## ABSTRACT

ROBERT, MICHAEL ANDREW. Mathematical Models of Genetic Strategies for Controlling the Dengue Vector, *Aedes aegypti*. (Under the direction of Alun L. Lloyd and Fred Gould.)

Because traditional efforts for controlling the primary mosquito vector of dengue fever, *Aedes aegypti*, have not led to significant decreases in disease cases, attention has turned to the development of novel methods of control, including genetic strategies. In genetic strategies, strains of genetically modified (i.e. transgenic) insects are released that are engineered to either have fewer viable offspring or to be less capable of transmitting disease pathogens, leading to either reduction of a wild-type population or replacement of a vector-competent population with one that cannot transmit disease pathogens. Significant progress in developing transgenic strains has been made in recent years, and the release of transgenic insects to control native populations is becoming more plausible; however, cautious evaluation, testing, and planning must occur before these measures are implemented on a large scale. Mathematical models are valuable tools that are used throughout the development of genetic strategies, particularly in the evaluation, testing, and planning stages. In this work, different types of models are utilized to study genetic strategies at different stages in the development process.

With a stochastic model designed to study cage experiments for testing a female-killing (FK) population reduction strategy, we underscore the utility of mathematical models in designing and evaluating experiments. We show that fitness disadvantages associated with transgenes can go undetected in experiments in which large numbers of transgenic individuals are released and that studying small population densities in cage experiments could be complicated by extinction that occurs as a result of the experimental design. We also illustrate how the model can be used to propose and explore experiments that have not previously been considered.

Utilizing an optimal control model, we explore integrated control programs that include FK releases. We show that optimal releases lead to lower costs of control than similar constant releases that have previously been studied. We show that integrated control approaches can be more cost-

effective than corresponding programs that involve single control strategies. We also explore the influence of costs of control on the total cost of integrated programs.

Employing a relatively simple deterministic model, we propose and evaluate a Reduce and Replace (R&R) strategy that aims to cause simultaneous population reduction and replacement by combining FK genes with anti-pathogen (AP) genes. We show that R&R releases are more effective in reducing competent vector densities long-term than similar FK releases. We show that releases including R&R females lead to greater reduction in competent vector density than male-only releases. Overall, the magnitude of reduction in total and competent vectors depends upon the release ratio, release duration, and whether females are included in releases.

With the same modeling framework, we evaluate an R&R strategy against AP-only and FK-only strategies, along with three hybrid strategies that combine FK, AP, and R&R releases. In most scenarios, we find that AP-only and R&R followed by AP strategies lead to the most reduction in competent vectors. While R&R releases often cause the most reduction while releases are being conducted, they are not as effective in reducing competent vectors long term in part due to the effects of linkage disequilibrium when population densities are low. We show that if fitness disadvantages are associated with the AP gene, fewer AP-only releases are needed to maintain low competent vector densities following an initial release period than similar R&R or FK-only releases.

The insights from the studies included in this dissertation can be and have been used to guide the development of genetic strategies in the laboratory, aid in the design and evaluation of experiments aimed at testing these strategies, and provide a general framework for guiding eventual release programs involving transgenic strains of *Ae. aegypti*.

Mathematical Models of Genetic Strategies for Controlling the Dengue Vector, *Aedes aegypti*

by
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## DEDICATION

To Mom, Dad, and Renee, for your endless love and support.

In memory of Nanny.

## BIOGRAPHY

Michael A. Robert was born in Pearl, Mississippi on March 10, 1986. After graduating from Pearl High School in 2004, he began studying Biological Sciences/Pre-Veterinary Medicine at Mississippi State University. After his freshman year, he found himself less interested in veterinary medicine and more interested in mathematics, and he changed his major to mathematics with the intention of teaching high school. In 2007, his best friend and fellow math major, Laura, invited him to join her in applying for a two-day undergraduate workshop at SAMSI. After being introduced to several examples of research in biomathematics, he became enamored with the subject. Michael worked on several biomath research projects as an undergraduate. In May 2008, he graduated magna cum laude with a Bachelor of Science in Mathematics with a minor in Spanish. He began graduate school the following fall at North Carolina State University, where he pursued a Doctor of Philosophy in Biomathematics under the direction of Drs. Alun Lloyd and Fred Gould. In October of 2013, Michael will join the lab of Dr. Helen Wearing at University of New Mexico as a postdoctoral researcher.

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# Chapter 1

# Introduction

## 1.1 Background

Dengue fever is a mosquito-borne viral disease that affects 50-390 million people each year throughout tropical and subtropical regions of the world [1,2]. Although significant progress towards vaccines has been made in recent years [3], there is not yet a licensed vaccine available for dengue. Prevention of dengue depends primarily upon control of its principal vector, *Aedes aegypti* [4-7], an urban mosquito species that prefers to lay its eggs in artificial containers in and around homes [8,9]. Although *Ae. aegypti* and dengue fever were effectively under control in the 1950s and 1960s due to intense campaigns to eliminate yellow fever, which is also vectored by this mosquito species, control efforts were not maintained and both *Ae. aegypti* populations and dengue fever cases have resurged in the past three decades [10,11].

Traditional control measures for reducing populations of *Ae. aegypti* include removal of sites where eggs are laid (i.e., source reduction), which targets immature mosquitoes, and application of pesticides, which targets adult mosquitoes [8]. In an attempt to provide more effective and sustainable control of the disease vector, much interest has been placed in the development of novel methods of control, including genetic strategies. In genetic strategies, the pest genome is altered to create a genetically modified (i.e., transgenic) strain of the pest species that either cannot reproduce as effectively as wild-type individuals or that is less able to transmit disease pathogens [12,13]. The former are "population reduction" strategies while the latter are "population replacement" strategies.

Development of genetic strategies for the control of disease vectors was inspired by the success of the traditional Sterile Insect Technique (SIT) in reducing, and in some cases eliminating, populations of some agricultural pests in the 1950s and 1960s [14]. In SIT, males of a pest species are irradiated and released into the wild [15,16]. Females that mate with irradiated males do not produce viable offspring, and populations decline with repeated SIT releases [16]. While SIT has been effective at reducing agricultural pest species, it has been less successful with mosquito species [14]. The promise of SIT sparked interest in developing more sophisticated methods for sterilization,

but the genetic technology necessary was insufficient at the time [12,13]. In the past two decades, however, genetic technology has advanced significantly, and interest in genetic strategies has been renewed [12,13].

Although genetic strategies hold promise for controlling *Ae. aegypti* populations, special care must be taken in the development and implementation of these approaches due in part to the novelty of the technology involved and the uncertainty of risks associated with introducing new genetic material into the environment [17,18]. For many strategies, one of the first steps is evaluating the feasibility of an approach with simple mathematical models of the population dynamics and/or the population genetics of a population subject to control (e.g. [19-26]). More complex mathematical models are used to evaluate the potential impacts of spatial movement, stochasticity, and complex biological processes on the ability of strategies to succeed (e.g. [27-32] ). Once transgenic strains have been successfully developed in a laboratory, experiments in an enclosed environment (such as a large cage) test the impact of the transgenic strain on a wild-type population (e.g. [18,33,34]). If these tests show promise, then releases can be conducted in a small, isolated area, and once the impacts on the isolated area are well understood, a transgenic strain can be a promising candidate for release in a larger population. Mathematical models can provide valuable insights in these latter stages of the development process by helping improve the design and understand the outcome of experiments as well as in planning releases in larger populations.

## 1.2 Dissertation Outline

In this dissertation, I develop and explore mathematical models that aim to assess the feasibility of genetic strategies at various stages of their development from idea to implementation. Chapter 2 discusses a stochastic model used to study large field cage experiments for testing a population reduction strategy. An optimal control model for exploring cost-effective integrated control strategies that include a population reduction strategy is discussed in Chapter 3. Chapter 4 introduces and explores the potential of a genetic strategy that combines population reduction and population replacement in a relatively simple deterministic model, and Chapter 5 utilizes this same model

to evaluate reduction, replacement, and combined strategies against one another.

## 1.3 Other Related Work

The work presented here has contributed to other projects outside of the scope of this dissertation, and the author of this dissertation has co-authored one paper and two currently unpublished manuscripts that have developed from these projects. The cage model of Chapter 2 was utilized in making decisions regarding the future of field cage experiments in Tapachula, Chiapas, Mexico, and once the experiments ended, the model was used to interpret the unexpected outcome of the experiments [34]. The work of Chapters 4 and 5 has developed in tandem with related explorations in a stochastic, spatially-explicit model that is calibrated to model mosquito populations in the city of Iquitos, Peru [Okamoto et al., submitted; Okamoto et al., in prep]. Furthermore, this work is currently being used by collaborators in the Gates Grand Challenge in Global Health initiative to develop and test new genetic strategies.

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# Chapter 2

# Mathematical models as aids for design and development of experiments: the case of transgenic mosquitoes [1]

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## ABSTRACT

We demonstrate the utility of models as aids in the design and development of experiments aimed at measuring the effects of proposed vector population control strategies. We describe the exploration of a stochastic, age-structured model that simulates field cage experiments that test the ability of a female-killing (FK) strain of the mosquito *Aedes aegypti* (L.) to suppress a wild-type population. Model output predicts that choices of release ratio and population size can impact mean extinction time and variability in extinction time among experiments. We find that unless fitness costs are greater than 80% they will not be detectable in experiments with high release ratios. At lower release ratios, the predicted length of the experiment increases significantly for fitness costs greater than 20%. Experiments with small populations may more accurately reflect field conditions, but extinction can occur even in the absence of a functional FK construct due to stochastic effects. We illustrate how the model can be used to explore experimental designs that aim to study the impact of density dependence and immigration; predictions indicate that cage population eradication may not always be obtainable in an operationally realistic time frame. We propose a method to predict the extinction time of a cage population based on the rate of population reduction with the goal of shortening the duration of the experiment. We discuss the model as a tool for exploring and assessing the utility of a wider range of scenarios than would be feasible to test experimentally due to financial and temporal restraints.

## 2.1 Introduction

In entomology, mathematical models have often been used as tools in Integrated Pest Management and Insecticide Resistance Management (Ruesink 1976, Worner 1991, Gould 2010). In this context, they are generally designed for predicting the spatial and temporal population genetics and dynamics of insect pests in the field environment (Stinner et al. 1983, Mayer et al. 1995). While it is easy to point out the potential for inaccuracies and artifacts in the predictions from these dynamical models, they are generally relied upon when empirical approaches for prediction are too expensive, not operationally feasible, or prohibited for regulatory or ethical reasons. For example, simulation models have been used by the U.S. EPA to develop resistance management strategies for transgenic insecticidal crops (U.S. EPA 1998, 2001).

Experimental design is often guided by statistical considerations; for instance, power calculations can guide choice of sample size. Such familiar calculations are based on simple statistical models (e.g., regression or ANOVA), but it is less widely appreciated that dynamical models can also be used to assess and improve the design of experiments (Curtis et al. 1976a, 1976b). In this context, models that include the essential parameters in an experiment can be used to tailor the experiment to answer the specific questions of most interest and to predict the limitations of an experiment before resources are invested. Here we demonstrate the application of simulation models for this purpose by describing a model developed to aid field cage testing of a transgenic strain of *Aedes aegypti* (L.), the major mosquito vector of dengue virus (Rosen et al. 1985).

Current strategies for dengue prevention rely on suppression or elimination of local *Ae. aegypti* populations (Gubler 1998, Morrison et al. 2008). There is no licensed, commercially available dengue vaccine, and anti-viral drugs are not expected to be used prophylactically (Scott and Morrison 2008, 2010). When implemented properly, mosquito control effectively prevents dengue (Morrison et al. 2008). Unfortunately, successful dengue vector control programs are the exception and where they do exist they are difficult to sustain. The urgent need to prevent the growing dengue public health problem has led to exploration of novel vector control approaches, including genetic

strategies, among which are methodologies based on the concept of the classical Sterile Insect Release method (Knipling 1955). In this modern genetic approach, mosquito strains are transgenically manipulated so that either all offspring or just the female offspring with a specific transgene will die under field conditions, but can be successfully reared in production facilities where tetracycline is added to the larval diet. Recent publications provide detailed descriptions of this pest control tactic and the molecular genetic methods used for strain development (see Heinrich and Scott 2000, Thomas et al. 2000, Alphey et al. 2008, Alphey et al. 2010, Fu et al. 2010).

In our study, we are specifically interested in the utility of female-killing (FK) strains of *Ae. aegypti*. In such strains, female offspring that inherit the FK transgene are unable to develop properly and die prior to pupation or are effectively removed from the population upon emergence because they cannot find mates or take bloodmeals. One such strain has been developed in which adult female *Ae. aegypti* that inherit the transgene are incapable of flight and therefore incapable of reproducing or obtaining a bloodmeal and transmitting virus (Fu et al. 2010). This effectively lethal flightless condition is intended to affect only females as a result of specific promoter sequences within the transgenic construct. The transgene that incapacitates female flight muscles is only turned on when tetracycline is absent from the diet. Although this success in developing a female-killing strain is promising, a series of tests must be conducted in order to ensure safety and effectiveness before genetically modified insects can be released (Benedict et al. 2008).

Results from laboratory cage trials indicate that a wild-type population can be suppressed successfully with regular introduction of FK individuals at a large release ratio (Wise de Valdez et al., 2011). Recently, large field cage trials in Tapachula, Mexico were conducted to study the ability of these FK individuals to suppress a wild population in a more environmentally natural setting (Facchinelli et al., 2013). The laboratory and field cage trials employed a similar experimental design which was aimed at testing whether releases of FK males into a target population could cause extinction under idealized release conditions. In order to quantitatively assess these experiments and determine what can be learned from their outcomes, we develop a stochastic model that incorporates the biological details of the experiments and enables a quantitative assessment of what

can and cannot be learned from the results of these cage trials.

We modify the model and use it to consider a number of other experimental designs and related experiments with the goal of illustrating the model's utility in assisting in the effective design of future cage studies of FK strains in which researchers seek to understand the impacts of mating fitness costs, density-dependent population regulation (Southwood et al. 1972, Legros et al. 2009), and immigration of wild-type individuals into the population being controlled (Dietz 1976, Prout 1978, Barclay 2005). While this model could also be used to conduct a detailed assessment of previous and ongoing experiments as well as to explore alternative experimental designs in detail, we do not pursue either avenue here.

In the following sections of this paper, we 1) describe the protocols used in the cage trials, 2) describe the characteristics of the mathematical model built to simulate the population dynamics and population genetics of the cage trials, 3) present results of model simulations, and 4) discuss the specific and broader implications of the model results.

## 2.2 The Experimental Protocol

The FK laboratory and field cage trials employ a design in which sets of control and treatment populations are maintained in separate cages throughout the experiment. Control populations consist solely of wild-type mosquitoes, provide baseline information on dynamics of a caged population of *Ae. aegypti*, and are used to calculate input of FK mosquitoes into treatment populations (see equation 2.1). Treatment populations consist of both wild-type and FK individuals. To reduce the impact of environmental influences on cage dynamics, a treatment population is paired with a control population with similar environmental conditions.

In both cages, wild-type populations are established and allowed to stabilize (i.e., reach a stable adult population density). Eggs laid in the cage are collected twice per week, counted, and hatched in a laboratory. At the beginning of each week a specific number, $N$, of second-instar larvae that hatch from collected eggs is returned to the cage from which they were collected. This is done in order to maintain a stable adult population size. Larvae are provided with adequate resources to

ensure that larval survival and development are not density-dependent. Using this method of population maintenance, a stabilization period of about 13 weeks is needed (Wise de Valdez, et al. 2011).

In the experiments, once both control and treatment populations are stabilized, the treatment phase of the experiment begins. Each week, in addition to the return of larvae hatched from eggs collected in the cage, $rN$ homozygous FK pupae are introduced into the treatment cage (pupae are introduced rather than larvae for operational reasons). Here, $r$ is the initial release ratio of homozygous FK pupae to wild-type larvae. For example, if a 10:1 initial ratio of homozygous FK pupae to wild-type larvae is desired ($r = 10$), then the number of FK pupae placed into the treatment cage each week is $10N$. (Note that since only male FK mosquito adults are expected to survive, the number of male mosquitoes released each week, assuming a 1:1 sex ratio, is approximately $\frac{1}{2}rN$.) The control cage continues to receive only larvae hatched from eggs collected in the cage.

If FK releases have the desired effect of reducing population size, the number of eggs laid in the treatment cage will decline over time, and so the number of larvae returned to the treatment cage is adjusted to reflect this. This is achieved by setting the input into the treatment cage at the beginning of week $w$, $N_w$, equal to the ratio of eggs laid in the treatment and control cages in the previous week ($E^{\mathrm{T}}_{w-1}$ and $E^{\mathrm{C}}_{w-1}$, respectively) multiplied by the fixed input into the control cage (Equation 2.1).

$$N_w = N\frac{E^{\mathrm{T}}_{w-1}}{E^{\mathrm{C}}_{w-1}} \tag{2.1}$$

Altering the input into the treatment cage in this manner ensures that the input is directly proportional to the numbers of eggs laid each week, and that population dynamics are not density-dependent.

In the experiments, once mating occurs between FK males and wild-type females, larvae that hatch from the eggs are screened for a physical marker indicating that they bear the FK genetic construct so that the frequency of the FK gene can be monitored. The larvae that are returned to the cage each week are hatched from a random sample of the eggs that are laid in the cage so that

the distribution of genotypes of the larvae is expected to reflect that of the eggs laid. This process of input, removal, and screening continues until no eggs are produced in the treatment cage for two consecutive weeks, at which point the population is declared extinct.

## 2.3 Description of the Model

We employ a discrete time stochastic model that links population genetics and population dynamics of caged *Ae. aegypti* populations and study expected extinction times and variation of extinction times that result from different experimental designs. For a detailed description of the mathematical details of the model, see Appendix A. We track integer numbers of the population each day subdivided by age, sex, and genotype. $M_{g,a,d}$ is the number of adult males of genotype $g$ and age $a$ on day $d$, $F_{a,d}$ is the number of adult females of age $a$ on day $d$, and $E_{g,d}$ is the number of eggs laid of genotype $g$ on day $d$. There are three possible genotypes: wild-type ($g = 1$), heterozygous FK ($g = 2$), and homozygous FK ($g = 3$); the only individuals of this latter genotype are the released males. Note that only wild-type females will be viable, so multiple adult female genotypes are not tracked.

### Emergence and Survival

Individuals in each larval cohort emerge as adults over several days according to the probability distribution presented in Fig. 2.1 (obtained from unpublished data from Facchinelli, Valerio, Ramsey, and Scott). Fig. 2.1 shows the emergence distribution for male larvae. We assume that females emerge one day later than males (Christophers 1960, Craig 1967), so female emergence is given by an identical distribution that is shifted by one day compared to that of males. Pupae emerge within the first two days of being placed into the cage, with a 32% chance of emerging the day after placement, a 65% chance of emerging two days after placement, and a 3% chance of dying before emergence (Rueda et al. 1990).

When mosquitoes are adults, the number that survives from one day to the next is determined by a sex-dependent daily survival probability: $s_m$ for males and $s_f$ for females. In the results pre-

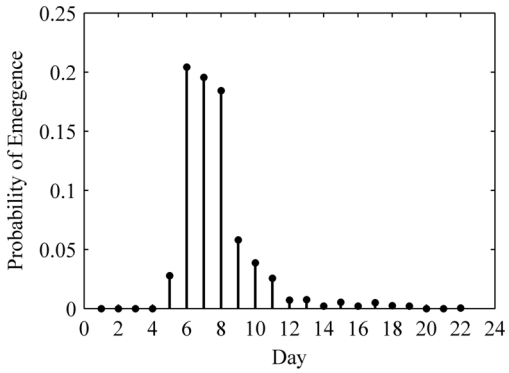


**Figure 2.1:** Probability distribution of adult male emergence times when second-instar larvae are seeded in containers in semi-field conditions. The horizontal axis is the number of days after second-instar larvae are placed in the cage on day 0, and the vertical axis is the probability that males will emerge on the day given on the horizontal axis. In this study, female emergence time distribution has the same shape, but is shifted so that female emergence occurs one day later than would male emergence. Note that the probability of mortality before emergence is 0.2318. (Obtained from Facchinelli, Valerio, Ramsey, and Scott, unpublished data)

sented below, we set $s_m = 0.72$ and $s_f = 0.9$ for all age cohorts (Muir and Kay 2007, Foque et al. 2006). In Appendix B, we explore variations in survival parameters (including age-dependent survival patterns), and we show that the effects of age-dependent survival are minimal unless the average lifespan is much longer than assumed here, underscoring that knowledge of the lifespan distribution is important in making predictions with this model (see Figs. B.1 and B.2 in Appendix B).

### Mating and Reproduction

We assume that adult males begin mating two days after emergence and that females mate one day after emergence with only one mate and do not mate again (Christophers 1960), although there is evidence that polyandrous mating occurs at a low rate (Williams and Berger 1980, Young and Downe 1982). A mating pair distribution (i.e., the distribution of mate genotypes) is determined for each female cohort on the day that it mates; this distribution determines the genotypes of the offspring of that cohort. In Appendix B, we further explore the effects that mating age and polyandrous mating might have on cage experiments. We assume that FK males can incur a fitness disadvantage in the form of reduced mating competitiveness. While fitness disadvantages could

also manifest themselves through a reduced number of offspring or survival disadvantages, we feel that studying fitness disadvantages only through reduced mating competitiveness captures the most likely effects of fitness disadvantages for this system. We assume that the mating fitness cost, if incurred by FK males, is additive, and denote this fitness cost by $c$. We define the mating fitness of each genotype as $\Phi_1 = 1$, $\Phi_2 = 1 - c/2$, and $\Phi_3 = 1 - c$. Hence on day $d$ a female chooses a male of genotype $k$ with probability $p_{k,d}$, where

$$p_{k,d} = \frac{\sum_a \Phi_k M_{a,k,d}}{\sum_a \sum_{k'} \Phi_{k'} M_{a,k',d}}. \tag{2.2}$$

We assume that females begin laying eggs five days after they mate and continue to lay eggs until their death. We assume that the daily number of eggs laid by a female mosquito is independent of age. (Age-dependent fecundity, which reflects changes in daily fecundity related to gonotrophic cycles, is explored in Appendix B). We assume the number of eggs laid by a cohort of age a each day follows a Poisson distribution with mean $\lambda F_{a,d}$ where $\lambda$ is the mean number of eggs that an individual female lays each day. Throughout this analysis, we set $\lambda = 10$ (Harrington et al. 2001, Styer et al. 2007). The genotypes of the offspring in eggs laid by each cohort on a given day are determined by the distribution of genotypes of the mating pairs formed on the day the cohort mated and the Mendelian probability, $P_{g,k}$, that an offspring of genotype $g$ results from the mating of a wild type female and male of genotype $k$. That is, the probability that an offspring produced by a female who mated on day $d$ is of genotype $g$ is $v_{g,d} = \sum_k p_{k,d} P_{g,k}$.

The distribution of genotypes among eggs laid is then used to determine the distribution of the genotypes of larvae input on a weekly basis as described in the experimental protocol. In the model, we select the number of larvae of each genotype by sampling from a multinomial distribution.

### Density Dependence

Density regulation of *Ae. aegypti* populations is a complicated process that is not yet well understood. Changes in population density can affect larval development time, larval survival, and adult size, and the impacts on these aspects of growth and development can depend upon factors

such as container size, food availability, and temperature (Barbosa et al. 1972, Gilpin and McClelland 1979, Agnew et al. 2002, Braks et al. 2004, Padmanabha et al. 2011). While the impacts of all of these aspects of density dependence on cage experiments are important to understand, the study of most of them would require drastic alterations of the current experimental protocol. Here, we focus on investigating the influence of density dependence on larval survival because such a study can be done using a simple variant of the current experimental protocol.

In this analysis, all aspects of the current design are maintained, with two exceptions. We consider the return of pupae rather than larvae in order to consider density-dependent regulation that occurs during the larval stages, and we put pupae into the cages according to the following function, adapted from Bellows (1981):

$$N_w(E_{w-1}) = \gamma E_{w-1} \exp\left(-\alpha E_{w-1}^{\beta}\right). \tag{2.3}$$

$N_w(E_{w-1})$ is the number of input pupae returned to the cage each week, determined as a function of the number of eggs, $E_{w-1}$, collected during the previous week. Parameters $\alpha$, $\beta$, and $\gamma$ determine the functional description of density-dependent larval survival. Although the parameter space available for exploration is vast, we focus on three parameter sets to provide a brief illustration of one way in which density dependence can be studied in the cage experiments. We choose these parameter sets because they give rise to three different descriptions of density dependence (see Fig. 2.2; values of the parameters $\alpha$, $\beta$, and $\gamma$ are given in the figure caption). In the absence of FK individuals, all three input curves give rise to an equilibrium at approximately $E_w^* = 4025$, which corresponds to the average weekly egg production in density-independent cage populations where the weekly pupal input is $N = 200$. The three forms differ primarily by location of the maximum relative to this equilibrium. Form 1 has a maximum near the equilibrium, so the number of pupae returned does not differ much when the number of eggs collected is near the equilibrium value. The maximum input for Form 2 results from a number of eggs being laid that is well beyond what is expected to be observed in cages. Pupal input for populations regulated by this form of density dependence can increase when the number of eggs collected surpasses the equilibrium value.

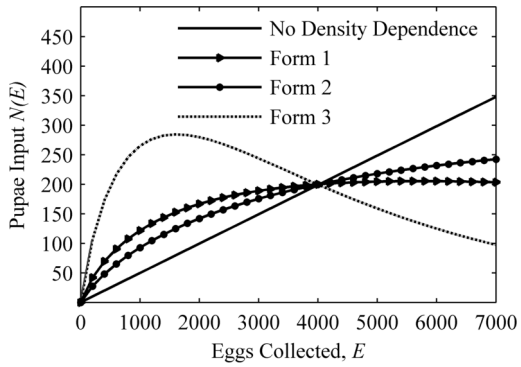


**Figure 2.2:** Functional forms for density-dependent survival. These forms determine the number of larvae placed into the cage each week in terms of the number of eggs collected in the previous week, mimicking density-dependent larval survival. For the function $N_w(E_{w-1}) = \gamma E_{w-1} \exp\left(-\alpha E_{w-1}^{\beta}\right)$, Form 1 (triangles): $\alpha = 0.0522$, $\beta = 0.4370$, and $\gamma = 0.3540$, Form 2 (circles): $\alpha = 0.0516$, $\beta = 0.4021$, and $\gamma = 0.2125$, and Form 3 (dotted): $\alpha = 0.0089$, $\beta = 0.6896$, and $\gamma = 0.7516$. The solid line represents input in the absence of density-dependent survival. Since a population resulting from regular input of 200 larvae will produce, on average, 4025 eggs in a week, populations resulting from each of the four input types have an equilibrium point at $E = 4025$, $N(E) = 200$.

The maximum value of Form 3 occurs below the equilibrium, so the number of pupae returned generally decreases when the number of eggs collected increases; however, as the number of eggs collected decreases from the equilibrium, the number of pupae returned first increases, but then decreases again as the number of eggs collected gets closer to zero. We assume that during a field release, the homozygous FK individuals produced for release will be provided with adequate resources, so their survival is not regulated by density dependence in the model.

## Immigration

We explore another variant of the current experimental protocol that could be used to study the potential effect of immigration of wild-type individuals into a population that is being controlled by releases of FK individuals. To study introduction of larvae that could occur as a result of movement of containers, we introduce additional wild-type larvae into both cages each week. These introductions occur for the duration of the experiment, including the stabilization period. In Appendix B, we consider the immigration of newly emerged, unmated adults and three day old, mated adult females and mating males.

### Simulations

We simulate a number of different experimental designs by varying the baseline wild-type input, $N$, and release ratio, $r$. We study the impacts that these experimental designs have on the wild-type population under various scenarios (e.g., considering fitness costs, immigration, and density dependence) by observing population decline and extinction. We focus primarily on mean time to extinction and variation in extinction times, which allows us to predict the range of total experiment times (i.e., stabilization period + time to extinction) that could result from different experimental designs. Throughout the results and discussion, we present the extinction time as the number of days until the treatment population reaches extinction following the initial FK release. Under some scenarios (e.g., with density dependence and immigration), population extinction does not always occur. In those cases, we observe reduction in population density as measured by the percentage of wild-type adult females remaining a given number of weeks following the initial FK release relative to the number of wild-type adult females present the day before releases begin. For illustrative purposes, we choose to measure the reduction 14 weeks following the initial release (14 weeks postrelease). Note that variability in our results is due only to the components outlined in the model description. There are a number of other potential sources of variability that we do not consider, such as that caused by environmental factors or individual-based variability in survival, mating, or reproduction. Unless otherwise stated, for each parameter set, we run 1000 simulations for a maximum of 1000 days each.

## 2.4 Results

Treatment cage dynamics from one simulation are shown in Fig. 2.3. The population in each cage was stabilized before releases were started, and population numbers oscillated with a period of seven days, which reflects the weekly release schedule. Females reached higher pre-FK release densities because they had a higher probability of survival than males. About three weeks after the first introduction of FK individuals, adult males heterozygous for FK began to appear, which

indicated successful matings between homozygous FK males and wild-type females. At the same time, the population of wild-type adults in the treatment cage began to decline, which followed the decline in the number of wild-type eggs. Comparison of the model output to data from Wise de Valdez et al. (2011) shows that the model captured the general dynamics observed in experiments (Fig. B.1); see Appendix B for further discussion.

