

# Modelling of the Abundance of Malaria Mosquitoes Using Poisson Mixed Model

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## Abstract

*Anopheles funestus* and *Anopheles gambiae* are malaria vector mosquitoes. Knowing their resting behavior is important for implementing control methods. The aim of this study was to investigate the resting behaviour of the two malaria mosquitoes. The study was conducted in Kilombero River Valley and mosquitoes were collected using indoor and outdoor traps from 2012-2015. Poisson mixed models were used to quantify the impact of environment variables on resting behaviour. A log ratio rate between the type of trap and its interaction with environmental variables was used to determine if there was a change over time in the resting behaviour. A total of 4696 mosquitoes were resting indoors of which 57% were *A. funestus* and 43% were *A. gambiae*. Similarly, a total of 12,028 mosquitoes were resting outdoor of which 13% were *A. funestus* and 87% were *A. gambiae*. Temperature was significant and affected the resting behaviour of *A. funestus*. Humidity, saturation deficit and temperature were significant variables influencing the resting behaviour of *A. gambiae*. *A. funestus* was resting indoor while *A. gambiae* was resting outdoor over time generally. The findings of this study on the effects of environmental variables and the variations in the resting behaviour of *A. gambiae* and *A. funestus* could be used as a guide to implementing appropriate intervention measures such as indoor residential spraying (IRS), insecticide treated nets (ITNs) and mosquito repellents.

## Keywords

*A. gambiae*, *A. funestus*, Resting Behaviour, Mosquito Density, Environmental Variables

## 1. Introduction

Mosquitoes are insects that can cause morbidity and mortality through the diseases they can carry. Beck-Johnson *et al.* [1] point out that mosquitoes not only carry diseases that affect humans but are also very efficient vectors of these diseases. Mosquitoes are responsible for transmitting some of the most devastating diseases today. For instance, they may cause several problems affecting activities that have a massive economic impact such as loss of economic productivity in the workforce through mortality of staff and also pose a threat to livestock [2]. Consequently, the cumulative effect in the long term may cause a decrease in national economic capacity and development. According to the World Health Organization (WHO) estimates, the number of cases and related deaths recorded in 2017 were 219 million and 435,000, respectively [3].

Mosquitoes feed on a range of different host vertebrates and some species have developed a characteristic host preference, feeding preferentially from humans, mammals other than human and birds [4]. In Africa, *A. gambiae* and *A. funestus* are found together in many areas of the continent [5]. Mboera *et al.* [2] observe that in Sub-Saharan Africa, there are variations in anopheline mosquito composition and malaria transmission which are within districts and between seasons. But, Smith and others cited in [3] argue that the differences in micro-ecological and socio-economic factors included vector density heterogeneity, mosquito survival, vector host contact and their innate feeding preference are likely to have contributed to these variations. The survival of Anopheles mosquitoes is dependent on climatic conditions such as rainfall pattern, temperature and humidity.

Understanding the biology and behavior of Anopheles mosquitoes can help understand how malaria is transmitted [3]. The abundance of Anopheles mosquitoes poses a threat to the population since they are vectors that transmit some deadly diseases of public health concern, of which one notorious example is malaria. Thus, knowing where they rest is important to assess the impact of existing control methods and develop new methods to eliminate them. Hence, understanding the distribution of Anopheles mosquitoes, how they interact or differ could give a guide for a critical aspect of our future ability to control malaria. Research in this area will provide the critical scientific base for the development of strategic planning in malaria control programmes and ensure that the most effective vector control intervention tools are used.

Kreppel *et al.* [6] used generalized linear mixed effects models (GLMM) in investigating the variation in proportion of malaria vectors. They considered *A. gambiae* and *A. funestus* within the total catch, fitted trap type as fixed effect and night, date and house as random effects. They found that *A. gambiae* (which they took as *Anopheles arabiensis*) was caught resting mainly outdoors while *A. funestus* was primarily caught resting inside.

In the study of Mayagaya *et al.* [7], the daily abundance variations of mosquito

vectors caught using different traps and resting collections, were analysed using generalized linear mixed models. Two separate analyses were carried out for *A. gambiae* and *A. funestus*, using the presence of livestock as a covariate. They found that the rate of *A. funestus* and *A. gambiae* resting indoor was lower and no significant association with the presence of livestock. They found that the proximity of livestock was associated with the abundance of *A. gambiae* but not with *A. funestus*.

Kabula et al. [4] argue that little is known about the distribution of Anopheles mosquitoes that are vectors of the disease that kills millions of people, how the species interact and differ across the country. Therefore, the abundance of the malaria vector (mosquito) would be a key determining factor for malaria risk which is a life-threatening disease. Thus, knowing their resting behavior is important for implementing control methods. The aim of this study was to investigate and determine if there was a change over time in the resting behaviour of *A. gambiae* and *A. funestus* and examine the effects of environmental variables. The remaining parts of this paper are organized as follows. We discuss the methodology considered in Section 2. The results and discussion are contained in Section 3 and lastly, we conclude the paper in Section 4.

## 2. Modelling Count Data

Count data refers to observations that can potentially take any non-negative integer value [8]. The aim of count data models is to explain the number of occurrences of an event. In this section, we review some of the most widely used statistical models for count data.

### 2.1. Poisson Model

The Poisson model can be considered as the standard model for count data [9]. The probability function of a Poisson variable  $Y_i$  is given by,

$$f(y_i, \mu_i) = \frac{\mu_i^{y_i} e^{-\mu_i}}{y_i!}, \quad y \geq 0 \quad y \text{ is an integer}, \quad (1)$$

where  $E(Y_i) = \mu_i$  and  $Var(Y_i) = \mu_i$ .

