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Computer Simulation of Genetically Modified *Aedes aegypti* Release Methods

Gregory Schwartz

Aedes aegypti mosquitoes are largely responsible for the spread of Dengue Fever and Zika virus. Field trials of genetically modified male *Aedes aegypti* which produce non-viable offspring are planned in the United States. It is critical to determine the best release method which controls the wild mosquito population and minimizes the use of modified mosquitoes. A computer simulation was developed to test various release methods under different conditions. The asimulation compared favorably with known field data from Brazil. All the variable release methods tested produced a 95% reduction ($p<0.001$) in the wild mosquito population. Both a slower changing release method and a more rapidly adjusting method without a minimum release allowed for a late increase in wild mosquito numbers. The rapidly adjusting method with a minimum released controlled the wild mosquito population while using 17% and 19% less genetically modified mosquitoes in dry and wet conditions respectively ($P < 0.001$).

Keywords: Aedes aegypti, mosquito, Zika, genetically modified

Introduction and Literature Review

Mosquito-borne diseases cause 700 million infections and over one million deaths each year. The increasingly mobile society and climate changes have led to a resurgence of mosquito-borne disease in previously disease-free areas. Dengue Fever is now reported in Texas and Florida. Vector control programs in the 1960's had largely eliminated Yellow Fever and Dengue Fever in Brazil.[1] In 2015 there were an estimated 1.5 million Dengue infections in Brazil. Fear of Zika and Dengue weighed on both athletes and spectators at both the FIFA world championships and 2016 Olympics.[2]

The mosquito species *Aedes aegypti* is largely responsible for the spread of Dengue Fever, Chikungunya, and Zika virus.[3] Female *A. aegypti* are highly evolved hunters that are particularly adept at spreading infection.[4] Female mosquitoes require a blood meal to obtain the necessary protein to reproduce. The mosquitoes live and breed around humans often travelling only a few meters during a lifetime. While able to feed on a variety of mammals, they prefer human blood. The female detects carbon dioxide, lactic acid, and octanol given off by humans. They bite frequently at ankles and back of knees to avoid observation. An anticoagulant in the saliva prevents clotting allowing for the rapid withdrawal of blood. *A. aegypti* are sip-feeders that take small amounts from several different bites greatly increasing their potential to spread infection. A single female can produce over 500 eggs during its one-month lifetime.

Mosquito control efforts have had varying degrees of success. Early efforts with mosquito netting and draining breeding areas were particularly effective against certain species of mosquitoes. Insecticides proved effective in controlling an explosion in spread after World War II. Unfortunately, the development of resistance to insecticides, adaptation, and globalization has allowed the spread of *Aedes aegypti*. According to the FDA even a well-organized mosquito control program is often ineffective.

Efforts to control mosquito populations and reduce use of potentially dangerous insecticides have led to several new techniques. Biological control using natural enemies such as mosquitofish has been used on a limited scale. Spores of bacterium Bacillus thuringiensis which disrupt larval digestion could be dropped from the air.[5] Mosquitoes have been infected with the bacterium Wolbachia pipientis rendering them infertile. Radiation which had been used to make other insects sterile damaged the mosquitoes making this technique ineffective.

A genetically engineered mosquito has been developed by Oxitec. The OX513A strain of *Aedes aegypti* has two genetic changes.[6] A fluorescent marker allows for rapid determination of native and modified mosquitoes. Inserted is a gene that produces a protein called tTAV (tetracycline repressible activator variant). When produced the protein ties up cellular machinery leading to cell death. Exposure to tetracycline in sufficient concentrations during breeding suppresses the protein allowing for normal reproduction. The males are separated from the females based on their smaller size. The males are released into the environment. The males fertilize native female mosquitoes and the eggs which have the tTAV gene don't survive.

Trials using OX513A to control *Aedes aegypti* have been done in the Cayman Islands, Panama, and Brazil.[7] Studies have shown a reduction of mosquito populations of 90% in small scale trials. One major hurdle has been the difficulty in producing and managing the large number of genetically modified mosquitoes needed. The study in Brazil had to be modified by cutting the test area in half to have sufficient OX513A mosquitoes.

The epidemic of Zika virus in Brazil and its spread to Florida has spurred interest in the planned field trial of OX513A in the Florida Keys.[8] The trial is designed to see if effective suppression can be achieved and if continued monitoring and adjustment of modified mosquito releases can suppress the population for up to 22 months.