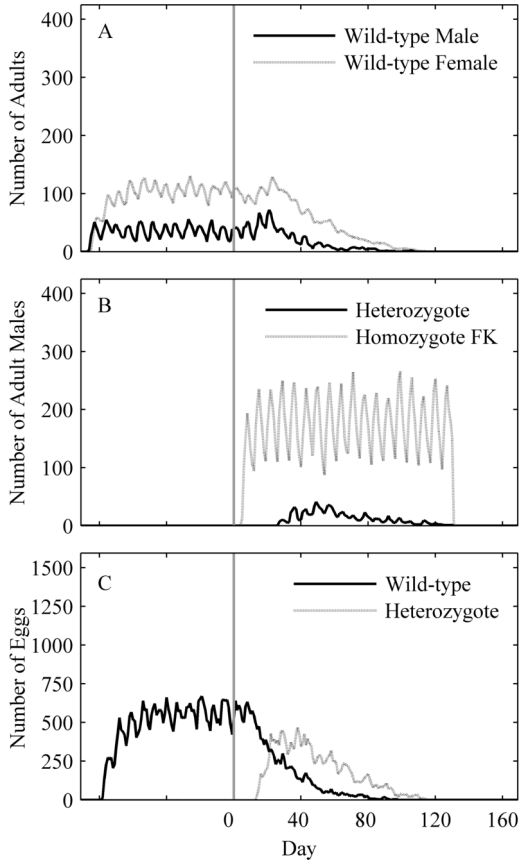


**Figure 2.3:** Treatment cage dynamics of the stabilization and postrelease period obtained from a single simulation. (A) The number of wild type adults, (B) The numbers of heterozygous and homozygous FK males, and (C) The number of eggs laid. Here, $N = 200$, $r = 5$, $\lambda = 10$, $c = 0$, $s_m = 0.72$, $s_f = 0.9$. The releases of FK individuals begin at the time marked by the vertical grey line.

While assessing the results presented here, it is important to remember two components of the experimental design that influence the extinction time in all of the results. First, heterozygote

adults typically began appearing three weeks following the initial input of FK males (see Fig. 2.3). This indicates that the presence of FK males began impacting the adult population about three weeks following the initial release. Second, extinction time is defined as in the experiments by Wise de Valdez et al. (2011): A population is considered to be extinct when no eggs have been laid for two consecutive weeks. With the influence of these two components, the very minimum extinction time will be more than 35 days postrelease.

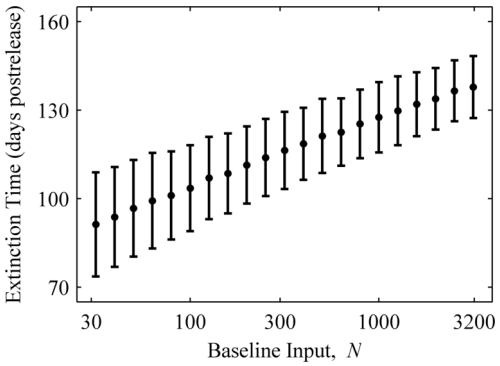


**Figure 2.4:** Extinction time (postrelease) for different values of baseline input, $N$. Circles represent mean time to extinction and error bars represent mean ± standard deviation. Here, $r = 10$, $\lambda = 10$, $c = 0$, $s_m = 0.72$, $s_f = 0.9$. Note the horizontal axis is on a log scale.

### Baseline Wild-type Input

We varied the baseline wild-type input, $N$, over a wide range of values. We found that as $N$ increased, the mean time to extinction increased gradually, and the variance generally decreased (Fig. 2.4). Even though mean extinction time did increase with larger values of $N$, the difference from $N = 100$ to $N = 1000$ was only about 24 days on average with a three day difference in standard deviation. An increase in $N$ by 100 individuals did not cause more than a few days change in average extinction time when $N \geq 400$, but decreasing $N$ from high values to low values (e.g. from 1500 to 50) did lead to a reduction of a few weeks in extinction time. We note, however, that small baseline input may lead to populations being incapable of persisting in the absence of FK introductions. We

return to this point in a subsequent subsection of the results.

### Release Ratio

Fig. 2.5 shows the effects of release ratio of FK to wild-type individuals on extinction time. We varied the release ratio, $r$, from 0.10 to 400, and we found that the mean and variance of time to extinction decreased as the release ratio increased. These decreases were most rapid for low release ratios and became more gradual with higher release ratios. In fact, in our simulations, the mean extinction time differed by about 209 days between $r = 0.1$ and $r = 1$ with a 51 day difference in standard deviation, whereas there was only a difference of eight days between average extinction times for $r = 25$ and $r = 400$ with a two day difference between the standard deviations.

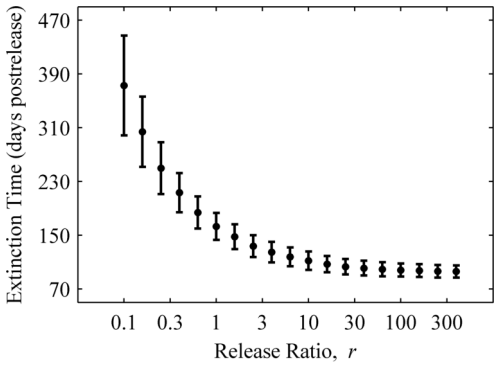


**Figure 2.5:** Extinction time (postrelease) for different release ratios, $r$. Circles represent mean time to extinction and error bars represent mean ± standard deviation. Here, $N = 200$, $\lambda = 10$, $c = 0$, $s_m = 0.72$, $s_f = 0.9$. Note the horizontal axis is on a log scale.

### Fitness Cost

We utilized the model to predict the effects that mating fitness cost, taken to vary from 0 to 0.9, could have on extinction time under four different release ratios ($r = 0.1, 1, 10, 100$). With all four release ratios, there were increases in the mean and variance of extinction times as the fitness cost was increased (Fig. 2.6). The differences in mean extinction time caused by increasing fitness costs

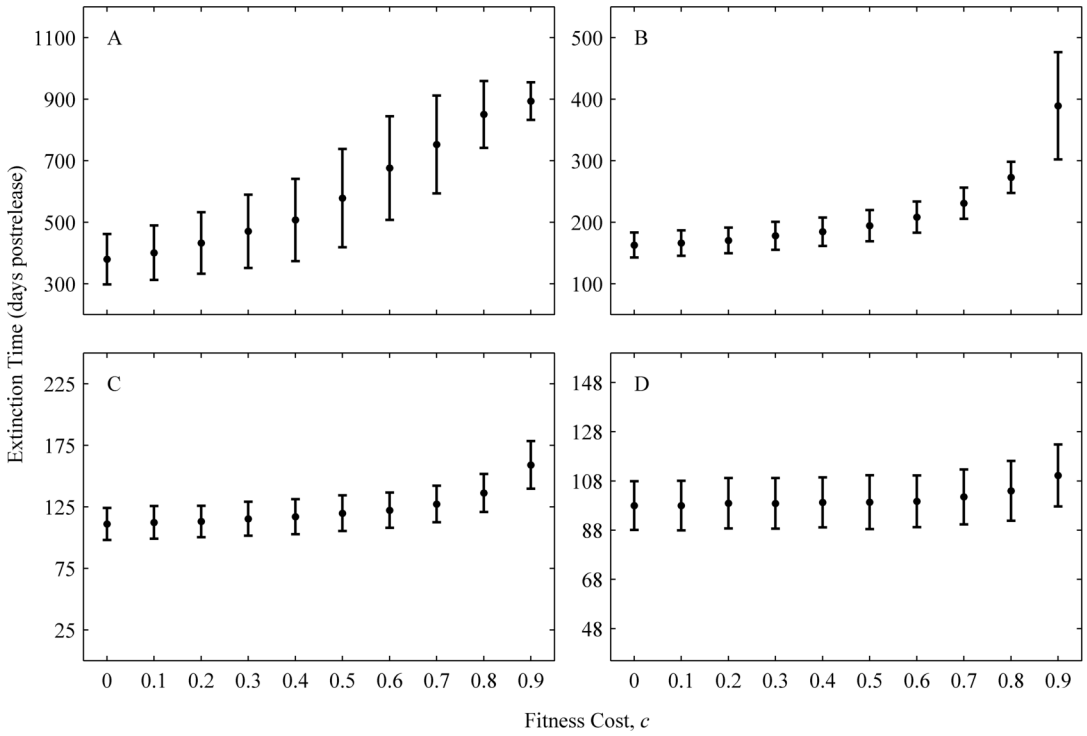


**Figure 2.6:** Extinction times (postrelease) for different fitness costs combined with different release ratios. Here, $N = 200$, $\lambda = 10$, $s_m = 0.72$, $s_f = 0.9$. Release ratios are $r = 0.1$ (A), $r = 1$ (B), $r = 10$ (C), and $r = 100$ (D). Solid circles indicate mean of 1000 simulations and error bars represent mean ± standard deviation. Note each panel has a different scale on the vertical axis, with panel (A) having the largest range and each subsequent panel having half the range of the previous one. Recall that we restrict the duration of each simulation to 1000 days following a release; this leads to the reduced variation in extinction time for high values of $c$ that is observed in panel (A).

were much greater with lower release ratios. As an example, under a high release ratio of $r = 100$, average extinction time differed by about 13 days between $c = 0$ and $c = 0.9$ (Fig. 2.6d), while for the low release ratio of $r = 1$ the average extinction time increased by 236 days between these two fitness costs (Fig. 2.6b). The standard deviations of extinction times exhibited similar patterns, increasing by just three days in the $r = 100$ case, but quadrupling in the $r = 1$ case. When $r = 10$, as in the laboratory and field cage experiments previously conducted (Wise de Valdez et al. 2011, Facchinelli et al., submitted), average extinction times differed by about 25 days between $c = 0$ and $c = 0.8$, with the average extinction time for $c = 0$ falling within the range of extinction times predicted by the model when $c = 0.8$ (98-199 days postrelease).

When fitness costs are high and release ratios are low (e.g., Fig. 2.6a, when $c = 0.8$ or 0.9 and $r = 0.1$), extinction in the treatment cage did not always occur within the 1000 day period allotted for the experiment. For example, when $r = 0.1$ and $c = 0.8$ or 0.9, extinction occurred in fewer than 32% of the simulations, and wild-type population persistence was observed in a handful of simulations for fitness costs as low as $c = 0.4$. These simulations indicate that extinction of populations in cages in which FK individuals are released at low ratios may take several years if released mosquitoes have a high reduction in fitness due to the presence of the FK gene.

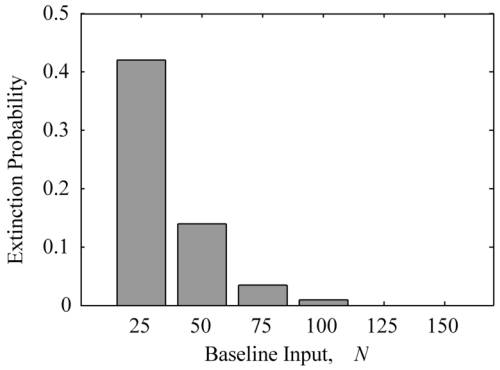


**Figure 2.7:** Extinction probability of a wild type population in the absence of FK introduction. Bars represent the proportion of 1000 simulations in which the population went extinct within one year when low-density populations were simulated. Here, $\lambda = 10$, $s_m = 0.72$, $s_f = 0.9$.

## Population Size and Natural Extinction

To date, cage experiments have used high population densities resulting from high baseline input values. Population densities of adult *Ae. aegypti* in many dengue-endemic areas can be rather low (Morrison et al. 2004, Koenraadt et al. 2008, Jeffery et al. 2009), so it would be informative to know how lower density populations react to introductions of FK individuals. Because the small cage populations needed to mimic low density natural populations are at greater risk than larger populations of going extinct even without the introduction of an FK population, it is important to know how small a population can be maintained in the cage experiment without it being likely to

go extinct due to environmental or demographic causes. We examined the capacity for small cage populations to persist in the absence of FK introductions by simulating experiments with baseline wild-type input numbers as low as $N = 25$. We simulated a wild-type cage population for one year following a 13-week stabilization period and calculated the proportion of 1000 simulations that went extinct before the end of that year (Fig. 2.7). The proportion decreased with increased larval input, with 42% of the simulated cages going extinct when $N = 25$, and less than 1% going extinct when $N = 100$.

## Density Dependence

We studied the effects of density-dependent survival, using the three descriptions of density dependence given above, under four different release ratios, $r = 0.10$, $r = 1$, $r = 10$, and $r = 100$. Density dependence had a marked effect on extinction times for the lower release ratios. For $r = 0.1$, there was no extinction within the 1000 day experimental time frame in any of the 1000 simulations for populations subject to any of the three forms of density dependence considered here. There was also no extinction for populations subject to Form 3 density dependence when $r = 1$. For all other combinations of release ratios and forms of density dependence, extinction was the typical outcome.

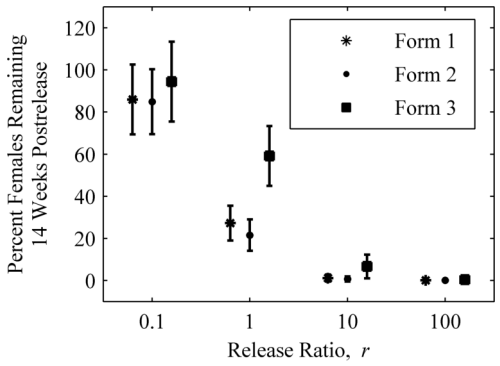


**Figure 2.8:** Effects of density dependent input for different release ratios. Vertical axis is the percentage of the wild-type adult female population remaining 14 weeks postrelease. Here, $c = 0$, $\lambda = 10$, $s_m = 0.72$, $s_f = 0.9$. Form 1, Form 2, and Form 3 density dependence from Figure 2 are represented by asterisks, circles, and squares, respectively. Solid asterisks, circles, and squares indicate mean of 1000 simulations and error bars represent mean ± standard deviation.

Because not all populations subject to density dependence went extinct in our simulated experiments, we compared the effects of density dependence on cage experiments by observing population reduction (see Fig. 2.8). We studied the percentage of the wild-type adult female population remaining 14 weeks postrelease, as previously defined. For populations subject to any of the three forms of density dependence considered here there was little population reduction when the release ratio was $r = 0.10$. When we considered larger release ratios, however, the populations were generally reduced. When $r = 1$, the mean percentage of females remaining 14 weeks postrelease ranged from 21.6% for density dependence described by Form 2 to 59.1% for that under Form 3. For $r = 10$, populations subject to density dependence of Forms 1 and 2 were at or near extinction after 14 weeks, whereas a mean of 6.7% of the female population remained when density dependence was described by Form 3. For releases with $r = 100$, all populations were reduced to, or very near to, extinction 14 weeks postrelease.

We compared the extinction time of cages subject to density-dependent input to those of experiments run with density-independent input. Here, we considered only $r = 10$ and $r = 100$. Extinction times when $r = 10$ were not much different when survival was density-dependent with the exception of populations subject to density dependence described by Form 3, where the average extinction time was more than 40 days greater. (Extinction times ranged between 80-167 days with no density dependence and between 81-393 days when density dependence was described by Form 3). Mean extinction times in our simulations for populations subject to Forms 1 and 2 density dependence were about 13 days greater and two days less, respectively, than when the population was not subject to density dependence. (Extinction times ranged between 82-193 days for Form 1 and 76-172 days for Form 2). When $r = 100$, the difference between density-dependent and density-independent cases was not as great; the mean extinction time was about the same for populations regulated by Form 3 density dependence, while populations subjected to Forms 1 and 2 density dependence were actually extinct a few days earlier, on average, than density-independent populations. (Extinction times ranged between 77-143 days when there was no density dependence, 73-153 days when density dependence was described by Form 1, 71-152 days for Form 2, and 69-

163 days for Form 3).

### Wild-type Immigration

For this analysis we assumed that larval input was not subject to density dependence. We considered the flow of 10, 20, 30, 40, or 50 larval immigrants per week under four release ratios ($r = 0.1, 1, 10, 100$). We found that the mean percentage of the wild-type adult female population remaining after 14 weeks increased approximately linearly with the weekly immigration rate (see Fig. 2.9). The variance decreased for the cases where $r = 0.10$ as the number of immigrants increased, but for all other release ratios, the variance increased with the number of immigrants. For higher release ratios ($r = 10$, $r = 100$) where the population, in the absence of immigration, was often extinct or nearly extinct 14 weeks postrelease, the percentage remaining corresponded roughly to the percentage of immigrants to the wild-type baseline (e.g., for the case of 10 immigrants per week, when $N = 200$ and $r = 100$, the mean percentage remaining was 7.64%, slightly larger than $10/200 = 0.05$, or 5%). This indicates that the population was being maintained primarily by immigrants.

### An Alternative Measure for Assessment of Population Reduction

In the previous two sections we studied population reduction that occurred in the first 14 weeks postrelease. Although extinction time is an important measure of the overall efficacy of a release strategy, extinction could take months or even years in some cases. By utilizing other measures of efficacy and defining a different endpoint of the experiment, overall experiment time can be shortened, and more experiments can be conducted.

As an illustration, we analyzed the model output for the scenario in which $c = 0.7$ and $r = 1$ (as presented in Fig. 2.6b) to assess the relationship between mean extinction time and wild-type adult female population reductions after 14 weeks. We obtained the correlation coefficient between time to extinction and percentage of the wild-type adult female population remaining 14 weeks postrelease (here, $n = 1000$ simulations was the total sample size). Fig. 2.10 shows a scatter plot of extinction time versus population reduction along with the line of best fit obtained via simple

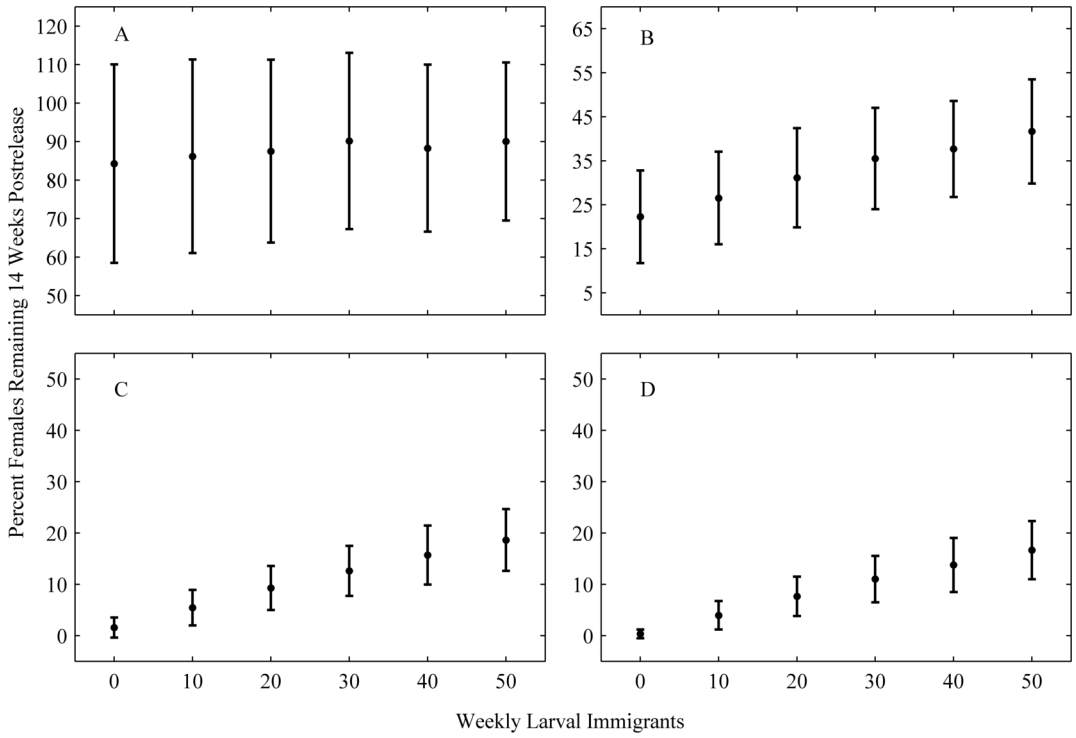


**Figure 2.9:** The effect of weekly larval immigration on wild-type adult female population reduction. The horizontal axis is the number of immigrants per week, and the vertical axis is the percentage of the wild-type adult female population remaining 14 weeks postrelease. Here, $N = 200$, $c = 0$, $\lambda = 10$, $s_m = 0.72$, $s_f = 0.9$. (A) $r = 0.1$, (B) $r = 1$, (C) $r = 10$, (D) $r = 100$. The solid circles indicate the mean of 1000 simulations and error bars represent the mean ± standard deviation.

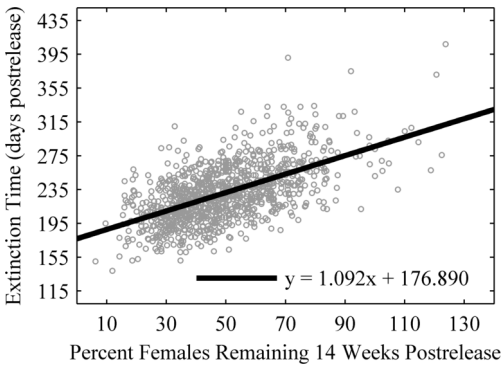


**Figure 2.10:** Scatter plot of days of treatment cage extinction time (postrelease) versus population reduction as measured by the percentage of wild-type adult females remaining 14 weeks postrelease. The equation for the line shown here is obtained from a linear regression model (coefficient of determination $R^2 = 0.339$). For these simulations, $N = 200$, $r = 1$, $\lambda = 10$, $c = 0.7$, $s_m = 0.72$, $s_f = 0.9$.

linear regression. The correlation between the percentage of the wild-type adult female population remaining after 14 weeks and extinction time was 0.582 ($R^2 = 0.339$, $p < 0.0001$).

In this case, the population reduction 14 weeks postrelease as defined here could be a good indication of cage extinction time. The time at which population reduction is measured (for the purposes of predicting extinction time) will depend upon the experimental setup chosen. For instance, if a larger release ratio is chosen, one may wish to observe the population reduction only a few weeks after the initial FK release rather than waiting 14 weeks because many of the cages will have reached extinction within 14 weeks postrelease (as in Fig. 2.6d). In general, one must be sure that the time chosen to observe reduction is neither before reductions in population size have begun nor after extinctions have begun. We further discuss how correlation depends on the time chosen to observe reduction in Appendix B.

## 2.5 Discussion

This study raises and addresses a series of important questions regarding the information that can (and cannot) be obtained about the qualities of FK transgenic mosquitoes from specific field cage experiments. Under which experimental designs can fitness costs be detected? Can populations of both low and high densities be studied in cages? Can information regarding the efficacy of FK introductions be obtained without waiting for extinction? As we noted, the experimental design that has been used so far leaves unanswered many important questions that will be need to be addressed before open field releases occur. How will immigration impact the population suppression ability of FK strains? Will density-dependent effects hinder population suppression, and how can such effects be overcome? What influence can cage experiments have on open field releases? Our study proposed and simulated variants of the current experimental design that could at least partially address these questions, although a more radical change to the experimental setup, and hence to the model, would be required to fully explore some of these questions, such as the complexities of density dependence. Such questions could easily be overlooked when temporal, financial, and personnel restraints limit the number and variety of experiments that are conducted, but models

can suggest which issues will be important to address in field cage experiments before open field releases are conducted. Models can provide guidance that will make the process of designing and implementing further experiments more directed and efficient.

We found one past example in which deterministic simulation models were used to assess experiments and examine how different release schedules within cages would affect experimental outcomes (Curtis et al. 1976a, 1976b). In contrast, our stochastic model explored a range of experimental parameters that transcends that which may be financially and temporally feasible to explore in field cage experiments, and considered at least some of the sources of variability that such experiments would experience.

For example, our model predicted that the size of the target population being studied in cages might not have a great effect on overall experiment time. The small differences that we found in extinction time due to population size can be beneficial from two perspectives. If one needs to reduce the effort of rearing large numbers of mosquitoes for an experiment, then it is useful to know that using a smaller population of mosquitoes will provide a similar result, in terms of overall experiment time, as using a larger population. In contrast, if one is interested in testing high density populations, a population that is increased by ten-fold in size by changing $N$ from 100 to 1000 can be driven to extinction without a substantial increase in time. Although experiment times may not differ much when large and small population densities are studied, one must be cautious when studying small population densities, because our model indicated that interpreting results of such experiments could be complicated by extinction that is due solely to demographic stochasticity. In cases where studying small populations is desired, such as when using cage studies to assess the ability of the released strain to find mates in the wild, short-term experiments that are not affected by population fluctuations could be more appropriate.

For a genetic control strategy to be successful, genetically modified mosquitoes must be able to compete with wild-type individuals for mates. The importance of mating competitiveness of males has been studied extensively in mathematical models for the sterile insect technique (see Barclay 2005, Ito and Yamamura 2005). Mating competitiveness similarly is expected to influence

strategies involving a female-killing mechanism (Schliekelman and Gould 2000). Results from our simulations of the impact of fitness costs predicted that a large range of costs will not be detected in field cage experiments that use high release ratios. This study revealed that when experiments are conducted with release ratios similar to those of laboratory and field cage experiments that have been conducted to date, fitness costs may not be detectable unless they are greater than 0.80. If an important goal of a field cage experiment is to assess fitness costs then low release ratios should be used.

The potential impact of density dependence on genetic control strategies has long been realized (Prout 1978, Foster et al. 1988, Schliekelman and Gould 2000). Within the constraints of the existing experimental design, we proposed a way to explore one facet of density dependence, namely larval survival. We found that when populations were subject to density dependence and FK releases occurred at low release ratios, eradication of the cage populations did not always occur within the 1000 days allotted for the modeled experiments. High release ratios could overcome the density-dependent regulation of the population and ultimately drive the wild-type population to extinction, but the time to extinction will be greater than when the population is not regulated by density dependence. Because density dependence in *Ae. aegypti* populations can be difficult to accurately quantify (Legros et al. 2009), we explored different forms of density-dependent survival in the model and found that the extent to which density dependence interferes with population extinction in the simulations depends on the particular form of density dependence used. Our results showed that the model can be used to determine if, for a given population, more accurate assessment of density dependence would be desirable. As mentioned previously, larval survival represents just one component of density-dependent population regulation. A more complete exploration of the impact of density dependence would likely entail radical changes to the experimental design and model, but would provide invaluable information to inform field releases.

Immigration of juveniles into a population subject to control by FK releases provided another scenario in which wild-type populations did not go extinct in our model, even in the presence of forceful control measures. Our simulations indicated that immigration of larvae could result in the

wild-type population being maintained, regardless of how many FK individuals were being introduced (see Prout 1978).

Once cage experiments have established that introduction of a specific FK strain can drive a population to extinction under ideal conditions, further experiments could be carried out to investigate the relationship between more realistic ecological factors and FK releases. Our model predicted that some of these more realistic experiments could take months or even years if extinction time is taken as the endpoint of the experiment. We found that as an alternative to the extinction endpoint, the efficacy of FK releases in cages could be assessed by observing the population reduction after a given time frame has passed. In our simulations, the proportion of the wild-type adult female population remaining 14 weeks postrelease was a good predictor in some instances of time to extinction and could lead to shorter and more manageable experiments. In other cases, population reduction after 14 weeks might not be a good predictor of extinction time, but as we show in Appendix B, our model can be used to determine beforehand the scenarios in which population reduction could provide information on extinction time.

Literature on previous field cage experiments indicates that population densities and release ratios used were often determined based on an intuitive feel for what would be logistically reasonable. In our exploration of the cage model, we illustrated why it is critical to design field cage experiments differently depending on goals for specific experiments. Clearly, an experiment aimed at assessing the impact of fitness costs should not use the same release ratio as might be considered optimal in the field because low or mid-range fitness costs may not be detectable when the wild-type population is inundated with FK males. Similarly, long-term field cage experiments may be inappropriate for assessing impacts of releases into low-density populations due to the effects of demographic stochasticity.

Our study of the impact that average lifespan may have on cage extinction time as well as our efforts to compare model output to data collected from laboratory experiments (see Appendix B) highlight the need to collect data on survival, fecundity, and emergence times before using this model to predict or assess experimental results. A detailed study of environment-specific values

for these types of demographic parameters should be carried out before an attempt is made to use this type of model to make predictions.

The modeling exercise presented here was focused on assessment of FK release strategies. The broader message from this work is that for long-term experiments aimed at evaluating population dynamics, simulation models can provide useful insights into substantial resource savings by fine tuning experimental designs to most effectively and efficiently address specific questions.

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# Chapter 3

# Optimizing integrated strategies for controlling the dengue vector, *Aedes aegypti*

## ABSTRACT

Dengue fever, a disease vectored by *Aedes aegypti* mosquitoes, is one of the most important viral diseases. Dengue fever control efforts rely upon control of the vector. Traditional vector control measures include applications of pesticides and removal of containers where eggs are laid. One novel control strategy involves the release of genetically modified Female-Killing (FK) mosquitoes. In this paper, we develop an ordinary differential equations model that describes an *Ae. aegypti* population that is being controlled with an integrated pest management program that includes both FK releases and larval-specific control measures. We utilize an optimal control framework to determine the best combination of the two control strategies for different ranges of costs associated with control measures. With numerical solutions to the optimality system, we compare optimal control releases to constant releases, and we compare the integrated approach to control programs using only one of the two strategies. We show that the optimal strategy is more cost-effective than the constant approach and that the integrated program can lead to more reduction and cost less than a single strategy programs. We use the results from this study to underscore the need for accurate information regarding costs associated with control programs.

## 3.1 Introduction

Dengue fever, a vector-borne viral disease, infects more than 50 million people each year throughout tropical and subtropical regions of the world [1]. Currently, there is neither a licensed vaccine nor a prophylactic drug treatment available to prevent dengue [1, 2]. Control efforts are focused on the principal vector, *Aedes aegypti* [2, 3, 4, 5], an anthropophillic mosquito species that prefers to lay its eggs in artificial containers located in and around homes in urban environments [6, 7, 8]. Traditional methods for controlling *Ae. aegypti* include applications of pesticides and source reduction (i.e., the removal of sites where eggs are laid).

Both pesticide application and source reduction are effective at reducing *Ae. aegypti* population densities; however, both require ongoing implementation and community participation in order to maintain low densities of the vector [8]. Furthermore, although research has produced pesticides that are low in toxicity, some can still be harmful to the environment, other species, and human health [9], and the mosquito species can develop resistance to the pesticides after long-term exposure [6, 10]. Motivated by these and other issues, much attention has been focused on the development of novel methods for controlling *Ae. aegypti* [11], which include genetic pest management(GPM) strategies.

GPM strategies involve the release of genetically modified (i.e. transgenic) mosquitoes to reduce or eliminate native mosquito populations or replace a native population with one that cannot transmit disease [12, 13]. GPM strategies have been studied for several decades following the success of the Sterile Insect Technique (SIT) in eliminating populations of the screwworm fly from North and Central America [14]. In SIT, sterilized males are released that compete with wild-type males for mates, and females that mate with sterile males do not produce offspring [15]. Although SIT has been successful in some cases, the radiation used to sterilize males typically causes high fitness disadvantages such as decreased mating competitiveness, which has led to research of improved methods for sterilization [12]. Fortunately, molecular technology has developed rapidly in recent decades, which has led to improved GPM approaches [13], including at least one genetic SIT

strategy in which individuals are genetically sterile [16].

