Modeling sustained transmission of *Wolbachia* among *Anopheles* mosquitoes: Implications for Malaria control in Haiti

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Abstract

Anopheles albimanus mosquitoes infected with the *wAlbB* strain of *Wolbachia* bacterium are less capable of spreading malaria. We develop and analyze an ordinary differential equation model to evaluate the effectiveness of different vector control strategies in establishing a sustained *Wolbachia* infection among wild Anopheles mosquitoes in Haiti. The model involves the male and female mosquitoes at different life stages, including egg, larva, and adult. It also accounts for critical biological effects, such as the maternal transmission of *Wolbachia*, where the offspring of infected female mosquitoes are infected, and cytoplasmic incompatibility, which effectively sterilizes uninfected females when they mate with infected male mosquitoes. We derive and interpret relevant dimensionless numbers, including the basic reproductive number. We then show that the system presents backward bifurcation, characterized by the disease-free, a complete-infection, and a threshold endemic equilibrium, which corresponds to the minimal infection that needs to be released. We use sensitivity analysis to rank the relative importance of the epidemiological parameters in Haiti during the

rainy season. Lastly, we simulate the integrated intervention strategies, where traditional vector controls are implemented to reduce the wild adult mosquitoes before releasing the *Wolbachia*-infected mosquitoes. Specifically, we compare two vector controls targeting the larvae stage (larviciding) and adult mosquitoes (thermal fogging), respectively, and we assess the efficacy of the controls by measuring the speed of establishment of *Wolbachia* among the mosquito population. 150 to 250 words.

Keywords: *Anopheles* mosquitoes, *wAlbB Wolbachia*, malaria control, mosquito control 4 to 6 keywords.

Contents

To do

- General
  ~~Update the manuscript to theoretical ecology journal template~~
  ~~Update abstract~~
- Miscellanous
- section 1
  ~~update the introduction, edit down the description on different wolbachia strategies, remove duplication of descriptions~~
  ~~edit down the tables 1 and 2 to fit the width.~~
- section 3
  ~~reorganize steady-states and $\mathcal{R}_0$ section in the following order~~
  ~~edit next generation numbers~~
  ~~edit DFE, CIE~~
  ~~$\mathcal{R}_0$, correct the interpretation~~
  ~~fix the next generation matrix mistakes~~
  ~~edit EE section, simplify the expression for L_u^*~~
- section 3:
  Stability analysis for all the steady states ($v_w = 1$ special case)
  - ~~show CIE and DFE are stable~~
  - ~~Write up the stability analysis for CIE and DFE~~
  - ~~show EE is unstable~~
  - Expand discussion on the bifurcation analysis
- ~~Update throughout the manuscript to remove the habitat manipulation~~
- section 4: numerical simulations
  - update the description of release scenarios (paragraphs before 4.1), only seasonality, wet vs. dry. Release males = females, no pregnant/single
    ~~Larviciding, any description? Reference?~~ ~~Remove the habitat manipulation.~~
  - Clean up the description on the "Role of seasonality" (cut down to maybe half page): describe the reduction in carrying capacity justified by the Haiti data (Alyssa describe the basics in data)
  - caveat to put in: temperature may affect the EIP of malaria in mosquitoes, but we don't track malaria infection, only the *Wolbachia*.
  - ~~Coding for finding the threshold release number needed~~
    ~~→ to establish *Wolbachia*~~
    ~~→ to establish and reach 90% infection within one month~~
  - ~~Run simulation results for the wet season interventions and fill in table 4~~
    ~~(table 4 - Mac will fill in)~~
    ~~Fix table format in table 4.~~
  - Pick and update figures (two illustrative plots to show the difference between different pre-release mitigation)
    describe and discuss numerical results
  - Implement results for the dry season interventions (seasonality)

discuss numerical results, notion of habitat manipulation

- section 5: sensitivity analysis (maybe combined with section 4)
  - The current QOI = $\mathcal{R}_0$ only. Update table 5 with the current model and parameters. What do we learn?
  - Other QOIs? Threshold level? Time to establishment?
  - other POIs? Relative fitness cost?
- section 6: Discussion
  - Summary of model proposed, innovation
  - major results, and significance
  - discuss model assumptions and limitation
    no spatial dynamics
    seasonality results are limited to Haiti
    ...

Notes

- I changed σ to π to represent the rate females become pregnant. I wonder if it would be better to just use τ_π or τ_p for the mean time for them to become pregnant and never define the rate since it doesn't appear in the equations. MH
- Be sure to include the first meaningful word in the title when creating a bibtex entry. For example, use xue2018comparing and not just xue2018. This will allow us to cite more than one paper a year from an author without confusion. MH

1 Introduction

Malaria is a febrile illness caused by several species of *Plasmodium* protozoan parasites through the bite of an infected female *Anopheles (An.)* mosquito. Falciparum malaria is a leading cause of death globally and the most lethal of the five known species of *Plasmodium* that can infect humans. Current efforts to control malaria typically focus on strengthening surveillance and case management, administering seasonal malaria chemoprophylaxis, or reducing mosquito populations, such as insecticide-treated bed nets (ITNs), larval control, indoor residual spraying (IRS). Due to increasing insecticide resistance and impact of climate change, among and other environmental factors, have on mosquito breeding and feeding behavior, more sustainable and effective mitigation strategies are necessary.