Hence, the Poisson distribution is characterized by a linear relationship between its variance and mean. To model  $\mu_i$  using explanatory variables  $x_r$ , the log link function is used, although other choices are also available. More specifically, we write

$$\log(\mu_i) = \beta_0 + \sum_{r=1}^p x_{ir} \beta_r, \quad (2)$$

where;

- $\beta_0$  is the model intercept.
- $\beta_r$  are the regression coefficients which regulate the effects of the explanatory variables  $x_r$ .
- $\log(\cdot) = \log_e(\cdot)$  computes the natural logarithm.

If the variance of the data is greater than the mean, the Poisson assumption is questionable. We refer to this case as over-dispersion. Ignoring over-dispersion can lead to invalid inferences on the regression relationship [10]. Probabilistic models that account for over-dispersion are the following.

## 2.2. Quasi Poisson Model

These models are only defined by specification of their mean, variance and a dispersion parameter  $k$  [4], that is;  $E(Y_i) = \mu_i$  and  $Var(Y_i) = k\mu_i$ .

We point out that a distributional form for the variable  $Y_i$  is not required for fitting of the model and making inference on  $\mu_i$ . If  $k = 1$ , we recover the identity relationship between the mean and the variance of the Poisson model. Another advantage of the quasi Poisson model is it can be fitted using the same algorithms for the Poisson model [11].

## 2.3. Negative Binomial Model

The negative binomial model is obtained by assuming that the mean  $\mu_i$  of the Poisson model is Gamma distributed [8]. The density function of the negative binomial is given by,

$$f(y_i; \mu_i, k) = \frac{\Gamma(y_i + k)}{\Gamma(k) \times \Gamma(y_i + 1)} \times \left(\frac{k}{\mu_i + k}\right)^k \times \left(1 - \frac{k}{\mu_i + k}\right)^{y_i} \quad (3)$$

where  $k$  is the dispersion parameter. The symbol  $\Gamma$  denotes the gamma function and is defined as  $\Gamma(y+1) = y!$ . The mean and variance of  $Y_i$  are now  $E(Y_i) = \mu_i$  and  $Var(Y_i) = \mu_i + \frac{\mu_i^2}{k}$ . The Poisson model is recovered as a limiting case for  $k \rightarrow \infty$ .

## 2.4. Poisson Mixed Model

This model is an extension of the standard Poisson model by the inclusion of the random effects in the linear predictor. One example is given by the random intercept model which is obtained as follows. Let  $Z_i$  for  $i = 1, 2, 3, \dots, n$  denote random variables, having joint multivariate Gaussian distribution with mean 0 and covariance matrix  $\Sigma_Z$ . We then assume that  $Y_i$ , conditionally on  $Z_i$  are mutually independent Poisson variables with mean  $\mu_i$  such that,

$$\log(\mu_i) = \beta_0 + \sum_{r=1}^p x_{ir} \beta_r + Z_i, \quad (4)$$

It can be shown that this model has larger variance than the standard Poisson model. This model can also be further extended by allowing the regression coefficients  $\beta_r$  to vary across the observations. In Equation (4),  $\beta_r$  would then be replaced by  $\beta_{ir}$  (assumed to follow a Gaussian distribution with some appropriate mean and variance). More importantly, this model assumes that the effect of  $x_r$  on the response is not homogeneous but might depend on both measured and unmeasured variables.

The main difference of the outlined modelling approach with respect to the previous models, is that over-dispersion is accounted for by specification of the conditional mean of  $Y_i$  for given latent variables (or random effects)  $Z_i$  and/or  $\beta_{ir}$ . Quasi-poisson and negative binomial models, instead, allow for over-dispersion through the parameter  $k$  which regulate the relationship between the marginal mean and variance of  $Y_i$ . These two different approaches are then used to answer substantially different research questions. The former approach is useful when it is of interest to model the different sources of heterogeneity inherent to the data. The latter techniques are instead more appropriate when inference should be drawn on the general population. For these reasons, we consider Poisson mixed models to be more suitable to address the specific objectives of the present analysis.

### 3. Methodology

The collection of the malaria vector, *A. funestus* and *A. gambiae* mosquitoes were collected in four villages of Kilombero River Valley by the use of indoor and outdoor traps over four years from 2012 to 2015. Each village was visited several times over the four years and each time, mosquitoes were trapped inside and outside the houses. Different houses were chosen each time a visit was made to the villages and collected mosquitoes over four (4) nights in each house. Information on temperature, humidity and saturation deficit were also collected from each visited household. To catch mosquitoes inside required a different trap than to catch them outside. Traps were set in the evening at 6 pm and emptied in the morning at 6 am. For every inside trap there are 10 outside traps.

#### 3.1. Description of Variables

The variables of interest used in this study were determined according to the study objectives and are shown in **Table 1**.

#### 3.2. Model Formulation

The collected data were subjected to verification for consistency, uniformity and accuracy with the researcher in charge. Data were then exported and analyzed in the R software environment [12]. The mixed effects model was used to quantify the association of mosquito abundance with environmental factors.

Let  $U_t \sim N(0, \sigma_u^2)$  and  $V_t \sim N(0, \sigma_v^2)$  be independently and identically distributed. Let  $Y_{it}$  denote the monthly counts of mosquitoes at village  $i$  at time  $t$ . We then assume that conditionally on  $U_t$  and  $V_t$ ,  $Y_{it}$  are mutually independent Poisson variables with mean  $\eta_{it}\mu_{it}$  where  $\eta_{it}$  is the number of traps used to catch the mosquitoes. Using a log link-function, we then write

$$\log(\mu_{it}) = \alpha_0 + \beta_0^T dt + \gamma_0^T x_{it} + \eta_t \delta_{it} + \mu_t, \quad (5)$$

$$\eta_t = \alpha_1 + \beta_1^T dt + V_t, \quad (6)$$

where,

- $x_{it}$  is vector of dummy variables identifying each of the four villages;
- $d_t$  is a vector of environmental variables;
- $\delta_{it}$  is a binary indicator of the type of trap, taking value 1 if outdoor and 0 if indoor;
- $U_t$  is a random intercept which accounts for extra-Poisson variation induced by unmeasured explanatory variables;
- $V_t$  is a random effect accounting for unmeasured explanatory variables which affect the log-ratio between the rate of mosquitoes outdoor and indoor;
- $\eta_t$  corresponds to the log ratio between the rate of indoor and outdoor mosquitoes at time  $t$ .