One variant of the genetic SIT strategy that has seen tangible progress in *Ae. aegypti* in recent years is female-killing (FK) [17, 18]. In FK strategies, males carrying two copies of a conditional dominant lethal FK gene are released into a wild-type population, and any female that inherits a single copy of the gene will not survive. Causing female-specific lethality is desirable in part because females are the only sex of the species that transmits disease pathogens. Males that inherit the gene are able to survive, and their offspring can inherit the gene, which is thought to make FK strategies more effective than SIT strategies [16, 19]. Conditional lethality of the FK gene allows for mass rearing of insects for releases. For example, FK strains of *Ae. aegypti* have been developed that are reared on a diet containing tetracycline, which deactivates the lethality of the FK gene; however, the offspring of released mosquitoes will not have tetracycline in their diet in the wild, and females inheriting the gene do not survive [20]. Both FK and SIT strategies have been engineered into strains of *Ae. aegypti* [16, 20] and well-studied with models [16, 21, 22, 23, 24].

Recently, lab and field cage tests of one specific FK strategy have shown that population reduction, and in some cases, extinction can occur with repeated releases of FK males [25, 26]. Small scale field releases of strains of *Ae. aegypti* modified to be genetically sterile (genetic SIT) have also led to reductions in wild-type populations [27]. Although FK and genetic SIT show promise for reducing or eliminating wild-type populations of *Ae. aegypti*, the cost associated with maintaining large scale transgenic releases may not be feasible for some regions where dengue is endemic. Furthermore, as with other control measures, community involvement and continuous implementation will be required for FK and similar genetic strategies to succeed in eliminating disease vectors.

Source reduction, pesticides, and trasgenic strategies can each independently lead to reductions in wild-type *Ae. aegypti* populations, yet each of these approaches has shortcomings. One way in which the strengths of each approach can be exploited while compensating for the weaknesses is to combine strategies in an Integrated Pest Management (IPM) program [28]. Such IPM programs have proven successful for other pest species [29, 30, 31], and hold promise for the control of *Ae. aegypti*. A successful IPM would consider the costs associated with each control measure

and aim to produce a cost-effective and sustainable program for maintaining control of the disease vectors.

We develop in this paper a mathematical model to assess the impacts of two different types of control measures: juvenile-specific control (such as larvae-specific pesticides or source reduction) and releases of FK males. We utilize techniques from optimal control theory to assess the roles that resource limitation and financial costs may place in developing strategies for controlling pest species and maintaining an integrated pest control program. We assess each approach individually and in combination with the other approaches, and discuss the potential for each strategy and combination of strategies to lead to a sustainable disease control program.

We remark that similar optimal control studies have been conducted previously for integrated programs for controlling *Ae. aegypti* with SIT. One previous work by Thome and co-authors studied optimal control of an *Ae. aegypti* population with integrated strategies that included releases of SIT males and applications of pesticides [32]. Another more general study by Fister and co-authors focused on combinations of SIT releases and increased mortality caused by habitat modification [33]. To our knowledge, this is the first study to assess optimal IPM approaches involving an FK strategy.

## 3.2 Methods

### General Model

We utilize a system of ordinary differential equations that describes the population dynamics and population genetics of an *Ae. aegypti* population in the presence of two different types of control measures: juvenile-targeted control and releases of homozygous FK males. We divide the population into juveniles (larvae and pupae; egg production is modeled implicitly), adult females, and adult males, and we subdivide adult males and juveniles by genotype. We define $M_g$ to be the density of adult males of genotype $g$, $J_g$ to be the density of juveniles of genotype $g$ and $F$ to be the density of wild-type adult females. We consider late-acting lethality associated with the FK gene

[20, 22], which means that all individuals carrying FK genes will survive through the juvenile stage, but females carrying the gene do not emerge as viable adults. Thus, only wild-type adult females will be viable, so we do not track multiple adult female genotypes. Because only wild-type adult females are viable, we do not need to consider homozygous FK juveniles either. Throughout, we let $g = 1$ denote the wild-type genotype, $g = 2$ the heterozygous FK genotype, and $g = 3$ the homozygous FK genotype.

The rate at which juveniles are born is assumed to be proportional to the number of females in the population: adult females give rise to juveniles at a rate $\lambda F$. The genotypes of juveniles born depend upon the fraction of males of each genotype in the population as well as the mating fitness of each genotype at the time the juveniles are born. Insertion of the FK gene into the mosquito genome could interfere with normal function, causing decreased mating fitness (i.e. mating competitiveness) for males carrying the FK gene. In the model, we assume that mating fitness is decreased by a fitness cost, $c$. Although we could easily adapt the model to consider different types of fitness cost as well as the effects of fitness cost at other life stages, we assume here that males can incur an additive mating fitness cost, and we define the fitness of a male of genotype $g$ relative to wild-type males to be $w_g$. We let the fitness of wild-type males be $w_1 = 1$ and the fitness of heterozygous (one copy of the FK gene) and homozygous (two copies of the FK gene) males is $w_2 = 1-0.5c$ and $w_3 = 1-c$, respectively.

Matings between wild-type females and wild-type males will result in 100% wild-type offspring, matings between wild-type females and heterozygous males will result in 50% wild-type and 50% heterozygous offspring, and matings between wild-type females and homozygous males will result in 100% heterozygous offspring. The proportion of births that are wild-type is

$$\frac{M_1 + 0.5\,w_2 M_2}{M_1 + w_2 M_2 + w_3 M_3}\,, \tag{3.1}$$

and the proportion that are heterozygous is

$$\frac{w_3 M_3 + 0.5 w_2 M_2}{M_1 + w_2 M_2 + w_3 M_3}\,. \tag{3.2}$$

Note again that because we only consider male FK releases, homozygous FK juvenile genotypes are not possible.

Juvenile offspring are subject to density-dependent mortality at a per capita rate $\left(\alpha J^{\beta-1}\right)$, where $J = J_1 + J_2$, as well as density-independent mortality at per capita rate $\mu_J$. Juveniles emerge as adults at a per capita rate $\nu$. Because immigration is known to have an impact on SIT strategies [31, 34], it is assumed that FK strategies could also be impacted by immigration, so we consider the immigration of wild-type juveniles at a rate $\theta$. This models immigration that could occur as a result of movement of containers from one location to another. We do not explicitly model immigration of wild-type adults because our model considers a large population that would likely cover a large area, and adult *Ae. aegypti* typically do not travel very far [35, 36, 37].

We assume an equal sex ratio in the juvenile population so that one-half of emerging wild-type juveniles are males and the other half females. Because we consider late-acting lethality due to the presence of an FK gene, one-half of emerging heterozygous juveniles are males while the other half do not survive. Adult females and males experience mortality at per capita rates $\mu_F$ and $\mu_M$, respectively.

To model control measures in the population, we also consider mortality of juveniles that results from juvenile-specific control by including an additional mortality rate for juveniles. Additional juvenile mortality occurs at a per capita rate $U_J(t)$. Because the rate of additional mortality will depend upon the effort put into control, we assume that the additional mortality rate has an upper bound, $\sigma_J$, and we assume that the relationship between effort and $\sigma_J$ is linear. That is, if more effort is made to control juveniles, a higher maximum additional juvenile mortality rate is achieved.

In addition to control measures aimed specifically at juveniles, we consider the constant release of homozygous FK males. This release occurs at rate $U_{FK}(t)$. We assume that the maximum rate of FK releases is $\sigma_{FK}$, and this maximum rate reflects the effort put into deploying FK mosquitoes. As

with the maximum rate of additional juvenile mortality, we assume a linear relationship between the upper bound on the FK release rate and the level of effort.

The assumptions described above give rise to the following system of equations.

$$
\begin{aligned}
\frac{dJ_1}{dt} &= \lambda F \frac{M_1 + 0.5 w_2 M_2}{M_1 + w_2 M_2 + w_3 M_3} - J_1 [\alpha (J_1 + J_2)]^{\beta - 1} - (\mu_J + U_J(t) + \nu) J_1 + \theta \\
\frac{dJ_2}{dt} &= \lambda F \frac{w_3 M_3 + 0.5 w_2 M_2}{M_1 + w_2 M_2 + w_3 M_3} - J_2 [\alpha (J_1 + J_2)]^{\beta - 1} - (\mu_J + U_J(t) + \nu) J_2 \\
\frac{dF}{dt} &= \frac{1}{2} \nu J_1 - \mu_F F \\
\frac{dM_1}{dt} &= \frac{1}{2} \nu J_1 - \mu_M M_1 \\
\frac{dM_2}{dt} &= \frac{1}{2} \nu J_2 - \mu_M M_2 \\
\frac{dM_3}{dt} &= U_{FK}(t) - \mu_M M_3
\end{aligned}
\tag{3.3}
$$

Because we assume that both $U_J(t)$ and $U_{FK}(t)$ have maximum values, we impose the constraints $0 \leq U_J(t) \leq \sigma_J$ and $0 \leq U_{FK}(t) \leq \sigma_{FK}$. Table 3.1 lists the model state and control variables along with a brief description of each. Table 3.2 lists the parameters as well as default values and units for each parameter.

### Optimal Control Model

Here, we define the controls $U_J(t)$ and $U_{FK}(t)$ to be the time-varying additional juvenile mortality rate and FK male release rate, respectively. We wish to find combinations of FK releases and juvenile control that lead to reduction in the wild-type population at minimal cost. Specifically, we aim to minimize the population of competent vectors (i.e. wild-type adult females) with cost-

**Table 3.1:** States and controls for the optimal control model.

| State | Description |
|---|---|
| $J_1(t)$ | Wild-type juveniles |
| $J_2(t)$ | Heterozygote FK juveniles |
| $F(t)$ | Adult Females |
| $M_1(t)$ | Wild-type adult males |
| $M_2(t)$ | Heterozygote FK adult males |
| $M_3(t)$ | Homozygote FK adult males |
| Control | Description |
| $U_J(t)$ | Rate of effort of implementing larval control |
| $U_{FK}(t)$ | Release rate of homozygote FK males |

effective control implementation during the period of that begins at time $t = 0$ and ends at time $t = T$. That is, we wish to minimize the objective functional given in (3.4).

$$\phi(U_J, U_{FK}) = \int_0^T AF^2(t) + BU_J^2(t) + CU_{FK}^2(t)dt \tag{3.4}$$

Here, $A$ is a coefficient that describes the cost arising from infections that are a result of the competent vector density, and $B$ and $C$ describe the costs associated with larval-specific control and FK releases, respectively. Each of these cost coefficients is defined relative to the others, thus weighting the importance of minimizing each of the three components of the objective functional against one another. For example, if $A = 2$ and $B = 1$, the cost associated with having females in the population would be twice as important to minimze than that of juvenile control. For this study, we assume for simplicity that the costs of competent vectors and control are quadratic, but we remark that the functional relationships for the costs of competent vectors and controls should be further explored.

To obtain optimal control strategies, we employ Pontyragin's Maximum Principle [38]. We first introduce adjoint equations to append the cost function described in Equation 3.4 to System 3.3. We define $Y = (J_1, J_2, F, M_1, M_2, M_3)$ to be the vector of state variables, $U = (U_J, U_{FK})$ to be the vector of controls, and $P = (p_1, p_2, p_3, p_4, p_5, p_6)$ to be the vector of adjoint variables. We form the Hamiltonian, $H(Y, U, t)$, as

**Table 3.2:** Parameters for the optimal control model.

| Parameter | Description | Default Value | Units |
|---|---|---|---|
| $\lambda$ | Per capita rate of larval production by females | 8 | $\text{day}^{-1}$ |
| $\alpha$ | Density dependent parameter | 0.002 | $\text{juveniles}^{1-\beta} \cdot \text{day}^{-\frac{1}{\beta-1}}$ |
| $\beta$ | Density dependence exponent | 3.4 | unitless |
| $\mu_J$ | Per capita mortality rate for juveniles | 0.03 | $\text{day}^{-1}$ |
| $\mu_F$ | Per capita mortality rate for adult females | 0.10 | $\text{day}^{-1}$ |
| $\mu_M$ | Per capita mortality rate for adult males | 0.28 | $\text{day}^{-1}$ |
| $\nu$ | Per capita rate of emergence of juveniles to adults | 0.14 | $\text{day}^{-1}$ |
| $\theta$ | Rate of immigration of wild-type juveniles | 0 | juveniles / day |
| $w_i$ | Mating fitness of males of genotype $i$ relative to wild-type males | 1 | unitless |
| $c$ | Mating fitness cost associated with the FK gene | 0 | unitless |
| $\sigma_J$ | Maximum rate of additional juvenile mortality | 50 | $\text{day}^{-1}$ |
| $\sigma_{FK}$ | Maximum rate of FK releases | 1000 | adult males / day |
| $A$ | Relative cost associated with competent vectors | 1 | $\text{cost} / \text{female}^2$ |
| $B$ | Relative cost associated with juvenile-specific control | 1 | $\text{cost} \cdot \text{day}^2$ |
| $C$ | Relative cost associated with FK releases | 1 | $\text{day}^2 \cdot \text{cost} / (\text{adult males})^2$ |

$$
\begin{aligned}
H(Y,U,t) = {} & AF^2 + BU_J^2(t) + CU_{FK}^2(t) \\
& + p_1\left(\lambda F\frac{M_1 + 0.5w_2M_2}{M_1 + w_2M_2 + w_3M_3} - J_1(\alpha J)^{\beta-1} - (\mu_J + U_J(t) + \nu)J_1 + \theta\right) \\
& + p_2\left(\lambda F\frac{w_3M_3 + 0.5w_2M_2}{M_1 + w_2M_2 + w_3M_3} - J_2(\alpha J)^{\beta-1} - (\mu_J + U_J(t) + \nu)J_2\right) \\
& + p_3\left(0.5\nu J_1 - \mu_F F\right) + p_4\left(0.5\nu J_1 - \mu_M M_1\right) \\
& + p_5\left(0.5\nu J_2 - \mu_M M_2\right) + p_6\left(U_{FK} - \mu_M M_3\right).
\end{aligned} \tag{3.5}
$$

For optimal $U^*$ and corresponding optimal state $Y^*$, there exists $P^*$ that satisfies the following adjoint equations, obtained by taking $\frac{\partial H}{\partial Y} = -\frac{dP}{dt}$.

$$
\begin{aligned}
\frac{dp_1}{dt} &= -p_1\left(-(\alpha(J_1+J_2))^{\beta-1} - X - \mu_J - U_J - \nu\right) + p_2X - 0.5\nu\left(p_3 + p_4\right) \\
\frac{dp_2}{dt} &= \left(p_1J_1X - p_2J_2X - \mu_J - U_J - \nu\right) - 0.5p_5\nu \\
\frac{dp_3}{dt} &= -2AF - p_1\lambda\frac{Z_1}{W} - p_2\lambda\frac{Z_2}{W} + p_3\mu_F \\
\frac{dp_4}{dt} &= -p_1\left(\frac{\lambda F}{W} - \frac{\lambda FZ_1w_1}{W^2}\right) + \frac{p_2\lambda FZ_2w_1}{W^2} + p_4\mu_M \\
\frac{dp_5}{dt} &= -p_1\left(0.5\frac{\lambda Fw_2}{W} - \frac{\lambda FZ_1w_2}{W^2}\right) - p_2\left(0.5\frac{\lambda Fw_2}{W} - \frac{\lambda FZ_2w_2}{W^2}\right) + p_5\mu_M \\
\frac{dp_6}{dt} &= \frac{p_1\lambda FZ_1w_3}{W^2} - p_2\left(\frac{\lambda Fw_3}{W} - \frac{\lambda FZ_2w_3}{W^2}\right) + p_6\mu_M
\end{aligned} \tag{3.6}
$$

Here,

$$X = \frac{J_1(\alpha(J_1+J_2))^{\beta-1}(\beta-1)}{J_1+J_2}$$

$$Z_1 = (M_1 + 0.5w_2M_2)$$

$$Z_2 = (w_3M_3 + 0.5w_2M_2)$$

$$W = w_1M_1 + w_2M_2 + w_3M_3.$$

Since there is no dependence on the state at the final time in the objective functional, $P(T) = 0$, which is the transversality condition. Next, we obtain the optimal control $U^*(t)$ by taking $\frac{\partial H}{\partial U} = 0$.

$$U_J^*(t) = \max\left(0, \min\left(\sigma_J, \frac{p_1(t)J_1(t) - p_2(t)J_2(t)}{2B}\right)\right)$$

$$U_{FK}^*(t) = \max\left(0, \min\left(\sigma_{FK}, -\frac{p_6(t)}{2C}\right)\right) \tag{3.7}$$

The state equations, adjoint equations, and control equations form the optimality system, which we solve numerically. To do so, we employ a gradient algorithm described in [39]. This algorithm follows the following steps.

1. Define $s = 1$, $k_{\max}$, and $\epsilon$, where $k_{\max}$ is the maximum number of allowable iterations and $\epsilon$ is the tolerance level that determines convergence of solutions.
2. Guess an initial control trajectory, $U_0(t)$.
3. Solve $Y_0(t)$ forward in time with $U_0(t)$ and calculate $\phi_0(U_0)$.
4. Set $k = 1$. While $k \leq k_{\max}$

(a) Solve $P_k(t)$ backward in time with $Y_{k-1}(t)$ and $U_{k-1}(t)$.

(b) Set $U_k(t) = U_{k-1}(t) - s\left(\frac{\partial H}{\partial U}(Y_{k-1}(t), P_k(t))\right)$.

(c) Solve $Y_k(t)$ forward in time with $U_k(t)$ and calculate $\phi_k(U_k)$.

(d) If $\phi_k(U_k) \geq \phi_{k-1}(U_{k-1})$, set $s = 0.5s$ and return to step 4b

(e) If $\phi_k(U_k) < \phi_{k-1}(U_{k-1})$, then calculate $R = \left|\phi_k(U_k) - \phi_{k-1}(U_{k-1})\right| / \left|\phi_k(U_k)\right|$.

(f) If $R \geq \epsilon$, set $s = 1.5s$ and $k = k+1$ and return to step 4a.

(g) If $R < \epsilon$, the optimal solution has been obtained.

This is an iterative method which approximates $Y(t)$, $P(t)$, and $U(t)$ by $Y_k(t)$, $P_k(t)$, and $U_k(t)$. We use the standard Matlab routine `ode15s` to solve for $Y_k(t)$ and $P_k(t)$. We calculate $\phi_k(U_k)$ with the Matlab routine `trapz`, which utilizes the trapezoidal rule for approximation of the definite integral in (3.4).

## 3.3 Numerical Results

Here we present optimal control solutions for a variety of parameter combinations that lead to different scenarios for control programs that occur over a 100 day period. As this study is primarily concerned with understanding the general effects of parameter values on optimal control solutions, we choose for the relative cost parameters the default values $A = 1$, $B = 1$, and $C = 1$. The default values for all parameters are listed in Table 3.2. In a more detailed study aimed at understanding the financial effects of a control program, the values for the relative cost should be chosen to reflect the financial cost associated with each component of the objective functional if such data is available.

### Constant versus Optimal Control

We compared an optimal control solution to solutions obtained from maintaining constant control rates. For each comparison, we set the constant control rates to be equal to the average

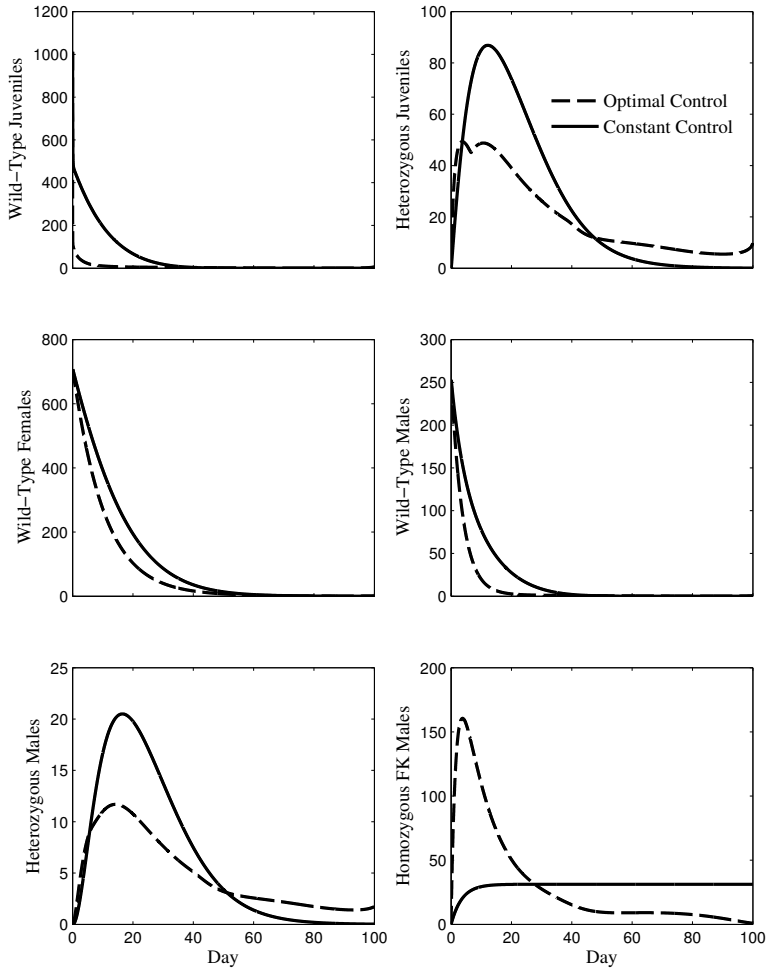


**Figure 3.1:** Outcome of an optimal control (dashed lines) strategy compared against a constant control (solid lines) strategy. Population dynamics of wild-type and heterozygous juveniles (top row), wild-type adult females and adult males (middle row), and heterozygous and homozygous adult males (bottom row). All parameters are the default values given in Table 3.2.

value of the optimal control rate. In this case, when the three components of objective functional (3.4) are weighted equally, the constant control rates lead to more reduction in the population size; however the total cost is more expensive (Figures 3.1 and 3.2). The constant control strategy did not change as the population decreased and this strategy ultimately led to a lower density of the wild-type population than the optimal control strategy. The controls in the optimal strategy began at values much higher than the average value utilized in the constant control scenario, but decreased with time. We observed this general shape for the additional juvenile mortality rate and the FK

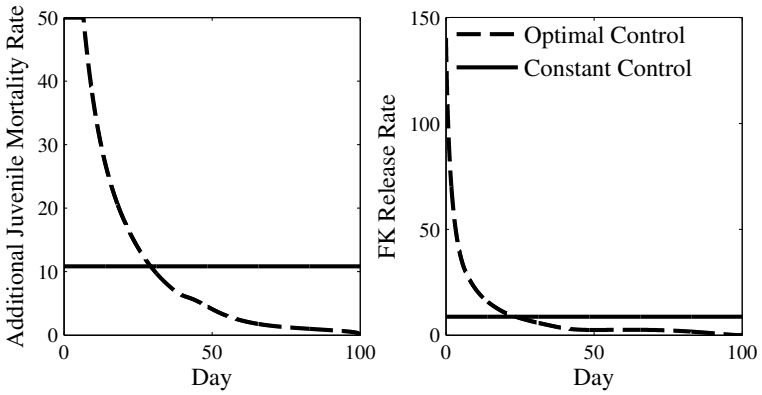


**Figure 3.2:** Control trajectories during constant and optimal control strategies. The control trajectories when the control strategy is optimal (dashed line) or constant (solid line). All parameters are the default values given in Table 3.2.

release rate for many of the remaining scenarios that we considered. That the constant strategy led to more reduction at the end of the time interval than the optimal strategy is in part an artifact of the finite-time horizon of the optimal control problem: as the end of the time period nears, the optimal controls go toward zero because control efforts near the end of the time interval do not have much influence on the dynamics, but do have costs of implementation.

The constant strategy resulted in a lower cost associated with wild-type females in the population; however, significant costs were accrued from continued control efforts. In the optimal strategy, the controls reacted to decreases in the population: once the wild-type population density fell to a significantly lower level than the pre-release equilibrium, low densities of wild-type females were maintained with less intensive control efforts. Note that Figure 3.1 shows the dynamics for wild-type and heterozygous juveniles, wild-type females, and wild-type, heterozygous, and homozygous males. Throughout the rest of the paper, we choose to simplify the presentation of results by showing only the wild-type juvenile and female population densities. The female population density in particular is of importance because females are responsible for transmission of disease pathogens.

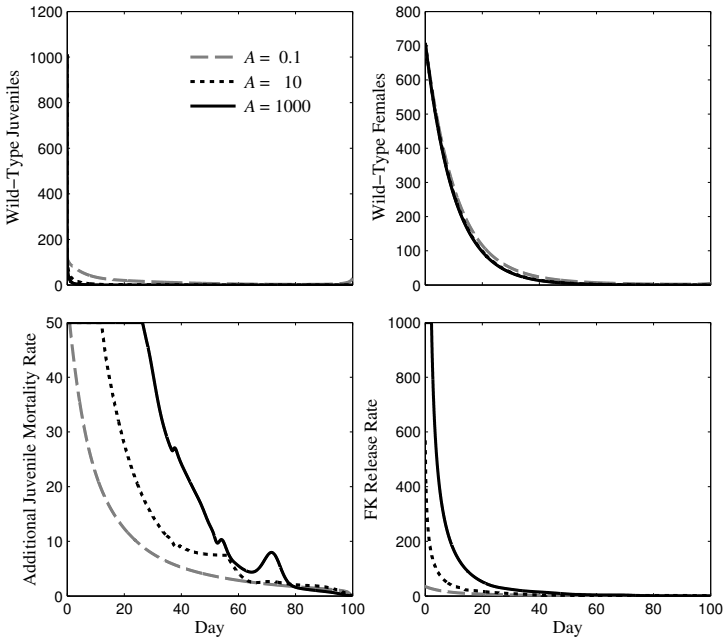


**Figure 3.3:** The effect of the cost associated with wild-type females, $A$. Population dynamics of wild-type juveniles and females (top row) when control is defined by the additional juvenile mortality rate and FK release rate given here (bottom row). All parameters are the default values given in Table 3.2.

## Relative Cost Parameters

We study the influence of relative costs on optimal control solutions by varying each of the parameters $A, B$, and $C$. Of each of these three parameters, changes in $A$ had the most profound effect on optimal control dynamics. When $A$ was small ($A = 0.1$), the additional juvenile mortality rate began at the maximum value and declined with time (Figure 3.3). The FK release rate also declined, although the initial value was well below the maximum release rate. The juvenile population declined rapidly from the initial value, and the wild-type female population declined at approximately an exponential rate. For larger values of $A$, this general behavior was observed, although the initial FK release rate increased towards the maximum ($\sigma_{FK} = 1000$) as did the initial value of the additional juvenile mortality rate ($\sigma_J = 50$). These rates were held at the maximum values for longer intervals with larger values of $A$. For the additional juvenile mortality rate, the optimal solution changed its qualitative form for very high values of $A$, (e.g. $A = 1000$). Instead of a somewhat

exponential decrease, the additional juvenile mortality rate decreased for a short time interval, but then briefly increased again before decreasing to zero. The total cost increased approximately linearly with increases in the value of $A$ (Figure 3.6, left panel).

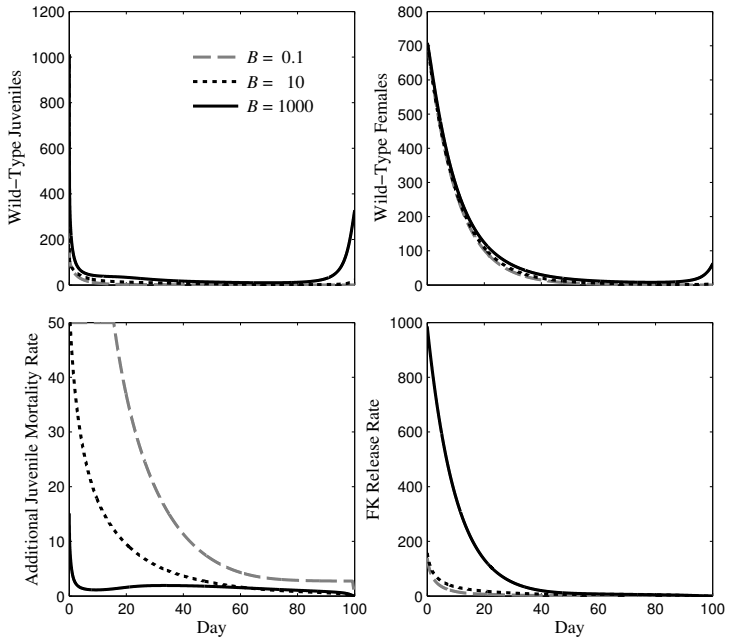


**Figure 3.4:** The effect of the cost associated with additional juvenile mortality, $B$. Population dynamics of wild-type juveniles and females (top row) when control is defined by the additional juvenile mortality rate and FK release rate given here (bottom row). All other parameters are the default values given in Table 3.2.

Increases in the cost associated with juvenile control had a less significant impact on the optimal control solutions. For lower values of $B$, the additional mortality rate began at the maximum value and was maintained there before decreasing (Figure 3.4). As $B$ increased, the control was at the maximum value for shorter intervals before decreasing. For very large values of $B$, the control began at a low value and decreased briefly before increasing to an intermediate value for a large interval of the control period. As $B$ increased, the control program focused more on releases of FK males than juvenile-specific control, with the highest rates of FK releases occurring for the highest values of $B$. For low values of $B$, the total cost was relatively similar, but when $B$ increased beyond

$B = 10$, the total cost increased rapidly with the value of $B$ (Figure 3.6, center panel).

