Optimal Release Conditions for *Wolbachia* Infected Male Mosquitoes in Sub-Saharan African Countries

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INTRODUCTION

In many developing countries, diseases such as dengue, malaria, zika, and other mosquito-borne viruses take a untenable toll on development. For example, many important infrastructural developments cannot proceed because they may increase malaria transmission: "dams contribute significantly to malaria risk, particularly in areas of unstable transmission" (Kibret *et al.*, 2015) Furthermore, malaria is still extremely common and lethal. According to the World Health Organization (WHO, 2016 World Malaria Report (2017), there were 214 million new cases of malaria and 438,000 malaria-related deaths. Of these, 92% of victims lived in sub-Saharan Africa. Currently, funding of the *WHO's Global Technical Strategy for Malaria 2016-2030* is $2.9 billion per year. To meet the 2020 funding milestone, contributions should be increased to $6.4 billion per year (WHO, 2016).

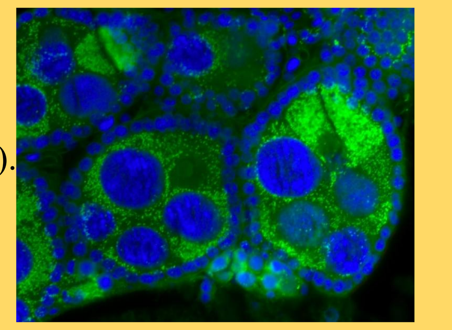

Figure 1: High magnification image of *Aedes aegypti* cells with *Wolbachia* shown in green. (World Mosquito Program, 2017)

To combat diseases, scientists have begun exploring the use of *Wolbachia*, a naturally occuring bacteria in insect populations, as a method of disease control. When *Wolbachia* infected male mosquitoes mate with uninfected females, the egg and sperm are cytoplasmically incompatible, resulting in non-viable offspring (Ross *et al.*, 2017). When both male and female are infected, or when an infected female mates with an uninfected male, the offspring produced are also infected (Jiggins, 2017). In this way, *Wolbachia* both limits the population and propagates from generation to generation.

Infected mosquitoes are more resistant to viral infection, making them less likely to transmit diseases like zika, dengue, and yellow fever. *Wolbachia* has also been shown to significantly inhibit the development of Plasmodium parasites, the organisms that cause malaria (Sinkens *et al.,* 2010).

Due to the beneficial characteristics of *Wolbachia* infection, our project aims to analyze the optimal release conditions for male mosquito release as a means for population control.

Figure 2: A stained wasp egg, with visible (glowing) *Wolbachia*

THE CODE

Incorporating equations, debugging the code, and creating an appealing user interface were the most challenging and time consuming aspects of this project.

Figure 3a-b: The code that calculates the mosquito population (based on a clutch size of 75 eggs). Code is based on the mating tendencies, shown in **Figure 4.**

```
//a denotes infected male, b denotes non-infected male
//c denotes infected female, d denotes non-infected female
    if (m < (tinfmale + infmale + 1)){
        mtemp = 'a';}
    else if (m >= (tinfmale + infmale)){
        mtemp = 'b';}

    if (f < (tinffem + inffem +1)){
        ftemp = 'c';}
    else if (f >= (tinffem + inffem)){
        ftemp = 'd';}

    if (mtemp == 'a' && ftemp == 'c'){
        clutchcon = "infected";
        tinfmale += 75;
        tinffem += 75;
        Xcount += 1;
        supercount += 1;
        }

    else if (mtemp == 'a' && ftemp == 'd'){
        clutchcon = "none";
        acount += 1;
        supercount += 1;
        }

    else if (mtemp == 'b' && ftemp == 'c'){
        clutchcon = "infected";
        tinffem += 75;
        tinfmale += 75;
        Xcount += 1;
        supercount += 1;
        }

    else if (mtemp == 'b' && ftemp == 'd'){
        clutchcon = "normal";
        tnonfem += 75;
        tnonmale += 75;
        ucount += 1;
        supercount += 1;
        }

}
```

Figure 3a: Organization of the code into various classes and subclasses was the initial starting step in creating the simulation. This figure shows the basic structure and organization of our code with its classes subclasses.