One of the critical concerns is what system for releasing the modified male mosquitoes will control the wild mosquito population but minimize the use of this limited resource. A system for monitoring the varying number of released OX153A has been proposed based on previous trials and formula-based models.

There are two basic types of epidemiologic models. Formula modeling began in the late 18th century with Thomas Malthus.[9, 10] Formula-based models have been steadily improved and are heavily utilized in epidemiology.[11] With the advent of modern computers individual based modeling has been possible. In this type of modeling each individual in a population being studied is tracked.[12] These models are very data intensive and best for studying small populations.[13]

This study uses an individual-based computer model to study population control of *Aedes aegypti* in Key Haven, Florida using OX153A. It is hypothesized that monitoring and adjusting OX153A release will control a population of *Aedes aegypti* while minimizing the use of genetically modified mosquitoes.

Methods

Using Visual Studio2012 Release 4 C++, a program was written to simulate a human and mosquito environment. Multiple factors were used in the programming the simulation. Parameters included size of the environment, probability of biting and mating, aging of mosquitoes, number of offspring produced, immigration of mosquitoes, male detection range, and environmental dispersion.

The program first creates human and mosquito populations. Their locations, age, sex, and state of feeding are determined. As the simulation begins, the people move about their environment. The mosquitoes move based on multiple factors including a female proximity to human, male proximity to female, environmental changes such as wind and random movements. Then interactions within a 1 square meter area are checked for each mosquito including its success at biting or mating. If females have obtained enough human blood protein, then they can interact with males and produce offspring who are added to the mosquito population. At the end of each hourly cycle the mosquitoes age and eventually die.

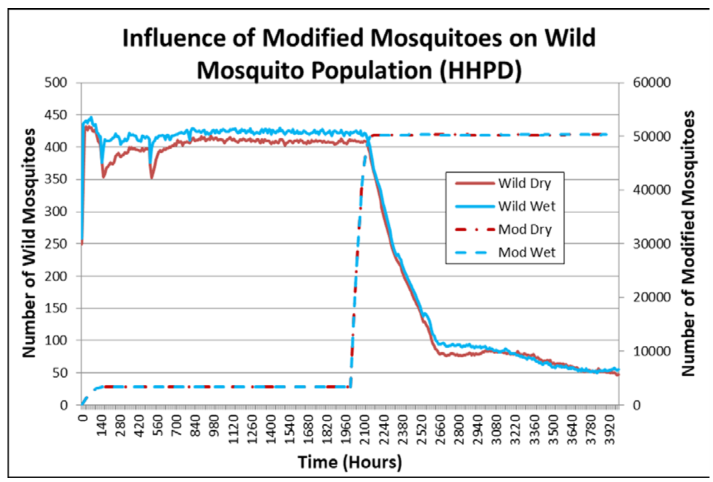
After 100 hours of simulation to allow for the simulation with humans and mosquitoes to reach homeostasis genetically modified mosquitoes are added to the environment. Of note, 100 hours were chosen because preliminary simulations showed 100 hours sufficient to create homeostasis. They are dispersed relatively evenly in the environment in numbers based on the method being tested for that simulation. Each hour the modified mosquitoes move around the environment and potentially mate with the female mosquitoes but the mating produces no viable offspring.

Simulations using high human population density were run for 4000 hours while the low population density environment was tested for 8000 hours. Methods tested in the low population density included fixed low releases of 75,000, 50,000, 25,000, and 10,000 (75K, 50K, 25K, 10K respectively) per hectare. Methods tested in the high population density environment, after the initial 100 hours, a fixed release at 20,000. After 2000 hours, the release was increased to 75,000. Variable method 10/10 releases 75,000 modified mosquitoes during the first 800 hours. After the initial 800 hours releases vary. If the ratio of modified matings is less than 2 times the number of wild matings than the number released is increased 10%. If the number of modified matings is 2 times or greater then number of wild matings, the number released is reduced by 10%. Method 50/20 No Floor (NF) also releases 75,000 during the first 800 hours. After which if the mating ratio is less than 2 times, the number released is increased by 50%. If the ratio is 2 times or greater, it is decreased by 20%. Method 50/20 is identical to Method 50/20 NF except the minimum release number is 7,500 due to the "Floor" parameter. In summary, 10/10 release method has a 10% increase or decrease with each adjustment while a 50/20 has a 50% increase or a 20% decrease of modified mosquitoes released.

The simulation was run with multiple different parameters including higher offspring production and different methods for determining modified mosquito release. Every 10 hours data was collected along with summary data at the end of the simulation run. The data was analyzed using Microsoft Excel. A P-value for significance was set at 0.01.