**Table 1.** Shows description of variables and the codes used.

Variable	Description of variable
$V_c$	The village visited (KID = Kidugalo, MIN = Minepa, LUP = Lupiro and SAG = Sagamaganga)
$T_m$	The type of mosquito trapped <i>i.e.</i> Ag = <i>A. gambiae</i> and Af = <i>A. funestus</i> .
$T_p$	Indicates the type of trap used that is in = Indoor and out = Outdoor.
$T_r$	The difference between the maximum and minimum temperature recorded in degree Celsius.
$H_r$	Average relative humidity per month.
$S_d$	Average monthly saturation deficit measured in pressure vapor.
$M_d$	The density is the number of monthly mosquito counts per trap in the study area and is given by mosquito counts divided by number of traps.

The standard error is computed by utilizing the formulation,

$$\text{Standard error} = \frac{\text{Standard deviation}}{\sqrt{\text{Sample size}}}$$

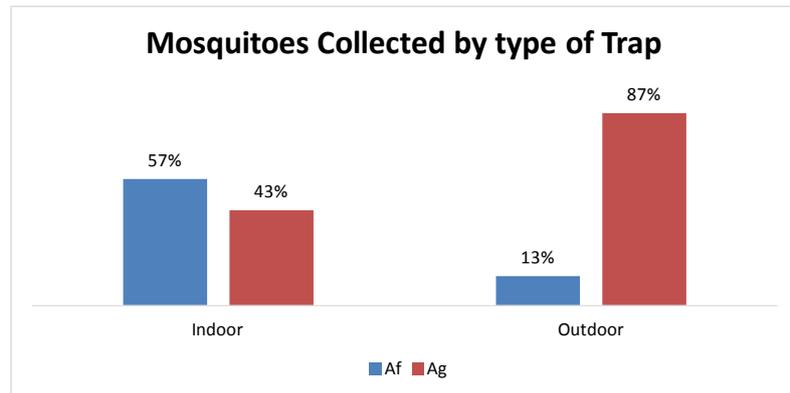
To understand how the resting behaviour of mosquitoes changes over time, we use Equation (6) which corresponds to the log-ratio between the rate of indoor and outdoor mosquitoes and therefore, represent our target for inference. The variable selection procedure was to start with a full model including interactions with all environmental variables and type of trap, *i.e.* indoor or outdoor. Then, the variables with a p-value greater than 5% (*i.e.* considering a 95% confidence level) are removed from the model. The process is repeated until all the variables have p-values less than 5%.

## 4. Results and Discussion

We now look at the total number of mosquitoes collected by type of mosquito and trap in the study area, that is, for *A. funestus* and *A. gambiae*.

In **Figure 1**, it is observed that there are differences in the numbers of mos-

quitoes caught by the type of trap for *A. funestus* and *A. gambiae*. Over the four years of mosquito collection in the study area, a total of 4696 mosquitoes were collected resting indoors of which 57% were *A. funestus* and 43% were *A. gambiae*. Similarly, a total of 12,028 mosquitoes were collected resting outdoor of which 13% was *A. funestus* and 87% were *A. gambiae*.

