Estimation of mortality among early developmental stages of tsetse flies (Glossina spp)

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July 31, 2015

1 Background

The potentially fatal diseases of sleeping sickness in humans and nagana in cattle are caused by the blood-borne protozoan pathogens of the genus Trypanosoma, transmitted in sub-Saharan Africa solely by tsetse flies (Glossina spp) [4]. Each year hundreds of thousands of people become clinically ill with sleeping sickness, and extreme cases lead to patient death, thus warranting public health concern [1]. It is estimated that the total African population at risk of contracting sleeping sickness is 69.3 million [10], and an annual estimated US$4.5 billion of livestock is lost to this killer disease [10]. It is evident that the negative and socio-economic impact of this disease is huge [33], and the global community has therefore invested heavily into the control and eradication of the disease, focusing on the tsetse fly vector.

Tsetse fly population dynamics are influenced by both abiotic factors such as temperature, precipitation and relative humidity, and biotic factors such as predation of adults by assassin flies of the family Asilidae, parasitisation of the pupal phase by various wasps, and (density dependent) competition between adults for hosts. Many control measures have been developed to reduce tsetse fly populations through the use of specialized insecticides and

chemicals, traps and screens, baits (both olfactory and visual, and the use of sterile insect techniques (SITs) [**?**, 2, 3, 5–9, 11–15, 25–32]. While these techniques have been successfully implemented, there are numerous open questions regarding tsetse fly lifecycle dynamics that could lead to novel control mechanisms.

It has been shown that gender, temperature, and age have large effects on tsetse fly mortality rate [16, 20, 21]. According to these past mortality estimate studies, males tend to have higher mortality rates than females; and mortality rate tends to increase with age and temperature. Until recently, however, there has been no way to investigate the mortality of the early developmental stages of the tsetse fly (pupal, and young adult) in the field. Ovarian dissection data have allowed for mortality estimation that wasn't possible before, but there are three major assumptions that are commonly considered when estimating mortality of tsetse flies from such data.

First, the probability of capturing a fly should not depend on the age of the fly. Second, the age distribution of the population of flies under study should be stable, also stationary. Last, the adult mortality should not be a function of age. If all of these assumptions are valid, then the estimation of the mortality is usually carried using either non-linear least squares [23, 24] or a maximum-likelihood approach [18]. However, due to sparsity of the data, these assumptions are usually not checked or valid [19]. Furthermore, a recent study suggests that the age distribution of G. pallidipes in the Zambezi Valley of Zimbabwe was seldom stable, and that led to a hypothesis that serious departures from stability were largely due to disproportionate increase in the mortality of pupae and young adults at the hottest times of the year [18]. Therefore, age and temperature may play a significant role in pupal mortality.

In our study, we therefore aim to model the influence of temperature and age on the mortality of the early stages of the tsetse fly life cycle. This will allow for novel estimation of tsetse fly mortality rates, and could lead to improved control measures for tsetse fly populations and the diseases they carry.

2 Methodology

Simulation of the tsetse fly population was implemented by following cohorts of adult flies indexed by their respective day of emergence, tracking female egg deposition throughout their lives [16].

3 Survivability of Adult Flies

Mortality was calculated for each day of the simulation period, until the final day, D_f. Cohort survivability on day $1 \leq d \leq D_f$ was derived from a daily mortality equation, which factored in cohort age, sex, and maximum daily temperature (T_d). Individual members of a particular cohort were assumed to face identical risk factors. Daily survivability, $\phi_d^{S,i}$, of members of cohort i of a particular sex, was defined as follows:

$$\phi_d^{S,i} = e^{-\mu(i,d,S)} \qquad i, d \in \{1, 2, \ldots, D_f\}$$

where i is the cohort index, mu is the mortality function and S is either M (male) or F (female). Extinction of either the male ($C_d^{M,i}$) or female ($C_d^{F,i}$) part of the cohort occurred:

1. For males when age exceeds 100 days (i.e. $a > 100$)
2. For females when age exceeds 200 days (i.e. $a > 200$)
3. For males or females when $C_d^{S,i} < 1$

where $a = d - i$ is the cohort age and , $C_d^{S,i}$ is the number of cohort i members of sex S on day d. The mortality function μ is of the same form for both genders but with different coefficient values. The full mortality function is defined as:

$$\mu(i, d, S) = (c_1^S + c_2^S T_d) \exp(a(c_3^S + c_4^S T_d)) + (c_5^S + c_6^S T_d)a$$

where the c_i^S are coefficients to be fitted. A reduced mortality function that depends only on sex and daily temperature (but not on age) was initially fitted in order to establish a basis for further model fits. This function, μ^* varies exponentially with temperature and is defined as:

$$\mu^*(d, S) = k_1^S \exp(k_2^S T_d) \tag{1}$$

where k_1^S and k_2^S are coefficients to be fitted.

The cohort size for males and females is defined recursively by the survival proportion provided the population does not meet any of the extinction criteria and the simulation has not reached D_f.

$$C_{d+1}^{S,i} = \phi_d^{S,i} C_d^{S,i}$$

4 Gestation and Pupal Development

New cohorts arise through the emergence of new flies produced via an egg that hatches in the uterus of a female which gives rise to a first instar larva. Development continues via two further instars and the female ultimately deposits a mature third instar larvae that weighs as much, or even slightly more, than she does. The larva burrows into the soft substrate on which it is deposited and forms around itself, in matter of minutes, a hard chitinous puparial case. Inside the case the pupa goes through the developmental changes that give rise to the adult. The duration of the pupal [22] and egg/larval phases [17] occur in a temperature dependent manner. Since all gestating females in a given cohort experience the same environmental temperature, births in a cohort are assumed to occur simultaneously. The same reasoning holds for their larvae of the same sex deposited on the same day which are thus assumed to emerge simultaneously on a given day according to the daily temperatures they experience during their development into adult flies.

4.1 Gestation

The daily gestational rate also depends upon whether the female has previously deposited larvae thus there are two different gestational rates for first time gestating $G_1(d)$ and previously gestated $G_2(d)$ females.

$$G_1(d) = \frac{1}{33.38 - 0.690T_d} \qquad G_2(d) = \frac{1}{18.39 - 0.352T_d}$$

gestation is completed and one pupae deposited (one larva per female) when the sum of the rates of successive gestation days is first above 1. In other words birth occurs when n is the smallest number satisfying one of the following conditions:

1. $\sum_{d=b}^{n} G_1(d)$ if the female has not previously gestated

2. $\sum_{d=p}^{n} G_2(d)$ if the female has previously gestated

where b is the day that the female emerged and p is the day that the female last gave birth.

The sex of larvae is assigned when a female members of a cohort deposit them as a group. The number of larvae of each sex is distributed evenly for an even number of larvae with the remaining larvae assigned to be female when the number is odd.

4.2 Pupal Development

Once the larvae have been deposited the female immediately begins a new gestational phase. The pupae's daily development rate depends on their sex as well as temperature. The two rates D_d^M (males) and D_d^F (females) are given by:

$$D_d^M = \frac{0.05415}{1 + \exp(4.8184 - 0.2149T_d)} \qquad D_d^F = \frac{0.05884}{1 + \exp(4.8829 - 0.2159T_d)}$$

The same conditions apply for emergence of new immature flies as for their births, with emergence occurring on the minimum day at which the relevant cumulative developmental exceeds one. On that day the pupae that emerge constitute the cohort associated with that particular day.

4.3 Initial Conditions

Initial conditions were chosen to mimic those that were used in the Antelope Island Experiment [20]. The population was seeded with an initial number of pupae P which were assigned a randomly selected day of emergence between 1 and 110. This was done to account for the lack of prior knowledge regarding the stages of development of pupae in the sample. Pupal sex was chosen by dividing the sample of size P in the same manner as for pupae born in the simulation. Each pupa was then assigned a day of emergence by selecting a uniformly generated random integer between 1 and 110 (inclusive). For our simulation $P = 400$.