Wolbachia pipientis is a gram-negative intracellular endosymbiotic bacterium that naturally infects over 75% of all arthropods Pan et al (2017); Mustafa et al (2016), including some mosquitoes that spread human diseases. Transinfection of *Aedes sp.* mosquitoes has show to be effective at controlling Dengue fever, Chikingunya and Zika virus transmission. Recently there is evidence to suggest that similar approaches can be used to control the spread of *P. falciparparum* malaria. In some *Anopheles* species, *Wolbachia* has also been shown to reduce the number of *P. falciparum* oocysts and sporozoites Bian et al (2013), and *Wolbachia*-infected *Anopheles* mosquitoes are less effective in transmitting the parasite. Passing of such anti-pathogenic traits to offspring is achievable as *Wolbachia* exhibits high rates of maternal transmission in both *Aedes* and *Anopheles* sp. mosquitoes Pan et al (2017); Joshi et al (2014, 2017). This leads to the development of population replacement strategy that involves introducing *Wolbachia*-infected mosquitoes into the field to replace the wild population. This innovative approach could be effective in controlling malaria Mustafa et al (2016); Zhang et al (2015); Bourtzis et al (2014); Mousson et al (2012).

Wolbachia has also been used as a population suppression strategy by releasing only infected-male mosquitoes. Evidence suggests that presence of the bacteria somehow modifies the paternal chromosomes during spermatogenesis Hancock et al (2011), and, when mating with an uninfected female, the sperm of the *Wolbachia*-infected male is unable to form viable offspring during the egg fertilization process, resulting eggs do not hatch Gomes and Barillas-Mury (2018). This *Wolbachia*-induced cytoplasmic incompatibility (CI) phenomenon provides an alternative approach that acts similarly to the adulticiding. This population suppression strategy requires constant introduction of infected male mosquitoes to sustain the suppression, and it suffers the same sustainability issue as the traditional approaches: when the intervention stops, mosquito population may re-emerge. Moreover, the strategy can be hard to deploy in practice due to accidental release of infected females, which may produce infected offspring and undermine the process Zheng et al (2019).

We summarize both strategies in table 1. For our study, we focus on the population replacement strategies, which we believe could serve as be a more self-sustained approach.

Infecting *Anopheles* mosquitoes with *wMelPop* and *wAlbB* strains of *Wolbachia* has shown to reduce *P. falciparparum* sporozoite and oocyst levels in specific species (table 2), and the *wAlbB* strain has shown to exhibit perfect maternal (vertical) transmission in *An. stephensi* Joshi et al (2014, 2017). Since almost all offspring of *wAlbB*-infected females will be infected, this specific strain is an excellent choice to create a sustained population of *Wolbachia*-infected wild *Anopheles* mosquitoes. Our focus will be to identify strategies for creating a sustained *wAlbB* infection within a population of wild *Anopheles* mosquitoes using the population replacement disease control approach.

Mathematical models that evaluate the impact of *Wolbachia* infection on mosquito capacity and disease transmission have focused primarily on arboviruses spread by *Aedes spp.* mosquitoes. Xue et al (2018) compared the effectiveness of infecting *Aedes aegypti* and *Aedes albopictus* mosquitoes with *wAlbB*and *wMel* in reducing the transmission of Dengue, Chikingunya, and Zika viruses. This study analyzed a system of seven ordinary differential equations (ODEs) that accounted for the reduced fitness of *Wolbachia*-infected mosquitoes, the reduced transmissibility of infected mosquitoes, and the behavior changes of infected humans caused by disease. This model was based on previous studies to establish a population of wild *Wolbachia*-infected *Aedes* mosquitoes. Qu et al (2018); Qu and Hyman (2019) and Xue et al (2015) created a series of two-sex heterosexual transmission compartmental models for *Wolbachia* transmission in *Aedes* mosquitoes. They used the models to quantify the effectiveness of different approaches in ensuring the sustained transmission of *Wolbachia* within wild *Aedes* mosquito populations.

These studies for non-malaria transmitting mosquitoes were the starting point for our *Anopheles* mosquito model. We extended their model by subdividing the aquatic stage into egg and larval/pupae stages. We assume that

Table 1 Role of *Wolbachia* in mosquito population replacement versus population suppression

	Population Replacement	Population Suppression
Goal	Replace wild mosquito population with *Wolbachia*-infected ones that have significantly lower competence and cannot transmit parasite as efficiently	Introduce male mosquitoes that cannot produce viable offspring, which limits the ability of mosquito to reproduce and reduces mosquito population
Role of CI	Infected females can mate successfully with infected males providing them with an evolutionary advantage over uninfected females	The sperm of the infected male is unable to form viable offspring during the egg fertilization process, and resulting eggs do not hatch
Release	Release infected males and females	Release infected males only

Table 2 *Wolbachia* strains, *Anopheles* species, and corresponding impact on vector and *P. falciparum* parasite replication.

Wolbachia strain	Anopheles species	Impact on vector	Impact on *P. falciparum*	Reference
wAnga	*coluzzii*	No CI, increases egg laying rate	Reduces sporozoite prevalence	Shaw et al (2016); Childs et al (2020); Sicard et al (2019); Gomes et al (2017)
	funestus	No CI	Unknown	Sicard et al (2019)
	gambiae	No CI	Unknown	Sicard et al (2019)
	arabiensis	No CI	Unknown	Sicard et al (2019)
wAlbB	*stephensi*	Almost complete CI, reduces egg hatching rate, perfect maternal transmission, no impact on female lifespan	Reduces sporozoite and oocyst levels	Joshi et al (2014); Bian et al (2013)
wPip	*gambiae*	CI, reduces egg development rate	Unknown	Adams et al (2021)
wMelPop	*gambiae*	No effect on lifespan	Significantly reduces oocyst level	Hughes et al (2011)

the time it takes for a newly born mosquito to be impregnated is small compared to the lifetime of the mosquito; therefore, we eliminated the impregnated mosquito compartments Qu et al (2018).

