Investigating the effects of temperature and humidity on mosquito abundances within Kilombero Valley, Tanzania

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Abstract

Malaria prevention is based on research performed on the vectors that transmit it- mosquitoes. Environmental factors that influence mosquito biting rates and abundances can help understand population control, which in turn can help control malaria transmission. Within four villages of the Kilombero Valley in Tanzania, mosquitoes were trapped, identified, sexed, and classified as fed, unfed, or gravid. At each trapping site, humidity and temperature data were daily collected. This paper intends to investigate the effects of temperature and humidity on mosquito abundances in this Sub-Saharan African region. Linear models and mixed effect models were generated using R and RStudio to look at which predictors captured variation within abundance data. The best fit model is a saturated mixed model, which considers the effect of both temperature and humidity on abundance, while taking into account status (fed, unfed, gravid), species (*An. funestus* and *An. gambiae*) and village as a random effect. Results show the interaction of temperature and humidity to have a significant effect on mosquito abundances for the fed and unfed states but not for the gravid state. These results, which can be further enhanced by other studies assessing the directionality of the interactions or other climatic factors can help predict mosquito feeding habits and malaria transmission rates.

Introduction

Malaria is a significant disease of global concern in many underdeveloped regions of the world, as it kills approximately 429,000 people annually (WHO, 2016). Malaria is a disease caused by parasites of the *Plasmodium* genus, which is transmitted through vectors. Vectors are living organisms that transmit infectious agents, such as parasites, to another animal or human. Malaria can only be transmitted by female mosquitoes of the genus *Anopheles* by biting humans while feeding (WHO, 2022). Since the *Plasmodium* parasite requires 7 to 30 days to develop and become mature enough to infect humans (WHO, 2022), it is typically only older mosquitoes that transfer the disease. Hundreds of species in the genus *Anopheles* are capable of transmitting malaria, and so the key malaria vector tends to vary with different geographical regions, as well as different factors such as temperature, humidity, and altitude (Daygena et al., 2017). The key species involved in transmission include *An. arabiensis, An. gambiae,* and *An. funestus* (Kelly-Hope et al., 2009).

Mosquitoes possess a unique reproductive strategy in which prior to oogenesis; a female mosquito will consume a large blood meal and digest it (Genoud et al., 2019). The mosquito will then undergo gestation while digesting, and move very little during this time (OECD, 2018). Thus, if a mosquito is gravid, this indicates that they consumed a blood meal at least within the past 69 to 97 hours (Genoud et al., 2019). Examining the abundances of gravid mosquitoes and climatic factors can provide information of when new mosquitos are likely to become active which can help anticipate a risk for exposure to malaria (Genoud et al., 2019). Previous research has been conducted on the biting behaviours in response to environmental conditions such as temperature where they found lower vector productivity in regions of lower temperature (Yan et al., 2006). Environmental factors such as temperature and humidity are important to understand as they can create suitable breeding sites for mosquitoes, which then affect their abundance and biting patterns (Kelly-Hope et al., 2009).

Studies have demonstrated that there are correlations between temperature and humidity and whether a mosquito is fed or unfed (Agyekum et al., 2021). However, there is currently no research regarding whether temperature and/or humidity affect gravid (carrying eggs) abundances. The purpose of this paper is to further explore the relationship between climatic factors and gravidity within the *An. gambiae* and *An. funestus* species.

Thus, we will investigate two alternative hypotheses regarding the abundance of mosquitoes in the Kilombero Valley in Tanzania. Hypothesis one posits that temperature and humidity have different effects on the abundances of mosquitos between the fed, unfed, and gravid states of mosquitos. This will allow us to investigate if temperature and humidity affect biting behaviour by looking at the abundance differences in fed, unfed or gravid states. Hypothesis two postulates that temperature and humidity have different effects on the abundances of the two different mosquitoes species (*An. gambiae* and *An. funestus*) between the fed, unfed and gravid states. We will also determine if villages have a statistically-significant random effect on mosquito abundance.