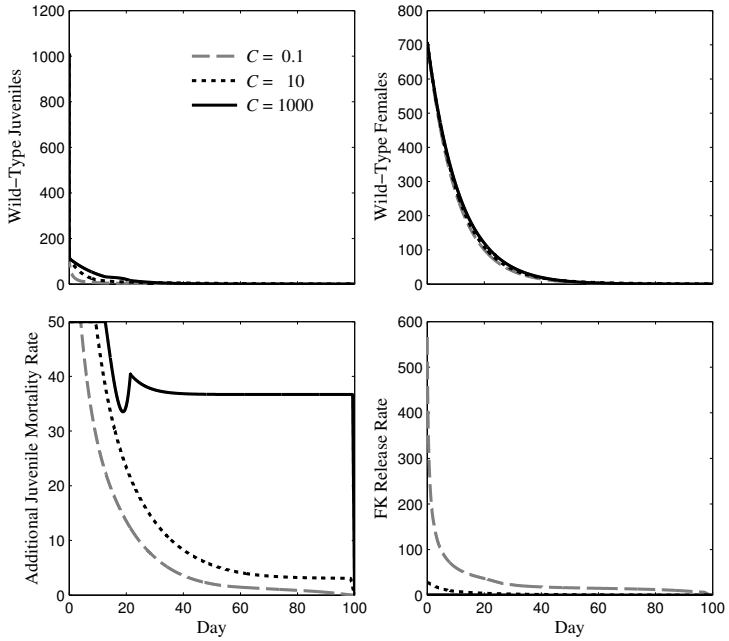


**Figure 3.5:** The effect of the cost associated with the FK release rate, $C$. Population dynamics of wild-type juveniles and females (top row) when control is defined by the additional juvenile mortality rate and FK release rate given here (bottom row). All other parameters are the default values given in Table 3.2.

That the juvenile control rates returned to an intermediate value after initially decreasing when $B$ was high suggests that continuous control efforts must be applied in order to maintain population reduction, regardless of the cost associated with control. The dramatic increase of the total cost of control that occurred when $B$ was increased from $B = 10$ to $B = 100$ reflects the change in the control program that resulted when the cost of juvenile control increased. That is, the cost associated with juvenile control became so high that more effort was put into FK releases, which contributed more to the cost function because the magnitude of FK releases was generally higher than that of the additional juvenile mortality rate. That, combined with the increase in cost associated with juvenile control caused the drastic increase in the total cost as $B$ increased.

Increases in the relative cost associated with FK releases had a significant impact on the optimal

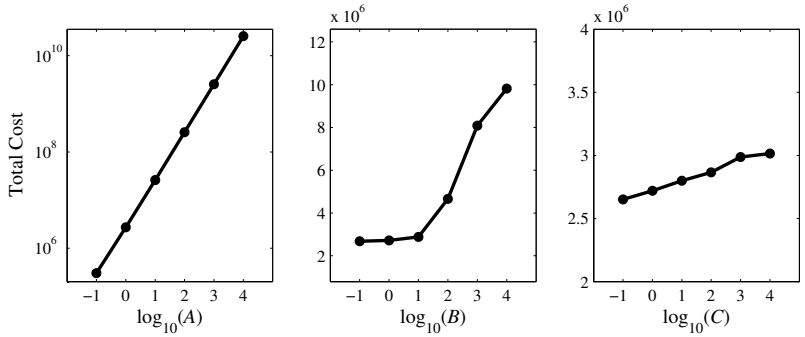


**Figure 3.6:** Total cost when $A$ (left), $B$ (center), and $C$ (right) are varied. Note that for each panel, the horizontal axis is on a log scale, and the vertical axis for the left panel is also on a log scale. All other parameters are the default values given in Table 3.2.

control solutions when $C$ was very high (Figure 3.5). For low values of $C$, the optimal controls for both the additional juvenile mortality rate and the FK release rate decreased exponentially with time. However, when $C$ was greater than $C = 1000$, the additional juvenile mortality rate decreased from the maximum and was maintained at a relatively high value for the duration of the control program. As $C$ increased, more effort was put into additional juvenile mortality than FK releases. The total cost increased slightly with $C$, although the differences were not as profound as when either $A$ or $B$ was increased (Figure 3.6, right panel).

### Single and Integrated Strategies

Finally, we compare the integrated strategy discussed throughout this paper to control strategies involving juvenile-specific control only and FK releases only (Figure 3.7). The integrated strategy led to the most reduction in the wild-type population throughout the duration of the control program and cost less than the other two programs. The juvenile-specific control only and FK release only programs cost about 7% and 96% more, respectively, than the integrated program. With the parameter values considered here, the FK release program cost about 84% more than the juvenile-specific control program. In the absence of juvenile-specific control, far more FK males had to be released to achieve population reduction similar to that observed when FK releases were combined with juvenile control. These results will differ depending on the relative costs associated

with control.

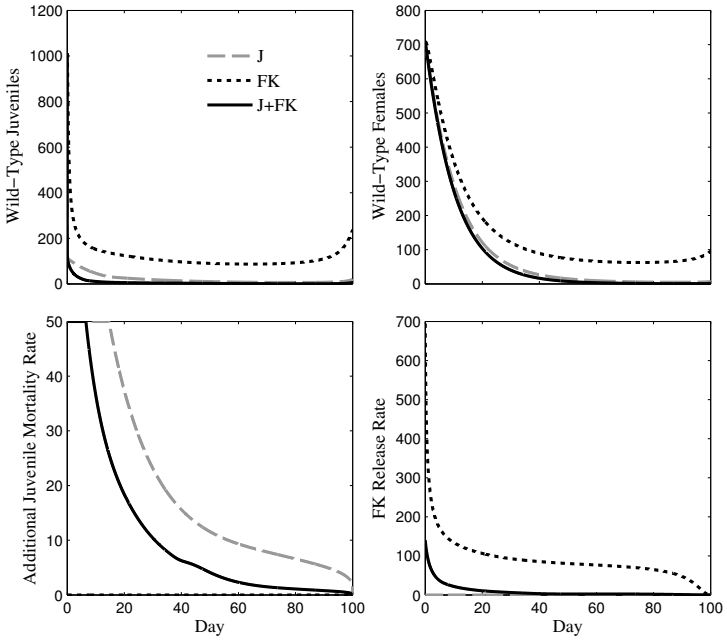


**Figure 3.7:** Population and control dynamics resulting from single and integrated control measures. Population dynamics of wild-type juveniles and females (top row) resulting from single and integrated controls strategies (bottom row). Here, we consider three control programs: juvenile-specific control (J, gray dashed line), FK releases (FK, dotted black line), and juvenile control combined with FK releases (J+FK, solid line). All parameters are the default values given in Table 3.2.

## 3.4 Discussion

In this paper, we presented a general study aimed at assessing optimal integrated control programs designed to reduce an *Ae. aegypti* population for a variety of scenarios. We found that changes in values within a biologically reasonable range for parameters such as fitness cost ($c$) and the strength of density dependence ($\beta$) did not lead to significant differences in optimal control strategies (results not shown). Although these results suggest that some optimal control strategies could be robust to parameter changes, we stress that further exploration with a model that accounts for realistic costs associated with control and more detail in biology is needed before a model of this

type could be used to inform control policy. In fact, we showed that the cost associated with competent vectors and controls can have a significant impact on the control program, which stresses the need to develop a more thorough understanding of the costs of control measures and the costs that result from having competent vectors in the population.

When we compared an optimal control strategy against a constant control strategy, we found that the constant strategy led to more reduction in the wild-type population, but resulted in a higher cost of control than the optimal control strategy. Many previous studies for FK and SIT have focused primarily on constant releases, and suggest that constantly releasing FK or SIT mosquitoes is a feasible approach to controlling the wild-type population. The constant control did provide significant reduction in the population; however, a more thorough cost-benefit analysis should be conducted to determine whether the additional reduction caused by the constant control compared to the optimal control is worth the additional cost.

Finally, we showed that the integrated control program led to more reduction in competent vectors at a lower cost than control programs that focused solely on FK releases or juvenile-specific measures. These results are dependent upon the costs associated with control, and, importantly, do not take into account fixed costs that may be associated with control such as the baseline cost of managing a rearing facility for FK mosquitoes. Although such additional costs would not change with the number of FK mosquitoes being released, they would have a large impact on the total cost. The results here do, however, provide an anecdote for theory in previous literature that suggests that an IPM approach will likely lead to better and more sustainable control of the pest population than single approaches [8, 28]. While this study only considered a combination of two control measures, one could design a study that combines many of the feasible approaches available for control of disease vectors.

In this paper, we assumed high maximum values for both additional juvenile control and FK releases. In particular, the maximum value of juvenile-specific control represents an optimistic situation in which extremely high rates of mortality would result from control implementation. While a carefully managed and well-funded control campaign could possibly achieve such high rates of

mortality, it is unlikely, and further exploration of this model should include contraints imposed on this control that better mimic mortality rates that are more plausible. The FK release rates observed in this study are more plausible given that large numbers of FK mosquitoes can be continuously reared relatively easily, although a more thorough study would consider the effects of stricter constraints on FK releases as well.

Throughout, we emphasized that this is a first-pass study aimed at providing a general qualitative overview of designing IPM strategies with optimal control theory. Indeed, this study brings to attention information that is lacking but necessary for models such as these to be useful for informing control policy, such as the expected control costs, functional forms and other effects of density dependence, potential fitness disadvantages of modified insects, and resources needed in order to implement an effective control program. While some of the data needed will be difficult to collect, the effort to do so could have important effects on control programs and could lead to cost-effective control measures that could be implemented in areas that are incapable of making significant financial investments. Cost-effective control programs will likely be more sustainable in the long-term and thus would have a lasting impact on disease cases.

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# Chapter 4

# A Reduce and Replace strategy for suppressing vector-borne diseases: insights from a deterministic model [1]

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## ABSTRACT

Genetic approaches for controlling disease vectors have aimed either to reduce wild-type populations or to replace wild-type populations with insects that cannot transmit pathogens. Here, we propose a Reduce and Replace (R&R) strategy in which released insects have both female-killing and anti-pathogen genes. We develop a mathematical model to numerically explore release strategies involving an R&R strain of the dengue vector *Aedes aegypti*. We show that repeated R&R releases may lead to a temporary decrease in mosquito population density and, in the absence of fitness costs associated with the anti-pathogen gene, a long-term decrease in competent vector population density. We find that R&R releases more rapidly reduce the transient and long-term competent vector densities than female-killing releases alone. We show that releases including R&R females lead to greater reduction in competent vector density than male-only releases. The magnitude of reduction in total and competent vectors depends upon the release ratio, release duration, and whether females are included in releases. Even when the anti-pathogen allele has a fitness cost, R&R releases lead to greater reduction in competent vectors than female-killing releases during the release period; however, continued releases are needed to maintain low density of competent vectors long-term. We discuss the results of the model as motivation for more detailed studies of R&R strategies.

## 4.1 Introduction

In recent decades, a number of vector-borne diseases have experienced a global resurgence due to changes in disease management strategies, development of insecticide and drug resistance, changes in social behaviors, pathogen evolution, and other factors [1,2]. For some vector-borne diseases there are no vaccines or prophylactic drugs. This leaves vector control as the primary method for disease suppression [3,4]. Traditional forms of vector control, such as source reduction and insecticide treatments, have sometimes been successful at reducing vector densities, but it is difficult to maintain these control programs indefinitely, and despite widespread applications of such programs, vector-borne diseases remain endemic in many regions of the world [1,5-7].

A number of alternative methods of vector control have been proposed, including genetic pest management (GPM) approaches that aim to either reduce or eliminate the vector population or replace the native population with insects that cannot transmit a pathogen [8,9]. Genetic constructs have been proposed and explored theoretically for either reduction [10-14] or replacement [15-20] strategies, and the transgenes necessary for both types of strategies have been developed for some species [21-24]. GPM approaches are being considered for a number of disease vectors, but one species for which there has been tangible progress is *Aedes aegypti*, the principal vector of dengue fever [25]. For this reason, we focus on the *Ae. aegypti* system throughout this paper.

One population reduction strategy that has been built and tested in *Ae. aegypti* is based on transgenes that cause female-specific mortality [21,26,27]. In order to rear large numbers of the transgenic mosquitoes for releases, the transgene that codes for lethality is engineered to have conditional expression [12,14]. In one current transgenic strain of *Ae. aegypti*, the lethal transgenes are repressed when mosquitoes are reared on a diet containing tetracycline [21]. In the absence of tetracycline, females that inherit the transgene are incapable of surviving to reproduce, while males inheriting the gene will survive, and their offspring can inherit the female-specific lethality gene. Repeated releases of this *Ae. aegypti* Female-Killing (FK) strain into large laboratory cages with wild-type *Ae. aegypti* populations resulted in extinctions [26]. In a similar experiment in out-

door cages in Mexico there was a reduction in mosquito density but extinction did not occur [27].

Several mathematical models have been developed to assess the feasibility of FK strategies. Simple models predict that repeated releases of FK mosquitoes into wild-type populations will cause extinction in a time frame of about 1-2 years under ideal conditions [28]. Because males pass on the FK gene to their offspring, this strategy is expected to be more effective in reducing a population than strategies involving mortality of all offspring (e.g., the classical Sterile Insect Technique) [29,30]. More detailed models demonstrate that biological complexities not addressed by the cage experiments, particularly density-dependent population regulation and spatial heterogeneity, can affect the success of FK and other population reduction strategies [13,29-34].

A failure of FK strategies to entirely eliminate a native population of disease vectors could have severe economic and public health consequences. Maintenance of the wild-type population at low levels would require the continuous production and release of mosquitoes. If releases were stopped after a number of years, the vector population would recover, potentially causing a severe epidemic in a human population lacking herd immunity.

Until now, most proposed transgenic strategies have focused on either vector reduction or vector replacement. In this paper, we propose a Reduce and Replace (R&R) strategy in which released insects have both an FK gene and an anti-pathogen (AP) gene. We theoretically assess the efficacy of potential R&R release scenarios with a system of ordinary differential equations that models both the population dynamics and population genetics of an *Ae. aegypti* population. We model R&R releases in a population for which vector elimination is difficult due to the strength of density-dependent larval population regulation. Because elimination in such a population is unlikely, it would be an ideal candidate for an R&R strategy. We show that it would be possible to reduce a population in a realistic time frame while ensuring that, when releases end, the reestablished population would have a low frequency of competent vectors.

## 4.2 Methods

The R&R strain we consider in this paper has one FK allele and one AP allele located on two different chromosomes. We track modified and wild-type alleles ('K' and 'k' for FK, 'A' and 'a' for AP, respectively) at two independently segregating loci, which results in nine possible genotypes (Table 4.1). We divide the population into juveniles, adult males, and adult females. The juvenile class includes larvae and pupae in one class; egg production is modeled implicitly. Each of the three classes is further divided by genotype. We let $J_i(t)$, $M_i(t)$, and $F_i(t)$ represent the density of juveniles, adult males, and adult females, respectively, of genotype $i$ at time $t$. Our model tracks matings between adults, births of juveniles, adult emergence, and deaths.

We assume random mating between adult males and females and that inheritance is Mendelian. The rate at which females produce viable larvae of genotype $i$ is given by $B_i(t)$, where

$$B_i(t) = w_i \sum_m F_m(t)\lambda \sum_n Pr(i|m,n)\frac{M_n(t)}{\sum_g M_g(t)} \tag{4.1}$$

Here, $\lambda$ is the per capita rate at which females produce larvae, $Pr(i|m,n)$ is the Mendelian probability that an offspring of genotype $i$ arises from a mating between a female of genotype $m$ and a male of genotype $n$, and $w_i$ is the fitness of an offspring of genotype $i$ relative to that of wild-type offspring (fitness is defined here as the fraction of eggs that survive and hatch into larvae). Throughout, we assume that fitness costs are additive at a given locus and multiplicative across loci. We define the fitness cost associated with an individual that is homozygous for the FK allele to be $c_K$ and for the AP allele to be $c_A$. The resulting fitness values for each genotype are listed in Table 4.1. Although we assume additive fitness costs that reduce egg viability, the model can easily be adapted to consider other types of fitness costs (e.g., dominant or recessive), as well as fitness disadvantages at other life stages (e.g., mating or adult viability).

Juvenile mortality is assumed to have both density-independent and density-dependent components, represented as per capita mortality rates $\mu_J$ and $(\alpha J)^{\beta-1}$, respectively. Here $J$ is the total density of juveniles, and $\alpha$ and $\beta$ are parameters that determine the strength of density-dependent

**Table 4.1:** Properties of genotypes resulting from R&R releases. Each possible genotype resulting from R&R releases is listed with corresponding fitness values ($w_i$) and female viability coefficients ($\gamma_i$). *These females are, however, viable when released as adults due to conditional lethality.

| $i$ | Genotype | $w_i$ | $\gamma_i$ |
|---|---|---|---|
| 1 | KKAA | $(1-c_A)(1-c_K)$ | 0* |
| 2 | KkAA | $(1-c_A)(1-0.5c_K)$ | 0 |
| 3 | kkAA | $(1-c_A)$ | 1 |
| 4 | KKAa | $(1-0.5c_A)(1-c_K)$ | 0 |
| 5 | KkAa | $(1-0.5c_A)(1-0.5c_K)$ | 0 |
| 6 | kkAa | $(1-0.5c_A)$ | 1 |
| 7 | KKaa | $(1-c_K)$ | 0* |
| 8 | Kkaa | $(1-0.5c_K)$ | 0 |
| 9 | kkaa | 1 | 1 |

mortality, and along with other parameters, the equilibrium population density [35,36]. The strength of density dependence refers to how quickly the population returns to equilibrium after perturbation away from equilibrium density: higher strengths of density-dependent mortality (i.e., larger values of $\beta$), lead to a more rapid rate of recovery. While the model could be altered to consider density-dependent effects in other life stages, we choose to consider only larval density-dependent mortality because of the observed relationship between high-density *Ae. aegypti* larval populations and increased mortality [37-40]. Juveniles emerge as mature adults at a per capita rate, $\nu$. We assume a 1:1 sex ratio at birth so that, in the absence of FK effects, one-half of the juveniles that emerge to adulthood are female and the other half male. Lethality induced by the FK allele is assumed to occur as adults emerge [13,21]. We multiply the rate of emergence of female adults by a binary constant, $\gamma_i$, where, $\gamma_i = 1$ for viable genotypes and $\gamma_i = 0$ otherwise (see Table 4.1). Adult males and viable females die at per capita rates $\mu_M$ and $\mu_F$, respectively. Adult males and females of genotype $i$ are introduced at rates $u_M^i$ and $u_F^i$, respectively. We consider the introductions of two of the listed genotypes: FK only (KKaa, $i = 7$) and R&R (KKAA, $i = 1$). Note that releases of transgenic adult females are possible because of conditional lethality: released females that are fed on a diet containing tetracycline as juveniles do not experience additional mortality due to carrying transgenes (in the absence of fitness costs) [12,14,21].

**Table 4.2:** R&R model parameters. Description of model parameters with default values and references for default values.

| Parameter | Description | Default Value | Reference |
|---|---|---|---|
| $\mu_J$ | Density-independent juvenile mortality rate (per capita) | 0.03 $\text{day}^{-1}$ | [41] |
| $\mu_M$ | Male mortality rate (per capita) | 0.28 $\text{day}^{-1}$ | [42,43] |
| $\mu_F$ | Female mortality rate (per capita) | 0.10 $\text{day}^{-1}$ | [42,43] |
| $\lambda$ | Average rate of larval production by females (per capita) | 8 $\text{day}^{-1}$ | [44,45] |
| $\nu$ | Rate of emergence to adulthood (per capita) | 0.14 $\text{day}^{-1}$ | [42] |
| $\alpha, \beta$ | Density dependence parameters | $2 \times 10^{-4}$, 3.4 | - |
| $c_A$ | Fitness cost associated with anti-pathogen allele | 0 | - |
| $c_K$ | Fitness cost associated with female-killing allele | 0 | - |
| $w_i$ | Fitness of genotype $i$ | See Table 4.1 | - |
| $\gamma_i$ | Female viability coefficient of genotype $i$ | See Table 4.1 | - |
| $r$ | Release ratio of R&R individuals to wild-type males | varied | - |
| $T$ | Duration of release | varied (day) | - |

We simulate releases into a wild-type population that is at equilibrium. Continuous releases of males of genotype $i$ occur at a rate $u_i^M = (rM_9^*)/7$, where $M_9^*$ is the equilibrium wild-type male population density, $r$ is ratio of transgenic adults released each week to the wild-type adult males in the population at the beginning of the release, and the factor 7 converts from a weekly to a daily rate. Continuous releases of females are defined similarly $\left(u_i^F = (rM_9^*)/7\right)$. We note that the same number of transgenic individuals is released each week regardless of changes in the number of individuals in the mosquito population over time.

We obtain the system of ordinary differential equations, wher $i$ represents the genotype of each class.

$$\begin{aligned}
\dot{J}_i &= B_i(t) - \mu_J J_i - J_i\left(\alpha \sum_g J_g\right)^{\beta-1} - \nu J_i \\
\dot{F}_i &= \frac{1}{2}\nu\gamma_i J_i - \mu_F F_i + u_i^F \\
\dot{M}_i &= \frac{1}{2}\nu J_i - \mu_M M_i + u_i^M
\end{aligned} \tag{4.2}$$

Since the only viable female genotypes are those with no FK alleles and released females, we need not track all female genotypes. Table 4.2 lists all model parameters described in this section with their default values.

## 4.3 Results

Due to the complexity of the system, this model does not lend itself to algebraic analysis. We can, however, obtain the wild-type population steady state solutions; we use these values as the initial values in our simulations. For the derivation of these values, we refer the reader to Appendix C. We conducted numerical simulations using the ordinary differential equation solver `ode15s` in Matlab (Version 7.12, Mathworks, Natick, Massachusetts, U.S.A.) to explore the model. We begin by presenting the general dynamics of the R&R system and evaluating the long-term impact of R&R

releases compared to FK releases. We then present a variety of release scenarios that result from varying release ratios and release durations, as well as from considering female-only and bi-sex releases. Finally, we assess the effects of fitness costs associated with the AP gene.

Because an R&R strategy seeks to decrease the number of disease cases, we are specifically interested in the effects that releases have on the overall density of adult females capable of vectoring the pathogen (hereafter referred to as competent vectors). We present the trajectories of the density of all adult females and competent vectors relative to the initial density of the adult female population. We also present time series for the frequencies of the FK and AP alleles in the juvenile population. Illustrating allele frequency dynamics in the juvenile population allows us to disentangle the contribution of released individuals in the adult population where allele frequencies are highly elevated during releases.

### Dynamics of the R&R System

We first considered a year-long release of R&R males. Male-only releases of GPM strains are generally preferred because released males do not contribute to additional population growth nor can they contribute to disease transmission [46]. For each of four different release ratios ($r = 1$, $r = 2$, $r = 3$, $r = 4$), once releases of R&R males began, the density of adult females decreased, with the density of competent vectors decreasing at a more rapid rate than that of total females. This more rapid decrease of competent vectors was due to the increase in the AP allele frequency (Figure 4.1). When releases ended, the female population density recovered to the pre-release size, but the competent vectors, even after increasing slightly in density once releases ended, remained at a negligibly low density (Figure 4.1A). The juvenile FK allele frequency increased once releases began, but the FK allele was removed from the population after releases of R&R mosquitoes ended (Figure 4.1B shows allele frequencies for the case when $r = 2$). The long-term frequency of the AP allele depended upon the release ratio as well as the duration of release.

As the release ratio increased, the reduction in the adult female population density during releases increased. However, the marginal long-term reduction in competent females resulting from

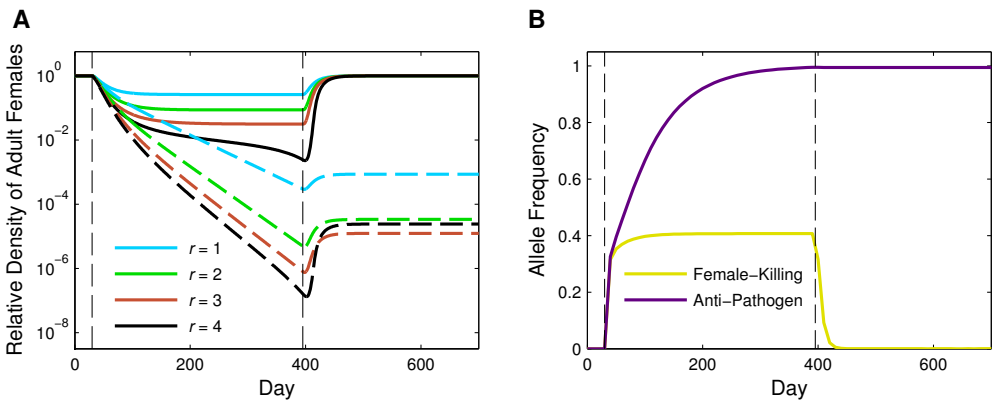


**Figure 4.1:** General R&R dynamics. Dynamics of an *Ae. aegypti* population when continuous male-only R&R releases occur for one year ($T = 365$). (A) Relative female population density for releases occurring at four different release ratios. Dashed lines indicate the relative density of competent vectors, and solid lines represent the relative density of the total adult female population. Solid lines also indicate the relative density of the total (and thus competent) adult female population during FK releases. Density is relative to the density of the total adult female population before releases begin. Note the vertical axis is on a log scale. (B) Juvenile FK and AP allele frequencies for a 2:1 ($r = 2$) release. For both panels, the first vertical dashed line represents the first day of release (30) and the second vertical dashed line represents the last day of release (395). All other parameter values are the default values listed in Table 4.2.

increasing the release ratio declined as this ratio increased, with high release ratios eventually resulting in higher densities of competent vectors than lower release ratios. For example, there was a greater difference in the reduction of competent vector density (log scale) between $r = 2$ and $r = 1$ than there is between $r = 3$ and $r = 2$, and for $r = 4$, the long-term density of competent vectors was actually higher than when $r = 3$ (Figure 4.1A). At high release ratios, the population was inundated with R&R males, and once the population was reduced, the FK and AP genes were almost always inherited together, resulting in very high linkage disequilibrium. Even if the competent vector population density was very low at the end of releases, some of the few females that remained in the population were likely to be competent vectors because most of the females that inherited the AP gene also inherited the FK gene.

In the absence of fitness costs associated with the AP allele, an R&R release had the same impact on total population density as the corresponding FK release. In the latter, however, all adult females were competent vectors: the impact of FK on competent vectors was identical to the impact of R&R

on the total number of adult females (solid lines in Figure 4.1A). Consequently, an R&R release had a more rapid and longer lasting impact on the competent vector population than the corresponding FK release (compare solid and dashed lines in Figure 4.1A).

### Release Ratio and Duration

We next considered releasing R&R mosquitoes for different periods of time. For each duration ($T = 120$, $T = 240$, $T = 360$), we simulated releases at a 2:1 weekly release ratio. As the duration of the release increased, the density of competent vectors remaining when releases ended was lower (Figure 4.2); however, the reduction in total adult female population density was the same throughout the release period. This was due to the effects of density dependence. Because density dependence was strong, releases at a 2:1 release ratio, regardless of the duration of the release, could not drive the population to extinction and instead reduced the population to a new intermediate equilibrium density. For a weaker form of density dependence (or a higher release ratio), the total female population density may not have this intermediate equilibrium (see Appendix D and Figure D.1).

Thus far, we have shown that the magnitude of reduction of total and competent female population densities depended upon the release ratio and release duration. In each of the previous cases, a different total number of R&R individuals was released depending on the combination of release ratio and release duration. We examined the reduction in competent and total female densities for different combinations of release ratio and duration that resulted in the same total number of R&R mosquitoes being released. Releases ranged from $r = 4$ for 20 days to $r = 0.16$ for 500 days, each totaling $80M_9^*/7$ transgenic males. We measured the competent vector density once the total population returned to the pre-release density (Figure 4.3a) and the total adult female population density (Figure 4.3b), at the time at which the total female population density reached a minimum (note the minima in the curves in Figures 4.1 and 4.2). Scenarios in which male-only releases were conducted over longer periods resulted in greater reductions of competent vector population densities than shorter, more intense, releases (blue lines, Figure 4.3A). The reduction in the density of the total female population was greatest for release durations and ratios that were intermediate

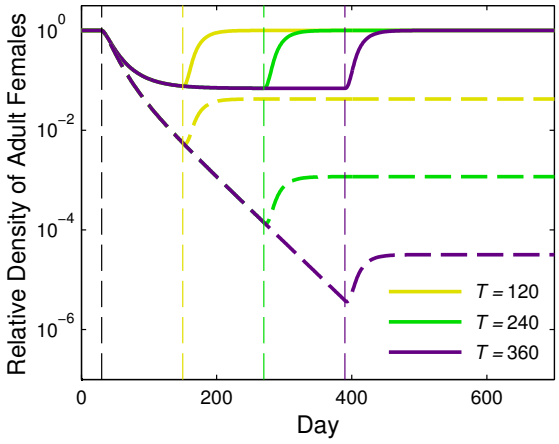


**Figure 4.2:** The effects of release duration on R&R releases. Relative adult female population density when continuous male-only R&R releases occur at a 2:1 ($r = 2$) release ratio for different release durations. Dashed lines indicate the relative density of the competent vectors, and solid lines indicate the relative density of the total adult female population. Each release begins on day 30, and release durations are $T = 120$, $T = 240$, and $T = 360$ days. The black vertical dashed line marks the beginning of releases, and the end of each release is indicated by a vertical dashed line of corresponding color. All other parameter values are the default values listed in Table 4.2. Note that the vertical axis is on a log scale.

among the combinations we considered (blue lines, Figure 4.3B). The release ratio and duration at which the intermediate optimum occurs will depend upon the strength of density dependence (see Appendix D and Figure D.2).