We focus on the malaria-specific interventions conducted before releasing various mosquito strategies, including larviciding, ultra-low volume spraying, and thermal fogging. These interventions can accelerate establishing an endemic infection of *Wolbachia* among *Anopheles* mosquitoes. We simulate the combination of these vector control strategies for different release scenarios of *Wolbachia*-infected mosquitoes.

We evaluate the impact that seasonality can have on the releasing time for reaching a stable high-infection among wild mosquitoes. We will parameterize our model based on the local data in Haiti, where the heterogeneous temporal transmission of falciparum malaria and highly focused seasonal interventions offer an excellent opportunity for evaluating new control measures, including releasing *Wolbachia*-infected mosquitoes.

After defining our compartmental differential equation model, we analyze the model by introducing the next generation numbers, $\mathbb{G}_{0u}$ and $\mathbb{G}_{0w}$, for the uninfected and infected mosquito populations. We derive the reproductive number, $\mathbb{R}_0$, for the spread of *Wolbachia* in the mosquito population, which are interpreted in terms of the next generation numbers. We then describe in detail different release scenarios, and we numerical investigate the effect of concurrent malaria vector control interventions and the impact of seasonality.

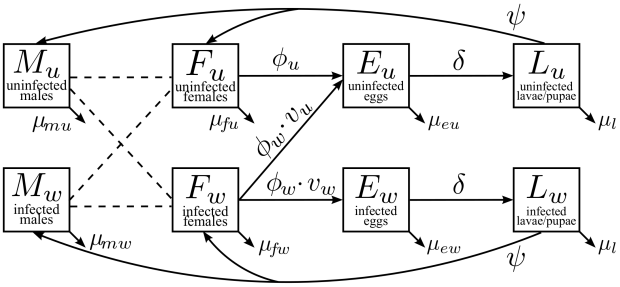

Fig. 1 Vertical transmission of *wAlbB* in mosquitoes. The adult population of male and female mosquitoes is divided into compartments based on their infection status. Uninfected females (F_u) produce uninfected eggs (E_u) at an egg-laying rate of ϕ_u. Infected females (F_w) produce a fraction of v_w infected eggs (E_w) with a rate of ϕ_w. Then, eggs develop into the larval stage at a hatching rate of δ. Larvae-stage mosquitoes emerge at a rate of ψ and develop into adult mosquitoes; the fraction of larvae that develop into adult male or female mosquitoes are the same, b_m and b_f, respectively.

2 Model Description

Our multi-stage, two-sex model partitions the mosquito population based on their life stages and *Wolbachia*-infection status, and fig. 1 gives the vertical transmission of *wAlbB* in mosquitoes. The adult stages include uninfected males, M_u, infected males, M_w, uninfected females, F_u, and infected females. We include the eggs, uninfected E_u and infected E_w, and combined larvae/pupae stages for the aquatic-stage mosquitoes, denoted by L_u and L_w for the uninfected and infected groups.

Male adult mosquitoes (M_u and M_w):

Uninfected and infected male adult mosquitoes have a mean lifespan of τ_{mu} or τ_{mw} days. We assume an exponential survival rate, which leads to constant daily death rates of $\mu_{mu} = 1/\tau_{mu}$ or $\mu_{mw} = 1/\tau_{mw}$. The uninfected and infected adult male mosquitoes randomly mix and impregnate the female mosquitoes. We assume that *Wolbachia*-infection minimally affects mating behavior of mosquitoes, and infected males are nearly as competent as the uninfected males Joshi et al (2014). The probability that a random impregnating male mosquito is uninfected or infected is determined by the proportions

$$\mathbb{M}_u = \frac{M_u}{M_u + M_w}, \text{ and } \mathbb{M}_w = 1 - \mathbb{M}_u = \frac{M_w}{M_u + M_w} . \tag{1}$$

Female adult mosquitoes (F_u and F_w):

Wolbachia infection may affect the lifespan of the female mosquitoes Joshi et al (2014). Suppose the uninfected and infected female mosquitoes have a mean lifespan of τ_{fu} and τ_{fw} days, respectively, which gives constant daily death rates $\mu_{fu} = 1/\tau_{fu}$ and $\mu_{fw} = 1/\tau_{fw}$.

For simplification, we don't distinguish between the nonpregnant and pregnant stages in female mosquitoes (see also Qu and Hyman (2019); Xue et al (2015)). This assumes that all female mosquitoes are impregnated soon after they hatch and start producing offspring in batches. We approximate the average daily egg-laying rates by ϕ_u and ϕ_w for uninfected and infected females, respectively.

Egg stages (E_u and E_w):

Regardless of the infection status of the males, among the eggs produced by the infected females F_w, a fraction v_w of them are infected, which is referred to as the maternal transmission rate, and the rest $v_u = 1 - v_w$ of eggs are uninfected.

When an uninfected female is impregnated by an infected male (F_u cross M_w, with probability $\mathbb{M}_w$), the *Wolbachia*-induced CI may cause a fraction, c_i, of the impregnated females to lay nonviable eggs. Thus, a fraction $c_i\mathbb{M}_w$ of uninfected females are sterile, and the rest $(1 - c_i)\mathbb{M}_w$ is fertile, producing viable uninfected eggs. Thus, the birth rate of the uninfected eggs is given by

$$\mathbb{M}_u\phi_u + \mathbb{M}_w(1 - c_i)\phi_u + \mathbb{M}_w c_i \cdot 0.$$

If there was no *Wolbachia*-induced CI, $c_i = 0$, the birth rate of the (viable) uninfected is ϕ_u per day. For the baseline considered in this study, we assume a complete CI, $c_i = 1$, and the birth rate for E_u is $\phi_u\mathbb{M}_u$.