For hypothesis one, we predict that temperature and humidity will affect the fed and gravid abundances since prior research indicates that increased fluctuations in temperature and humidity results in more efficient digestion, causing more frequent feedings (Suh et al., 2020. Literature also states that the abundance of *An. gambiae* is positively associated with moisture index and *An. funestus* abundance is higher in the dry than rainy season in Sub-Saharan Africa (Minakawa et al., 2002). For hypothesis two, we predict that temperature and humidity will affect the abundance of *An. gambiae*, and will not affect the abundance of *An. funestus,* since *An. gambiae* abundance is known to rise dramatically in response to small temperature increases, likely due to accelerating the larval life stage and gonotrophic cycles (Charlwood, 2017). Ultimately, this research can serve to help predict biting behaviours of mosquitoes, allowing for better prevention measures against malaria.

Methods

Field Work

The data was collected in the Kilombero Valley, a global malaria hotspot in Tanzania. Since 2007, entomologists from the University of Glasgow have trapped malaria vectors from four households across four villages (KID for Kidugalo, MIN for Minepa, LUP for Lupiro and SAG for Sagamaganga) within the valley (see Figure 1). Vectors were trapped using MET and CDC trap designs. The data collected ranges from June 2016 to September 2017. The individual trapped mosquitoes were sexed, identified as *An. gambiae* or *An. funestus*, and classified into three states: fed, unfed, and gravid. A number of climatic factors including mean temperature and humidity were recorded daily at each vector trapping site. Standard CDC traps were left overnight inside the households within each village. Each household within each village was visited at least 5 days per month over the project.

Data Manipulation

This dataset was collected by the University of Glasgow, in collaboration with the NERC Environmental Information Data Centre; this was not open-source community data. Due to sampling difficulties with the MET equipment, the CDC (Centre for Disease Control) trap dataset was used for analysis. Six rows of data consisted of NAs due to house changes or issues encountered with the traps overnight. These rows were removed as they were missing abundance values. Columns regarding information on abundance of male mosquitoes were removed as only female mosquitoes are able to transmit malaria, making them the population of interest in this case. The dataset was also manipulated to generate 2 new columns, one for species and one for status, in order to make statistical tests possible to run. The data originally listed abundance based on species and status as one variable.

Data Analysis

Version 4.2.1 of R and R Studio was used for all data analysis. The packages lme4 (Bates et al., 2015), lmerTest (Kuznetsova et al., 2017), MuMIn (Barton, 2020), sjmisc (Lüdecke, 2018), tidyverse (Wickham et al., 2019) and lsmeans (Lenth, 2016) were used in our analysis. To test hypothesis one, we generated a linear model to investigate if mean temperature and mean humidity differently affect the abundance of mosquitoes in fed, unfed and gravid states. Assumptions of homogeneity and normality were met. To test hypothesis two, another linear model was generated to take species into consideration to see if there is a difference in abundances of the mosquito status between species *An. gambiae* and *An. funestus*. These candidate linear models included the same fixed effects as the models created for hypothesis one, but species was added as a fixed effect.

This is nested data, which includes random effects of both village and household (see Figure 1). We chose to focus on the random effect of villages instead of the random effects of households as mosquitos may travel between villages due to biological factors such as breeding location proximity (Al-Thukair et al., 2022). Mixed effect models also took into account if mosquito abundances differ by villages. In all models (linear and mixed), we chose to look at the interaction between mean temperature and mean humidity because we believed that differences captured in humidity and temperature are biologically important. Temperature and humidity were found to be highly correlated, and so were systematically dropped during model generation. In all models we kept status as a fixed effect, due to the specific investigation of the abundances of mosquitoes across the three different states. We used an AICc (Akaike, 1974) to rank our twelve candidate models and identified the top model based on the AICc value (Burnham & Anderson, 2002) to investigate which predictors explain the variation in abundance in mosquitos. Results were considered significant if $p \leq 0.05$.

Results

The first linear model considers the effects of mean temperature and mean humidity on abundance, while taking into account status (fed, unfed, and gravid). The following linear model was fit: abundance ~ MeanTemp*MeanHum + status. Mean temperature and mean humidity were systematically dropped to create two more linear models. The results of the model (see Table 1) find that the interaction between mean temperature and mean humidity have a significant effect on fed and unfed states ($t = 14.5$, $p = < 0.001$). This model also finds that there is not a significant interaction between mean temperature and humidity on the gravid state (t = -0.43, p = 0.666). Mean temperature (t = -3.43, p = < 0.001) and mean humidity (t = -2.92, p = < 0.001) also has a significant effect on mosquito abundance. This linear model consists of: F(5, 5070 residuals) = 61.19, p = < 0.001. Across the timespan of the data collection, unfed mosquitoes were more abundant than the gravid or fed (see Figure 2).