### Releases including Females

Because R&R females are assumed to be incapable of transmitting disease, we considered the option of bi-sex or female-only releases and compared the results to male-only releases. Here, we simulated male-only, bi-sex, and female-only releases for $T = 100$ days at a release ratio of $r = 1$. If carried out for longer periods of time, bi-sex or female-only releases at this release ratio typically led towards population extinctions (see Appendix D Figure D.1). For the bi-sex case, the releases were of the same total number of individuals as in the single-sex releases, but half of the released individuals were female and half were male. In general, female-only releases resulted in the greatest reduction in the competent vector population density (Figure 4.4A). Female-only releases also led

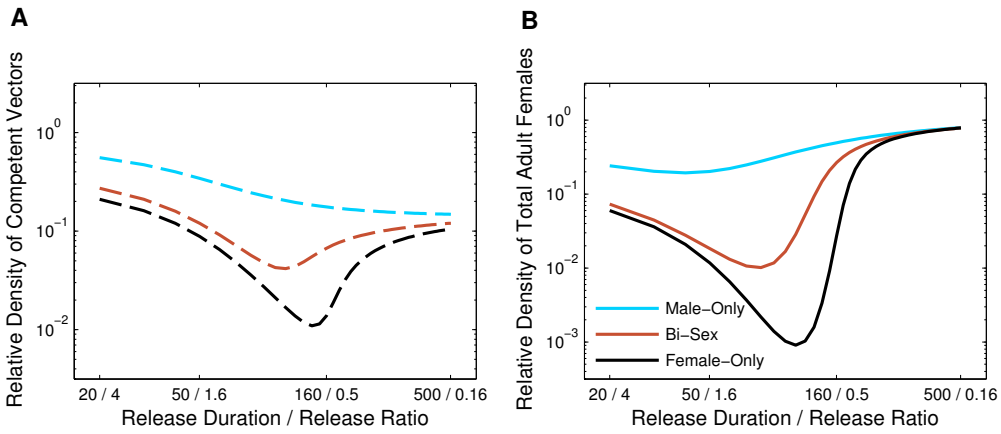


**Figure 4.3:** The effects of release ratio and release duration on R&R releases. Relative adult female population density following releases of R&R individuals with release scenarios involving different combinations of release ratios and durations. (A) Relative density of competent vectors is measured once the total population returns to its pre-release density following male-only, bi-sex, and female-only releases. (B) Minimum relative density of total adult females (not including released females) is measured on the day in which the minimum occurs for corresponding release scenarios. The horizontal axis for both panels is labeled as release duration / release ratio, with release durations increasing from left to right but release ratios decreasing from left to right. Each scenario results in the release of the same total number of mosquitoes. All other parameter values are the default values listed in Table 4.2. Note the both axes are on a log scale.

to the most reduction in the total adult female population density during the transient period (i.e., between the time releases began and the time when the total population was near equilibrium density again), even though when releases first began the total female population density increased noticeably due to the introduction of additional females.

When we compared male-only, female-only, and bi-sex releases for release scenarios that arise from different combinations of release ratios and durations with the same total number of released mosquitoes (as in section 4.3B), we found that for all combinations considered, female-only releases were the most effective at reducing the density of competent and total adult females; however, as R&R individuals were released for longer periods of time (fewer mosquitoes per release), the differences in the impacts of the three different types of releases became less noticeable (Figure 4.3). The combination of release duration and release ratio that resulted in the maximum reduction in total adult female density differed for each release type (Figure 4.3B). As with male-only releases,

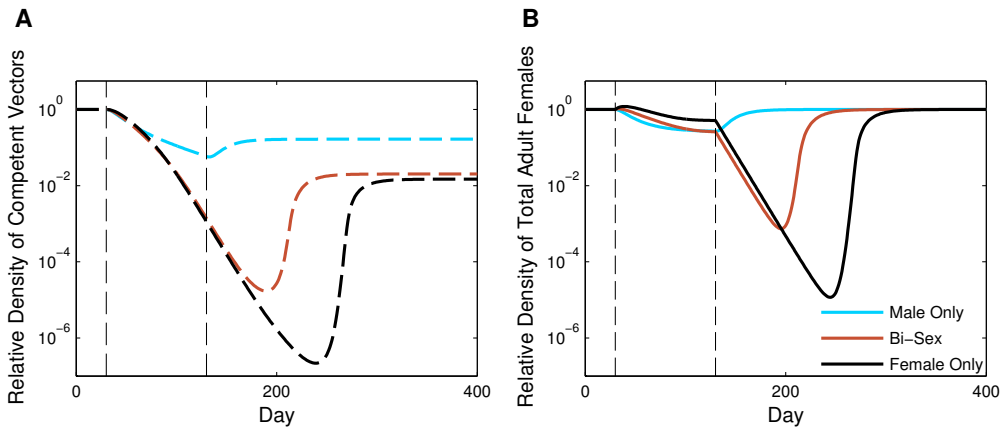


**Figure 4.4:** The effects of R&R releases including females. The relative female population density when releases are conducted at a 1:1 ($r = 1$) release ratio for $T = 100$ days with releases of only males, males and females, and females only. (A) Relative density of competent vectors. (B) Relative density of total adult females (including released females). All other parameter values are the default values listed in Table 4.2. Note the vertical axis is on a log scale.

the intermediate optimum for each release type resulted from the trade-off between the release ratio and density dependence effects. Bi-sex and female-only releases, however, had intermediate optimums for longer, less intense releases because releases including females had a stronger impact at lower release ratios than male-only releases. In contrast to male-only releases, intermediate combinations of release ratio and duration for bi-sex and female-only releases led to the greatest reduction in competent vectors as well (Figure 4.3A).

### Effects of Fitness Costs

If fitness costs are associated with carrying the AP gene, it is not expected to remain in the population indefinitely after R&R releases end. We examined the impacts of fitness costs associated with the AP gene by simulating year long male-only R&R releases at a 2:1 release ratio when $c_A = 0.1$ and $c_A = 0.2$. For higher $c_A$, the AP gene was not able to reach as high a frequency and went extinct more quickly. Increased fitness cost of the AP gene also led to less reduction in the competent vector population density, both during releases and after releases ended (Figure 4.5). However, even with $c_A = 0.2$, there was more rapid reduction in competent vectors than seen with releases of FK

only males (compare to the solid green line in Figure 4.1A).

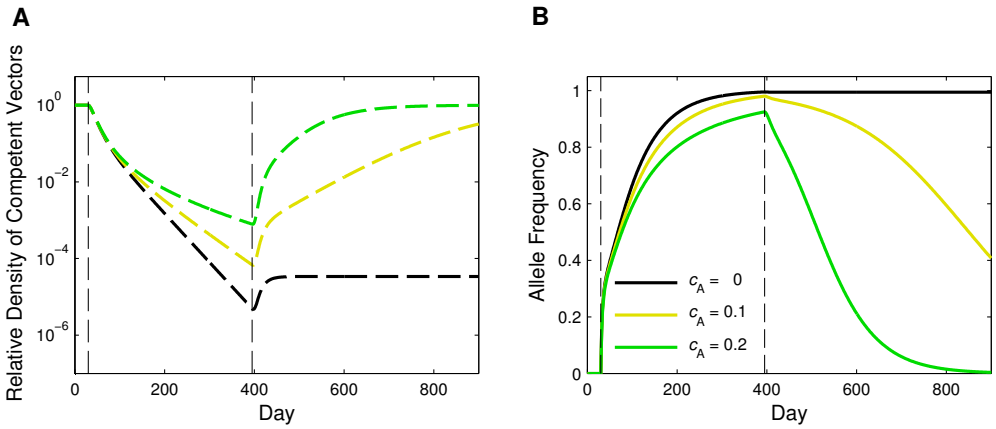


**Figure 4.5:** The effects of fitness cost on R&R releases. Dynamics of an *Ae. aegypti* population subject to continuous male-only R&R releases at a 2:1 ($r = 2$) release ratio for one year ($T = 365$) when there is an additive fitness cost associated with the AP allele. The fitness costs considered here are $c_A = 0$, $c_A = 0.1$ , and $c_A = 0.2$, where $c_A$ is the fitness cost of carrying two AP alleles. (A) Relative density of competent vectors. Note the vertical axis is on a log scale. (B) AP allele frequency in the juvenile population. All other parameter values are the default values listed in Table 4.2.

## 4.4 Discussion

Using a relatively simple deterministic model, we theoretically assessed the utility of an R&R strategy, basing our analysis on hypothetical releases of the transgenic strain into a population of the dengue vector *Ae. aegypti*. We showed that repeated releases of R&R mosquitoes led to reductions in total population density, but more importantly, to both transient and long-term reductions in the density of competent vectors. In fact, within the system modeled, if the release ratio and release duration are chosen carefully, competent female vectors could effectively be eliminated from the population entirely.

We compared the R&R strategy to the FK strategy, in which transgenic mosquitoes carrying only the FK component of the R&R strain were released. We showed that in the absence of fitness costs associated with the AP gene the reduction in the total population was the same for the FK and

R&R strategies throughout releases for equivalent release ratios, but the reduction in total competent vectors was much greater with the R&R strategy during releases as well as following the end of releases. Even when there was a modest fitness cost associated with the AP gene, the transient reduction in competent vectors was greater with R&R than with FK alone. This suggests that for populations in which elimination is not feasible by either strategy, R&R is superior in offering insurance against a resurgent pathogen if a program is less effective at population reduction than planned, or if the cost of sustaining releases indefinitely is not acceptable.

We showed that bi-sex and female-only releases were more effective at reducing densities of females than comparable male-only releases. In bi-sex releases, the first generation of offspring could receive two copies of both the FK and AP alleles. This facilitated both a more rapid increase in the AP allele frequency and a greater decrease in the population than comparable levels of male-only releases. When R&R females were released, whether in bi-sex or female-only releases, they quickly dominated the population of mating females as the FK allele spread in the population so that most offspring were those of the R&R females and had both FK and AP alleles. Moreover, releases of females resulted in increased offspring production, which in turn led to additional larval competition and thus higher rates of larval mortality. The combination of the more rapid rise in the frequency of the FK and AP genes and the increased larval mortality resulted in greater population declines that affected both competent and total female population density than comparable male-only releases.

We showed that while increased ratios and increased durations of R&R male-only releases led to greater reduction in competent vectors, release duration was the more important factor when density dependence was strong. Similar to results seen in previous modeling exercises for other GPM strategies, completely inundating a population with R&R males for a short period of time was not optimal primarily because the number of wild-type females was limited [33,47]. Additionally, releasing at high release ratios could have resulted in linkage disequilibrium when population density was low, causing the FK and AP alleles to be inherited together too frequently. This led to increased mortality of vectors that would otherwise be incompetent, and thus many of the vectors remaining when the total population density was low were competent vectors. We found that the

greatest reduction in the total female population density for male-only, bi-sex, and female-only releases, as well as the greatest reduction in competent vector density for bi-sex and female-only releases, was observed for intermediate combinations of release ratio and duration. This is due to the interaction between density-dependent population regulation and the population reduction caused by the FK gene. High-intensity releases conducted over a short period of time became wasteful because the marginal benefit from releasing more R&R individuals waned as the frequency of the FK allele increased in the population. On the other hand, when low-intensity releases were conducted over a long period of time, each release had less impact because density dependence had time to counteract any population reduction. The long-term density of competent vectors for R&R releases reflected the impact of both FK and AP components of the strain. For the release of a neutral AP only strain (i.e., without the FK component), the ultimate density of competent vectors would decrease monotonically with increasing release duration (A.L. Lloyd, unpublished results).

For the strong density dependence considered in the main text of this study, we observed a non-monotonic pattern in the long-term density of competent vectors following both female-only and bi-sex releases, but a strictly decreasing density with increased release duration for male-only releases. For weaker density dependence, the ultimate densities of competent vectors after male-only releases also exhibited this non-monotonic pattern (refer to Appendix D and Figure D.2).We also found that under conditions of weaker density dependence the R&R releases still caused a more rapid reduction in the density of competent vectors than was seen with comparable FK releases (compare the two panels of Figure D.1). Furthermore, because a population regulated by weak density dependence could not recover from population reduction as quickly as one which is regulated by stronger density dependence, there was a greater reduction in total female population density for populations regulated by weak density dependence as a result of R&R releases (see the right panel of Figure D.1). These results, although obtained from a simple treatment of density dependence, underscore the importance of understanding the density-dependent processes underlying population dynamics before beginning any vector control program.

Despite our findings that releases including females are expected to result in greater reduction

in competent vectors, the introduction of females may face opposition from local communities because wild females bite and transmit disease. Although all released females and their offspring should be incapable of transmitting disease, the potential for increased biting nuisance or even a small chance of disease transmission could make female releases less acceptable than male releases. However, the recent release of female *Ae. aegypti* in Australia infected with *Wolbachia* [48] makes the case that female releases could be acceptable when the released individuals are not competent vectors.

All GPM strategies can be hampered if there is a fitness disadvantage associated with the inserted transgenes. Such fitness costs can manifest themselves in a number of ways, including reductions in attractiveness to mates, fecundity, and survival. Here, we considered an additive fitness cost associated with the AP gene that reduced the survival of offspring during the egg stage. We showed that higher fitness costs slowed the rate of competent vector population reduction and reduced the amount of time that the AP allele remained in the population once releases ended. However, even with a substantial fitness cost to the AP gene, the R&R releases reduced the density of competent vectors more rapidly than the FK releases without a fitness cost. If there are any fitness disadvantages associated with the AP gene of the R&R strain, releases would need to be conducted continuously, perhaps in the form of smaller maintenance releases. Our results highlight that in order for an R&R strategy to reach its full promise as a solution to the logistical difficulties of an FK strategy, minimizing any such fitness costs is important.

As with other strategies, the efficacy of R&R could be reduced by immigration of wild-type mosquitoes. The impact of immigration on population-wide vector competence depended on the magnitude of the rate of immigration (see Appendix D.2). Higher wild-type immigration rates accelerated the recovery of competent vector densities to pre-release equilibrium levels shortly after R&R releases ended. If immigration rates were lower, however, much more time was needed for the competent vector population to increase to the pre-release density (Figure D.4). The predicted impacts of wild-type immigration on the success of an R&R strategy are similar to those expected based on previous modeling studies of population reduction strategies [31,49]; however, a more de-

tailed exploration of the potential impacts of immigration on an R&R strategy is warranted before R&R releases are conducted.

In this paper, we demonstrated that an R&R strategy is likely to be preferable to an FK strategy under a variety of scenarios due to its ability to cause a greater, sustainable reduction in competent vectors. We emphasize this comparison because FK strategies and related GPM population reduction strategies have seen notable progress, and large-scale releases are becoming increasingly possible [26-27, 50]. Although strategies for introducing AP genes without the aid of gene drive have been considered [51], they have received less attention; however, developments in molecular technology that lead to sustained inhibition of pathogen transmission via transgenes and minimal fitness costs associated with transgenes could make such AP-only strategies more feasible. While it is beyond the scope of this paper, R&R releases could potentially be evaluated against AP-only strategies as well strategies that combine FK, AP, and R&R releases.

The R&R strategy led to greater long term reduction in competent vectors because of the combination of population reduction and replacement genes. Most previous studies have focused either on population reduction or population replacement. Some population replacement strategies would have some degree of transient population reduction due to the genetic load imposed during the replacement process (e.g., *Wolbachia* [52], *Semele* [16]), and it has been proposed that insecticides be used to reduce populations before beginning a population replacement strategy. In contrast, the R&R strategy is specifically designed to cause population reduction while simultaneously spreading an AP gene to reduce competent vector density, and continuous releases of R&R mosquitoes would lead to continuous reduction. Although many gene drive strategies have been proposed and theoretically assessed, successful implementation of these strategies for disease vectors has proven difficult. An R&R strategy, however, could take advantage of the advances that have been made in the independent development of AP and FK strategies.

Although the study we presented here has demonstrated the potential utility of an R&R strategy, we emphasize that the model used is very general and is only intended to provide an introduction to, and broad overview of, a novel strategy that can be useful in the fight against vector-borne dis-

eases. As with any vector control strategy, a more thorough species-specific assessment should be considered before implementation of an R&R strategy. The role of stochastic effects, spatial heterogeneity, immigration, and abiotic factors such as meteorological fluctuations in the dynamics of a population subject to R&R releases should be carefully evaluated using models that incorporate more details than have been considered here.

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# Chapter 5

# Anti-pathogen genes and the replacement of disease vector populations: a model-based evaluation of hybrid strategies[1]

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## ABSTRACT

In recent years, genetic strategies aimed at controlling populations of disease vectors have received considerable attention as alternatives to traditional control measures. Theoretical studies have shown that Female-Killing (FK), Anti-Pathogen (AP), and Reduce and Replace (R&R) strategies can each cause reductions in competent vector populations. In this paper, we utilize a relatively simple mathematical model to evaluate the impacts on competent *Aedes aegypti* vector populations of FK-only, AP-only, and R&R-only releases as well as hybrid strategies that result from combinations of these three approaches. We show that while the ordering of efficacy (from most to least effective) of these strategies depends upon the release ratio, release duration, inclusion of females, and switch time in hybrid strategies, AP-only releases lead to the greatest reduction in competent vectors in most scenarios. R&R-only releases often cause the largest reduction in competent vectors during releases, but are less effective at reducing competent vector density long-term than AP releases or R&R followed by AP releases. In all scenarios we consider, FK and FK followed by R&R releases lead to the least long-term reduction in competent vectors. We show that the strength of density dependence and fitness costs that could be associated with AP genes due to genetic modification of the insect do not have a significant impact on the ordering of efficacies of the six strategies, and that reduction in competent vector density caused by AP-only releases is easier to maintain than that caused by FK-only or R&R-only releases when the AP gene confers a fitness cost. We discuss the roles that linkage disequilibrium and inclusion of females play in the relative efficacy of the strategies. We motivate the continued development of AP strategies by discussing their benefits over other strategies.

## 5.1 Introduction

Because many insect-vectored diseases remain endemic despite traditional control measures, many novel genetic pest management (GPM) vector control strategies have been proposed [1-3]. These strategies have generally aimed to achieve either reduction [4-8] or replacement [9-11] of populations of disease vectors by field release of engineered strains of the vector . Both population reduction and population replacement strategies have been explored theoretically [11-23], and several engineered mosquito strains have been developed [24-27].

Among the GPM approaches that have seen tangible progress are female-killing (FK) strategies[4,6]. In an FK strategy, a transgene is inserted into the pest genome that causes conditional female-specific lethality, but does not affect males. To enable the rearing of the transgenic strain, the female-specific lethal element is designed to be conditionally expressed. For example, certain FK strains of the mosquito species *Aedes aegypti* and *Aedes albopictus* are reared on a diet containing tetracycline that deactivates the lethal effects of the FK gene; tetracycline is not found in their diet in the wild, so offspring of the released individuals are subject to the effects of the FK gene [25,26]. When FK males are released and mate with wild-type females, female offspring that inherit a single copy of the FK gene do not survive. Males inheriting the FK gene can survive and pass the gene to their offspring, which is thought to make FK strategies more efficient than the Sterile Insect Technique (SIT) and similar genetic strategies in which neither male nor female offspring survive [16,28]. At least one FK strategy has been engineered in mosquito species [25,26], and laboratory and field cage tests have shown that repeated releases of FK mosquitoes into a caged wild-type population of *Ae. aegypti* can lead to either reduction or elimination of the wild-type population [29,30].

In a previous paper, we proposed and evaluated a Reduce and Replace (R&R) strategy that leads to both reduction and long-term replacement of a population of disease vectors by releasing individuals that are homozygous for FK genes as well as anti-pathogen (AP) genes that render the vector incapable of transmitting disease pathogens [31]. In that study, we explored a number of

potential R&R release scenarios and showed that R&R releases are more effective at reducing the number of competent vectors (i.e., adult females capable of transmitting disease) than comparable FK releases. In a related study, we explored R&R releases in a stochastic, spatially explicit model that simulated a neighborhood in the city of Iquitos, Peru [Okamoto et al., in revision]. In contrast to the results of the simple model, we found that substantial replacement of the native population with a population incapable of transmitting disease via R&R releases is unlikely, in part because of the influences of genetic drift when population size is low and spatial heterogeneity as the population recovers from reduction.

Although R&R may be more effective at reducing competent vector populations than FK releases alone in comparable release scenarios, the lack of AP allele fixation observed in the complex stochastic model raises the need to further evaluate R&R against related strategies that exploit the strengths of R&R, FK, and AP strategies. FK-only strategies have been well-studied theoretically [14-16,32], while AP-only strategies (in the absence of gene-drive mechanisms) have rarely been subject to detailed modeling, with at least one exception [32]. In addition to R&R, FK-only, or AP-only strategies, control programs could be developed that use combinations of the three strategies. For instance, a program that releases FK individuals prior to beginning releases of AP or R&R individuals would reduce the population before attempting to replace the population with incompetent vectors. Similarly, R&R releases prior to AP-only releases allow for population replacement to begin while reduction occurs, but could be timed so that AP releases begin before population density is too low (in attempt to avoid the effects of low population size observed in Okamoto et al.). While FK, AP, and R&R releases have been explored independently in previous models [14,23,33], to our knowledge, programs involving combinations of releases of FK, AP, and R&R strategies have not yet been considered.

In this paper, we use a deterministic model based on the general biology of the dengue vector *Ae. aegypti* to compare R&R releases to FK-only, AP-only, and hybrid releases. We explore the effects of different release strategies on transient and long-term dynamics to better understand how release ratio, release duration, and female-inclusion in releases lead to different magnitudes of re-

duction in competent vectors. We also explore the influence of fitness cost and density dependence on the ability of releases to reduce competent vector density.

## 5.2 Model Description

This model tracks genes at two different loci in the *Ae. aegypti* population. We assume that the FK and AP genes are located at two independently segregating loci, and we denote the FK and AP alleles as 'K' and 'A' respectively; the corresponding wild type alleles at each locus are denoted 'k' and 'a'. This leads to nine possible genotypes (see Table 5.1). We utilize a model identical to that described by Robert et al. in [31]. We briefly describe the model here in the context of this study.

Let $J_i(t)$, $F_i(t)$, and $M_i(t)$, be the density of juveniles (larvae and pupae; egg production is modeled implicitly), females, and males, respectively, of genotype $i$ at time $t$. We assume random mating between adults and Mendelian inheritance. The rate at which females produce viable larvae of genotype $i$ is

$$B_i(t) = w_i \sum_m F_m(t)\lambda \sum_n \Pr(i|m,n)\frac{M_n(t)}{\sum_g M_g(t)} \tag{5.1}$$

where $\lambda$ is the per capita rate at which females produce larvae, $\Pr(i|m,n)$ is the Mendelian probability that an offspring of genotype $i$ arises from a mating between a female of genotype $m$ and a male of genotype $n$, and $w_i$ is the fitness of an offspring of genotype $i$ relative to that of wild-type offspring (Table **??**. Here, we assume that fitness costs affect the fraction of eggs that survive to the larval stage, and that fitness costs are additive at a single locus and multiplicative across loci. We denote the fitness cost associated with homozygous FK individuals as $c_K$ and that associated with homozygous AP individuals as $c_A$. We note that the model could be altered to consider other types of fitness costs (e.g. dominant or recessive) acting at different life stages (e.g. mating or adult viability).

Let $\mu_J$, $\mu_F$, and $\mu_M$ be the per capita mortality rates of juveniles, adult females, and adult males, respectively. In addition, juveniles experience density-dependent mortality at per capita

**Table 5.1:** Properties of genotypes resulting from R&R, AP, and FK releases. Each of the possible genotypes resulting from R&R releases is listed with corresponding fitness values ($w_i$) and female viability coefficients ($\gamma_i$). *Conditional lethality allows for the release of these females as adults.

| $i$ | Genotype | $w_i$ | $\gamma_i$ |
|---|---|---|---|
| 1 | KKAA | $(1-c_A)(1-c_K)$ | 0* |
| 2 | KkAA | $(1-c_A)(1-0.5c_K)$ | 0 |
| 3 | kkAA | $(1-c_A)$ | 1 |
| 4 | KKAa | $(1-0.5c_A)(1-c_K)$ | 0 |
| 5 | KkAa | $(1-0.5c_A)(1-0.5c_K)$ | 0 |
| 6 | kkAa | $(1-0.5c_A)$ | 1 |
| 7 | KKaa | $(1-c_K)$ | 0* |
| 8 | Kkaa | $(1-0.5c_K)$ | 0 |
| 9 | kkaa | 1 | 1 |

rate $(\alpha J)^{\beta-1}$, where $J$ is the total density of juveniles and $\alpha$ and $\beta$ are parameters that determine the strength of density dependence and, together with other parameters, the equilibrium population density [39]. The strength of density dependence impacts how quickly the population returns towards equilibrium following perturbations. Strong density dependence (e.g., higher values of $\beta$) leads to more rapid return. Juveniles emerge as mature adults at per capita rate $\nu$, and we assume that, in the absence of FK effects, the emerging adults are 50% female and 50% male. We multiply the rate of emergence of female adults by a viability coefficient, $\gamma_i$, where, $\gamma_i = 1$ for viable genotypes and $\gamma_i = 0$ otherwise (see Table 5.1).

We assume transgenic releases occur continuously. Let $u_M^i = (rM_9^*)/7$ and $u_F^i = (rM_9^*)/7$ be the rates at which releases of adult males and females, respectively, of genotype $i$ occur. Here, $r$ is the initial weekly release ratio of transgenic individuals to the equilibrium wild-type male population density $M_9^*$ (the factor 7 converts from a weekly to daily release rate). Note that the rate at which individuals are released is taken to remain constant even if population densities decline. Release rates that vary as the population declines could be considered as has been done in some previous work [15], though we do not do so here. In this paper, we consider the releases of three distinct genotypes: homozygous FK (KKaa, $i = 7$), homozygous AP (kkAA, $i = 3$), and homozygous R&R (KKAA, $i = 1$). Note that conditional lethality allows for the release of females carrying FK genes.

**Table 5.2:** Model parameters. Description of model parameters with default values and references for default values.

| Parameter | Description | Default Value | Reference |
|---|---|---|---|
| $\mu_J$ | Density-independent juvenile mortality rate (per capita) | 0.03 $\text{day}^{-1}$ | [34] |
| $\mu_M$ | Male mortality rate (per capita) | 0.28 $\text{day}^{-1}$ | [35,36] |
| $\mu_F$ | Female mortality rate (per capita) | 0.10 $\text{day}^{-1}$ | [35,36] |
| $\lambda$ | Average rate of larval production by females (per capita) | 8 $\text{day}^{-1}$ | [37,38] |
| $\nu$ | Rate of emergence to adulthood (per capita) | 0.14 $\text{day}^{-1}$ | [35] |
| $\alpha$ | Density dependence parameters | $2 \times 10^{-4}$ $\text{juveniles}^{\beta-1} \cdot \text{day}^{-1/(\beta-1)}$ | - |
| $\beta$ | | 3.4 | - |
| $c_A$ | Fitness cost associated with anti-pathogen allele | 0 | - |
| $c_K$ | Fitness cost associated with female-killing allele | 0 | - |
| $w_i$ | Fitness of genotype $i$ | 1 | - |
| $\gamma_i$ | Female viability coefficient of genotype $i$ | See Table 5.1 | - |
| $r$ | Weekly release ratio of transgenic individuals to wild-type males | 1 | - |
| $T$ | Duration of release | 100 days | - |
| $T_s$ | Time at which combination releases switch | 50 days | - |

The model description above leads to the following system of 27 ordinary differential equations, where $i$ represents the genotype of each class; parameter descriptions along with default values are listed in Table 5.2.

$$\begin{aligned}
\dot{J}_i &= B_i(t) - \mu_J J_i - J_i \left( \alpha \sum_g J_g \right)^{\beta-1} - \nu J_i \\
\dot{F}_i &= \frac{1}{2} \nu \gamma_i J_i - \mu_F F_i + u_i^F \\
\dot{M}_i &= \frac{1}{2} \nu J_i - \mu_M M_i + u_i^M
\end{aligned} \tag{5.2}$$

In this study, we consider six different release approaches that arise from combinations of FK, AP, and R&R strategies. Along with releases that include only one strategy (R&R-only, AP-only, and FK-only), we consider three approaches that switch from one strategy to another at a certain switching time ($T_s$). In the FK/AP strategy, FK releases are conducted first, followed by a period of AP-only releases. In the R&R/AP strategy, R&R releases are conducted and followed by AP-only releases. In the FK/R&R strategy, FK-only releases are conducted before R&R releases. We compare combination approaches against single-strategy approaches that have the same total release duration.

Throughout, we measure the efficacy of release strategies by observing changes in the competent vector population that result from releasing transgenic mosquitoes into an entirely wild-type population that is at equilibrium density. We compare each of the strategies against one another by considering the order of efficacy from most to least effective at reducing competent vector (i.e., wild-type adult female) density. We consider male-only, bi-sex (50% male, 50% female), and female-only releases and compare releases of each type that lead to the same total number of mosquitoes being released. We remark that while releases of R&R and AP females would be possible because these females could not transmit disease, releases of FK-only females are unlikely because they are competent vectors. For this reason, in scenarios that would involve the release of FK females, we replace FK females with FK males for our analyses, even when comparing strategies that include female-only and bi-sex releases of the R&R and AP-only strains. For example, in a "female-only

FK/AP release", FK males are released followed by AP females.

## 5.3 Results

Here we present results for a number of scenarios in which each of the six different release types are conducted. In these results, the values for release ratio, release duration, and switch time are held at the default values in Table 5.2 unless noted otherwise. In Appendix E, we briefly discuss the influence that these parameters have on our results.

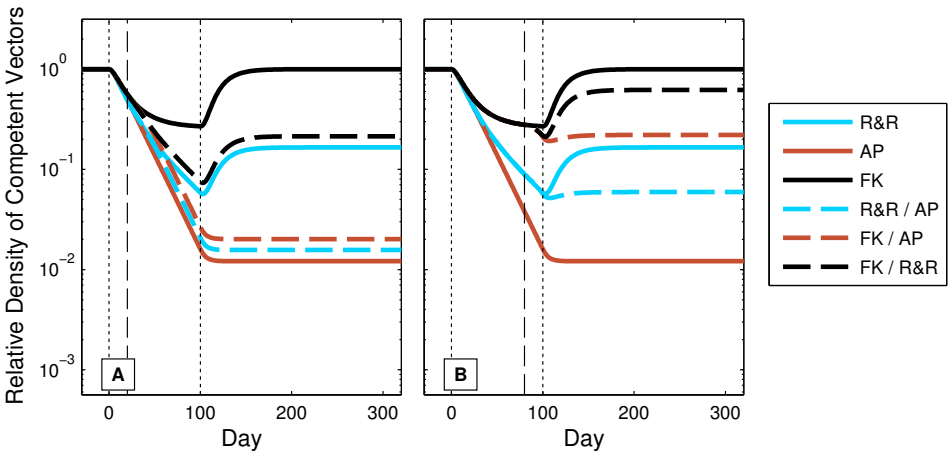


**Figure 5.1:** Comparison of six control strategies. Relative competent vector population density when male-only releases are conducted at a 1:1 release ratio ($r = 1$) for $T = 100$ days. Vertical black dotted lines represent the time at which releases begin ($t = 0$) and end ($t = 100$). For releases that combine strategies, the middle black dashed vertical line represents the time at which a switch is made between strategies. Here, the time of switch is $T_s = 20$ (A) and $T_s = 80$ (B). All other parameter values are the default values listed in Table 5.2. Note the vertical axis is on a log scale.