Wolbachia infection may reduced the fecundity of the female due to the reduced egg surivorship. Thus, we assume that the uninfected and infected eggs have the daily death rates of μ_{eu} and μ_{ew}. We assume that the eggs hatch at rate δ, regardless of the infection status, if they survive and progress to larvae stage.

Larvae/pupae stages (L_u and L_w):

We assign a combined compartment for the larvae and pupae life stages, and we impose a carrying capacity constraint, the value of which, denoted by K_l, depends on the availability of water and food resources. This is incorporated in the model by applying the following logistic constraint

$$\mathbb{K} = 1 - \frac{L_u + L_w}{K_l} \tag{2}$$

to the birth rate of larvae/pupae group. The carrying capacity will also account for seasonal variations in the mosquito populations.

The adult mosquitoes emerge from the larvae stages with constant rates ψ, which is not significantly different among between the wild and infected cohort Joshi et al (2014), and fractions of b_f and $b_m = 1 - b_f$ of them become female and male mosquitoes.

The system of ODEs that satisfies the above assumptions is given by

$$\begin{aligned}
\frac{dM_u}{dt} &= b_m \psi L_u - \mu_{mu} M_u \ , \\
\frac{dM_w}{dt} &= b_m \psi L_w - \mu_{mw} M_w \ , \\
\frac{dF_u}{dt} &= b_f \psi L_u - \mu_{fu} F_u \ , \\
\frac{dF_w}{dt} &= b_f \psi L_w - \mu_{fw} F_w \ , \\
\frac{dE_u}{dt} &= \phi_u \mathbb{M}_u F_u + \phi_w v_u F_w - \delta E_u - \mu_{eu} E_u \ , \\
\frac{dE_w}{dt} &= v_w \phi_w F_w - \delta E_w - \mu_{ew} E_w \ , \\
\frac{dL_u}{dt} &= \delta \mathbb{K} E_u - \psi L_u - \mu_l L_u, \\
\frac{dL_w}{dt} &= \delta \mathbb{K} E_w - \psi L_w - \mu_l L_w \ ,
\end{aligned} \tag{3}$$

where $\mathbb{M}_u$ and $\mathbb{K}$ are non-dimensional nonlinear function of the state variables, defined in eqs. (1) and (2), respectively. We discuss in detail how we obtain the baseline values of the parameters in section 4.1, and the results are summarized in table 3.

Table 3 Model parameters and the baseline values. All rates have the unit day^{-1}.

	Description	Value	Reference
	***Anopheles spp.*-specific**		
δ	Hatching rate for eggs ($= 1/\tau_\delta$)	1/3	CDC (2022)
ψ	Emergence rate for larvae ($= 1/\tau_\psi$)	1/18.3	Joshi et al (2014)
μ_{fu}	Death rate for uninfected females ($= 1/\tau_{fu}$)	1/13	Joshi et al (2014); CDC (2022)
μ_{fw}	Death rate for infected females ($= 1/\tau_{fw}$)	1/15	Joshi et al (2014); CDC (2022)
μ_{mu}	Death rate for uninfected males ($= 1/\tau_{mu}$)	1/7	Joshi et al (2014); CDC (2022)
μ_{mw}	Death rate for infected males ($= 1/\tau_{mw}$)	1/7	Joshi et al (2014); CDC (2022)
μ_{eu}	Death rate for uninfected eggs	0.12	Joshi et al (2014)
μ_{ew}	Death rate for infected eggs	0.33	Joshi et al (2014)
μ_l	Death rate for larvae	0.01	Joshi et al (2014)
ϕ_u	Per capita egg laying rate for wild females	3.8	Joshi et al (2014)
ϕ_w	Per capita egg laying rate for infected females	3.3	Joshi et al (2014)
v_w	*wAlbB* maternal transmission fraction	1	Bian et al (2013)
c_i	*wAlbB* CI fraction	1	
	non-*Anopheles spp.*-specific		
b_f	Fraction of larvae emerge to females	0.5	Tun-Lin et al (2000)
b_m	Fraction of larvae emerge to males	0.5	Tun-Lin et al (2000)
K_l	Carrying capacity of larvae/pupae stages	2×10^5	Assume

3 Model Analysis

We analyze the model eq. (3) by first defining two *next-generation numbers*, $\mathbb{G}_{0u}$ and $\mathbb{G}_{0w}$, for the uninfected and infected population cohorts. As proposed in Qu et al (2018), these factors provide key information on mosquito reproduction rates and the reflect the competition between the two cohorts during the population replacement process.

3.1 Next-Generation Numbers

Among the uninfected mosquitoes, when there is no *Wolbachia* infection in the population, the average number of uninfected eggs that an uninfected female F_u lays throughout its entire life span is ϕ_u/μ_{fu}. A fraction $\delta/(\delta+\mu_{eu})$ of these uninfected eggs can survive and develop into the larvae/pupae stage L_u. With probability $\psi/(\psi+\mu_l)$, larvae progress to adults, b_f of which are uninfected females F_u. The product of these factors give the number of new uninfected individuals generated by one uninfected individual during the entire life cycle,

$$\mathbb{G}_{0u} = b_f \frac{\psi}{\psi+\mu_l} \frac{\delta}{\delta+\mu_{eu}} \frac{\phi_u}{\mu_{fu}}, \tag{4}$$

which we define as the next-generation number for the uninfected population. Near the baseline parameter values (table 3), we have $\mathbb{G}_{0u} > 1$, indicating that the wild mosquito population can persist when there are no *Wolbachia*-infected mosquitoes.

Similarly, we define the next-generation number for the infected population,

$$\mathbb{G}_{0w} = v_w b_f \frac{\psi}{\psi+\mu_l} \frac{\delta}{\delta+\mu_{ew}} \frac{\phi_w}{\mu_{fw}}, \tag{5}$$

where v_w is the maternal transmission rate, which gives the fraction of infected eggs produced by the *Wolbachia*-infected females.