The second linear model intends to observe differences between the two mosquito species *An. gambiae* and *An. funestus*. The following linear model was fit: abundance ~ MeanTemp*MeanHum + status + species. Similar to the first linear model investigating status abundance, the results show (see Table 2) the interaction of mean temperature and mean humidity to also have a significant effect on abundance between the fed and unfed state (t = 14.5, p = < 0.001) as well as a non-significant interaction affecting gravid abundance (t = -0.43, p = 0.666). We find a significant effect between species on mosquitos abundance (t = 5.06, p = < 0.001). This linear model consists of: F(6, 5069 residuals) = 55.51, p = < 0.001. For all three states, mosquitoes of the *An. gambiae* species had a higher mean abundance than mosquitoes of the *An. funestus* species (see Figure 3).

Six mixed models were generated, systematically dropping each fixed effect except status. The saturated model fit all predictors: abundance ~ MeanTemp*MeanHum + status + species + (1| village) (see Table 3). Similar to both linear model, we find the interaction of mean temperature and mean humidity to also have a significant effect on abundance between the fed and unfed state (t = 13.64, p = <0.001**)** as well as a non-significant interaction affecting gravid abundance (t = -0.44, p = 0.663). We find a significant effect between species on mosquitos abundance (t = 5.06, p = < 0.001). We also find a strong correlation between mean temperature and humidity (0.98). Village (n=4) accounts for 11.65 units of the variance, with a standard deviation of 3.41.

Model selection was done using an AICc on all models, and the best performing model is the saturated mixed model: abundance ~ MeanTemp*MeanHum + status + species + (1| village) with an AICc of 48618.85 (see Table 4). All models which include species as a predictor perform better than models without species as a predictor.

Discussion

Hypothesis one postulates that temperature and humidity influence the abundances of fed-, unfed-, and gravid-state mosquitoes. This hypothesis is supported by prior research indicating that increased fluctuations in temperature and humidity results in more efficient digestion, causing more frequent feedings (Day, 2016; Daygena et al., 2017). Despite this, our results failed to reject the null hypothesis. While the results align with the known relationship between temperature and humidity and whether a mosquito is fed or unfed (Agyekum et al., 2021), we found no evidence for a relationship between temperature and humidity and gravid-state mosquitoes. This has interesting epidemiological implications, as it suggests that gravidity may not be an important factor to consider when looking at biting rates and malaria transmission when considering shifts in climatic factors, despite the state duration being temperature-dependent (Day, 2016).

As for hypothesis two, we postulated that climate-caused effects on state abundance are species specific. This too is supported by literature, specifically, that *An. gambiae* is positively associated with moisture index (recall: likely due to accelerated larval life stage and gonotrophic cycles) and *An. funestus* with higher abundances in the Sub-Saharan dry season (Minakawa et al., 2002). While we did expect interaction of mean temperature and mean humidity on fed versus unfed state abundances, our results found no significant interaction between the climatic factors and gravid abundance from any of the models (linear or mixed) created. The effects of climate on both species overall abundance as well as their fed and unfed abundances is unsurprising given *An. gambiae*'s success in moist climates and previous literature (Minakawa et al., 2002). For each state, *An. gambiae* had significantly higher abundances than *An. funestus*. Given the results of testing hypothesis one, it is unsurprising that species-specific gravidity is unaffected by mean temperature and humidity.

Conclusion

Limitations and Future Investigations

Mosquitoes are very particular when it comes to selecting a location for oviposition (Kelly-Hope et al., 2009), which often means oviposition is delayed. Thus, females may remain gravid for longer periods of time than what is developmentally necessary if they are not in a suitable environment (Day, 2016). For this reason, gravid mosquito abundance may somewhat reflect the suitability of the environment for oviposition, rather than direct climatic effects on gravid abundance. This is worth noting when considering our results, because although we assessed the effects of temperature and humidity on the abundances of gravid individuals, we could not break down the gravid category by the time spent in that state because that was not part of the dataset. Thus, we cannot be sure that there are no indirect climatic effects.