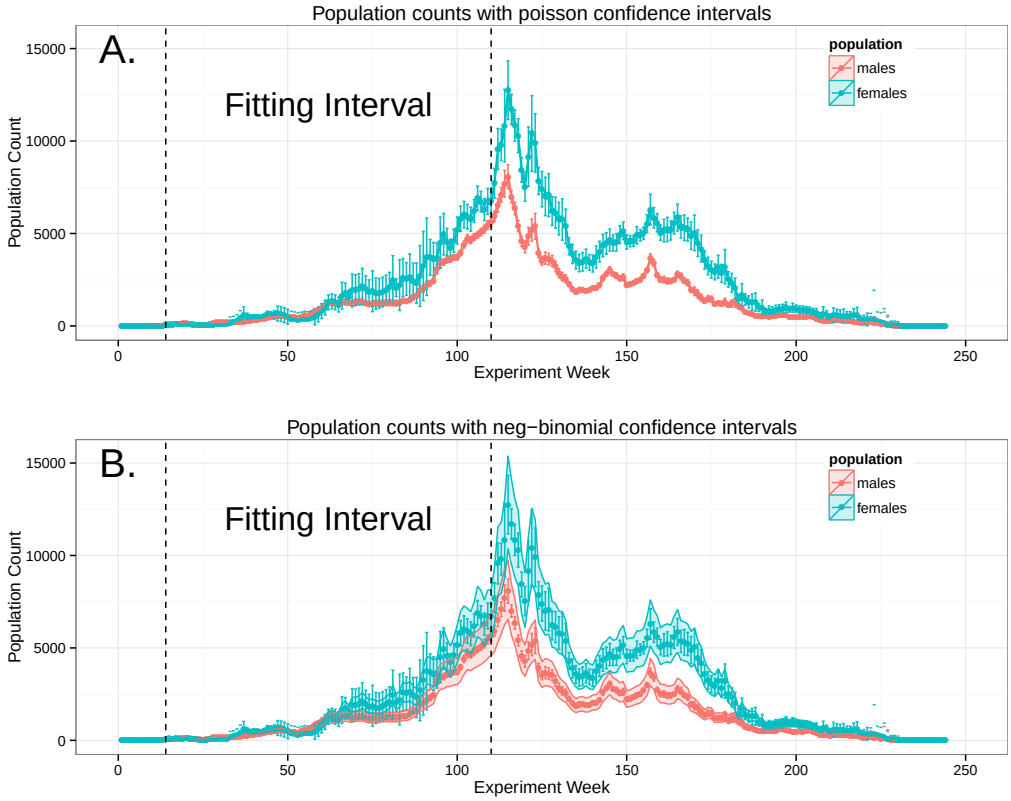

Figure 1: Plot of mark-recapture population estimates (dots and error bars). Ribbons around population estimates are 95% confidence intervals for the respective likelihood function. The dotted line interval indicates the actual estimates that were used for fitting, selected because no traps were used during this time interval which would have added extra variables to the mortality function. The figure shows (A) variance using a Poisson likelihood function and (B) variance using a negative binomial likelihood function.

4.4 Fitting Procedures

Fitting was performed in R using the *optim* function. We fit the reduced model to data obtained during a Tsetse fly population experiment on Antelope Island [30]. The data include mark recapture birth, mortality, and population estimates for male and female tsetse flies as well as daily temperatures for the study location. We first attempted to fit the model to

the overall population estimates of males and females assuming that they arose from a poisson distribution. However, we find that the variance in the poisson distribution does not fully capture the variance from the data, suggesting that our data are overdispersed Figure 1a. We therefore assume our data arose from a negative binomial distribution of size 100, as the distribution appears to reflect the correct variance we see in our data Figure 1b. We fit all models by minimizing the negative log-likelihood between our data and simulated outputs. Our final model estimates for the reduced model were derived as follows: (1) Estimate female mortality parameters (only 2) fitting our model to only female population data using SANN and then Nelder-Mead minimization; and (2) estimate male mortality parameters fitting to male population estimates in the same manner.