3.2 Nontrivial Equilibria and Basic Reproductive Number

There are three types of nontrivial equilibrium points for the model: the disease-free equilibrium (DFE), the complete-infection equilibrium (CIE), and the endemic equilibrium (EE).

3.2.1 Disease-free equilibrium (DFE)

We derive the DFE by setting the populations in all infected stages equal to zero in the system of equations eq. (3), i.e $E_w = L_w = F_w = M_w = 0$, and the corresponding equilibrium solution gives the DFE, which is denoted by

$\boldsymbol{X}^0 = (E_u^0, 0, L_u^0, 0, F_u^0, 0, M_u^0, 0)$,

$$\begin{aligned} E_u^0 &= b_f \frac{\psi}{\mu_{fu}} \frac{\phi_u}{\delta + \mu_{eu}} L_u^0, \\ L_u^0 &= K_l \left(1 - \frac{1}{\mathbb{G}_{0u}}\right), \\ F_u^0 &= b_f \frac{\psi}{\mu_{fu}} L_u^0, \\ M_u^0 &= b_m \frac{\psi}{\mu_{mu}} L_u^0 \ , \end{aligned} \tag{6}$$

and $\mathbb{G}_{0u}$ is the next-generation number for the uninfected population defined (see eq. (4)).

3.2.2 Complete-infection Equilibrium (CIE)

The CIE exists when assuming perfect maternal transmission, that is $v_w = 1$, and all the mosquitoes are infected. We derive CIE by setting all the uninfected compartments equation to zero, i.e. $E_u = L_u = F_u = M_u = 0$, in the system eq. (3), and the corresponding equilibrium solution gives the CIE, which is denoted by $\boldsymbol{X}^c = (0, E_w^c, 0, L_w^c, 0, F_w^c, 0, M_w^c)$,

$$\begin{aligned} E_w^c &= b_f \frac{\phi_w}{\delta + \mu_{ew}} \frac{\psi}{\mu_{fw}} L_w^c, \\ L_w^c &= K_l \left(1 - \frac{1}{\mathbb{G}_{0w}}\right), \\ F_w^c &= b_f \frac{\psi}{\mu_{fw}} L_w^c, \\ M_w^c &= b_m \frac{\psi}{\mu_{mw}} L_w^c, \end{aligned} \tag{7}$$

and $\mathbb{G}_{0w}$ is the next-generation number for the infected population (see eq. (5)).

Derivation using the Next Generation Matrix

Consider the infected compartments in the model eq. (3), denoted by $\boldsymbol{X}_w = (E_w, L_w, F_w, M_w)^T$, and define a subsystem for these variables,

$$\begin{aligned}\frac{d\boldsymbol{X}_w}{dt} &= \frac{d}{dt}\begin{pmatrix} E_w \\ L_w \\ F_w \\ M_w \end{pmatrix} = \boldsymbol{F}(\boldsymbol{X}_w) - \boldsymbol{V}(\boldsymbol{X}_w) \\ &= \begin{pmatrix} v_w\phi_w F_w \\ 0 \\ 0 \\ 0 \end{pmatrix} - \begin{pmatrix} (\delta+\mu_{ew})E_w \\ -\delta(1-\frac{L_u+L_w}{K_l})E_w + (\psi+\mu_l)L_w \\ -b_f\psi L_w + \mu_{f_w}F_w \\ -b_m\psi L_w + \mu_{mw}M_w \end{pmatrix},\end{aligned} \tag{8}$$

where the vectors $\boldsymbol{F}$ and $\boldsymbol{V}$ represent the rate of new infections and the rate of transition among the infected compartments. We then linearize the equation at the DFE and obtain the Jacobian matrices $\boldsymbol{J_F}(\boldsymbol{X}^0)$ and $\boldsymbol{J_V}(\boldsymbol{X}^0)$,

$$\boldsymbol{J_F} = \begin{pmatrix} 0 & 0 & v_w\phi_w & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}, \quad \boldsymbol{J_V} = \begin{pmatrix} \delta+\mu_{ew} & 0 & 0 & 0 \\ -\delta/\mathbb{G}_{0u} & \psi+\mu_l & 0 & 0 \\ 0 & -b_f\psi & \mu_{fw} & 0 \\ 0 & -b_m\psi & 0 & \mu_{mw} \end{pmatrix}, \tag{9}$$

and the reproductive number is given by

$$\mathbb{R}_0 = \text{spectral radius of } \boldsymbol{J_F}\boldsymbol{J_V}^{-1} = v_w\frac{\mu_{fu}\phi_w(\delta+\mu_{eu})}{\mu_{fw}\phi_u(\delta+\mu_{ew})}. \tag{10}$$

Derivation using heuristic approach and interpretation

One of the advantages of the heuristics approach is that we can interpret each factor in the basic reproductive number using the biological meanings.

The basic reproductive number, $\mathbb{R}_0$, of the *Wolbachia* transmission among the mosquitoes describes the initial invasion of the *Wolbachia*-infected population and its competition with the natural population, thus we define the $\mathbb{R}_0$ as

$$\mathbb{R}_0 := \frac{\mathbb{G}_{0w}}{\mathbb{G}_{0u}} = v_w\frac{\mu_{fu}\phi_w(\delta+\mu_{eu})}{\mu_{fw}\phi_u(\delta+\mu_{ew})}, \tag{11}$$

which matches the result in eq. (10). Recall the definition of the next generation numbers $\mathbb{G}_{0u}$ and $\mathbb{G}_{0w}$, which represent the numbers of new offspring reproduced per generation among the uninfected and infected cohorts. Thus, the ratio of the two factors estimates the average number of infected offspring generated per infected individual at the DFE. It also measures how the ratio of new infected versus new uninfected population changes from one generation to the next and determines the future spreading of *Wolbachia* in the

wild mosquito population, assuming the system is near the DFE. This quantity serves as a threshold condition for the species invasion, given a small introduction of the infected population.