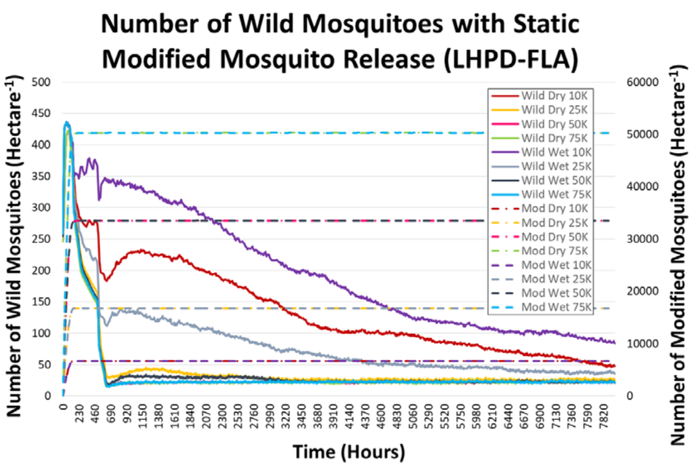
Results

The initial simulation used a high human population density (HHPD) of 460 per hectare. The simulation was run during wet and dry periods for 4000 hours. There was a statistically significant decrease in the wild mosquito population within 40 hours for both wet and dry situations (Dry 424 to 406 $p < 0.001$, Wet 433 to 415 $p<0.001$) after the release of the modified mosquitoes. The wild mosquito population plateaued between hours 1000 and 2000 with a change of less than 1% in both dry ($p=0.3$) and wet ($p=0.8$) simulations (Graph 1). The increase in genetically modified mosquitoes at hour 2000 caused a precipitous statistically significant drop in the mosquito population. The final mosquito counts were down 89% in the dry simulation and 88% in the wet simulation both which were statistically significant ($p<0.001$).



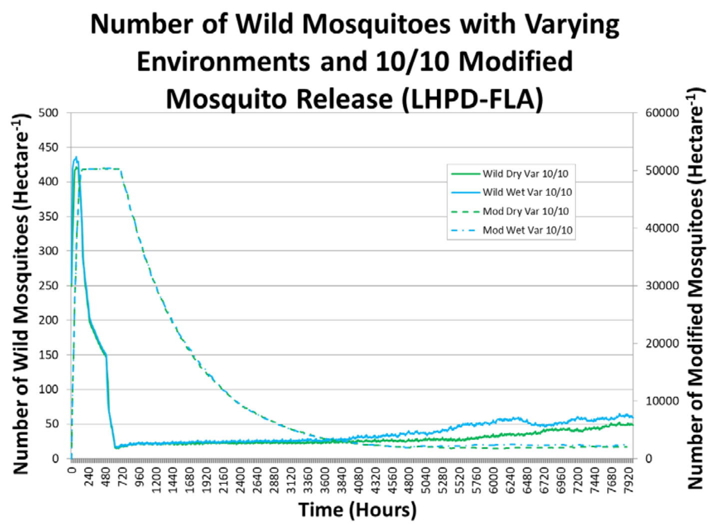
Graph 1- Introduction of a low number of genetically modified mosquitoes resulted in a small but statistically significant decrease in wild mosquito populations. Increasing the number of released modified mosquitoes caused a rapid decrease in wild mosquito populations by almost 90% (p-value < 0.001). Both wet and dry had similar results.

Next, the simulation was changed to a low human population density (LHPD) environment run for 8000 hours. Four fixed release strategies of modified mosquitoes were tested (75K, 50K, 25K, 10K) and produced a statistically significant decrease in wild mosquito populations within the first 20 hours ($p< 0.001$) (Graph 2). The higher release methods continued to cause a rapid and sustained decrease in wild mosquito populations which leveled off around 600 hours. The lower fixed release methods (25K, 10K) also decreased the wild mosquito populations but at a slower rate which was slowed further during wet conditions. At 8000 hours there was a statistically significant difference between method 10K and both 50K and 75K ($p<0.003$). There was also a statistically significant difference between 10K wet conditions and 25K wet conditions ($p = 0.002$).