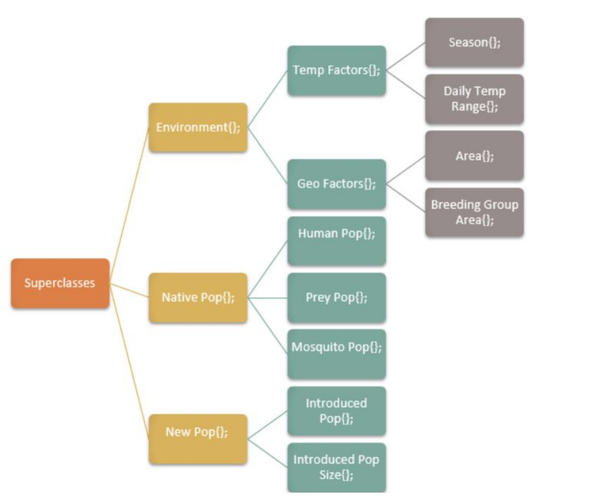

Figure 3b: Code structure and subclasses

RESEARCH METHODOLOGIES

We used a case study approach to collect quantitative data to create a simulation. Our simulation models the spread of *Wolbachia* and is adaptable to many scenarios and environments. We attempted to make the simulation maximally realistic by incorporating many environmental conditions. Data on actual *Wolbachia* releases is relatively limited due to the novelty of this technique, so some of the *Wolbachia* spread equations are based on both mathematical models and real world data. Our simulation is built on the C++ development environment, the Universal Simulator software, and the R console. To the right is an example of one of the equations we researched and used for our simulation.

$$A_f = (c * t * \sqrt{\pi} + \sqrt{A_r}\ )^2$$

A_f = Final Area (meters)
A_r = Release Area (meters)
t = total generations (# of generations)
c = wave speed (meters/generation)

$$c = \sigma(\tfrac{1}{2} - \widehat{p})$$

$\widehat{p}$ = Threshold Frequency
σ = Dispersal Parameter

Threshold frequency is different for every environmental condition, and has not been studied in depth for many areas. The best estimable range we have is that the frequency falls between 0.25 and 0.5 for most areas.

The dispersal parameter is similarly based on environmental conditions, and needs to be evaluated on a case by case basis to eliminate the potential influence of confounding variables in estimations.

Understand — Initially, we researched conditions affecting our accounted for factors and found data regarding conditions and how they interacted with our factors.

Create — Next, we created an equations by comparing data for our conditions with matching data from our factors, or modified equations to fit with our data of interest.

Implement — Finally, we coded the equations relating to the various factors into the simulation, taking into account the effects they have on each other.

EQUATIONS

Factors Taken Into Account

Temperature coefficients:
If location is North of the equator, temp = (-0.008)*(latitude)^2+27
If location is South of the equator, temp = (-0.005)*(latitude)^2+27

Mosquito Fitness:
Based on factors such as humidity, temperature, wind speeds, infection of *Wolbachia,* rainfall, and other factors.

Mating Probabilities and Results:
Figure 4 shows the offspring of the possible crosses between infected and uninfected mosquitoes.

Release Patterns:
Size of release area, speed of infection spread, shape of infection spread, and the effects of obstacles preventing spread in one direction.

Mosquito Feeding:
Time since last meal and prevalence of plant based and animal based feeding.