When $\mathbb{G}_{0w} > \mathbb{G}_{0u}$, that is $\mathbb{R}_0 > 1$, more infected population can be reproduced than the uninfected population, thus the small infection is expected to spread in the population. When $\mathbb{R}_0 < 1$, which is the practical case at the baseline values, the infected population suffer from the fitness cost, and a small introduction of infected population will be wiped out by the natural uninfected population.

3.2.3 Endemic Equilibrium (EE)

When having imperfect maternal transmission, $v_w < 1$, some infected females produce uninfected offspring, and CIE is not achievable. Instead, there exists EE, where the infected and uninfected mosquitoes coexist.

We first define r_{wu} as the ratio between the infected and uninfected larvae/pupae stages, i.e, $r_{wu} = L_w/L_u$, which is a key dimensionless quantity in the derivation. Then EE, denoted by $\boldsymbol{X}^* = (M_u^*, M_w^*, F_u^*, F_w^*, E_u^*, E_w^*, L_u^*, L_w^*)$, can be written in terms of this ratio as follows (assume $\mu_{mu} = \mu_{mw}$)

$$
\begin{aligned}
M_u^* &= b_m \frac{\psi}{\mu_{mu}} L_u^*, \\
M_w^* &= r_{wu} b_m \frac{\psi}{\mu_{mw}} L_u^*, \\
F_u^* &= b_f \frac{\psi}{\mu_{fu}} L_u^*, \\
F_w^* &= r_{wu} b_f \frac{\psi}{\mu_{fw}} L_u^*, \\
E_u^* &= \frac{b_f \psi}{\delta + \mu_{eu}} \left(\frac{\phi_u}{\mu_{fu}} \frac{1}{1 + r_{wu}} + \frac{v_u \phi_w}{\mu_{fw}} r_{wu} \right) L_u^*, \\
E_w^* &= r_{wu} \frac{b_f \psi}{\delta + \mu_{ew}} \frac{v_w \phi_w}{\mu_{fw}} L_u^* = r_{wu} E_u^*, \\
L_u^* &= \frac{1}{1 + r_{wu}} K_l \left(1 - \frac{1}{\mathbb{G}_{0w}} \right), \\
L_w^* &= r_{wu} L_u^*,
\end{aligned}
\tag{12}
$$

where the ratio r_{wu} satisfies the following quadratic relation that involves the maternal transmission rate v_w, the infection leakage rate $v_u = 1 - v_w$, and the basic reproductive number $\mathbb{R}_0$,

$$
\frac{v_u}{v_w} \frac{\delta + \mu_{ew}}{\delta + \mu_{eu}} r_{wu}^2 + \left(\frac{v_u}{v_w} \frac{\delta + \mu_{ew}}{\delta + \mu_{eu}} - 1 \right) r_{wu} + \frac{1 - \mathbb{R}_0}{\mathbb{R}_0} = 0. \tag{13}
$$

This relation will change depending on the values assigned to v_w. Under the special case of perfect maternal transmission ($v_w = 1$), some of the terms in eq. (13) vanish, and we obtain a linear relation,

$$r^*_{wu} = \frac{L^*_w}{L^*_u} = \frac{1 - \mathbb{R}_0}{\mathbb{R}_0}.$$

In order to have a physically relevant endemic equilibrium, we need to impose $r^*_{wu} > 0$. This implies that the basic reproductive number must satisfy $0 < \mathbb{R}_0 < 1$. Our best estimate for the basic reproductive number is $\mathbb{R}_0 \approx 0.683$.

3.3 Stability and Bifurcation Analysis

The stability analysis of the three equilibrium points allows us to characterize the dynamics of the solution by the threshold conditions that establish a *Wolbachia*-infected mosquito population.

The stability of an equilibria is determined by the signs of the eigenvalues of the Jacobian, linearized about the equilibria. We present the conclusions on the stability analysis below. The proofs of Theorem 1 and Theorem 2 can be found in appendices A.1 and A.2, and we conjecture Theorem 3, which is then verified numerically in the bifurcation analysis. We note that these conclusions are similar to the ones in Qu et al (2018), which is expected due to the similar model structure.

Theorem 1 (Stability of the Disease-free Equilibrium)**.** The DFE (eq. (6)) of system eq. (3) is locally asymptotically stable provided that $\mathbb{G}_{0u} > 1$ and $\mathbb{R}_0 < 1$.

Theorem 2 (Stability of the Complete Infection Equilibrium)**.** The CIE (eq. (7)) of the system eq. (3) is locally asymptotically stable provided that $\mathbb{G}_{0w} > 1$.

Theorem 3 (Stability of the Endemic Equilibrium)**.** When having the perfect maternal transmission ($v_w = 1$), the physically relevant EE (eq. (12)) of the system eq. (3) exists for $\mathbb{R}_0 < 1$ and $\mathbb{G}_{0w} > 1$, and it is an unstable equilibria.

We then generate the bifurcation plot (fig. 2), which summarizes the stability of different equilibrium and highlights the critical threshold condition for establishing *Wolbachia* infection among mosquitoes. We have varied the parameter ϕ_u to trace out different steady states and kept other parameters at the baseline values.