### Release Switch Time for Male-Only Releases

We simulated 100-day male-only releases at a 1:1 weekly release ratio. For the combined approaches, we switched from the first to second strain after 20 (Figure 5.1A) or 80 (Figure 5.1B) days. Regardless of the switch time, the AP-only strategy always led to the greatest reduction in compe-

tent vectors during and after releases, followed by the R&R/AP combination. The impact on competent vector density of each of the combination strategies differed for each time of switch. For the earlier switch time, the FK/AP combination led to more reduction in competent vectors than R&R (Figure 5.1A), but for the later switch time, R&R reduced the competent vector density more than the FK/AP strategy (Figure 5.1B). The FK-only strategy always had the smallest impact of the six approaches on competent vectors during and after releases. The FK/R&R combination strategy led to better reduction than FK-only but still performed more poorly than any of the other approaches. These impacts of the FK-only and FK/R&R strategies relative to other strategies remained consistent for all release scenarios we describe throughout this paper, so henceforth we omit these results to simplify the presentation and discussion of results.

### Releases Including Females

We simulated 100-day male-only, bi-sex, and female-only releases at a 1:1 (total engineered adults:total wild-type males) release ratio of the six approaches (Figure 5.2). For each of the combination approaches, we considered a switching time 50 days after releases began. When releases included females, R&R releases led to the greatest reduction during the transient period; however, R&R/AP releases followed by AP-only releases led to the greatest long-term reduction in competent vectors for bi-sex and female-only releases. For the switch time considered here, R&R releases including females had much more similar long-term impacts on competent vector densities as FK/AP releases than did corresponding male-only R&R and FK/AP releases. As with male-only releases, increasing the switch time for bi-sex and female-only releases led to the FK/AP strategy having less impact on the competent vector population density than the R&R strategy (results not shown). Bi-sex and female-only releases of each of the approaches led to greater reduction in competent vectors than comparable male-only releases.

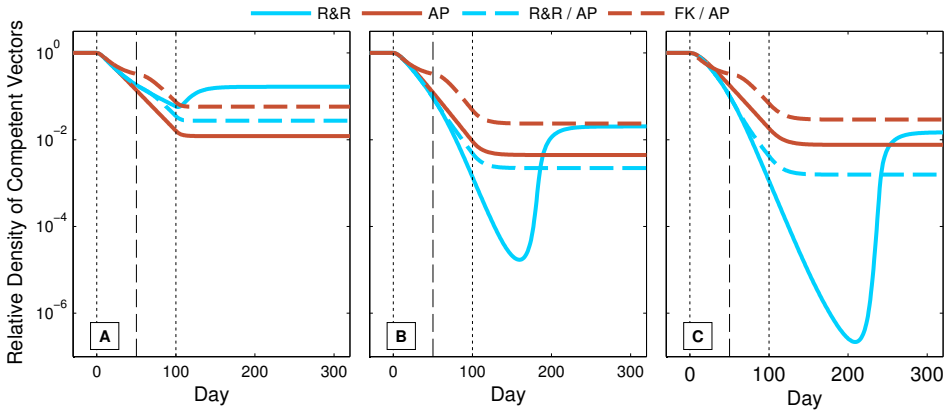


**Figure 5.2:** Effects of female releases on R&R and AP releases. Relative density of competent vector population when releases are conducted at a 1:1 release ratio ($r = 1$) for $T = 100$ days with male-only (A), bi-sex (B), and female-only (C) releases. Vertical black dotted lines represent the time at which releases begin ($t = 0$) and end ($t = 100$), and the vertical black dashed line represents the time at which the switch between combination strategies occurs ($T_s = 50$). All other parameter values are the default values listed in Table 5.2. Note the vertical axis is on a log scale.

## Fitness Cost

We simulated male-only, bi-sex, and female-only releases for 100 days at a 1:1 release ratio with combination releases switching after 50 days, and we observed the impact of releases on competent vectors when the AP gene carried an additive fitness cost (Figure 5.3). To measure the impact, we calculated the average density of competent vectors over the time period beginning the day releases start and ending one year after the last day of releases relative to the wild-type equilibrium density of females in the absence of control ($F_9^*$). That is, since $F_9(t)$ represents the density of wild-type female adults in the population subject to control, the relative average density is given by

$$\bar{f} = \frac{\frac{1}{t_f - t_0} \int_{t_0}^{t_f} F_9(t)\, dt}{F_9^*} \tag{5.3}$$

where $t_0$ is the day that releases begin and $t_f$ is one year after releases end.

For all release types, the relative average density of competent vectors increased as the value of the fitness cost increased. For male-only releases, the AP strategy, followed by the R&R/AP and

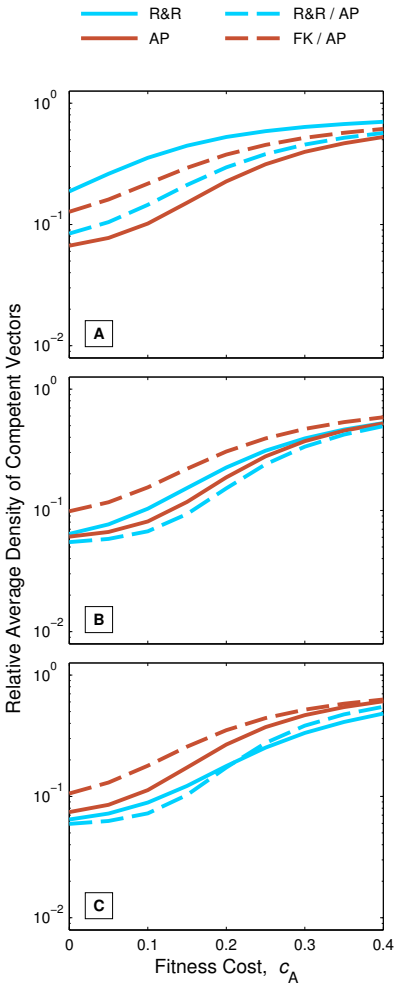


**Figure 5.3:** The effects of fitness costs on R&R and AP releases. Relative average density of competent vectors between the time releases began ($t = 0$) and one year after the final day of releases when the anti-pathogen gene carries a fitness cost. Releases were conducted at a 1:1 ($r = 1$) release ratio for $T = 100$ days with male-only (A), bi-sex (B), and female-only (C) releases. For combination strategies, the switch occurred after $T_s = 50$ days. All other parameter values are the default values listed in Table 5.2. Note the vertical axis is on a log scale.

FK/AP combination strategies, led to the greatest reduction in average competent vector density for the values of fitness cost we considered (Figure 5.3A). R&R-only releases led to the least reduction in relative average density of these four strategies. The impact of each strategy as measured by the relative average density became more similar as the values of fitness cost increased. For bi-sex releases, the R&R/AP combination strategy led to the lowest relative average density of competent

vectors followed by the AP-only and R&R strategies (Figure 5.3B). For both bi-sex and female-only releases, the FK/AP strategy led to the least reduction in relative average density of these four strategies. For female-only releases, the AP-only strategy led to more reduction in relative average density than the FK/AP strategy, but not as much reduction as the R&R and R&R/AP strategies. For values of fitness cost lower than approximately $c = 0.15$, the R&R/AP strategy led to more reduction in relative average density of competent vectors than R&R, but the opposite was true for higher values of the fitness cost.

### Maintenance Releases

If AP genes have a fitness cost, continuous maintenance releases will be needed in order to maintain reduction in competent vectors in a population indefinitely. To compare maintenance releases, we first simulated releases of R&R-only, AP-only, and FK-only males at 1:1 release ratio when the anti-pathogen gene had an associated fitness of $c = 0.2$. In reality, maintenance releases might begin after a fixed duration of releases or once the population density falls below some predetermined value. In our simulations, we followed the latter approach by allowing the original releases to occur until competent populations under each release strategy reached the same density, and we considered maintenance releases at different proportions of the original release size (Figure 5.4). FK releases required maintenance releases at at the original release ratio in order to maintain the reduction, and as the size of the maintenance releases decreased, the relative density was maintained at higher values, although still lower than those of the pre-release density (Figure 5.4B). Smaller maintenance releases were required to maintain the reductions caused by either R&R or AP releases (Figure 5.4 A,C). Reduction caused by R&R releases was maintained by releases at 30-50% of the original release ratio, and reduction caused by AP-only releases was maintained by releases at about 10% of the original release ratio. R&R and AP-only maintenance releases at higher percentages led to additional reduction in competent vectors.

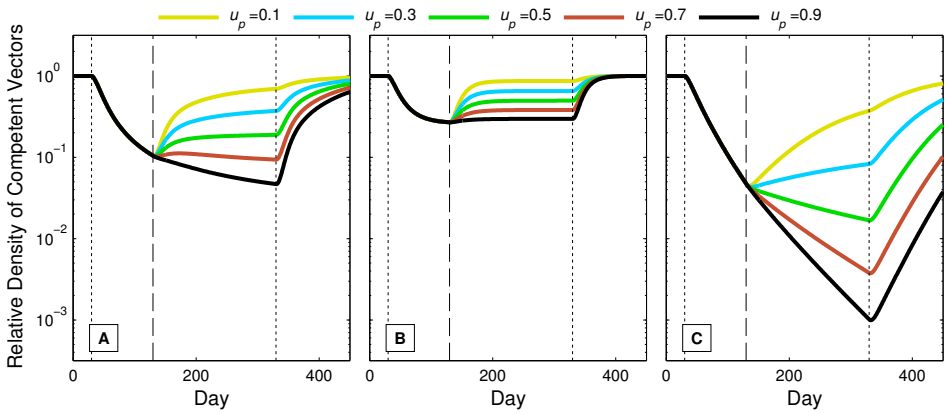


**Figure 5.4:** Maintenance releases for FK R&R, and AP. Relative density of competent vectors in populations where maintenance releases occur when the anti-pathogen gene has a fitness cost. Male-only R&R (A), FK (B), and AP (C) releases were conducted at a 1:1 release ratio. The original releases were ended at different times so that the population density at the beginning of maintenance releases is the same in each scenario. The first dotted line represents the beginning of the original releases, the dashed line represents the end of the original releases, and the last dotted line represents the end of maintenance releases. Here, $u_p$ is the fraction of the original releases being released. That is, $u_p = 1$ represents continued releases at the same intensity as the original releases. Here, $r = 1$ and $c = 0.2$, and all other parameter values are the default values listed in Table 5.2. Note the vertical axis is on a log scale.

### Role of Density Dependence

We simulated male-only, bi-sex, and female-only releases of each of the release strategies for 100 days at a 1:1 release ratio for populations regulated by different strengths of density dependence, and set the switch time to $T_s = 50$ days. We observed both the long-term (Figure 5.5) and minimum (Figure E.2 in Appendix E) relative competent vector population density resulting from each release scenario. In general, the strength of density dependence did not affect the ordering of efficacy of the strategies on reducing competent vectors for bi-sex and female-only releases. The exception was that for very weak density dependence FK/AP releases led to greater long-term reduction in competent vectors than AP-only, while for stronger density dependence, AP-only led to lower long-term density of competent vectors (Figure 5.5). For bi-sex and female-only releases, the R&R strategy led to the greatest reduction during the transient period for most strengths of density dependence considered here (Figure E.2 B,C), and invariant of the strength of density dependence,

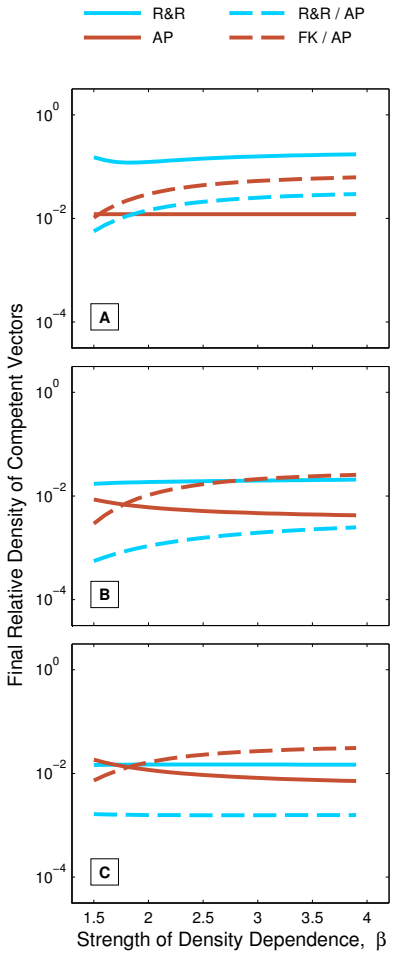


**Figure 5.5:** The effects of density dependence on R&R and AP releases. Long-term relative density of competent vectors for different strengths of density dependence when releases were conducted at a 1:1 ($r = 1$) release ratio for $T = 100$ days with male-only (A), bi-sex (B), and female-only (C) releases. For combination strategies, the switch occurs after $T_s = 50$ days. All other parameter values are the default values listed in Table 5.2. Note the vertical axis is on a log scale.

R&R/AP releases led to the most long-term reduction (Figure 5.5 B,C).

For male-only releases, the strength of density dependence determined which of the strategies was most effective at reducing the population density in the transient, but had less impact on long-term reduction. For weak density dependence, R&R led to the greatest reduction in the population while AP and FK/AP releases led to the least reduction (Figure E.2 A); R&R/AP, AP-only, and FK/AP releases caused the most long-term reduction in competent vectors when density de-

pendence was weak (Figure 5.5A). For stronger density dependence, AP releases led to the greatest reduction during the transient period, followed by R&R/AP releases (Figure E.2 A) while FK/AP and R&R releases had approximately the same effect on minimum relative density, with FK/AP leading to slightly more reduction than R&R for the strongest density dependence we considered. AP-only releases led to the greatest long-term reduction when density dependence was strong, followed by R&R/AP releases (Figure 5.5A). For a longer switch time ($T_s = 80$), the long-term reduction caused by FK/AP and R&R became approximately the same as density dependence strengthened (results not shown).

## 5.4 Discussion

Because a number of GPM approaches for controlling populations of competent disease vectors are in development, emphasis needs to be placed on evaluating different strategies against and in concert with one another in order to determine where resources may be best invested. In this paper, we showed that vector control strategies involving FK, AP, and R&R strains of *Ae. aegypti* can have very different impacts on transient and long-term competent vector populations. Indeed, the most effective strategy for control depended upon whether females were included in releases, the strength of density-dependent mortality, fitness costs, the combination of release ratio and release duration, and in some cases, the time at which combination approaches switched from one strategy to the next. While the results presented here were obtained for a population whose structure and dynamics are relatively simple, they bring to attention the important roles that population dynamics and design of release strategies play in the success of control programs involving GPM approaches.

In many of the scenarios that we considered, we found that AP-only releases led to the most long-term reduction of competent vectors of all of the six strategies at the focus of this study. This result was rather unexpected in part because it has previously been assumed that population reduction, whether prior to or in conjunction with releases of individuals carrying AP genes, would aid the spread of the gene, resulting in higher frequencies of individuals incapable of transmitting

disease pathogens. While simultaneous releases of FK and AP genes (as in the R&R releases) led to reduction in competent vectors, once the population density was low the two genes were inherited together more frequently, which led to many of the AP genes being lost due to the lethal effects of the FK gene. Although an R&R strain would ideally be designed so that FK and AP genes are not physically linked, this linkage disequilibrium at low population densities would be difficult to avoid. AP-only releases, however, did not suffer from these lethal effects and AP genes were able to rapidly propagate through the population. Reduction of the population prior to AP releases, as in the FK/AP strategy, did not lead to more reduction in the scenarios we considered in part because strong density dependence impeded significant reductions in total population density, and the period of reduction left less time for AP releases, resulting in a higher long-term density of competent vectors.

AP-only approaches in the absence of gene drive have been given little attention previously because it has been assumed that they would be infeasible. Early development of population replacement strategies assumed that fitness costs associated with introducing AP genes into a genome would be too high for AP genes to establish in a population, and successful suppression of a population of disease vectors would require the rearing of large numbers of insects [1,38]. To counter this, a number of gene drive systems that cause super Mendelian inheritance of AP genes have been suggested that hope to increase the chances of having AP genes fix in a population despite fitness cost [9,11,41-44]. That large numbers of insects would need to be reared is a concern shared with many GPM approaches, including FK and SIT strategies which are currently in development and testing. In this paper we showed that despite a fitness cost, the number of competent vectors can be reduced more quickly by release of AP-only strains compared to the release of a similar number of FK mosquitoes, and that the maintenance of competent vector population reduction with AP releases requires fewer mosquitoes than FK releases. In fact, in terms of long-term competent vector reduction, the FK strategy was least effective of all of the strategies considered.

Although population reduction did not generally benefit the spread of AP genes in the population, we found some scenarios in which R&R/AP releases led to more reduction in competent vec-

tors than AP-only releases. While AP-only releases typically led to the greatest long-term reduction when male-only releases occurred, bi-sex and female-only R&R/AP releases typically caused more long-term reduction than corresponding AP-only releases. After the R&R phase of this hybrid strategy caused population reduction, strong density dependence led to the population's attempt to recover rapidly; however, the additional offspring resulting from releases of AP-only females maintained high density-dependent mortality. This led to slower overall recovery of the population and allowed time for the removal of AP genes linked to FK genes, which aided introgression of AP genes. In these R&R/AP releases including females, population reduction during the R&R phase actually aided the spread of AP genes throughout the entire R&R/AP release. When male-only releases were conducted, however, the population was reduced to a much lower density because no additional offspring resulted from released individuals. Rapid population recovery, coupled with linked FK and AP genes, impeded the ability of AP genes to spread during the AP phase of releases, causing the AP-only releases to more effective than the R&R/AP combination.

In general, female-only AP releases led to greater long-term reduction than similar male-only releases but less reduction than similar bi-sex releases. Releasing females in addition to males increased the rate of spread of the AP gene into the population in part because releasing both sexes reduced the competition for mates. That is, when multiple males were released they had to compete with one another as well as with wild-type males, but when half the number of males are released with an equal number of females, the competition is not as strong. The rate of spread of the AP gene during releases including females was also aided by the additional density-dependent mortality that resulted from increases in the total number of offspring. Female-only releases did not lead to more reduction than bi-sex releases in part because the increased density-dependent mortality caused by the increase in females began to work against the spread of the AP gene as more offspring, including those with the AP gene, died before reaching the adult stage due to high larval density. While releases of females are not generally considered because females are a biting nuisance and transmit disease pathogens, release of females that cannot transmit pathogens could become more likely, as recent releases of both males and females of *Wolbachia*-infected strains of

*Ae. aegypti* have occurred in northeastern Australia and have not yet been found to cause any additional problems [45]. While releasing females would be possible, male-only releases of GPM strains will most likely remain the standard, and such releases have in fact occurred in trials [46].

Although we found that neither the strength of density dependence nor the magnitude of fitness cost associated with the AP gene had a significant effect on the ordering of long-term efficacy, from most to least effective, of each of the strategies, understanding the role that each plays in a control program is still important. While populations regulated by weak density dependence saw greater reductions in the transient period when FK, FK/R&R, and R&R releases were conducted, R&R/AP and AP-only strategies still led to the most long-term reduction for most values of the strength of density dependence we considered. This indicates that the strength of density dependence may not play a vital role in determining whether control strategies succeed in replacing the population; however, a failure to understand density regulation in a population may result in driving a population to elimination rather than replacing the population, so a clear understanding of density-dependent regulation in the population is especially important if the latter is the preferred goal. While elimination may seem more desirable, if a population is assumed to be eliminated and control measures cease, immigrant mosquitoes could re-establish the population and cause an outbreak in disease in a human population with decreased herd immunity.

Our study of the effects of fitness cost and maintenance of competent vector population showed that maintaining the reduction caused by AP releases was easier than doing so for reduction caused by R&R or FK releases. Since FK releases caused reduction in the population, density dependence acted quickly to increase the population density as soon as releases ended, which caused maintenance of reduction to be more difficult than after AP or R&R releases. AP releases did not affect population size, and maintenance of reduction caused by AP releases was easier because the loss of AP alleles caused by the fitness cost, which would lead to an increase in competent vectors, occurred slowly. Although R&R releases caused some reduction in the population, rebound of competent vectors did not occur as quickly as in FK releases because of the presence of AP alleles. R&R releases were, however, harder to maintain than AP releases because of the combination of the loss

of alleles due to the fitness cost and the population rebound. For any GPM control program to be successful in the long term, maintenance releases of modified strains are a likely reality, regardless of whether the strains suffer from a fitness disadvantage. The results presented here could have important implications for the relative costs associated with maintaining different types of control programs.

This study has evaluated the relative efficacy of a number of existing and potential genetic strategies for controlling disease vectors by comparing strategies in a simple, single-population, deterministic ordinary differential equation model. While more complex models should be used to further evaluate all of the strategies, our work provides substantial motivation for the utility of AP, R&R, and hybrid strategies in the fight against disease vectors. In particular, we stress the further theoretical and empirical study of AP-only strategies because of their predicted ability to lead to substantial long-term reduction in competent vectors relative to other strategies. While a number of AP strains of *Ae. aegypti* and other pests have already been developed and could be potential candidates for eventual AP-only releases [24,47,48], serious consideration should be given to the continued development and improvement of genetic constructs not requiring gene drive that interfere with the ability to transmit disease pathogens.

## 5.5 Acknowledgements

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# APPENDICES

# Appendix A

# Stochastic Cage Model Mathematical Details [1]

## Mathematical Model

We present a discrete time, discrete state age-structured stochastic model that links population genetics and population dynamics to simulate the experimental design described in the main text. Our cohort-based model tracks, on a day-by-day basis, the (integer) numbers of adult mosquitoes, classified by age, sex, and genotype. We let $M_{g,a,d}$ be the number of adult males of genotype $g$ and age $a$ on day $d$, $F_{a,d}$ be the number of females of age $a$ on day $d$, and $E_{g,d}$ be the number of eggs of genotype $g$ that are laid on day $d$. We consider three possible genotypes: wild-type ($g = 1$), heterozygous FK ($g = 2$), and homozygous FK ($g = 3$). As discussed in the main text, eggs can be either wild-type or heterozygous, adult males can be of any genotype, although the only source of homozygotes are from FK releases, and the only reproductively-viable adult female genotype is wild-type (this assumes, as we do throughout this study, that the female killing mechanism is 100% effective).

[1] This appendix is included as supplementary online material for the publication Robert, M.A., M. Legros, L. Facchinelli, L. Valerio, J.M. Ramsey, T.W. Scott, F. Gould, and A.L. Lloyd. (2012) Mathematical Models as Aids for Design and Development of Experiments: The Case of Transgenic Mosquitoes. J. Med. Entomol. 49, 1177-1188.

An individual model simulation represents one experiment with one treatment and one control cage. For the duration of the experiment, $N$ wild-type larvae are placed into the control cage on the first day of each week. The number of larvae placed in the treatment cage varies over time, reflecting the numbers of eggs laid in the treatment cage relative to the control cage. The same baseline number, $N$, is used throughout the stabilization period, but beginning with the week of the initial release, the number is determined by finding the input necessary to make the ratio of larvae input to eggs laid in the previous week equal in both the treatment and control cages. That is, the number of larvae placed in the treatment cage each week is $N_w$, where, at the beginning of week $w$,

$$N_w = N \cdot \frac{E^T_{w-1}}{E^C_{w-1}}$$

where, $E^T_{w-1} = \sum_{g=1}^{2}\sum_{d=1}^{7} E^T_{g,d}$ and $E^C_{w-1} = \sum_{d=1}^{7} E^C_{1,d}$ represent the total number of eggs laid in the treatment (T) and control (C) cages in the previous week. For the remainder of this section, we will discuss only the treatment cage unless otherwise noted, so we drop the superscripts that indicate the cage type.

As discussed in the main text, the genotype distribution of the $N_w$ larvae introduced into the treatment cage in a given week reflects the genotype distribution of eggs laid in the cage in the previous week. The numbers of wild-type and heterozygous larvae, which we write as $n_1$ and $n_2$ respectively, are chosen from a multinomial distribution: $n = (n_1, n_2) \sim \text{Multinomial}(N_w, H_w)$, where $H_w = (h_{1,w}, h_{2,w})$, with

$$h_{g,w} = \frac{E_{g,w-1}}{E_{1,w-1} + E_{2,w-1}}.$$

Here, $h_{g,w}$, represents the frequency of genotype $g$ in the eggs laid during week $w$.

Releases of homozygous FK pupae begin at week 14; these releases occur in addition to the $N_w$ larvae returned from eggs laid in the cage. We take the release number to be a constant multiple of the baseline input. That is, the number of homozygous FK mosquitoes placed into the cage each week is $rN$. The release ratio, $r$, describes the ratio of FK pupae to wild-type larvae input. For example, for an input of 10:1 (FK : wild-type), $r = 10$.

A larval cohort comprises the individuals placed into a cage on a given day. The model simulates the emergence of these larvae based on an emergence time distribution, i.e. day-by-day probabilities that an individual will emerge, as shown in Figure 2.1 of Chapter 2. These probabilities, together with a nonzero probability of juvenile mortality, sum to one. The numbers of individuals that emerge on each day following the placement of the cohort, or that die before emerging, is chosen from a multinomial distribution with these probabilities. (Figure 2.1 presents the emergence distribution for males. We assume females emerge one day later than males, therefore the distribution of female emergence is simply shifted by one day). The sex ratio of eggs laid is assumed to be 1:1, so the sexes of the emerging adults are determined by sampling from a binomial distribution with probability parameter 0.5. We remark that alternative experimental designs in which other immature age classes, i.e. eggs or pupae, are introduced into the cage could be modeled by an appropriate modification of the emergence time distribution.

For adult cohorts, we assume that there is a day-to-day survival probability for all mosquitoes in an age class. In the simplest model, the number of mosquitoes in an age class that survives from one day to the next is chosen from a binomial distribution with a constant daily survival probability for males ($s_m$) or females ($s_f$). More generally, the survival probability can be taken to be age-dependent.

The model keeps track of the genotype distribution of the offspring of a given female cohort. This is determined from the distribution of genotypes of males of mating age on the day (or days) on which the female cohort mated, weighted by the mating fitness cost of each genotype. We call this the **mating pair** distribution. Notice that we treat the offspring genotype distribution as a property of the cohort: this simplifies our model as it means we do not have to track individual females and their offspring genotype distributions, although it means we underestimate the genetic variability of eggs laid by the members of the cohort, particularly when only a few females remain in the cohort.

We first describe how the calculation of the offspring genotype distribution is undertaken when we assume females mate once. Using the mating fitness costs discussed in the main text (additive

fitness cost of FK, cost per FK allele equal to $c/2$), the mating fitnesses of each genotype are $\Phi_1 = 1$, $\Phi_2 = 1-c/2$, and $\Phi_3 = 1-c$. Hence the probability that a female who mates on day $d$ will mate with a male of genotype $k$, which we denote by $p_{k,d}$, is given by

$$p_{k,d} = \frac{\sum_a \Phi_k M_{a,k,d}}{\left(\sum_a \sum_{k'} \Phi_{k'} M_{a,k',d}\right)}.$$

Here, the sum is taken over all male age classes of mating age. Writing the Mendelian probability that an offspring of genotype $g$ is born to a wild-type female who mates with a male of genotype $k$ as $P_{g,k}$, the probability that an offspring of a female who mates on day $d$ is of genotype $g$, denoted by $v_{g,d}$, is given by

$$v_{g,d} = \sum_k P_{g,k} p_{k,d}.$$

We assume that the number of eggs laid by a female mosquito each day is independent of her age and is Poisson distributed with mean $\lambda$. The total number of offspring of females of a given age class on a given day, $f_{a,d}$, is therefore Poisson distributed with mean $\lambda \cdot F_{a,d}$. The genotypes of these offspring are determined by sampling from a multinomial distribution: denoting the numbers of eggs of genotype $g$ laid by females of age class $a$ on day $d$ by $e_{g,a,d}$, we have that $(e_{1,a,d}, e_{2,a,d}) \sim$ Multinomial$(f_{a,d}, (v_{1,\hat{d}}, v_{2,\hat{d}}))$, where $\hat{d}$ is the day on which the female cohort mated.

For our simulations involving multiple matings, we consider the offspring distributions that result from matings on both days and form a weighted average of the two, imagining that a fraction $z$ of offspring result from sperm from the first mating and the remaining $1-z$ of offspring result from sperm from the second mating. We consider $1-z$ to be the the **degree of polyandry** since the fraction $1-z$ determines the influence of the second mating on the genotype distribution of the offspring. The numbers and genotypes of the eggs laid by the female cohort of age $a$ on day $d$ are given by $(e_{1,a,d}, e_{2,a,d}) \sim$ Multinomial$(f_{a,d}, (z v_{1,\hat{d}_1} + (1-z) v_{1,\hat{d}_2}, z v_{2,\hat{d}_1} + (1-z) v_{2,\hat{d}_2}))$, where $\hat{d}_1$ and $\hat{d}_2$ are the first and second days on which the female cohort mated.

The total number of eggs of a given genotype laid each day is then the sum of the offspring of that genotype from all age classes of females. The distributions of genotypes among eggs laid

are then used to determine the distribution of the genotypes of larvae input on a weekly basis as described previously in this section.

# Appendix B

# Stochastic Cage Model: Comparison to Data and Exploration of Alternative Model Assumptions [1]

## Comparison to Data

We compare model output to data published in Wise de Valdez et al. (2011), whose laboratory experiments follow the same protocol outlined in our manuscript. We emphasize that although we can use our model to simulate the experimental design of these experiments, there are a number of parameter values for these experiments that are unknown (e.g. daily survival in the cage environment, average daily fecundity, and distribution of emergence times). Before using the model to simulate potential experiments, it is critical to obtain this information. For a given parameter set, we see that the model captures the general dynamics seen in the laboratory experiments by Wise de Valdez et al. (Figure B.1a). Their data, however, exhibit more variability than the model, which reflects sources of variation that are not considered in our model, such as overdispersion in the

[1] This appendix is included as supplementary online material for the publication Robert, M.A., M. Legros, L. Facchinelli, L. Valerio, J.M. Ramsey, T.W. Scott, F. Gould, and A.L. Lloyd. (2012) Mathematical Models as Aids for Design and Development of Experiments: The Case of Transgenic Mosquitoes. J. Med. Entomol. 49, 1177-1188.

daily egg production. The extinction times of two of the three populations in laboratory cages do fall within the distribution of extinction times predicted by the model for the parameters used here (Figure B.1b).