5 Results

Current successful fitting has been accomplished on a reduced mortality model (see (1)). This mortality model has only 4 parameters to estimate, which made fitting possible. Figure 2 shows the data used for fitting along with confidence intervals and the simulated model fits. We were able to get reasonable model fits to the data with the optimized parameter estimation as seen in Figure 2. Further exploration of parameters not used for fitting (*e.g.* initial pupae numbers and emergence distribution) still must be explored with these reduced models to investigate their impact, but past work on these models suggests that they will have little influence on the fit model parameters.

The mortality curves corresponding to the optimized parameter estimates are shown in Figure 3. Oddly enough, the original most optimal mortality curves represent a negative relationship between mortality and temperature, suggesting that tsetse fly mortality is greatest at the coldest temperatures. As we know this to be incorrect based on past research, we have restriced these mortality curves to be positive exponential curves, resulting in Figure 3. This discrepancy needs to be better understood, however our parameter restriction estimate for mortality curves appears reasonable based on past knowledge suggesting male mortality is higher than female mortality.

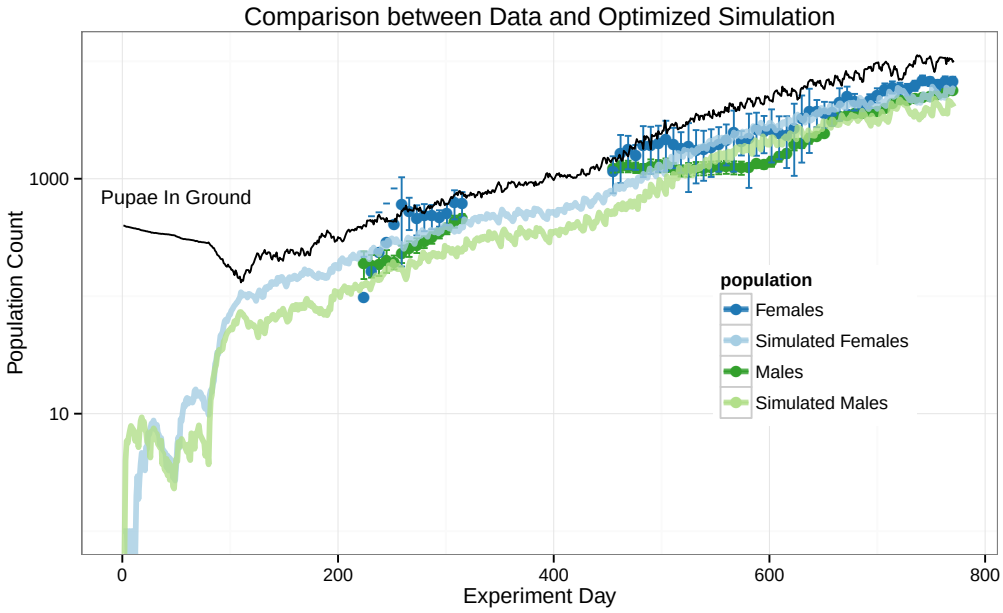

Figure 2: Plot of simulated daily population counts for male and female tsetse flys (colored lines) and total pupae in ground (black line) based on optimized parameters (lighter colours) along with available population count estimates from data (darker colours)

6 Issues

In fitting the full model (12 parameters), we were unable to get any reasonable model fit, even using informed parameter choices from previous work. We spent significant time setting up these full model fitting methods on the Texas Advanced Computing Center supercomputers, so that these methods could be run for the necessary time with hopes that reasonable estimates could be obtained. Initial attempts to fit slightly reduced models to work up to the full model did not give reasonable estimates for the full model. However, our recent attempts to fit extremely reduced models have been successful. The reduced model does not answer our initial question, so we are working on fitting methods that will allow for fitting a slightly more complex model that allows for age stratification of mortality along with temperature dependence. We are also pursuing fitting to other mark-recapture estimates rather than total gender segregated population estimates such as estimated births and