*put in some discussions on the bifurcation plot, outlined below. ZQ

- describe the threshold condition, bistable behavior, etc.
- Within the biologically-relevant regime ($0 < R0 < 1$), when R0 increase/decrease, the threshold decreases/increases. Give biological interpretation, R0 is the ratio between G0w and G0u, competition between two cohorts.
- When $R0 > 1$, we have unstable DFE, which means...
- R effective (threshold when $R_e = 1$)

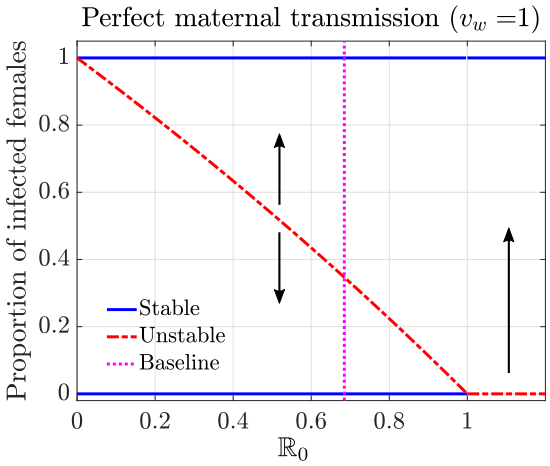

Fig. 2 Bifurcation diagram characterizing the threshold condition for establishing a stable infection in mosquitoes for the perfect maternal transmission rate ($v_w = 1$). The solid blue curves represent the stable equilibrium. The red dashed curve corresponds to the unstable equilibrium, which serves as the threshold condition. At the baseline case (vertical dotted line, $\mathbb{R}_0 = 0.68$), the threshold infection rate among females is 0.34.

4 Numerical Simulations

Our simulations address several integrated mosquito management strategies to establish a sustained *Wolbachia* infection. We consider larviciding and thermal fogging/ultra-low volume spraying as pre-release mitigation strategies to reduce the population of wild uninfected mosquitoes. Along with these, we have also evaluated different scenarios for the releasing of a large number of *Wolbachia*-infected mosquitoes after a certain amount of days in our simulation. The releasing approaches chosen are: *Infected pregnant females* F_w *and infected males* M_w, *Single infected females* F_w *and infected males* M_w and *Infected pregnant females* F_w only *to be updated: we don't distinguish between the pregnant/single now. Also, do we only consider the approach for releasing both infected males and females together?.

Seasonality plays an important role on one of our baseline parameters: the regional carrying capacity of aquatic stages K_l. For this reason, it is essential to evaluate our target scenarios under the seasonal conditions of Haiti. We have run the simulations of our target scenarios for both rainy and dry seasons with the aim of providing answers to the following questions:

Q1. Which pre-release scenario (Larviciding, Thermal Fogging/ULV Spraying) is more effective during the rainy season?

Q2. What is the best mix of infected mosquitoes to release during the rainy season?See my comments above, do we consider different release approaches?

4.1 Parameter Estimations

Most of our estimates are based on the work by Joshi et al. Joshi et al (2014), which characterize the life parameters of the mosquitoes in an ideal lab setting. These parameters can vary depending on temperature, rainfall, and food supply. We will consider the impact of seasonality on the *Wolbachia*-releasing strategies in either the dry or the rainy season. As previously mentioned, we only vary the carrying capacity (see section 4.1), as the death rates, egg hatching rate, emergence rate are not significantly impacted Christiansen-Jucht et al (2014); Beck-Johnson et al (2013).

Vertical transmission from infected females to their offspring is the primary mechanism that *Wolbachia*is transmitted to other mosquitoes. *Wolbachia* (*wAlbB*) infected females have close to perfect (100%) vertical transmission where almost all of the offspring of infected females are infected.

Mosquito lifespan

Wolbachia does not impact the longevity of males, and the median lifespan under the laboratory condition is about 16 days Joshi et al (2014). However, in the competitive field environment, the lifespan can be much shorter Centers for Disease Control and Prevention (2022), and we use a more realistic estimate on the lifespan of male mosquitoes $\tau_{mu} = \tau_{mw} = 7$ days. Both infected and uninfected females can live for approximately 22 days in the lab, when feeding on human blood. However, the infected females show better survivorship during the first two weeks Joshi et al (2014). Thus, we assume a shorter lifespan in the realistic setting, $\tau_{fu} = 13$ days and $\tau_{fw} = 15$ days.

Egg-laying rates

Wolbachia infection does not significantly impact the total number of eggs a female can produce throughout her lifespan, which is about 50 eggs/female Joshi et al (2014). Thus, the daily egg-laying rates for the uninfected females $\phi_u = 50/\tau_{fu} \approx 3.8$ eggs/day, and for the infected females $\phi_w = 50/\tau_{fw} \approx 3.3$ eggs/day.

Egg-hatching rate and death rates

We assume that *Wolbachia* infection does not impact the length of egg-hatching period, and on average, it takes about three days for the eggs to hatch Centers for Disease Control and Prevention (2022), and we set $\tau_\delta = 3$ days and $\delta = 1/\tau_\delta = 1/3$. *Wolbachia* infection reduces the fecundity in female mosquitoes, where the fraction of eggs hatch and survive to first-instar larvae is much lower among the infected population than the uninfected one (50% vs. 73%) Joshi et al (2014). Thus, we have $\delta/(\delta+\mu_{eu}) = 0.73$ and $\delta/(\delta+\mu_{ew}) = 0.5$, which gives the egg death rate estimates $\mu_{eu} \approx 0.12$ and $\mu_{ew} \approx 0.33$.

Larvae/pupae emergence rate and death rate

Wolbachia has no significant impact on the life traits of the *Anopheles* mosquitoes, including the emergence time from larvae to adults and the

survivorship during the process. On average, it takes about $\tau_\psi = 18$ days (sum of pupation time and emergence time, see Joshi et al (2014)), which gives the emergence rate $\psi = 1/\tau_\psi = 1/18$. The fraction of larvae survive to adult is $\psi/(\psi + \mu_l) \approx 80\%$, thus the daily death rate for the larvae/pupae stage is estimated as $\mu_l = 0.01$.

