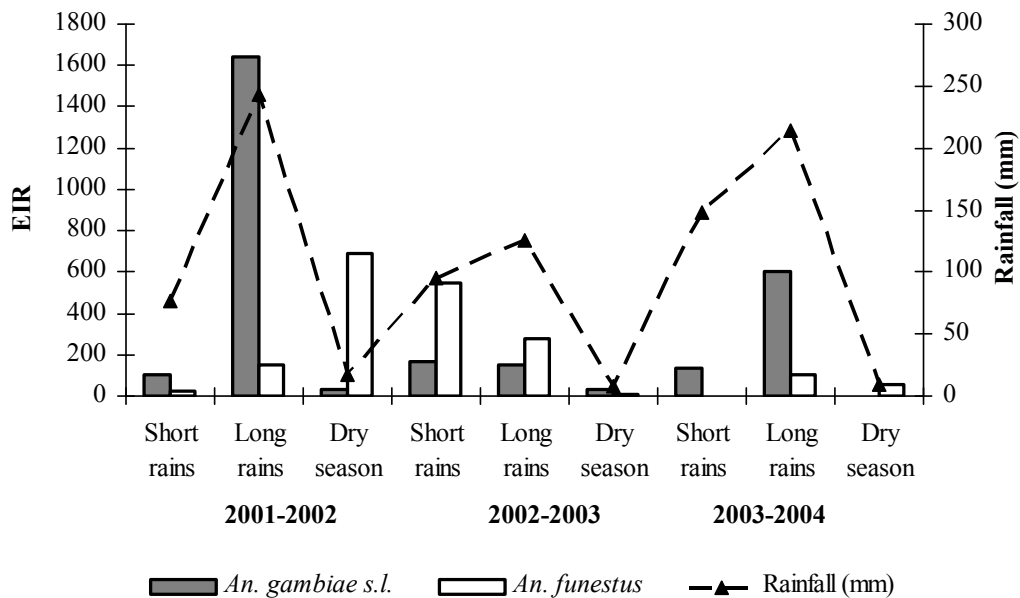


Figure 3: Seasonal variations of the EIR in Rufiji, October 2001 to September 2004

Discussion

The results of the present study show clear differences in mosquito density between wards, and between seasons. Density was higher during the rainy season (when there were more breeding habitats) and low during the dry season (when breeding habitats were scarce). The spatial and temporal distribution of malaria vectors observed here corroborate with studies elsewhere in Tanzania (Biro, 1987; Smith *et al.*, 1993; Temu *et al.*, 1998; Mboera *et al.*, 2010)

and other tropical areas with similar climatic conditions (Charlwood & Graves, 1987; Mendis *et al.*, 2000). *An. gambiae* density is always high near human dwellings surrounded by temporary water bodies, while *An. funestus* is linked with closeness to permanent water bodies (Ilboudo-Sanoga *et al.*, 2001).

The present study was an attempt to confirm the identity of the members of the *An. gambiae* complex that occur in Rufiji District, which were confirmed as *An. gambiae* s.s., *An. arabiensis* and *An. Merus*. Several studies have reported the occurrence of *An. merus* on the north-eastern coast such as Muheza (Bushrod, 1981; Mnzava & Kilama, 1986) and Bagamoyo (Temu *et al.*, 1998). The succession of *An. gambiae* s.s. and *An. arabiensis* (i.e. an explosive increase in the population of *An. gambiae* s.s at the start of the rainy season, decreasing gradually towards the end of the rainy seasons and the predominance of *An. arabiensis* at the end of the rainy season), was evident in Rufiji. Similar findings have been reported by other authors in Tanzania (Charlwood *et al.*, 2000). The population succession of the members of the *An. gambiae* s.l. during the rainy season and *An. funestus* at the end of the rains and during the dry season (Charlwood *et al.*, 2000) was again clearly observed. *An. funestus* is likely to be the most-important long term vector of malaria in Rufiji River Basin owing to its sustained output from more stable breeding sites during the dry season. The spatial and seasonal variations in abundance and species composition of malaria vectors observed in the present study further confirm the heterogeneities in the population dynamics of malaria vectors within a small ecologic area (Mboera *et al.*, 2010). This further shows the need for understanding local dynamics of malaria vectors if appropriate efforts to be invested in controlling malaria transmission.

Estimates of malaria transmission observed in the present study are one of the highest in Tanzania (Smith *et al.*, 1993; Temu *et al.*, 1998; Mboera & Magesa, 2001; Maxwell *et al.*, 2003; Mboera *et al.*, 2010). Recently, Mboera *et al.*, (2010) reported an overall human biting rate in *An. gambiae* s.l. and *An. funestus* of 175.3 and 35.8 bites per person per night among rural communities of Mvomero district in central Tanzania (Mboera *et al.*, 2010). In Rufiji District, it seems that the high malaria transmission rates result from a combination of factors, which include climatic, ecological and socio-economic. The climatic (e.g. rainfall) and ecological (e.g. availability of breeding habitats) factors were the major cause for the high Anopheles densities, which inevitably leads to increased malaria transmission. There are many favourable habitats for mosquito breeding throughout the year within the flood plain from where migrating mosquitoes could quickly spread to nearby areas or even the plateau. The spatial and temporal variations in mosquito abundance, mosquito species composition and transmission indices of malaria vectors observed in the present study confirm the observations that heterogeneities in the population dynamics of malaria vectors and transmission indices occur even within a small ecologic area (Dolo *et al.*, 2004; Okello *et al.*, 2006; Ye' *et al.*, 2007; Mboera *et al.*, 2010).

In conclusion the study has provided basic data, which could be useful for the evaluation of the relationship between transmission and morbidity of malaria in the study area. Moreover, establishment and strengthening of vector surveillance mechanisms to monitor surges in vector density and transmission indices would be invaluable in making informed malaria control decisions.

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