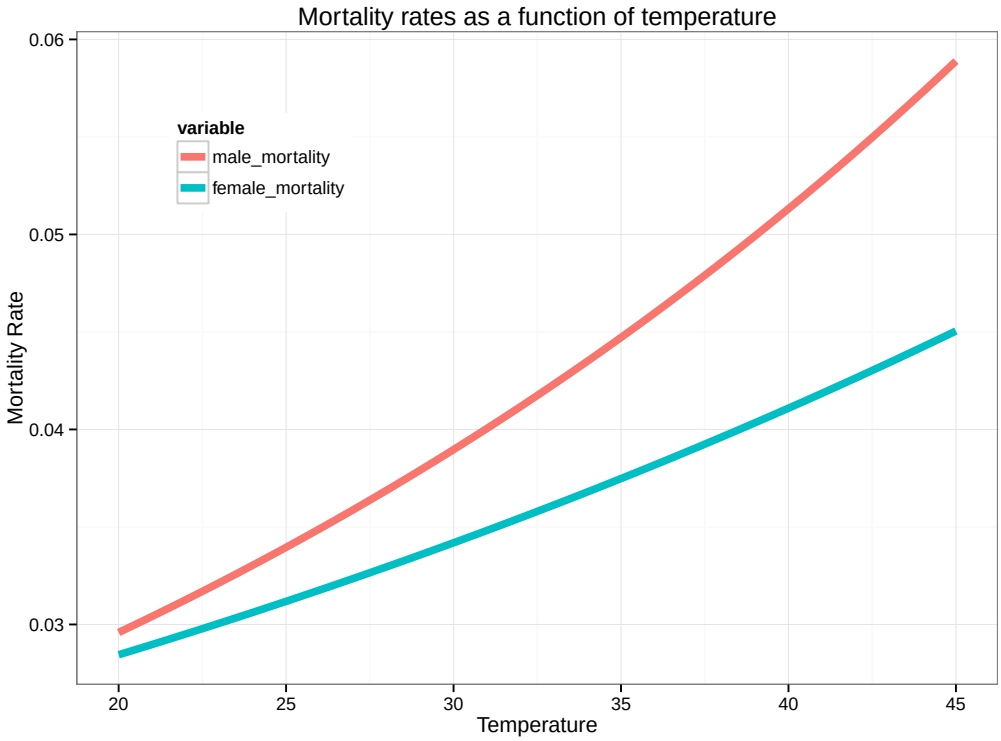

Figure 3: Fit mortality curves for male and female tsetse as a function of temperature based on reduced model fitting procedure

estimated survival, though these have higher uncertainty than the population estimates. This would be a preferred method though, because the total population estimates are not independent samples, and we would instead prefer to fit to birthing incidence.

7 Plans

We are currently planning to refine fitting methods to obtain reasonable parameter estimates to answer our initial question. Our goal is to take those obtained estimates to publication. We have a meeting scheduled in the near future that will allow us all to get on the same page for moving forward with the project in terms of task allocation and time expectations.

8 Contributions

OO and KM completed a literature review, JW contributed to methodological details and organization, SJF ran models and produced plots. All authors wrote and gave comments on the manuscript.

9 Supplemental

All code can be found on our team github page: ICI3D/tsetseProject.git, though we apologize for the current state of affairs of the repository as things got a bit hairy there towards the end of our project. We hope to clean up the code over the next couple of weeks.

10 Acknowledgements

We thank Eva for help organizing our team meetings and plan. Without her management we would have been lost. We also thank John Hargrove for his insight, direction, and time working with the project. We also thank the ICI3D program and faculty and SACEMA for organizing the workshop that brought our group together and facilitated our project completion. Finally we would like to thank Stellenbosch University, as it was with their help that we obtained the data necessary for our project.

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