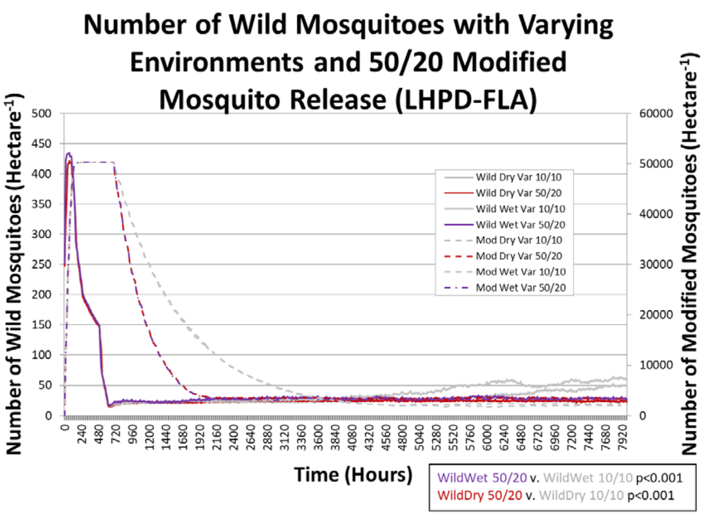


Graph 2- All produced statistically significant drop in the wild mosquito population (p-value < 0.001). High fixed release (75K, 50K) caused rapid sustained suppression of the wild mosquito population. Low fixed release (25K, 10K) produced a slower decrease in the wild mosquito population. Wet conditions did not affect high fixed release methods but further slowed suppression with low fixed release methods.

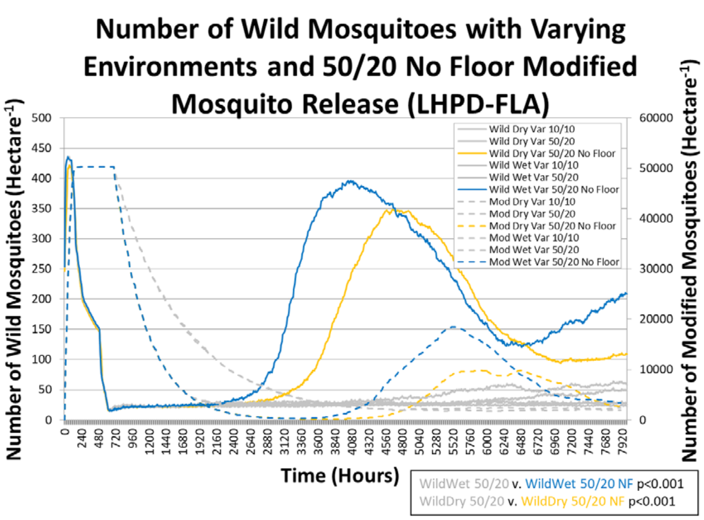
All six variable release strategies for the genetically modified mosquitoes caused a precipitous decrease in wild mosquito populations. (Graphs 3a, Graph 3b, Graph 3c and Graph 4) Within the first 20 hours after the modified mosquitoes were released there was a statistically significant drop in all scenarios ($p < 0.001$). By 1000 hours there was a greater than 95% decrease in the wild mosquito population. Release method variable 10/10 developed a statistically significant increase in both Dry and Wet scenarios ($p < 0.001$) late in the simulation. The release method 10/10 during wet condition had a more rapid increase in the wild mosquito population that plateaued between 6000 and 8000 hours, while the dry condition had a slower increase which was statistically significant ($p<0.001$) between 6000, 7000, and 8000 hours. The release method 50/20 NF (no floor) method achieved the same initial decrease but rebounded as the number of released modified mosquitoes decreased. The increase in wild mosquito numbers caused an increase in the number of genetically modified mosquitoes released but never reduced it to the level of the release method variable 50/20 with floor ($p < 0.001$).