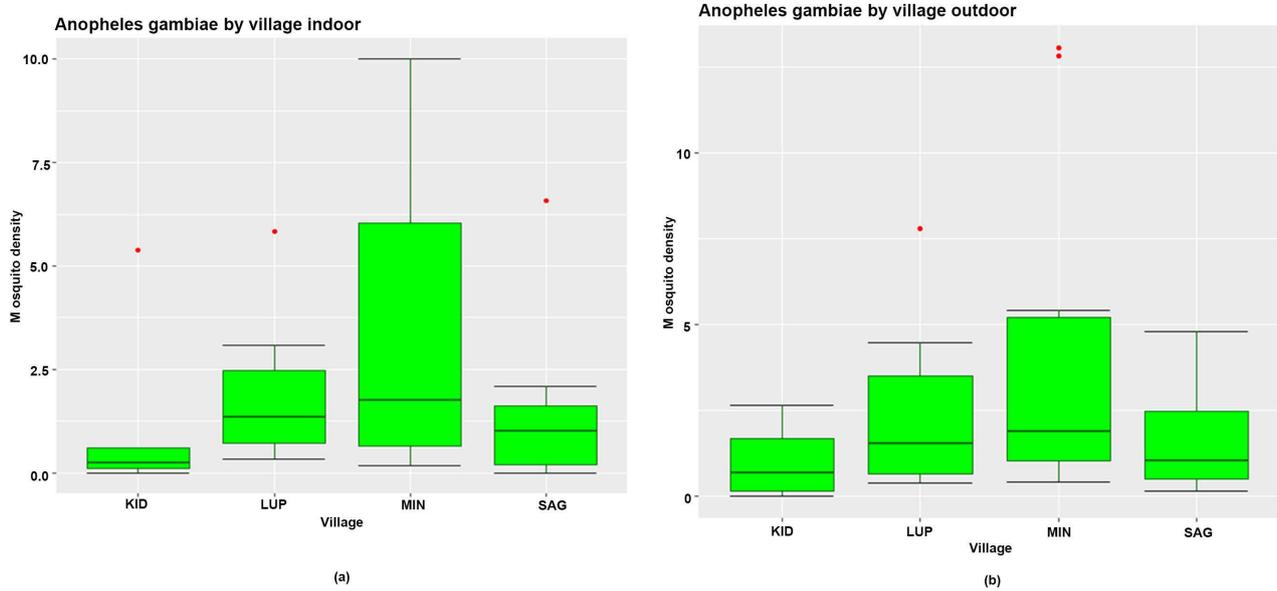


**Figure 1.** Mosquitoes collected by type of mosquito and trap in the study area.

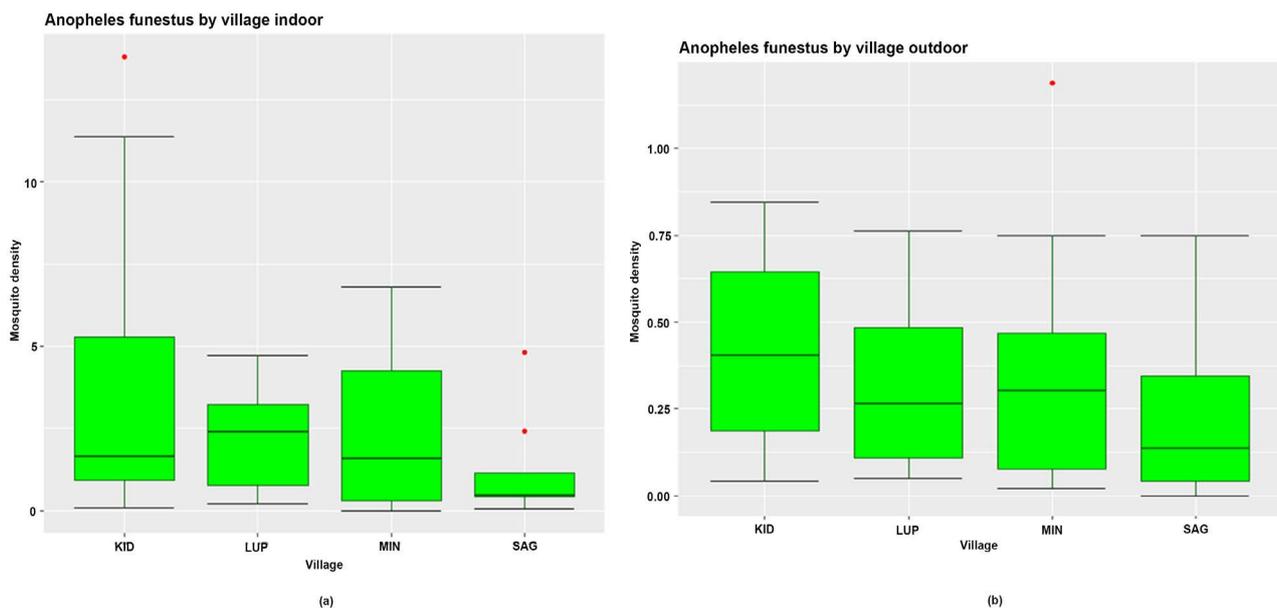
**Figure 2(a)** and **Figure 2(b)** shows the mosquito densities of *A. gambiae* by village for indoor and outdoor trap. It is observed that the median is different for all the villages for both indoor and outdoor trap. The highest density of *A. gambiae* was by indoor trap in Minepa village. We also note that there were three reported large densities by indoor trap each within Lupiro (LUP), Sagamaganga (SAG) and Kidugalo (KID) and in Minepa (MIN) by outdoor trap. We observe that by both indoor and outdoor trap for *A. gambiae*, the mosquito densities seem to have a similar pattern. Kidugalo and Sagamaganga village have lower densities of *A. gambiae* mosquitoes as compared to Lupiro and Minepa village for both indoor and outdoor trap. This can be attributed to village specific environmental characteristics. For instance, Kidugalo and Sagamaganga are on a higher altitude than Minepa and Lupiro villages.

**Figure 3(a)** and **Figure 3(b)** shows the densities of *A. funestus* mosquito indoor and outdoor by village. The highest density of *A. funestus* was by indoor trap in Kidugalo village. All the villages had the lowest density of *A. funestus* mosquito approximately being zero for indoor and outdoor trap. We observe large densities of Anopheles mosquitoes in villages Kidugalo, Sagamaganga and Minepa respectively by indoor and outdoor trap. In this case, we observe different patterns by village in *A. funestus* mosquitoes indoor and outdoor.

For both *A. funestus* and *A. gambiae*, we observe a similar pattern in the resting behaviour outdoor while the pattern seems different indoors. In almost all the villages, *A. funestus* was mostly caught indoor while *A. gambiae* was caught primarily resting outdoor. This implies that *A. funestus* is likely to be more exposed to insecticide treated nets (INTs) and other chemicals applied to the walls such as indoor residual spraying (IRS). However, for *A. gambiae* mostly resting outdoor reduces the risk of exposure to chemicals and bites humans and animals outside.



**Figure 2.** (a) A plot of *A. gambiae* mosquito density indoor by village. (b) A plot of *A. gambiae* mosquito density outdoor by village.



**Figure 3.** (a) A plot of *A. funestus* mosquito density indoor by village. (b) A plot of *A. funestus* mosquito density outdoor by village.

In order to quantify the association between mosquito abundance and environmental factors and trap location, we carried out the analysis separately for *A. funestus* and *A. gambiae*.

#### 4.1. Model for *Anopheles funestus*

The first model considered was for *A. funestus*. After applying the variables selection procedure described in Section 3.2, only temperature range showed a significant association. The final model is shown in **Table 2**.

**Table 2.** Output for *A. funestus* with environmental factors.

Parameter	Estimate	Std.Error	P value
$\alpha_0$	0.3265	0.2810	0.2451
LUP	-0.2733	0.0576	2.09e-06
MIN	-0.4816	0.0496	2e-16
SAGE	-0.5815	0.0682	2e-16
$T_p(out)$	-0.9108	0.2697	0.0007
$T_r$	0.0991	0.0188	1.36e-07
$T_p(out):T_r$	-0.1971	0.0302	6.81e-11

The coefficient of Lupiro village indicates that the mosquito density is 0.7609 times the density of *A. funestus* mosquitoes in Kidugalo village. The density of *A. funestus* mosquitoes in Minepa village is 0.6178 times the density of mosquitoes in Kidugalo village. In Sagamaganga, the *A. funestus* mosquito density is 0.5591 times that in Kidugalo village. 40% of the density of *A. funestus* mosquitoes were caught outdoor implying that 60% were caught indoor. The percentage change in the *A. funestus* density is by 10% for every 1°C increase in the temperature range. The interaction effect between temperature and type of trap was significant. This suggests that the effect on the density of *A. funestus* resting outdoors is a change by 0.8211 compared to resting indoors for every 1°C change in temperature range.