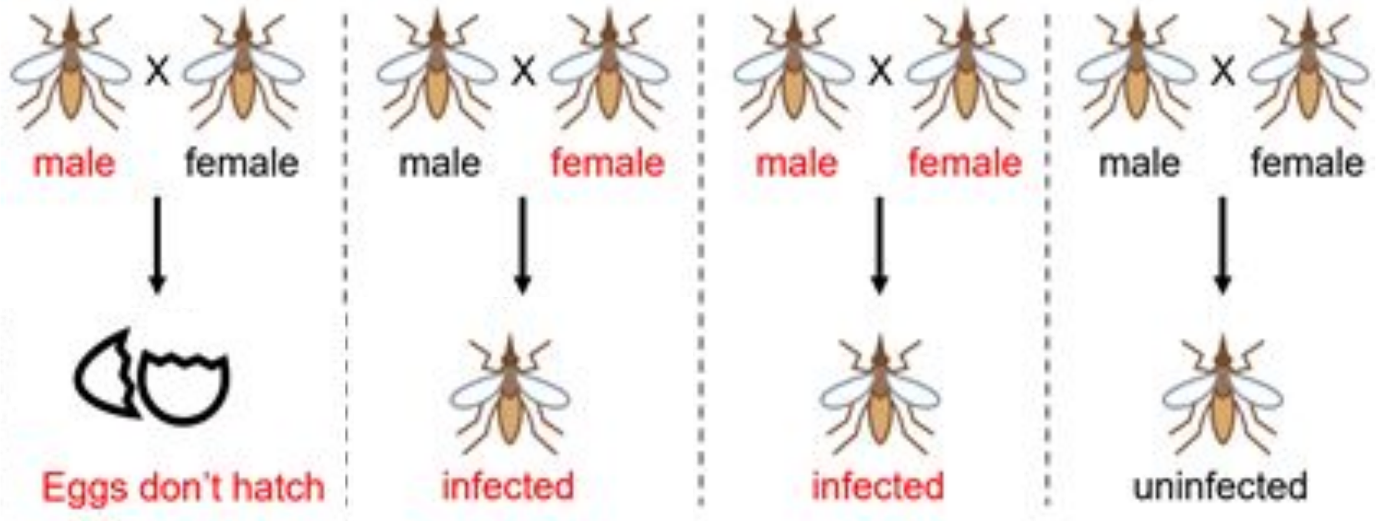

Figure 4: Results of mating between *Wolbachia* infected and uninfected mosquitoes. Eggs not hatching is akin to cytoplasmic incompatibility.

ASSUMPTIONS

Our simulation uses real world data to estimate the effects of future releases. Because it is based on past data, several assumptions must be made, including but not limited to:

- ❖ Cytoplasmic Incompatibility occurs in 100% of infected-male/uninfected-female crosses.
- ❖ Native mosquito population distributions are homogenous.
- ❖ Mosquito travel patterns stay the same.
- ❖ Human activity does not impact mosquito mating patterns.
- ❖ *Wolbachia* fitness and transmission rates are static and are not affected by environmental conditions.
- ❖ The area modeled with has a *Wolbachia* frequency over 0.5 for each sample taken.

Figure 5: The Aedes aegypti mosquito, one of the mosquitoes of interest due to its role in transmitting dengue, malaria, yellow fever, and other diseases.

DATA

Release Area	$\widehat{p}$ = 0.3	$\widehat{p}$ = 0.3
0.25 km	0.808 km	1.064 km
0.5 km	1.223 km	1.535 km
1 km	1.957 km	2.346 km
2 km	3.287 km	3.787 km
5 km	6.943 km	7.661 km

Data Table 1 models the asymptotic wave speed of the spread of *Wolbachia* over the course of one year. The dispersal parameter was assumed to be $\sigma \approx 100$ m/(gen)½, as per Schmidt et al. This wave speed is the growth of the area where at least 50% of mosquitoes are infected with *Wolbachia*, or where p-hat is 0.50 or greater.

Each generation is assumed to last approximately 24.33 days. The true time/generation can vary between 17 and 60 depending on environmental conditions, and would need to measured continuously in the field to achieve accurate results. This 24.33 day long generation is a realistic average for the purposes of generating this sample data.

CONCLUSIONS, IMPLICATIONS, AND NEXT STEPS

Future work on this project would involve a deeper investigation of environmental conditions that impact mosquito populations. Also, reducing the list of assumptions made would make the simulation more useful for modelling for situations as there is less variation and inaccuracy from unaccounted variables.

One assumption that could be elaborated is being able to calculate total time (days) per generation of mosquito. We operated under the assumptions shown below in Data Table 2.

Life Cycle Factors	Optimal Conditions	Suboptimal Conditions
Larval Development Time	7-10 days	20-50 days
Egg Formation and Oviposition	4 days	4 days
Egg Embryonation	3 days	3 days
Mating and Blood Feeding	2-3 days	2-3 days

Data Table 2: Assumed timing for each life cycle stage

In future studies, we would analyze how different conditions are related to the dispersal parameter, then create an equation to estimate this factor.

Threshold frequency is very case specific, so it is unlikely that we would be able to create equations to estimate this factor.

Based on the equations and information collected through our case studies, several general conclusions can be drawn:

Given limited time constraints, it is better to choose a release area with a lower threshold frequency. This factor does not affect long term effectiveness of spread, but in the short term, a lower threshold frequency will allow the rate of change in infected area to reach its asymptote faster. Greater dispersal parameters lead to faster wave speed. Consequently, a larger area can be infected in the same amount of time.

The main benefit from this simulation is its fluidity, if enough data is incorporated and enough factors are accounted for, the simulation can model the effectiveness of *Wolbachia* release in many areas, and the only dependencies will be collecting the environmental and topographical data.

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