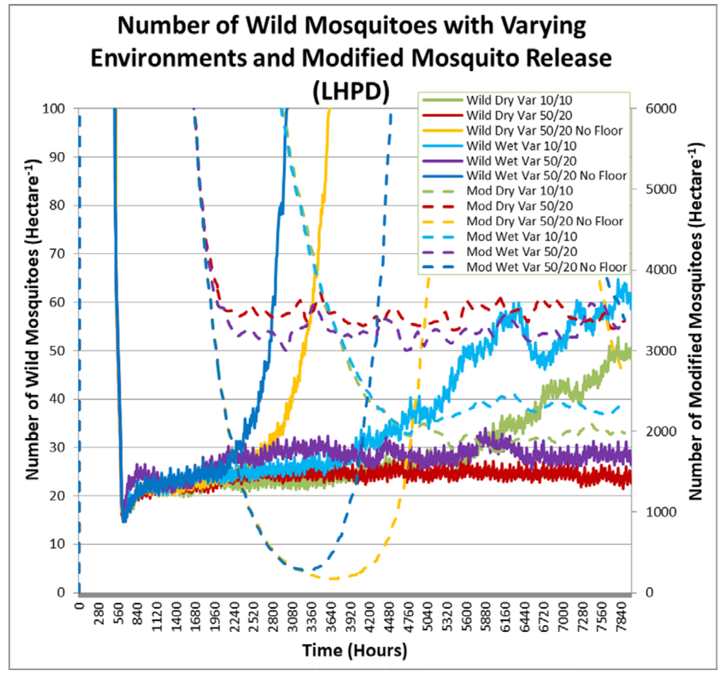
Graph 3a- Release method 10/10 allowed for a statistically significant late increase in wild mosquito populations (p-value < 0.001). Wet conditions allowed for an earlier and more rapid late increase with the 10/10 method that plateaued between 6,000-8,000 hours.



Graph 3b- Method 50/20 produced a rapid sustained suppression in both wet and dry conditions using the least number of genetically modified mosquitoes.



Graph 3c - Method 50/20 No Floor (NF) produced a rapid decrease in the wild mosquito population with a large rebound in mosquito population as the number of released genetically modified mosquitoes decreased. Under wet conditions, method 50/20 NF had a more rapid and larger increase in the wild mosquito population.



Graph 4 - Looking closer, the 50/20 No Floor release model produced a rapid decrease but allowed for a large rebound in wild mosquitoes. The 10/10 release model allowed for a late increase in the wild mosquito population.

The number of genetically modified mosquitoes used by each technique varied considerably. The variable methods used less modified mosquitoes then the fixed methods except 10K. Variable method 50/20 used 17% less genetically modified mosquitoes in dry conditions and 19% less in wet conditions compared to variable method 10/10 ($p<0.001$). Variable method 50/20 NF in dry conditions showed a trend to increased use of modified mosquitoes (4%, $p=0.060$) compared with variable method 50/20 and significantly more under wet conditions (24%, $p < 0.001$). The wet and dry conditions with floor changed the need for sterilized males by 1 to 2 percent.

Discussion

Computer simulation of the low human population density environment produced several results. The fixed releases using large numbers (75K and 50K) of modified mosquitoes were effective at controlling the population but used extensive resources. The lower fixed releases did significantly reduce the wild mosquito populations but it was such a slow process that even after 8000 hours there were still significantly more wild mosquitoes compared to the high fixed methods. The 10K method used the least modified mosquitoes but had persistently elevated wild populations.

Variable method 50/20 suppressed the wild mosquito population throughout but Variable method 10/10 did not. After 4000 hours, there was a significant increase in the wild mosquito population during wet conditions. After 6000 hours, the wild mosquito population increased during dry conditions. Variable method 10/10 did not suppress the wild population as well and wet conditions allowed for a more rapid recovery of the wild mosquito population. Variable method 50/20 without a floor produced the same initial reduction in mosquito populations seen with the other variable methods. As the number of modified mosquitoes released dropped to low levels the wild mosquito population increased quickly with a more rapid increase during wet conditions. This caused a compensatory increase in the number of modified mosquito released which reduced the population but it did not achieve the low numbers seen with the other variable methods. Variable method 50/20 with floor was effective and used the least number of genetically modified mosquitoes.

The high-density simulations, similar to the field trail in Brazil during 2011-2012, demonstrated several key aspects of the population model. During the Brazil trial the number of genetically modified mosquitoes released was limited. The trial initially studied an 11-hectare area. The limited number of modified mosquitoes and large area caused the density of released mosquitoes to be low. During this time, there was limited suppression of the wild mosquito population. A similar response was shown in the computer model during the first 2000 hours of the simulation. In the Brazil field trial the area was reduced to 5.5 hectare and the concentration of released mosquitoes was sharply increased leading to a dramatic reduction of 90% in the number of wild mosquitoes. This was also seen in the high population density model after 2000 hours when the number of released mosquitoes was increased a similar amount. The similarity between the Brazil field trial and high population density simulations supports the accuracy of this computer model.

The simulations using low population density are based on an environment for a planned field trial in Key Haven, Florida. The planned study involves a "Range finding Phase" to reduce the population and a "Suppression phase" to maintain the effect. The first phase involves a fixed release based on initial human and wild mosquito populations. The second phase releases are to be based on ratio of modified to wild larvae found in traps. The low population density computer model suggests a rapid adjustment and higher floor in the number of released modified mosquitoes is likely to be more effective at controlling the wild mosquito population.