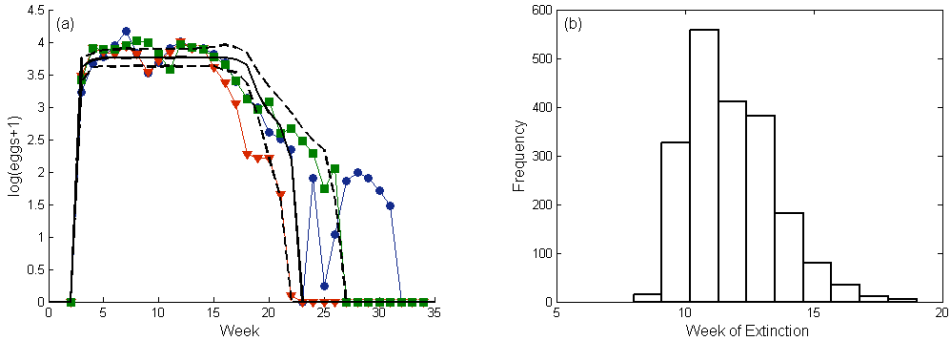


**Figure B.1:** Comparison of cage model output to lab cage data. (a) Dynamics of Eggs from data collected in laboratory experiments by Wise de Valdez et al. (2011) compared to model output. Blue, Red, and Green lines represent data. The solid black line represents the median behavior of 1000 simulations and the black dashed lines represent the 5th and 95th percentiles of 1000 simulations. (b) Histogram of extinction times from 1000 simulations with the same parameters used in (a). Note that the extinction times observed in Wise de Valdez et al. (2011) were 10, 15, and 20 weeks post release. For these simulations, $N = 200$, $r = 10$, $c = 0$, $s_f = 0.85$, $s_m = 0.72$, and $\lambda$ varies with female age,$a$, according to the function $\lambda(a) = -0.1543a + 35.694$ for $a = 6, ..., 35$ and $\lambda(a) = 0$ for $a < 6$ and $a > 35$, which is obtained by fitting the model to weekly egg counts obtained by Wise de Valdez et al.

## Exploration of Alternative Model Assumptions

In Chapter 2, we present results from a variety of simulations that are aimed at addressing the design of cage experiments. Throughout, we make simplifying assumptions regarding survival, mating behavior, and fecundity; however, the model is designed in such a way that these assumptions can be relaxed in order to study scenarios that incorporate more biological detail. In this appendix, we present results from simulations in which more detailed descriptions of relevant biological processes (i.e., survival, mating, and fecundity) are considered. The aim here is to understand potential impacts of biological complexities not considered in the main text that could have played a role in the outcomes of previous experiments. Further, we consider a variant of the cur-

rent experimental protocol that would allow for delay periods between input of wild-type and FK individuals, a change that might ease the daily work load of personnel responsible for rearing and managing the mosquito populations. We revisit immigration of wild-type individuals by studying the immigration of adults, and we study how correlation between extinction time and reductions in the wild-type adult female population depend upon the time at which reduction is measured.

## Survival

In the main text, we assume constant daily adult survival. Here, we consider age-dependent survival based on data presented in Figure 1 of Styer et al. (2007). We consider four different release ratios, $r = 0.1, r = 1, r = 10, r = 100$. To begin studying the effects of age-dependent survival, we take average daily survival probabilities for females and males that lead to average lifespans equivalent to those calculated in Styer et al. (2007), about 54 days for females and 30 days for males. We obtain $s_f = .9818$ and $s_m = .9679$. We note that the survival curve when the mortality rate is constant must have a longer tail in order to obtain an average lifespan equivalent to that that results from mortality that is age-dependent. We compare cage experiments when survival is assumed to be age-dependent with those in which survival is held constant (Figure B.2). We find that, for all four release ratios considered, extinction times are greater when constant survival is considered. While the average lifespan is the same for both cases, the long-tailed survival curve resulting from constant daily survival leads to a higher percentage of mosquitoes with long lifespans. For both age-dependent and constant survival, the extinction times predicted here are far greater than those predicted in the main text because the average lifespans observed in the Styer et al. (2007) data are much longer than those seen in the field and assumed in the main text.

In order to make a better comparison with the main text, we scale the age-dependent survival probabilities from Styer et al. (2007) to obtain lifespans more similar to those resulting from the constant probabilities of survival used throughout the main text and the remainder of the supplementary material. For the constant probability of survival, we take $s_f = .9$ and $s_m = .79$. We take this value of $s_m$ (rather than taking $s_m = .72$ as in the main text) to match the ratio of the male and

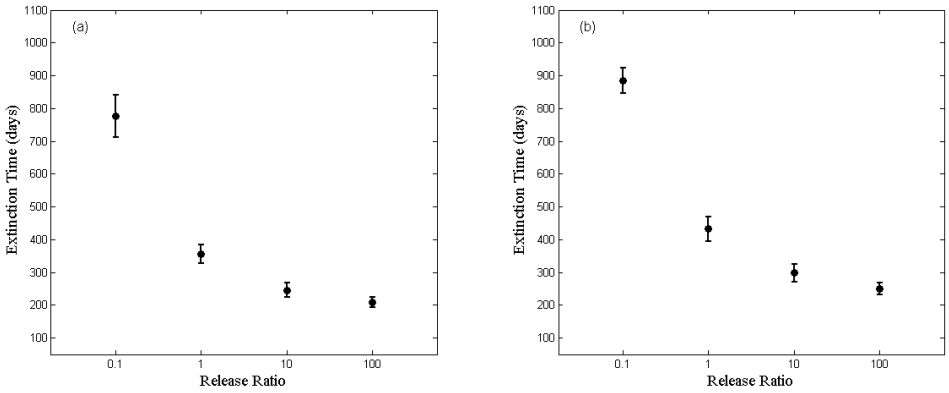


**Figure B.2:** Effects of lifespan on cage model results. Extinction time for two different survival scenarios with experiments conducted with four different release ratios. (a) Survival is age-dependent with daily survival probability obtained from data presented in Styer et al. (2007). (b) Survival is constant with respect to age. Circles are mean extinction time and error bars represent mean ± standard deviation. For these simulations, $N = 200$, $\lambda = 10$, and $c = 0$.

female average daily survival obtained from Styer et al. (2007). With constant daily survival given by these values, average lifespan is about 10 days for females and 4.76 days for males, which reflects average lifespans observed in the field. We multiply the age-dependent survival probabilities in Styer et al. (2007) by a constant ($k_f = 0.9021$ for females and $k_m = 0.7928$ for males) in order to equate the average lifespan with that resulting from constant daily survival. Results are presented in Figure B.3 below.

For all four release ratios, we find that the mean and variation of extinction times are slightly higher when daily survival is taken to be constant across age classes. The maximum difference in mean extinction time is about 10 days when $r = 0.1$. This indicates that the simplifying assumption of constant daily survival does not have a significant impact on the extinction times that the model predicts when lifespans are short.

We note, however, that average lifespan can have significant impacts on the extinction times predicted by the model. For instance, if we use the age-specific daily survival probabilities directly obtained from Styer et. al (2007), we find that extinction times are approximately double those in which daily survival is adjusted for a shorter lifespan (refer to Figure B.2). This result underscores

the need for an accurate assessment of the environment-specific life history characteristics of the pest species being studied in cage experiments. Knowledge of the average lifespan and survival curve of the species will improve the predictive ability of the model.

### Mating Behavior

Here, we look at the impact on extinction time of polyandrous mating of females. Females are considered to be polyandrous if they mate on two or more occasions in their lifetime. We consider only the case in which a female mates twice. We assume that each mating occurs on separate days and that the female mates with two different males. First, we assume that sperm from each mating has an equal probability of fertilizing an egg, and we consider matings that occur 7, 14, 21, 28, and 35 days apart under four different release ratios. Results are presented in Figure B.4.

Overall, we find that there is little difference in mean extinction time between simulations in which females only mate once and those in which two matings occur. For larger release ratios, the mean time to extinction decreases slightly when matings occur 7 and 14 days apart, but for longer periods between mating, the mean extinction time is similar to the cases when females only mate

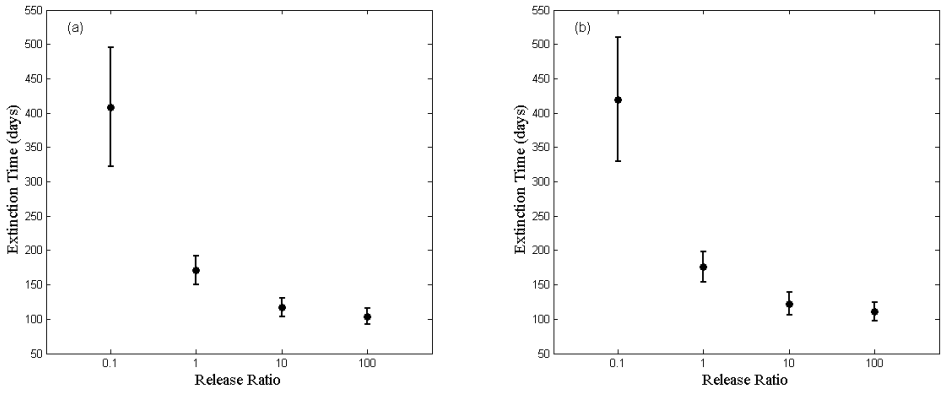


**Figure B.3:** Effects of lifespan on cage model results (2). Extinction time for two different survival scenarios with experiments conducted with four different release ratios. (a) Survival is age-dependent with adjusted daily survival obtained from data presented in Styer et al. (2007). (b) Survival is constant with respect to age. Circles are mean extinction time and error bars represent mean ± standard deviation. For these simulations, $N = 200$, $\lambda = 10$, and $c = 0$.

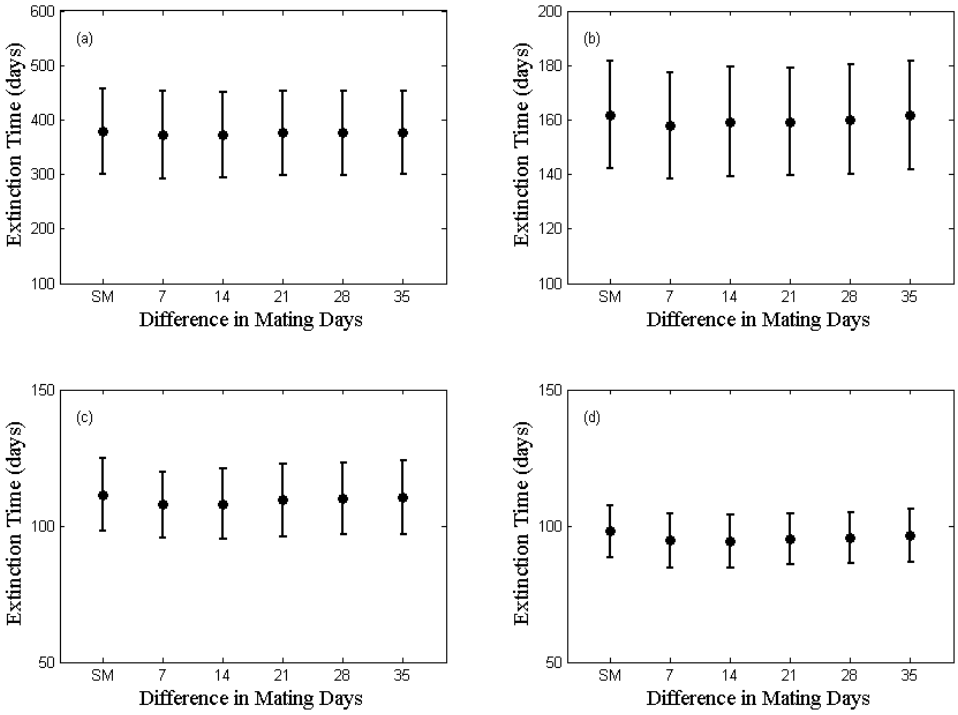


**Figure B.4:** Effects of mating behavior on cage model results. Extinction time for treatment cages for experiments conducted with four different release ratios. Females are assumed to be polyandrous and sperm from each of the females' two matings have an equal probability of fertilizing eggs. (a) $r = 0.1$, (b) $r = 1$, (c) $r = 10$, and (d) $r = 100$. Circles are mean extinction time and error bars represent mean ± standard deviation. The label 'SM' denotes the case in which only a single mating occurs. In these simulations, $N = 200$, $\lambda = 10$, $c = 0$, $s_f = 0.9$, and $s_m = 0.72$.

once.

The overall lack of differences in extinction times for different periods between the two matings could be due to the low impact that the two matings have on the population over the duration of the experiment. While females who mate twice should be more likely to mate with homozygous FK males when they mate a second time than when they first mate, this increase in likelihood will be most significant just after FK males have been initially released. Wild-type frequency decreases most rapidly during the early weeks of releases and this rate of decrease gradually slows until the wild-type population is extinct. The reduced impact of the longer periods between matings is also

a result of mortality. That is, fewer females survive to mate a second time when the period between matings is longer.

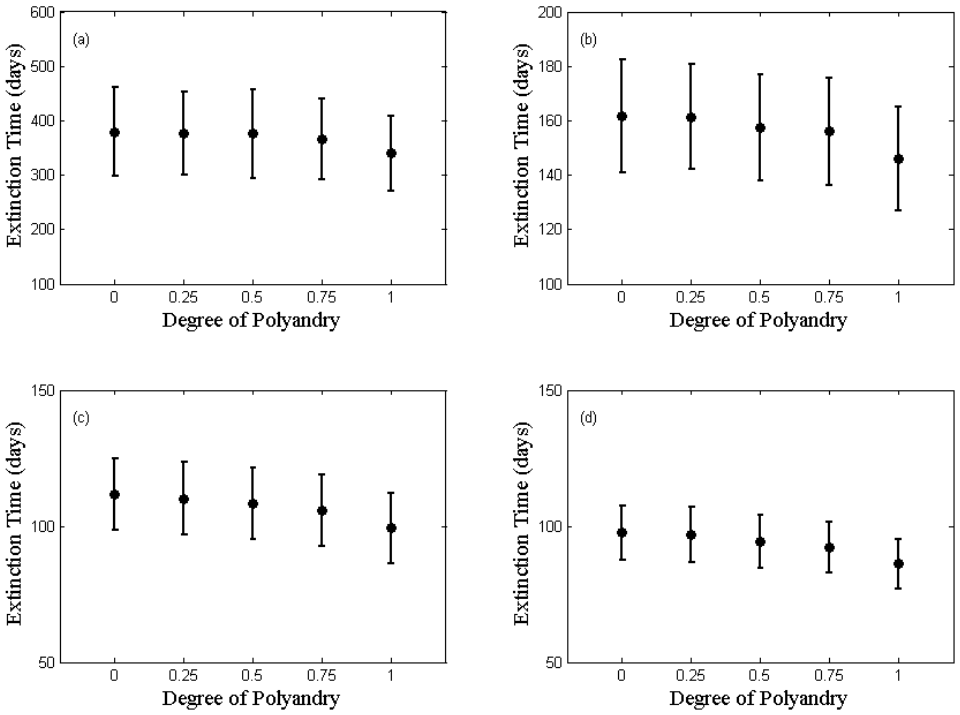


**Figure B.5:** Effects of mating behavior on cage model results (2). Extinction time for treatment cages for experiments conducted with four different release ratios. Females are assumed to be polyandrous and sperm from each of the females' two matings have a different probability of fertilizing eggs, which is determined by the degree of polyandry. (a) $r = 0.1$, (b) $r = 1$, (c) $r = 10$, and (d) $r = 100$. Circles are mean extinction time and error bars represent mean ± standard deviation. In these simulations, $N = 200$, $\lambda = 10$, $c = 0$, $s_f = 0.9$, and $s_m = 0.72$.

The contribution of polyandrous mating seems rather minimal when sperm are used equally from each mating; however, the probability that sperm from one mating fertilize eggs need not be the same as that of the other mating. We consider experiments in which offspring are more likely to receive sperm from one mating than the other. We define the **degree of polyandry** to be ratio of sperm from the second mating to the total sperm available to fertilize eggs. For example, if the

degree of polyandry is 0, none of the sperm used in fertilization are from the second mating; if the degree of polyandry is 1, all of the sperm used in fertilization are from the second mating. We study the effects of different degrees of polyandry on extinction time when the two matings occur 7 days apart. Results are shown in Figure B.5.

In general, extinction time decreases as the degree of polyandry increases from 0 to 1. This decrease is greater when release ratios are smaller. For all four release ratios, when all of the offspring have fathers from the second mating (i.e., the degree of polyandry is 1), the mean extinction time is the lowest. With increased degree of polyandry, a greater portion of the offspring have fathers from the second mating, which results in faster decline of the wild-type population.

### Fecundity

Next, we consider age-dependent fecundity. Rather than assuming females of all ages produce the same number of offspring on average, we allow for different fecundity values based on the age of the female. We use age-dependent fecundity values taken from data presented in Figure 3 of Styer et al. (2007). In the results presented here for the constant fecundity, we take $\lambda = 8$. We take this average from the data by calculating the average of the expected number of offspring a female of a certain age has given that she survives to that age. That is, if $l_x$ is the probability that a female lives to age $x$ and $m_x$ is the expected number of total offspring of a female of age $x$, we take $\lambda = \lceil \frac{1}{L} \sum_{x=1}^{n} l_x m_x \rceil$. Here, $L = \sum_{a'} l_{a'}$ is the average lifespan of a reproductive female (i.e. the average number of days a female is able to lay eggs) and $\lceil \cdot \rceil$ denotes the ceiling function. Results are shown in Figure B.6.

For all four release ratios considered, the mean extinction time and variation in extinction time is slightly lower when fecundity is constant across age classes. This is likely the case because, based on the data obtained from Styer et. al (2007), young females have more offspring on average than older females. The average value used for constant daily survival is averaged across all age classes and is thus influenced by the low number of eggs laid by older females. The increase in the number of offspring results in larger wild-type populations that must be suppressed.

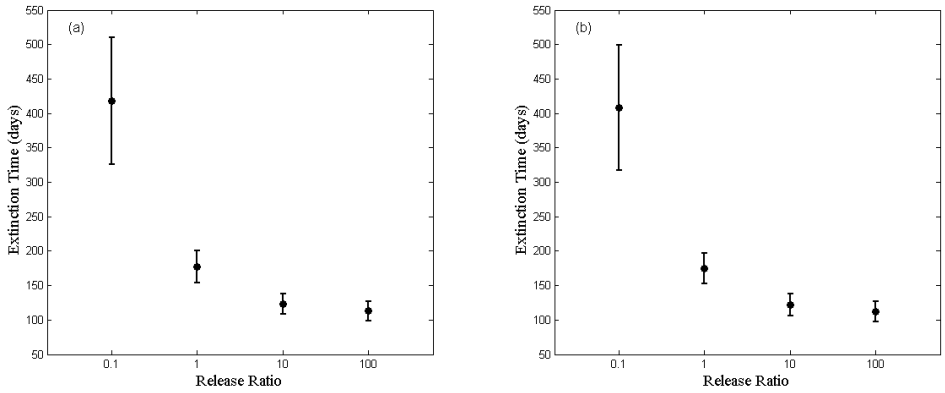


**Figure B.6:** Effects of fecundity on cage model results. Extinction time for treatment cages for two different fecundity scenarios when experiments are conducted with four different release ratios. (a) Age-dependent fecundity is taken from Styer et al. (2007) and (b) Fecundity is constant across age classes. Circles are mean extinction time and error bars represent mean ± standard deviation. In these simulations, $N = 200$, $c = 0$, $s_f = 0.9$, and $s_m = 0.72$.

## Input Delay

In some cases, the logistics of carrying out the experiment may make it desirable to separate the releases of larvae hatched from eggs laid in the cage and those of FK mosquitoes by delaying the release of FK mosquitoes by several days. However, doing so could lead to female mosquitoes mating with wild-type males before FK males are introduced. Here, we examine the impact of FK introduction delays under the four release ratios. We consider delays of between 1 and 6 days and compare to the case in which there is no delay between introductions. See Figure B.7 for the results.

We find that under all release ratios except $r = 100$, mean extinction time increases to a maximum for delays mid-week, then decreases as the delays increase. This trend is a result of the overall proximity of the releases of FK individuals to the release of the other individuals. That is, input delays of 1 day or 6 days have less impact than delays of 3-4 days because the release of FK males in the former two cases occur within a day of the input of other individuals. When FK males are released mid-week, they are less likely to mate with those individuals that are released at the beginning of the week or at the beginning of the next week, which leads to a slower decrease in the population. Input delays of 2-3 days seem to have a greater impact on experiments conducted with smaller

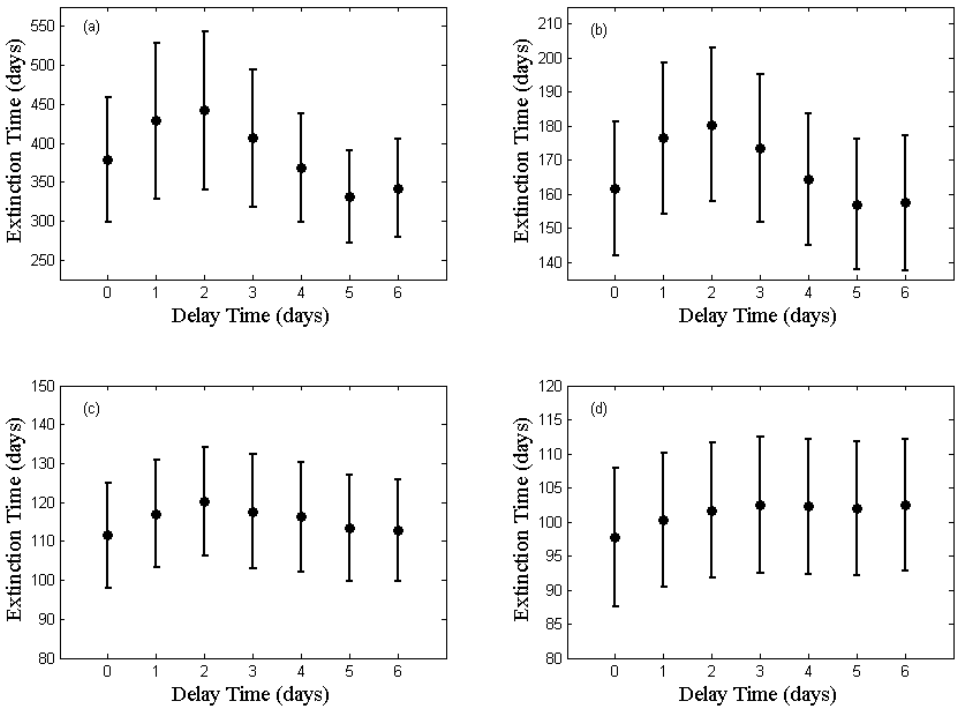


**Figure B.7:** Effects of input scheduling on cage model results. Extinction time for treatment cages when there is a delay between the input of homozygous FK males and non-homozygous FK individuals with experiments conducted with a range of four different release ratios. (a) $r = 0.1$, (b) $r = 1$, (c) $r = 10$, and (d) $r = 100$. Circles are mean extinction time and error bars represent mean ± standard deviation. For these simulations, $N = 200$, $c = 0$, $s_f = 0.9$, and $s_m = 0.72$.

release ratios. For $r = 0.1$, extinction occurs earlier on average with delays of 5-6 days. This likely occurs because FK pupae that are released at the end of the week emerge and are ready to mate at the same time as the female larvae that are released earlier in the week. This is less noticeable for larger release ratios. For $r = 100$, the increase in extinction time is almost linearly increasing with delay time. In this case, after the first few releases, the population is inundated with FK males. The delay in releases does not have a great impact on the population because the ratio of FK males to wild-type males is always very large. The delays then lead to extinction times that correspond to a scenario in which all releases are shifted by the number of days of delay. For example, if the mean extinction time with no delays is 100 days, the mean extinction time for a two-day delay would be

roughly 102 days.

### Immigration

In the main text, we considered an experimental design in which the effects of juvenile immigration can be studied in a cage experiment. Here, we extend this study further to consider the effects of adult immigration. We study the introduction of newly emerged, unmated adults and three day old, mated adult females and mating males by introducing additional adults of the age cohort under consideration. To make fair comparisons with larval immigration, we account for mortality of a cohort between larval and adult stages. We do this by selecting the number of migrating mosquitoes from a binomial distribution with parameters $N_l$ and $p_x$, where $N_l$ is the number of migrating larvae considered in the main text (10, 20, 30, 40, or 50) and $p_x$ is the probability that larvae survive to adulthood and live to age $x$. For newly emerged adults, $p_0 = (1-0.2318)$ and for three-day old adults, $p_3 = (1-0.2318)(.5s_m^3 + .5s_f^3)$. Because immigrants are constantly being introduced into the population, we must observe population reduction as defined in the main text rather than extinction time. Results are presented in Figure B.8.

As in the case of juvenile immigrants, we see a gradual increase in the mean and variance of percentage of the wild-type adult female population remaining after 14 weeks as the number of immigrants increases (Figure 2.9). The only exception is, again, $r = 0.1$, in which the variance decreases as the number introduced increases. In general, immigration of the three day old adults seems to inhibit population reduction only slightly less than newly emerged adults, as the mean and variance of the percentage of the wild-type adult female population remaining is slightly lower in all cases when adult immigrants are three days old than when they are newly emerged. In many cases, however, the difference is less than one percent. In all cases, immigration of juveniles results in a higher percentage of females remaining than does immigration of adults.

Our model output indicates that if already-mated adults enter a population, the adult female population will be maintained at a low level, because all offspring of the mated immigrants will be wild-type. This differs slightly from the result when immigrants have not yet mated. In the latter

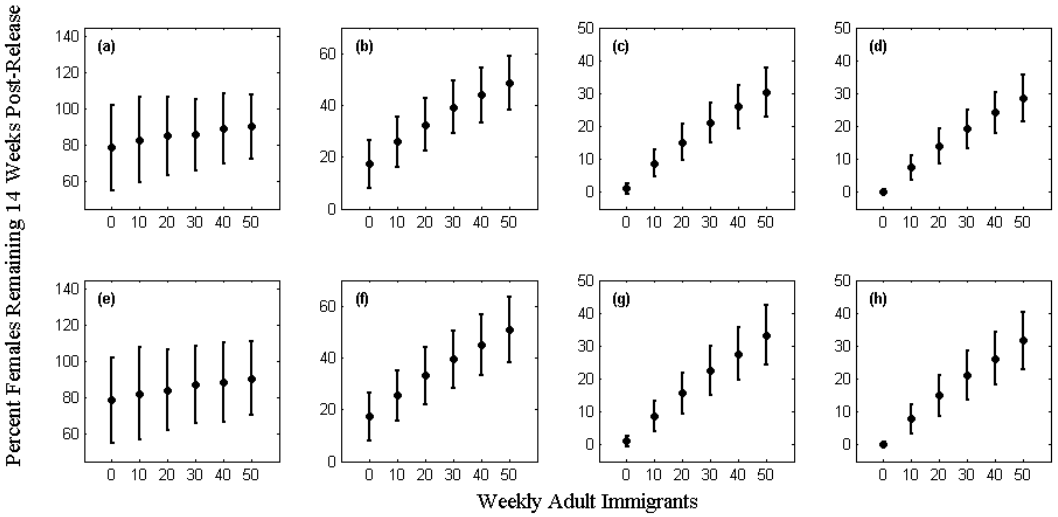


**Figure B.8:** Effects of adult immigration in the cage model. Percent wild-type adult females remaining 14 weeks post-release when wild-type immigrants are introduced to the cages every week. Experiments are conducted with four different release ratios. (a),(e) $r = 0.1$; (b),(f) $r = 1$; (c),(g) $r = 10$; and (d),(h) $r = 100$. (a)-(d) Immigrants are newly emerged, unmated adults. (e)-(h) Immigrants are three days old and females have mated before immigration. Circles are mean extinction time and error bars represent mean ± standard deviation. For these simulations, $N = 200$, $c = 0$, $\lambda = 10$, $s_f = 0.9$, and $s_m = 0.72$.

case, once the target population is sufficiently suppressed, the immigrant females would have a high probability of mating with FK males and the population would consist of only the immigrants themselves.

### An Alternative Measure for Assessment of Population Reduction

We analyze the results generated by the model for several different scenarios that arise from the combinations of fitness costs and release ratios (i.e., results presented in Figure 2.6) to assess the relationship between extinction time and wild-type adult female population reductions after a given period of time. We obtain the coefficient of determination $R^2$ from a linear regression of extinction time against percentage of the wild-type adult female population remaining after a given number of weeks (here, n = 1000 simulations is the total sample size). We present here four different scenarios that result from considering two release ratios ($r = 1$ and $r = 10$) and two fitness costs

($c = 0$ and $c = 0.6$).

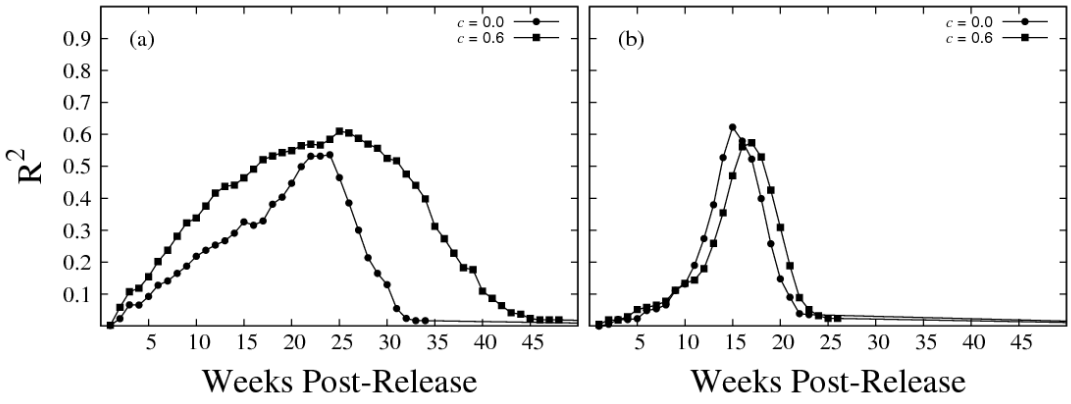


**Figure B.9:** Correlation between extinction and population reduction. The correlation between cage extinction time and population reduction after a given time period. Values of the coefficient of variation $R^2$ are plotted for each week post-release for (a) $r = 1$ and (b) $r = 10$. Circles represent simulations run with $c = 0$ and squares $c = 0.6$. For these simulations, $N = 200$, $\lambda = 10$, $s_m = 0.72$, $s_f = 0.9$.