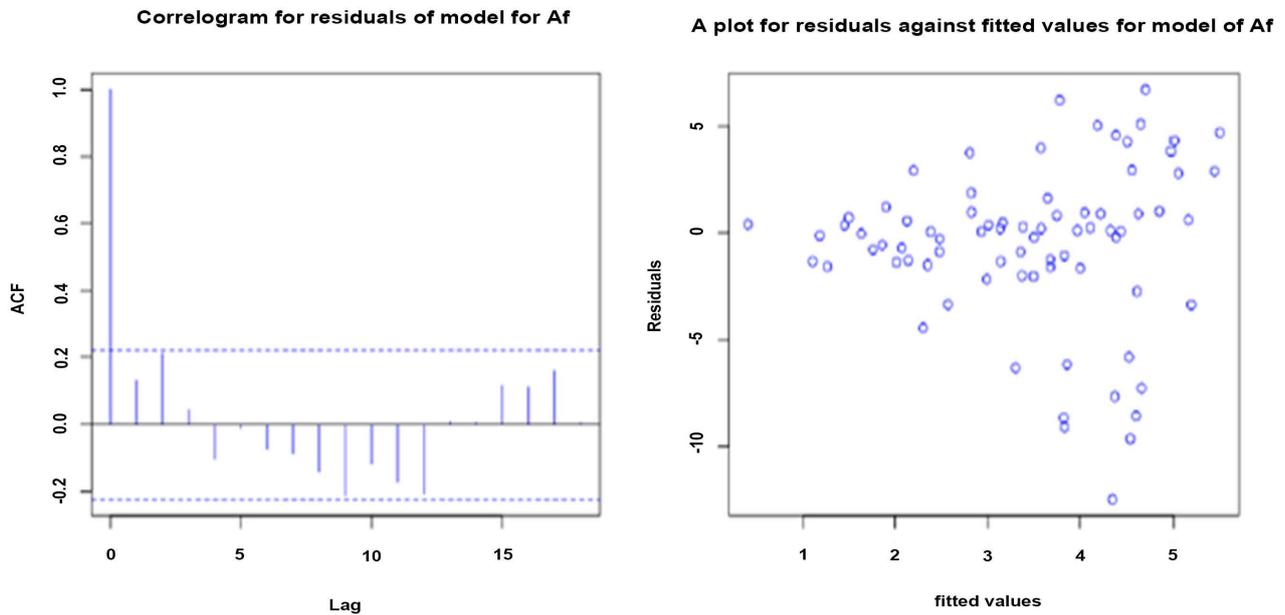
Temperature is an important environmental factor affecting the resting behavior of *A. funestus*. We observe a significant relationship between temperature and the density of *A. funestus*. This implies that, a higher temperature range explains the change in the resting behaviour of *A. funestus*. It suffices to note that temperature is dependent on several factors such as location of the village, surroundings and structure of the buildings in the area.

We now carry out diagnostic checks of the residuals.

We can observe in **Figure 4** that there is a fairly random pattern in a plot of residuals against the fitted values for the model of *A. funestus*. The random pattern indicates that the residuals appear to show homogeneity, normality and independence. This is an indication that the model provides a adequate fit to the data. As can be seen from the correlogram plot, we observe that there is no strong serial correlation in the residuals and the autocorrelation at all the lags is nearly close to zero.

#### 4.2. Model for *Anopheles gambiae*

We now look at the model for *A. gambiae*. In the final model, temperature range, humidity range and saturation deficit showed a significant association with mosquito density. Also, there were interactions between humidity range and saturation deficit with type of trap. The estimates are given in **Table 3**.



**Figure 4.** A plot and correlogram of residuals of the model for *A. funestus*.

**Table 3.** Output for *A. gambiae* with environmental factors.

Parameter	Estimate	Std.Error	P value
$\alpha_0$	-1.2632	0.3990	0.0016
LUP	1.2866	0.0596	2e-16
MIN	1.7102	0.0524	2e-16
SAG	0.5019	0.0494	2e-16
$T_r$	-0.8404	0.0297	2e-16
$H_r$	0.1349	0.0125	2e-16
$S_d$	2.8338	0.2172	2e-16
$H_r : T_p (out)$	0.0675	0.0116	6.17e-09
$S_d : T_p (out)$	-0.9438	0.2234	2.39e-05

The coefficient of Lupiro village indicates that the mosquito density is 3.6205 times the density of *A. gambiae* mosquitoes in Kidugalo village. The density of *A. gambiae* mosquitoes in Minepa village is 5.5301 times the density of mosquitoes in Kidugalo village. In Sagamaganga, the *A. gambiae* mosquito density is 1.6519 times that in Kidugalo village. The *A. gambiae* mosquito density decreases by 0.4315 for every 1 °C increase in the temperature range. The change in the density of *A. gambiae* mosquitoes is 1.1444 for a 1 unit change in the average relative humidity range. A one unit change in the average monthly saturation density changes the density of *A. gambiae* mosquito by 17.01. The effects of the density of *A. gambiae* mosquitoes resting outdoors is 1.0698 times the density resting indoors for a unit change in the average relative humidity range. The effect on the density of *A. gambiae* mosquito resting outdoor is a change in the

density by 0.3891 compared to resting indoors for a unit change in the average monthly saturation deficit.