While the data did provide support for the hypothesis there are several limitations with the model and this study. Because of limited published field trial data, the model was only compared to the Brazil trial 2011-2012. Comparison with other field studies may show major discrepancies with the computer model. The study only looked at wet and dry conditions which are not a dichotomy but occur to varying degrees over time. The study also raised concern that Variable method 50/20 may not respond quickly enough to suppress an increase in wild mosquito populations and a more aggressive response to changes in the wild population may be needed. This could be studied by repeated simulations under more challenging environmental conditions. The study did not take into account more complex geographic and weather features which alter mosquito populations. Studies have suggested that some genetically modified mosquitoes can survive and possibly procreate. While only male genetically modified mosquitoes were included in the study, research has shown that genetically modified females are also released and their effects are not considered in this model.

This study leads to several other possible areas of research. The computer model could look at several different variable methods besides the two that were tested in this study including the effect of minimum release number. This may allow for further refinement and provide an optimal release method for genetically modified mosquitoes for a particular environment. A comparison of this model with other field trials would allow for further modification of the model for different environments. The model could also be used to track infectious transmission such as Dengue Fever or Zika and the effect of genetically modified mosquitoes.

Conclusion

The study supports the utility of this individual based computer model and the hypothesis that variable methods of release for genetically modified mosquitoes can control wild Aedes aegypti mosquito populations and reduce resource requirements. Optimizing the modified mosquito release strategy will allow for both the effective control of wild mosquito populations and reduce the number of genetically modified mosquitoes that need to be released. Using computer models will allow for testing many different strategies reducing the number of expensive and difficult field trials.

References

1. Siqueira JB, Martelli CM, Coelho GE, et al. (2005) Dengue and Dengue Hemorrhagic Fever, Brazil, 1981–2002. *Emerging Infectious Diseases*.11(1):48-53. doi:10.3201/eid1101.031091.
2. Dengue Virus Net (n.d.) *Aedes aegypti* Retrieved from http://www.denguevirusnet.com/aedes-aegypti.html
3. Encyclopaedia Britannica (n.d.) *Aedes*. Retrieved from https://www.britannica.com/animal/Aedes
4. LaFrance, A. (2016, April 25). The Sneak-Attack Mosquito. *The Atlantic.* Retrieved from http://www.theatlantic.com/science/archive/2016/04/aedes-aegypti/479619/
5. Pollack, A. (2016, January 30). New Weapon to Fight Zika: The Mosquito. *The New York Times.* Retrieved from www.nytimes.com/2016/01/31/business/new-weapon-to-fight-zika-the-mosquito.html?_r=0
6. Oxitec (n.d.) Retrieved from http://www.oxitec.com/
7. Carvalho DO, McKemey AR, Garziera L, Lacroix R, Donnelly CA, Alphey L, et al. (2015, July 2). Suppression of a Field Population of Aedes aegypti in Brazil by Sustained Release of Transgenic Male Mosquitoes. *PLOS Neglected Tropical Diseases* Retrieved from http://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0003864
8. Center of Veterinary Medicine, United States FDA Department of Health and Human Services. (2016, August 5). *Environmental Assessment of Investigational use of Aedes Aegypti: QX513A* Retrieved from https://www.fda.gov/downloads/AnimalVeterinary/DevelopmentApprovalProcess/GeneticEngineering/GeneticallyEngineeredAnimals/UCM514698.pdf
9. Cohen, J. (1995, July 21). Population Growth and Earth's Carrying Capacity. Retrieved September 2, 2014, Retrieved from http://www.montana.edu/screel/Webpages/conservation biology/cohen.pdf
10. MacFarlane, A. (n.d.). Thomas Malthus and the Making of the Modern World. Retrieved September 2, 2014, Retrieved from http://www.alanmacfarlane.com/TEXTS/Malthus_final.pdf
11. Brauer, F., & Vez, C. (2001). *Mathematical models in population biology and epidemiology*. New York: Springer.
12. Lomnicki, A. (2011, January 1). Individual-based Models in Population Ecology. Retrieved September 2, 2014, Retrieved from http://www.els.net/WileyCDA/ElsArticle/refId-a0003312.html
13. Reynolds, C. (n.d.). Individual-Based Models. Retrieved September 2, 2014, Retrieved from http://www.red3d.com/cwr/ibm.html