This unexplored factor has important biological and epidemiological relevance because the longer oviposition is delayed, the more likely that an infected mosquito will transmit the pathogen to the next generation, thus rapidly expanding the pathogen-carrying population (Day, 2016). Such delays are due to the time it takes for the pathogen to mature, replicate, and invade the saliva and tissues being temperature-dependent, so if the environmental and climatic conditions are non-optimal for oviposition, oviposition will be delayed, and the more likely the next generation will be born already carrying the pathogen (Day, 2016). Therefore, there is a chance that there exists a relationship between the abundance of gravid-state mosquitos and the climatic factors of interest, but it is obscured by the variable time spent gravid. Investigating how to further break down the gravid state category would make possible more thorough research.

In terms of epidemiological relevance, research like this is essential to expanding our understanding of vector behaviour and biology so that these characteristics can be exploited for affordable and more widely generalizable disease control strategies. Specifically, understanding gravidity, oviposition, and other reproductively relevant processes and behaviours is imperative to the development of improved surveillance and control of malaria vectors (Mwingira et al., 2020). This study further developed knowledge of the relationship between climate conditions --temperature and humidity-- and mosquito life-states, which is crucial to the future of malaria vector control efforts in an ever-warming environment.

A major limitation of our study is directionality (increase or decreases) on abundances. Further studies are required to determine whether abundances of these states increase or decrease based on climatic factors such as temperature and humidity. Also, these results may not be generalizable to non-malaria hotspots or differing geographical and environmental conditions. For example, topographical barriers such as hills, rivers, and valleys may impact mosquito population movement and feeding behaviour (Ndoen et al., 2010; Soleimani-Ahmadi et al., 2015; Afrane et al., 2005). This certainly impacted our data given that the study site is a valley. Thus distribution of states (fed, unfed, and of most interest, gravid) and consequent malaria transmission rates may be more topologically relevant than we accounted for in our analysis. In this way, future research investigating topological distributions of mosquito fed, unfed, and gravid states would be valuable.

Appendix A: Figures and Tables

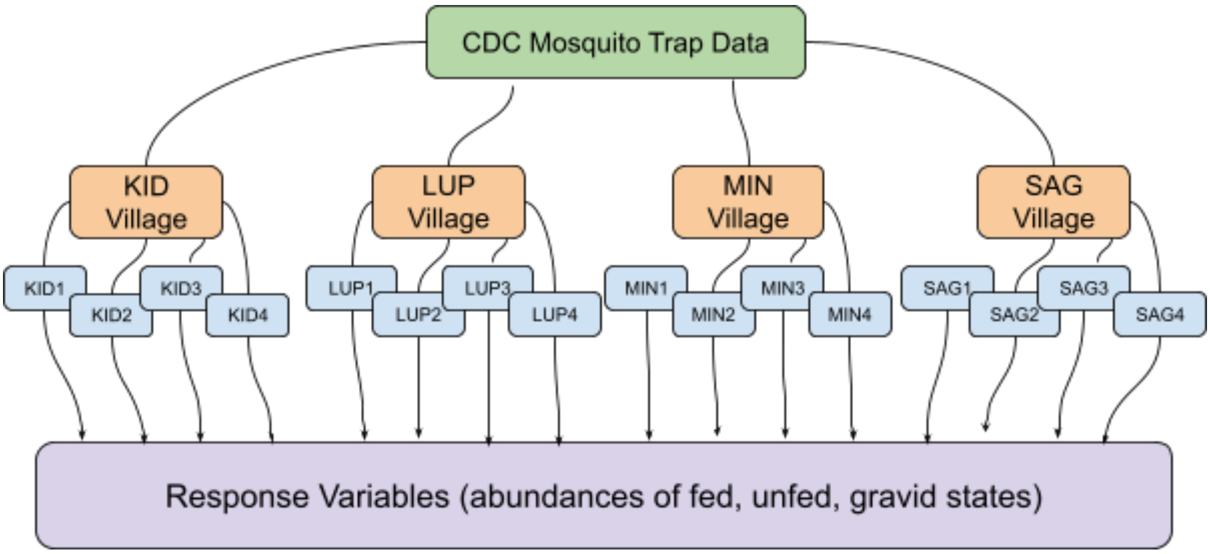

Figure 1. Nested method collection design using CDC traps from four villages, with four specific households within each village. Abundances of fed, unfed, and gravid mosquitoes were recorded, as well as their species and sex.