Figure B.9 shows that the value of $R^2$ can be very different depending upon the time at which population reduction is observed. In general, population reduction as measured by observing the percentage of wild-type adult females remaining 15-30 weeks post-release is a better predictor of extinction time than observing reduction earlier than 15 or later than 30 weeks when $r = 1$. When $r = 10$, that window is earlier and more narrow, around 10-20 weeks. These so-called windows of higher correlation correspond to periods of time after reductions in population size have begun but before a large number of extinctions have occurred. In fact, the peaks of the curves in Figure B.9 are partially a result of extinctions beginning to occur. That is, as $R^2$ increases to the maxima seen here, extinctions are beginning to occur before the time of observation of population reduction. The strength of predictions that can be made based on observing population reduction then decreases rapidly as the time post release gets longer. Therefore, observing population reduction before the times associated with these peaks would be best for using the model to make predictions.

## References

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Wise de Valdez, M.R., D. Nimmo, J. Betz, H. Gong, A. A. James, L. Alphey, and W.C. Black, IV. 2011. Genetic elimination of dengue vector mosquitoes. Proc. Natl. Acad. Sci. U.S.A. 108:4772-4775.

# Appendix C

# R&R model Equilibrium analysis [1]

In Chapter 4, we describe the development of a system of ordinary differential equations that we use to simulate population dynamics and population genetics of *Aedes aegypti* following the introduction of an R&R strain into a wild-type population. Analysis of this model is limited by the model complexity; however, we are able to obtain equilibrium population density of the wild-type population in the absence of transgenic releases.

In a completely wild-type population, the system

$$\begin{aligned}
\dot{J}_i &= B_i(t) - \mu_J J_i - J_i \left( \alpha \sum_g J_g \right)^{\beta-1} - \nu J_i \\
\dot{F}_i &= \frac{1}{2} \nu \gamma_i J_i - \mu_F F_i + u_i^F \\
\dot{M}_i &= \frac{1}{2} \nu J_i - \mu_M M_i + u_i^M \\
B_i(t) &= w_i \lambda \sum_m F_m(t) \sum_n Pr(i|m,n) \frac{M_n(t)}{\sum_g M_g(t)}
\end{aligned} \tag{C.1}$$

[1] This appendix is included as supplementary online material for the publication Robert MA, Okamoto K, Lloyd AL, Gould F. (2013) A Reduce and Replace Strategy for Suppressing Vector-Borne Diseases: Insights from a Determinstic Model. PLoS ONE 8(9): e73233. doi:10.1371/journal.pone.0073233

for $i = 1...9$, where $i = 9$ represents the wild-type genotype, reduces to

$$\begin{aligned}
\dot{J}_9 &= \lambda F_9 - \mu_J J_9 - \alpha^{\beta-1} J_9^{\beta} - \nu J_9 \\
\dot{F}_9 &= \frac{1}{2}\nu J_9 - \mu_F F_9 \\
\dot{M}_9 &= \frac{1}{2}\nu J_9 - \mu_M M_9.
\end{aligned} \tag{C.2}$$

Here, $\dot{M}_9$ is decoupled from the system, so we can analyze the reduced system

$$\begin{aligned}
\dot{J}_9 &= \lambda F_9 - \mu_J J_9 - \alpha^{\beta-1} J_9^{\beta} - \nu J_9 \\
\dot{F}_9 &= \frac{1}{2}\nu J_9 - \mu_F F_9 \,.
\end{aligned} \tag{C.3}$$

This system has a trivial equilibrium at $(J_9^{(1)}, F_9^{(1)}) = (0,0)$, and one non-trivial equilibrium at

$$\begin{aligned}
J_9^{(2)} &= \frac{1}{\alpha}\left(\frac{\nu\lambda}{2\mu_F} - \mu_J - \nu\right)^{\frac{1}{\beta-1}} \\
F_9^{(2)} &= \frac{\nu}{2\mu_F} J_9^{(2)} \,.
\end{aligned} \tag{C.4}$$

We rearrange the expression for $J_9^{(2)}$ by noting that

$$\begin{aligned}
\frac{\nu\lambda}{2\mu_F} - \mu_J - \nu &= (\mu_J + \nu)\left(\frac{\nu\lambda}{2\mu_F(\mu_J+\nu)} - 1\right) \\
&= (\mu_J + \nu)(R_0 - 1)\,,
\end{aligned} \tag{C.5}$$

where

$$R_0 = \frac{1}{\mu_F} \cdot \frac{\lambda}{2} \cdot \frac{\nu}{\mu_J + \nu} = \frac{\nu\lambda}{2\mu_F(\mu_J+\nu)} \,. \tag{C.6}$$

Here, $\frac{1}{\mu_f}$ is the average lifespan of adult females, $\frac{\lambda}{2}$ is the rate of production of female offspring, and

$\frac{\nu}{\mu_J+\nu}$ is the fraction of juveniles that survive to emerge as adults. Thus, $R_0$ is the basic reproductive number of the population. We rewrite (C.4) in terms of $R_0$.

$$\begin{aligned} J_9^{(2)} &= \frac{1}{\alpha}\left((\mu_J+\nu)(R_0-1)\right)^{\frac{1}{\beta-1}} \\ F_9^{(2)} &= \frac{\nu}{2\mu_F} J_9^{(2)} \end{aligned} \tag{C.7}$$

In order for population to have a positive equilibrium (i.e., $J_9^{(2)} > 0$), $R_0 > 1$. Thus, we analyze the stability of the equilibrium only for the case when $R_0 > 1$.

To verify the stability of the equilibrium in (C.7), we first find the Jacobian of system (C.3).

$$\text{Jacobian}(J_9, F_9) = \begin{pmatrix} -\left(\mu_J+\nu+\beta(\alpha J_9)^{\beta-1}\right) & \lambda \\ \frac{1}{2}\nu & -\mu_F \end{pmatrix} \tag{C.8}$$

We then evaluate the Jacobian at the equilibrium in (C.7).

$$\mathscr{J} = \text{Jacobian}(J_9^{(2)}, F_9^{(2)}) = \begin{pmatrix} -\left(\mu_J+\nu+\beta(\mu_J+\nu)(R_0-1)\right) & \lambda \\ \frac{1}{2}\nu & -\mu_F \end{pmatrix} \tag{C.9}$$

We now study the eigenvalues of $\mathscr{J}$ by studying the determinant and trace of $\mathscr{J}$. The equilibrium point $(J_9^{(2)}, F_9^{(2)})$ is stable when $\text{Tr}(\mathscr{J}) < 0$ and $\det(\mathscr{J}) > 0$ (i.e., both eigenvalues of $\mathscr{J}$ must be negative). First, we calculate $\text{Tr}(\mathscr{J})$.

$$\text{Tr}(\mathscr{J}) = -(\mu_J+\nu+\beta(\mu_J+\nu)(R_0-1)+\mu_F) \tag{C.10}$$

Since $R_0 > 1$, and because we require $\mu_J$, $\nu$, $\beta$, and $\mu_F$ to be positive, $\text{Tr}(\mathscr{J}) < 0$. Next, we calculate $\det(\mathscr{J})$.

$$\det(\mathscr{J}) = \mu_F\left(\mu_J+\nu+\beta(\mu_J+\nu)(R_0-1)\right) - \frac{\lambda\nu}{2} \tag{C.11}$$

Rearranging the terms, we get

$$\det(\mathscr{J}) = 1+\beta(R_0-1)-\frac{\lambda\nu}{2\mu_F(\mu_J+\nu)}$$
$$= 1+\beta(R_0-1)-R_0\ . \tag{C.12}$$

In order for $\det(\mathscr{J})>0$,

$$1+\beta(R_0-1)-R_0>0$$
$$\beta(R_0-1)>R_0-1 \tag{C.13}$$
$$\beta>1\ .$$

So we have that the equilibrium $(J_9^{(2)}, F_9^{(2)})$ is stable when $\beta>1$.

### Equilibrium Values for Model Runs

Here, we list the values of the equilibrium density of juveniles, adult males, and adult females that are used for model runs in the main text. Note that the release size of R&R individuals is always defined as a function of the equilibrium wild-type male population density so that release rates are always relative to the population density. This allows for a general study of R&R releases in an *Ae. aegypti* population. While changes in $\alpha$ will result in changes in the density of the population, the qualitative results for relative density are the same.

$$J_9^* = J_9^{(2)} = 10118.98$$
$$F_9^* = F_9^{(2)} = 7083.28 \tag{C.14}$$
$$M_9^* = M_9^{(2)} = 2529.74$$

# Appendix D

# Further exploration of R&R [1]

## Density dependence

Throughout Chapter 4, we consider a population in which the strength of density-dependent larval regulation is strong. Here, we vary the strength of density dependence by considering different values of $\beta$ ($\beta = 1.8$, $\beta = 2$, $\beta = 2.2$, $\beta = 2.8$, $\beta = 3$, and $\beta = 3.2$). These results are presented in Figure D.1. For lower values of $\beta$, the transient reduction in both the total female and competent vector population densities is greater than for higher values of $\beta$. This occurs because populations rebound more quickly from perturbations away from equilibrium density for higher values of $\beta$ (stronger density dependence). For these higher values of $\beta$, the total adult female population reaches an intermediate equilibrium density while releases occur before returning to the pre-release density. For lower values of $\beta$, however, including $\beta = 2$ (which would correspond to the familiar logistic term), the total population density decreases towards extinction, but eventually returns to pre-release densities after releases end. If population elimination is desired in a population in which density-dependent regulation is strong, release sizes would need to be increased to ratios high enough to overcome this strong density-dependent population regulation. In these

[1] This appendix is included as supplementary online material for the publication Robert MA, Okamoto K, Lloyd AL, Gould F. (2013) A Reduce and Replace Strategy for Suppressing Vector-Borne Diseases: Insights from a Determinstic Model. PLoS ONE 8(9): e73233. doi:10.1371/journal.pone.0073233

populations, R&R releases may be desired because even though population elimination is difficult to achieve, R&R releases lead to a lower density of competent vectors than FK only releases (compare each panel of Figure D.1).

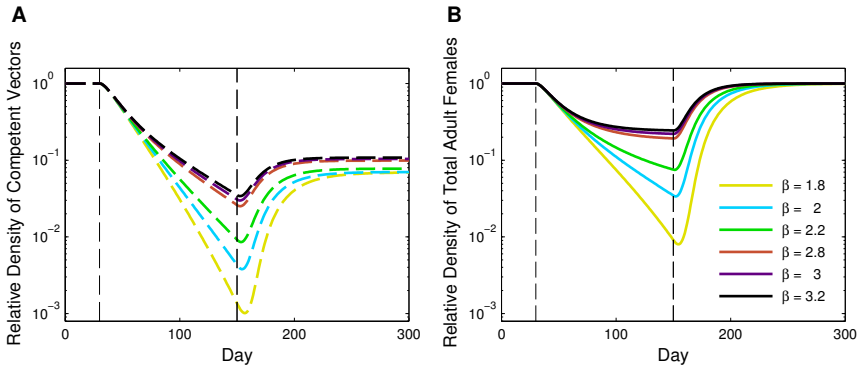


**Figure D.1:** R&R and density dependence. Dynamics of an *Ae. aegypti* population subject to continuous male-only R&R releases at a 1:1 ($r = 1$) release ratio for 120 days for different strengths of density dependence. (a) Relative density of competent vectors. (b) Relative density of total adult females. (Note that this panel also indicates the relative density of the total (and thus competent) adult female population during FK releases.) For both panels, the first vertical dashed line represents the first day of release (30) and the second vertical dashed line represents the last day of release (150). All other parameter values are the default values listed in Table 4.2 of Chapter 4. Note the vertical axis is on a log scale.

In Chapter 4, we consider the reduction in competent and total female vector densities that results from different combinations of release ratio and duration. In each release, the same total number of R&R males is released. Here, we consider the effects of density dependence on release scenarios that arise from these different combinations (Figure D.2). The intermediate combinations in which total population reduction is the greatest depends upon the strength of density dependence. As density dependence gets stronger (higher values of $\beta$), population reduction is greatest at lower intermediate release durations. This is because the population is capable of rebounding much more quickly than populations with weaker density dependence. For stronger density dependence, short but very intense releases are required to maximize population reduction, and smaller release sizes over long periods of time cannot overcome the strength of density depen-

dence.

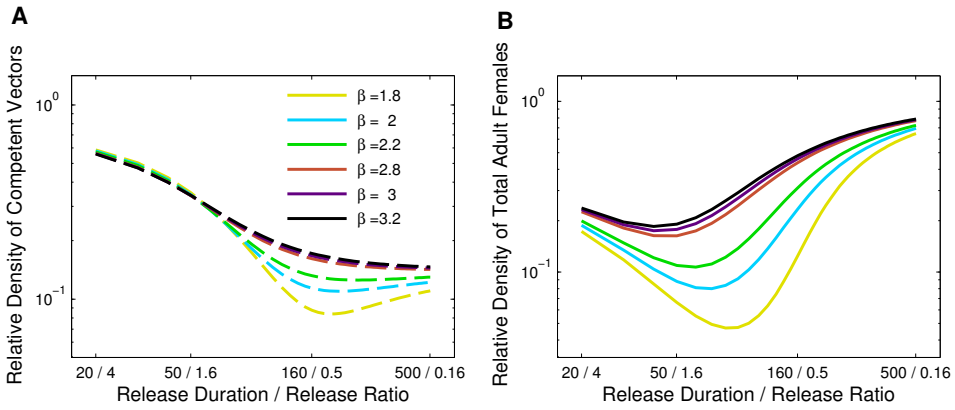


**Figure D.2:** Density dependence and release ratio and duration. Relative adult female population density following releases of R&R males into populations regulated by different strengths of density dependence with release scenarios involving different combinations of release ratios and durations. (A) Relative density of competent vectors is measured once the total population returns to its pre-release density following releases. (B) Minimum relative density of total adult females is measured on the day in which the minimum occurs for corresponding release scenarios. The horizontal axis for both panels is labeled as release duration / release ratio, with release durations increasing from left to right and release ratios increasing from right to left. Each scenario results in the release of the same total number of male mosquitoes. All other parameter values are the default values listed in Table 4.2. Note the both axes are on a log scale.

One should also note that the shape of the curves describing the reduction in competent vectors change slightly as density-dependent population regulation strengthens. As $\beta$ increases, the relative density of competent vectors changes from a non-monotonic to a monotonically decreasing curve for the combinations of release ratio and release duration considered here. For each of the non-monotonic curves, the minimum occurs for scenarios in which releases occur at low intensity over longer periods of time. As density dependence strengthens, the marginal benefit of increasing release duration is lost because the population can rebound more easily, and the smaller releases have less impact on the population.

## Release duration and female-only releases

In Chapter 4, we present results from male-only, bi-sex, and female-only releases conducted at a 1:1 ratio for only 100 days. We mention there that this combination of release duration and release ratio was chosen because longer durations of releases including females typically led to population extinctions. Here, we show that the total female population density decreases rapidly as the release duration is increased when female-only releases are conducted at a 1:1 ratio (Figure D.3). In fact, for the parameter set considered here, a release duration $T = 120$ days led to extinction of the population whereas a release for $T = 110$ days did not.

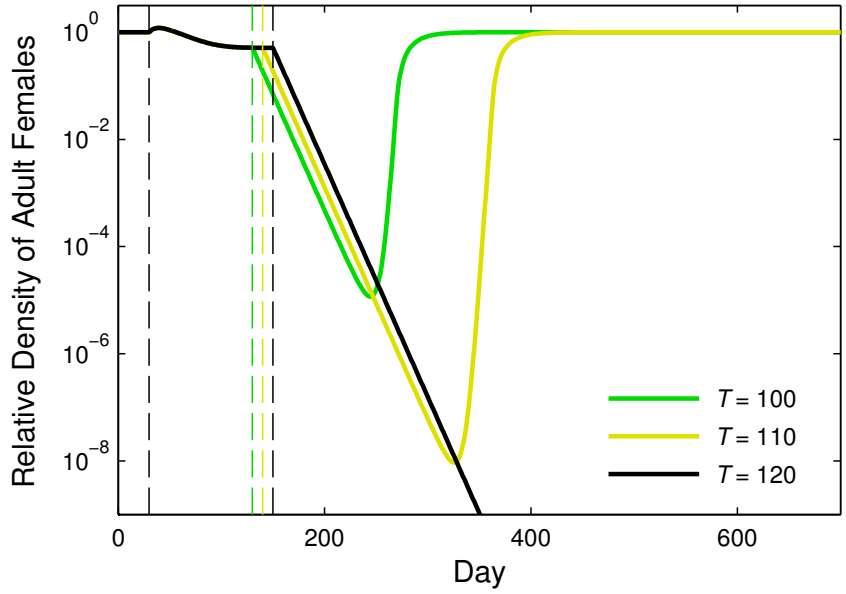


**Figure D.3:** Duration of female-only R&R releases. Relative total adult female population density when continuous male-only R&R releases occur at a 1:1 ($r = 1$) release ratio for different release durations. Each release begins on day 30, and release durations are $T = 100$, $T = 110$, and $T = 120$ days. The black vertical dashed line marks the beginning of releases, and the end of each release is indicated by a vertical dashed line of corresponding color. All other parameter values are the default values listed in Table 4.2. Note that the vertical axis is on a log scale.

**Wild-type immigration**

Here, we study the impacts of immigration of wild-type juveniles. We assume that immigrants enter the population at a constant daily rate, $\eta$, before, during, and after R&R releases occur. We note that this changes the equation describing the dynamics of wild-type juveniles to

$$\dot{J}_9 = B_9(t) - \mu_J J_9 - J_9 \left( \alpha \sum_g J_g \right)^{\beta-1} - \nu J_9 + \eta \, .$$

We define immigration rates as fractions of the equilibrium wild-type juvenile density per day. For example, if immigration occurs at a rate of 1% of the wild-type equilibrium density per day, then $\eta = 0.01 J_9^* \text{day}^{-1}$. We find that the impact of immigration on the density of competent vectors depends upon the magnitude of the immigration rate (Figure D.4). That is, larger immigration rates lead to smaller decreases in the competent vector population than smaller immigration rates. Furthermore, larger immigration rates result in the competent vector density returning towards the pre-release equilibrium much more quickly than smaller immigration rates. For the smallest immigration rate we consider here, the competent vector density does not increase much, even hundreds of days after R&R releases end, whereas the competent vector population is near pre-release equilibrium soon after R&R releases end for the largest immigration rates we consider. This is in part due to the inability to reduce the competent vector population as much in the presence of larger immigration rates. Although immigration does hinder R&R releases from leading to the same reduction in competent vectors as is observed without immigration, R&R releases still lead to more reduction in competent vectors than FK releases, regardless of the immigration rate (compare solid and dashed lines in Figure D.4).

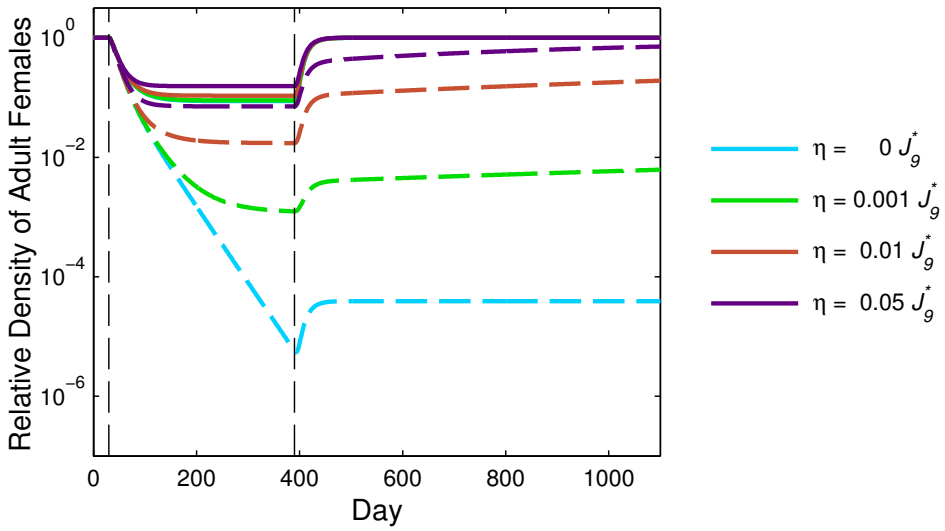


**Figure D.4:** R&R and immigration of wild-type juveniles. Relative total adult female population density (solid lines) and relative competent vector density (dashed lines) when continuous male-only R&R releases occur at a 2:1 ($r = 2$) release ratio for $T = 100$ days in the presence of wild-type juvenile immigration. The black vertical dashed lines mark the beginning and end of releases. Immigration rates are defined in terms of a fraction of the equilibrium juvenile density per day: No immigration, 0.001 $J_9^*$, 0.01 $J_9^*$, and 0.05 $J_9^*$. All other parameter values are the default values listed in Table 4.2. Note that the vertical axis is on a log scale.

# Appendix E

# Additional exploration of hybrid strategies [1]

## Release ratio, duration, and switch time

We studied the effects of release ratio and release duration on each of the six strategies by varying the release ratio and release duration while holding the total release number constant (Figure E.1). For combination approaches, we considered switch times of 1/4 and 3/4 the total release duration. We considered both male-only, bi-sex, and female-only releases. For all release types, longer releases of AP-only individuals led to greater long-term reduction than shorter AP-only releases, and among all of the release strategies, AP-only releases led to the greatest long-term reduction in competent vectors when the release duration was longer, whereas R&R/AP releases led to the greatest long-term reduction for short, intense releases when females were included in releases. For male-only releases, AP-only and R&R-only strategies led to more reduction as the release duration increased, as did R&R/AP approaches, regardless of the switch time for combination strategies. For early switch times, FK/AP strategies led to more reduction as the release duration increased, but as

[1] This appendix will be included as supplementary online material for the manuscript Robert MA, Okamoto K, Gould F, Lloyd AL. Anti-pathogen genes and replacement of disease vector populations: a model-based evaluation of hybrid strategies

the switch time increased, the most reduction caused by the FK/AP strategy resulted from an intermediate combination of a shorter release duration and larger release ratio. For longer switch times, there was a release ratio/duration combination for which R&R and FK/AP releases were equally effective, and as the release duration increased, R&R releases became more effective than FK/AP releases.

For releases including females, FK/AP dynamics were similar to those of male-only releases, with greater reductions at larger release ratios when the switch time was early and an intermediate optimum for later switch times. For early and intermediate switch times, there were two release ratio and duration combinations for which R&R and FK/AP were equally effective for female-only releases. Bi-sex releases exhibited similar behavior for intermediate switch times, but FK/AP was always more effective than R&R for early switch times. For both female-only and bi-sex releases with later switch times, FK/AP releases were less effective than R&R as the release duration increased and were as effective as R&R releases for one release ratio and duration combination. As discussed in Robert et al. (in press), R&R releases involving females had the most impact at intermediate combinations of release ratio and release duration. This intermediate optimum was reflected in releases involving R&R: R&R/AP releases also had the most impact on competent vector density for intermediate combinations of release ratio and release duration when switch times were longer.

For releases including females, there was at least one release ratio and duration combination for which R&R/AP and AP-only releases had a similar impact on competent vectors, regardless of the switch time. For all switch times, bi-sex releases of R&R/AP and AP-only had a similar impact for two combinations of release duration and ratio. For early and later switch times, female-only releases of R&R/AP and AP-only individuals had similar impacts for two different combinations of release duration and ratio. For female-only releases, there also were two combinations of release ratio and release duration for which R&R and AP-only strategies had similar impacts on competent vector density, with a small range of combinations for which R&R strategies led to more reduction than AP-only strategies.

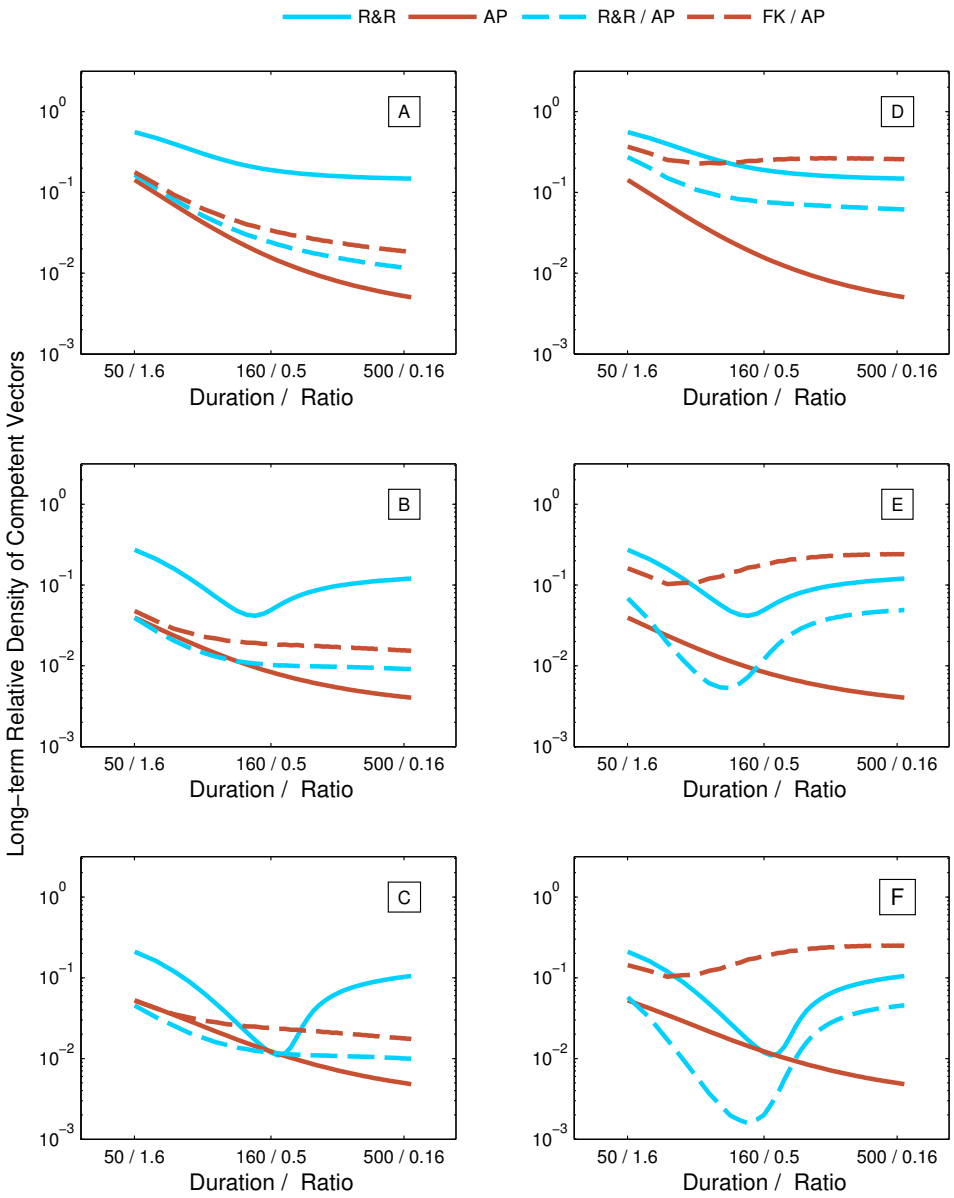


**Figure E.1:** The effects of release parameters on FK, R&R, and AP strategies. Long-term relative competent vector population density resulting from release scenarios that arise from different combinations of release ratio and release duration with male-only (A,D), bi-sex (B,E), and female-only releases (C,F). For combination strategies, the switch occurs after 1/4 the total release duration (A-C) or 3/4 the total release duration (D-F). All other parameter values are the default values listed in Table 5.2. Note the vertical axes are on a log scale.

## Density dependence and minimum population density

Figure E.2 shows the minimum relative density of competent vectors for different values of $\beta$.

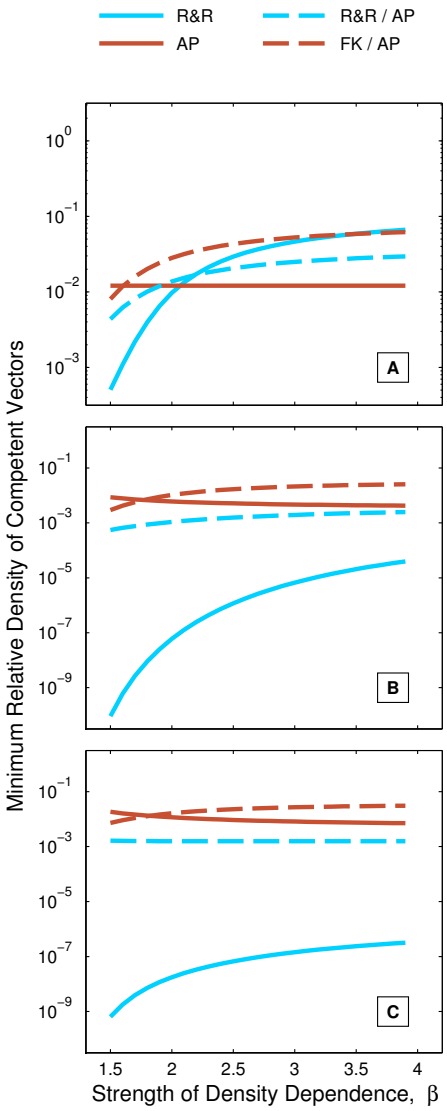


**Figure E.2:** The effects of $\beta$ on minimum relative density of competent vectors. Minimum relative density of competent vectors for different strengths of density dependence when releases were conducted at a 1:1 ($r = 1$) release ratio for $T = 100$ days with male-only (A), bi-sex (B), and female-only (C) releases. For combination strategies, the switch occurs after 50 days. All other parameter values are the default values listed in Table 5.2. Note the vertical axis is on a log scale.