All environment variables were statistically significant in explaining the suitability of the environment in the resting behaviour of *A. gambiae*. An increase in the average relative humidity implies more *A. gambiae* mosquitoes resting outdoor. [13] Argue that when relative humidity rises, mosquitoes survive better and is higher at night. Also, a high temperature decreases the density of *A. gambiae* resting indoors. [14] In their study state that *A. gambiae* mosquitoes have the ability to avoid high temperatures thus, move to more humid locations that facilitate their survival. Saturation deficit plays an important role in the resting behaviour of *A. gambiae* as it is a significant variable. The higher the average saturation deficit in the study area, the more the *A. gambiae* mosquitoes rest outdoor and this can also be attributed to the location of the study area.

We now check for model fit by carrying out a diagnostic plot of residuals.

As can be seen from the correlogram plot in **Figure 5**, we observe that there seems to be no serial correlation in the residuals and the autocorrelation at all the lags is nearly close to zero. We can also observe that there is a fairly random pattern in a plot of residuals for the model of *Anopheles gambiae*. The random pattern indicates that the residuals appear to show homogeneity, normality and independence and is an indication that the model provides an adequate fit to the data.

We now compare the resting behavior of *A. funestus* and *A. gambiae* by village and use of Equation (6) for the log ratio between the rate of indoor and outdoor mosquitoes. The analysis is based on a comparison of the abundance of *A. funestus* and *A. gambiae* trapped indoor and outdoor. When the value of the log ratio is zero *i.e.*  $\eta_i = 0$ , there is no difference in the log ratio between the mosquitoes resting indoors and outdoors. When  $\eta_i > 0$  implies that the log ratio of mosquito density outdoor is greater than the density indoor and conversely when  $\eta_i < 0$ .

We start by looking at the log ratio rate between indoor and outdoor resting of *A. funestus* mosquitoes. The log ratio relationship is shown in **Figure 6**.

As can be seen in **Figure 6**, the patterns are different over the villages implying that there is spatial heterogeneity in the log ratio between the rate of *A. funestus* mosquitoes indoor and outdoor. This implies that the densities of *A. funestus* are different in all the villages and we observe that the mosquitoes were not collected in 2015 for some villages. In Kidugalo village, we observe that the log ratio is negative implying that the mosquito density of *A. funestus* was more abundant indoor than outdoor. We observe that in both Kidugalo and Minepa villages, there seems to be no clear decrease or increase in the density of *A. funestus* mosquito in relation to the log ratio of the resting behaviour over time. Similarly, in Sagamaganga and Lupiro villages the density of *A. funestus* mosquitoes was more abundant indoor. We observe that in the two villages, the fluctuations in the mosquito densities for *A. funestus* are seen to almost have the

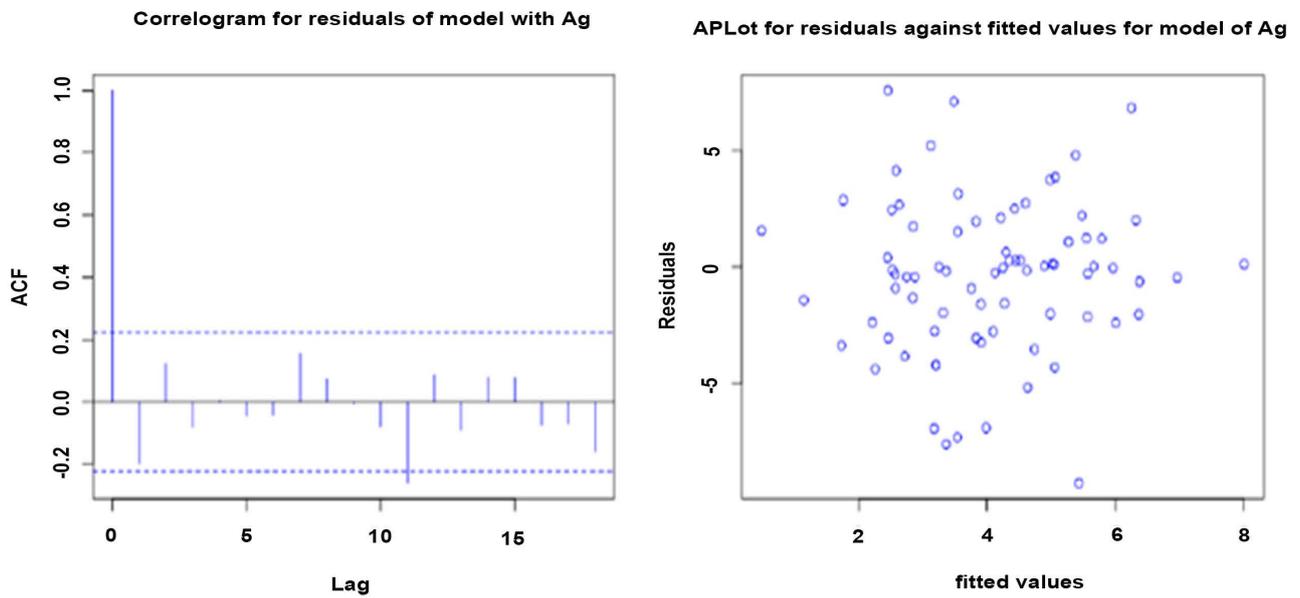


Figure 5. A plot and correlogram of residuals of the model for *A. gambiae*.