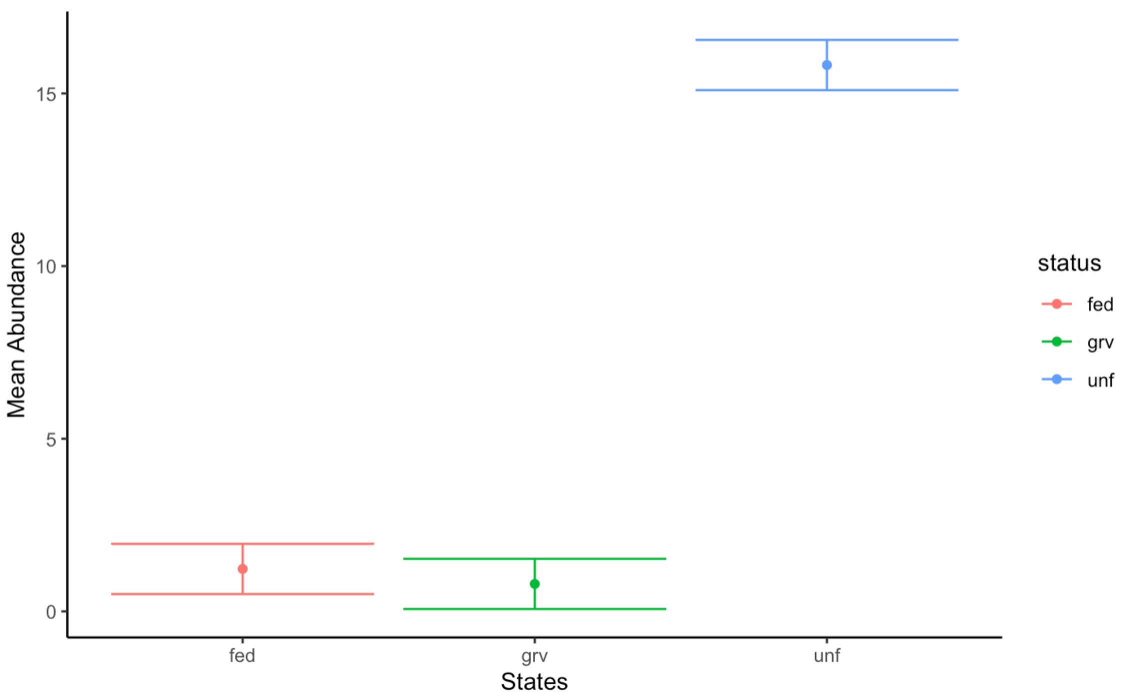

Figure 2. Mean abundance of mosquitoes separated into the three states. The error bars represent one standard error. We found that unfed mosquitoes were the most abundant, followed by fed and then gravid mosquitoes. There was a significant effect of mean temperature and humidity on the fed and unfed states, but an insignificant effect on the gravid state.