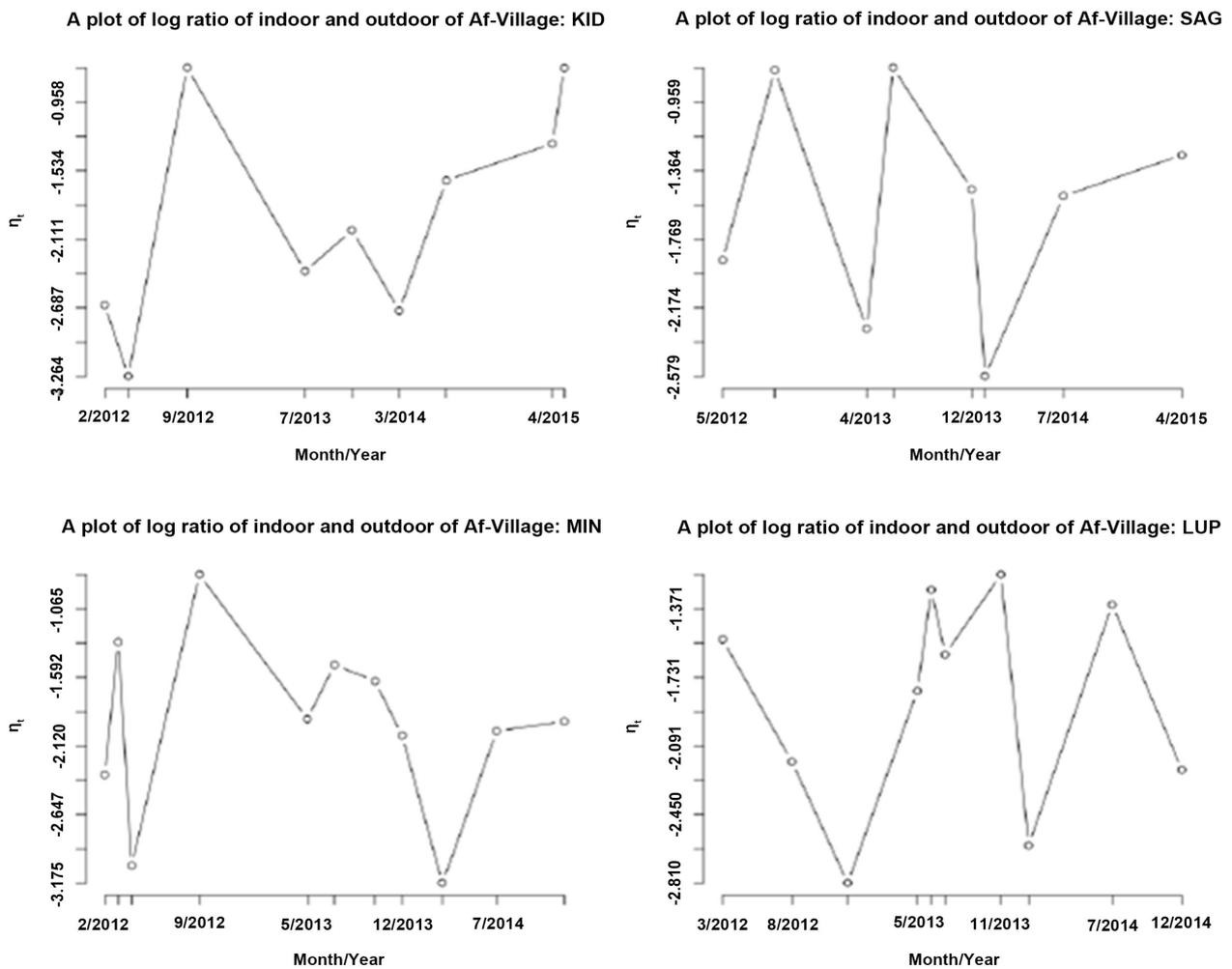
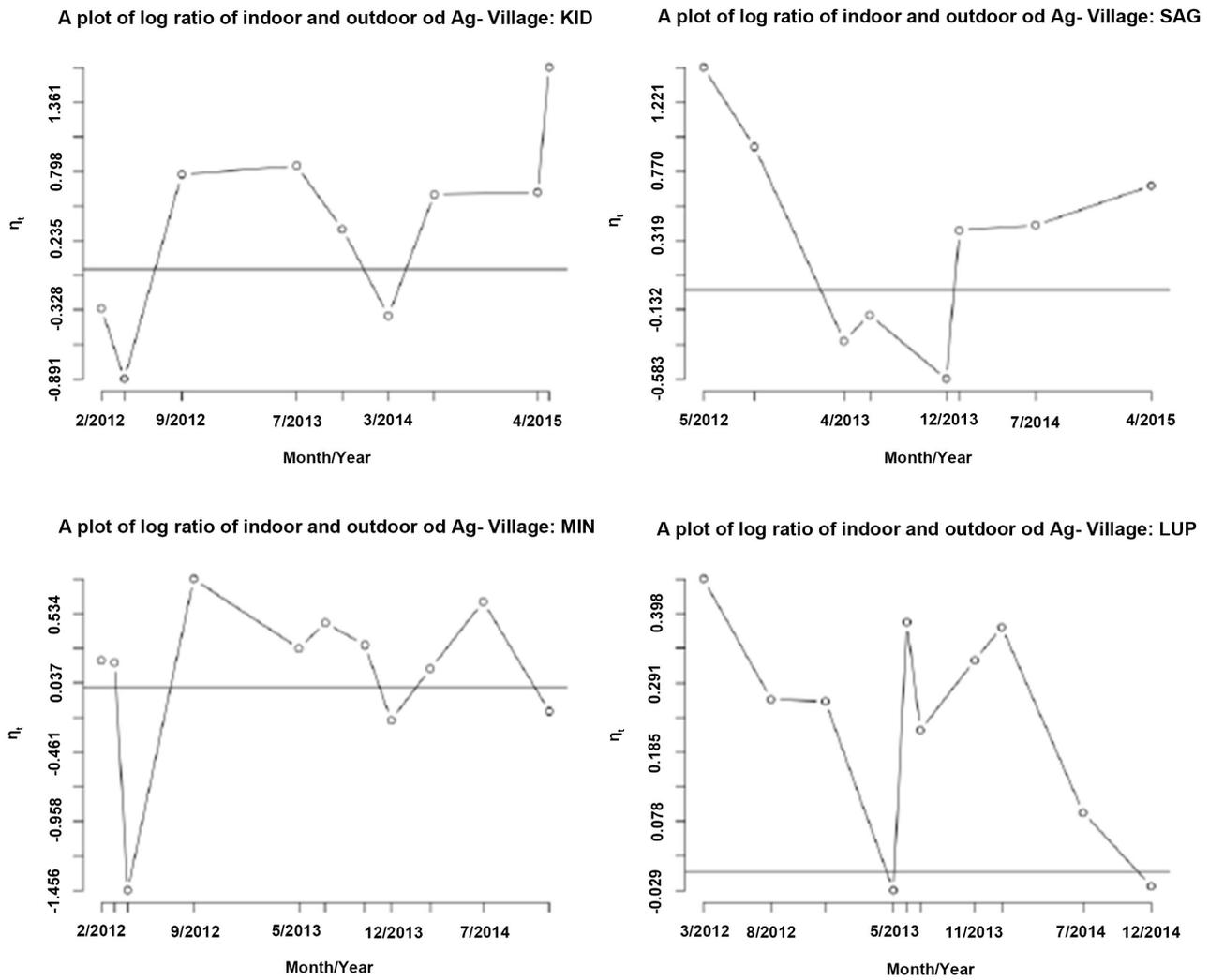


Figure 6. Plots of the log ratio of mosquitoes resting indoor and outdoor with time for *A. funestus*.



**Figure 7.** Plots of the log ratio mosquitoes resting outdoor and indoor with time for *A. gambiae*.

same pattern. Also, we observe that in all villages there are no clear signs of any shift in the resting behaviour of *A. funestus* mosquitoes from indoor to outdoor.

Investigating the resting behavior of *A. funestus* mosquito over time found that it was mostly resting indoor. There were no clear variations in the resting behaviour of *A. funestus* in all the four villages as observed in **Figure 6**. These fluctuations can be attributed to malaria control strategies in the area such as indoor residential spraying, use of insecticide treated nets and other several reasons that may affect interactions with environmental variables. This indicates that despite the high usage of antibodies for malaria in the study area, mosquitoes still enter houses as evident from the abundance of *A. funestus* indoor.

Now, we consider the log ratio rate of *A. gambiae* mosquito resting indoors and outdoors. The plot is shown in **Figure 7**

Similarly, it can be seen from **Figure 7** that the patterns over time for all the villages differ implying spatial heterogeneity in the log ratio between the rate of *A. gambiae* mosquitoes indoor and outdoor. We observe both positive and nega-

tive values of the log ratio between the rate of mosquitoes indoor and outdoor for all villages. The straight line represents a case when  $\eta_i = 0$  implying that the density of mosquitoes indoor and outdoor is equal. In Lupiro and Minepa villages, we observe a similar pattern in the resting behavior of *A. gambiae* mosquitoes as the density of mosquitoes is more outdoor than indoor. This indicates that maybe some *A. gambiae* mosquitoes were resting indoors as we have observed regardless of the presence of vector control indoors. Overall, *A. gambiae* mosquitoes seem to be more abundant outdoor in these villages. We observe significant temporal variation in the resting behaviour of *A. gambiae* in all villages.

The density of *A. gambiae* mosquitoes in all the villages was mostly more abundant outdoor over time. This can be attributed to the fact that *A. gambiae* breeds in places such as rice fields, puddles, sunlit pools and bites both human and animals. For instance, the shift in the resting behavior in some of the villages can be explained by malaria control programs. In Minepa and Lupiro, the main activities are cultivation of rice and thus, *A. gambiae* is mainly breeding in such fields. While in Sagamaganga and Kidugalo, they have a lot of cattle and therefore this explains the shift in the resting behavior of Anopheles to biting animals outdoor.

## 5. Conclusion

We conclude that *A. gambiae* and *A. funestus* show a remarkable temporal variation in their resting behaviour. *A. funestus* was the most abundant malaria vector indoor while *A. gambiae* was more abundant outdoor in the study area. The finding is in agreement with the results of [6]. Over four years of mosquito collection, 57% of mosquitoes collected indoor were *A. funestus* while 43% were *A. gambiae*. Similarly, 87% of the mosquitoes collected outdoor were *A. gambiae* and 13% were *A. funestus*. Minepa and Lupiro villages had large densities for both *A. gambiae* and *A. funestus* indoor and outdoor as compared to Kidugalo and Sagamaganga village. This can be due to their location as they are on the lower altitude of the Valley compared to Kidugalo and Sagamaganga, with possible presence of waterlogged areas. Also, the prominent activity in Minepa and Lupiro villages is farming while keeping cattle is the prominent activity in Kidugalo and Sagamaganga villages. Thus, the rice fields may be breeding sites for mosquitoes. There were variations in the resting behaviour of *A. gambiae* and *A. funestus* over time in all the villages. The patterns in the log ratio between indoor and outdoor densities of *A. gambiae* and *A. funestus* were different implying spatial heterogeneity in their resting behaviour. *A. funestus* was found to rest mainly indoor over time in all the villages. We found that *A. gambiae* was more abundant outdoor although there were some resting indoors in all the villages. Environmental variables had an impact on the abundance of *A. gambiae* and *A. funestus*. Temperature was found to significantly affect the resting behaviour of *A. funestus*. Whilst humidity range, temperature and saturation

deficit had an effect on the resting behavior of *A. gambiae* mosquitoes in the study area. The findings of this study could be used as a guide to implementing appropriate intervention measures. The use of indoor residential spraying (IRS), insecticide treated nets (ITNs) and other insecticides may then impact on the abundance of *A. funestus*. However, for mosquitoes outdoor, the use of mosquito repellents and other interventions would help reduce the abundance of *A. gambiae* which was more abundant outdoor. Ensuring that all possible mosquito breeding sites are eliminated is also a way to eliminate mosquitoes outdoor as this is prevention through source reduction.

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### Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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