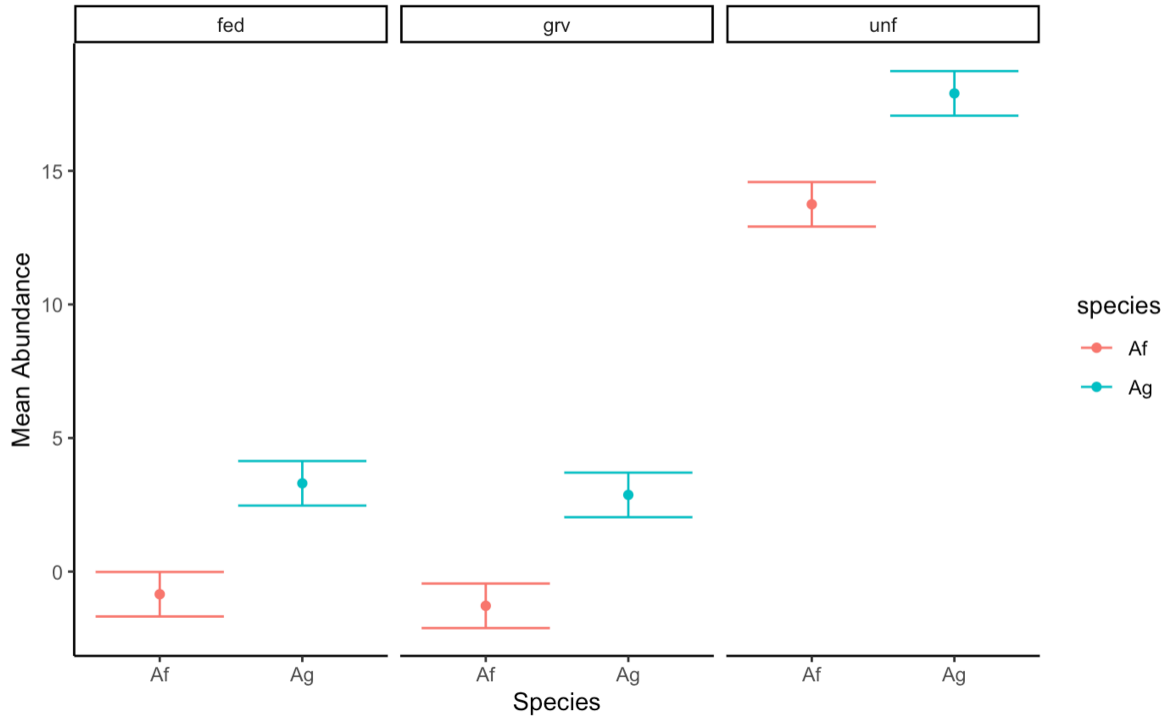

Figure 3. Mean abundance of mosquitoes across the two species separated into the three states. Af represents *An. Funestus* and Ag represents *An. gambiae*. The error bars represent one standard error. We found that unfed mosquitoes were the most abundant across both species, and that the abundance of gravid and fed mosquitoes were similar. There was a significant effect of mean temperature and humidity on the both species.

Table 1. Results from a linear model of the effect of temperature and humidity on mosquito status abundance.

	Estimate	Std error	*t*-value	*P*
Intercept	65.62	18.72	3.504	**<0.001**
Mean Temp	-2.44	0.71	-3.43	**<0.001**
Mean Hum	-0.82	0.28	-2.92	**0.003**
Gravid Status	-0.43	1.01	-0.43	0.666
Unfed Status	14.59	0.996	13.64	**<0.001**

Mean Temp*Mean Hum	0.03	0.01	2.86	**0.004**

Statistically significant results are in bold.

Table 2. Results from a linear model of the effect of temperature and humidity on mosquito status and species *An. gambiae* and *An. funestus* abundance.

	Estimate	Std error	*t*-value	*P*
Intercept	65.62	18.72	3.504	**<0.001**
Mean Temp	-2.44	0.71	-3.43	**<0.001**
Mean Hum	-0.82	0.28	-2.92	**0.003**
An. gambiae	4.15	0.82	5.06	**<0.001**
Gravid Status	-0.43	1.01	-0.43	0.666
Unfed Status	14.59	0.996	13.64	**<0.001**
Mean Temp*Mean Hum	0.03	0.01	2.86	**0.004**

Statistically significant results are in bold.

Table 3. Results from a mixed effects model on the effect of temperature and humidity on mosquito status, species *An. gambiae* and *An. funestus* abundance*,* using village as a random effect.

	Estimate	Std error	*t*-value	*P*
Intercept	49.19	18.76	2.62	**0.008**
Mean Temp	-1.87	0.71	-2.62	**0.008**
Mean Hum	-0.61	0.28	-2.197	**0.028**
An. gambiae	4.15	0.81	5.10	**<0.001**
Gravid Status	-0.43	0.996	-0.435	0.663
Unfed Status	14.59	0.996	13.64	**<0.001**
Mean Temp*Mean Hum	0.02	0.01	2.12	**0.034**

Statistically significant results are in bold.

Table 4. Model selection of the best fit model on the effect of temperature and humidity on mosquito abundance using all linear and mixed models.

Model	**AICc**	**ΔAICc**	***df***
abundance~MeanTemp*MeanHum + species + status + (1|village)	48618.85	0	9
abundance~MeanTemp + status + species + (1|village)	48620.20	1.35	7
abundance~MeanTemp+MeanHum + species + status + (1|village)	48621.31	2.46	8
abundance~MeanHum + status + species + (1|village)	48626.18	7.33	7
abundance~MeanTemp*MeanHum + status + (1|village)	48642.78	23.93	8
abundance~status + (1|village)	48648.08	29.23	5
abundance~MeanTemp*MeanHum + species + status	48669.86	51.01	8
abundance~MeanTemp + status + species	48674.54	55.69	6
abundance~MeanHum + status + species	48682.89	64.04	6
abundance~MeanTemp*MeanHum + status	48693.47	74.62	7
abundance~MeanTemp + status	48698.11	79.26	5
abundance~MeanHum + status	48706.41	87.56	5

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