The role of seasonality

Environmental and climactic covariates such as rainfall and temperature can affect multiple stages of the mosquito life cycle by influencing density and distribution of vector breeding sites, the number of eggs an adult pregnant female mosquito lays, the ability of larvae to emerge from eggs once they are laid (hatching or emergence rate), and the adult mosquito lifespan. Regional carrying capacity is also affected as this parameter is directly influenced by the number of available vector breeding and egg laying sites. It is important to account for the effect that variations in rainfall and temperature have on independent parameter values as these values influence the ability to achieve endemic, stable *wAlbB* transmission among the mosquito population.

R studio (Version 1.2.5033) was used to extract CHIRPS mean monthly rainfall values for years 2016-2020 for the department of Grand Anse, where the majority of the country's malaria transmission occurs. The mean value for each month over this 5 year time span was then calculated and plotted to visually identify trends in rainfall and identify months of the year that would be categorized as either a dry or rainy season. The majority of rainfall in the highest transmission region of Haiti occurs during two rainy seasons -April through June- and -September through November-, based on the mean rainfall in the department of Grand Anse, with an average duration of 90-100 days and 90 days respectively. In contrast, the dry season occurs within two periods, -December through March- and -July through August-, with an average duration of 120 days and 60 days respectively.

R studio version (Version 1.2.5033) was also used to extract monthly temperature in Grand'Anse for years 2018-2020. Mean monthly temperature across these years ranged from 25.7 to 29.8 degrees Celsius. Whereas temperature can influence egg laying rates, larval emergence rates, and adult mosquito lifespan, mean monthly temperature in our region of focus does not vary enough to influence rates for these parameters in our model Christiansen-Jucht et al (2014); Beck-Johnson et al (2013). We therefore rely exclusively on adapting the regional carrying capacity as a function of rainfall to simulate our model in both a dry and rainy season scenario.

**Double check the seasonality description below, remove duplicated description. The seasonal malaria transmission in Haiti coincides with the rainy seasons, particularly in the department of Grand Anse, where the majority of the country's malaria transmission occurs. The land surface monthly mean temperatures for Grand Anse between 2018-2020 range between 78.2 to 85.7 degrees Fahrenheit Funk et al (2015). We assumed that this small range in

temperature had a minor impact on the larval emergence rates, adult mosquito lifespans, egg-laying, and hatching rates compared to the impact of rainfall.

We analyzed the mean monthly rainfall Funk et al (2015) between 2016-2020 to identify the dry and rainy seasons. Based on this data, we defined a spring rainy season (March - May), summer dry season (June - July), fall rainy season (August - October), and winter dry season (November - February). We assumed that seasonally had the most impact on the regional carrying capacity for the aquatic stages. We accounted for this effect in the model by varying the carrying capacity, K_l, instead of explicitly including temperature and rainfall in the model.

♣ *include here description of how we derived carrying capacity figures-AY* ♣

4.2 Interventions strategies

To reduce the number of *Wolbachia*-infected mosquitoes needed to establish a stable infected wild population, we use vector control strategies to reduce the wild mosquito population before releasing the infected mosquitoes. Larviciding, thermal fogging, and ultra-low volume spraying can reduce wild mosquito populations by targeting the mosquitoes in different life stages. Our model can help quantify the effectiveness of different pre-release mitigation approaches in reducing the wild mosquito population before releasing the infected mosquitoes to establish a stable *Wolbachia*-infected mosquito population. We acknowledge that not all of our mitigation strategies are widespread primary vector control interventions in Haiti. Nevertheless, our results inform the potential effectiveness should such these intervention controls become prevalent in the area.

We account for the effectiveness of pre-release interventions by adjusting the initial conditions of the simulations, that is the disease-free equilibrium is modified to reflect the killing rate for the corresponding mosquito life stages. For example, for the larvicide intervention, we assume larval reduction occurs on the existing wild mosquito population (pre-release condition) and not on the released mosquito population infected with *Wolbachia*.

Larviciding

Larviciding treats mosquito breeding sites with bacterial or chemical insecticides to kill the aquatic stage of mosquitoes. Reducing aquatic forms lead to a reduction in the adult population of mosquitoes. Fewer adult mosquitoes can result in fewer bites and malaria infection in humans. Field studies of bacterial larvicide products, targeting *Anopheles* larval habitats, report larval reduction between 47% and 100% Derua et al (2019). Our model simulates thresholds as low as 40% to be representative of more challenging settings for implementing larvicides; other thresholds simulated were 60%, 80% and 90%.

Thermal Fogging and Ultra-Low Volume (ULV) Spraying

Space spraying or *fogging* refers to the process of dispersing a liquid fog of insecticide into an outdoor area with the aim to kill adult insects. The insecticide may be delivered using hand-held, vehicle-mounted or aircraft-mounted equipment Organization et al (2003). Space spraying requires two different mechanisms for generating fog: cold and thermal fogs. Cold fogging use ultra-low-volume (ULV) mixtures of insecticide and pass it through a mechanical structure such as a high-pressure nozzle or high-speed air flow Pryce et al (2018). Conversely, thermal fogging is formed with hot gas, which vaporizes a solution of insecticide in an oil-based carrier liquid. Upon spraying, the steam interacts with colder air and produce a dense fog. Both thermal and cold fog mechanisms are only effective while droplets remain airborne Organization et al (2003), for this reason it is recommended that the timing of spraying coincides with the biting activity period of the target species. For Anopheles mosquitoes, this period occurs early morning, in the evening and at night. Space sprayings that are conducted during the day can also target malaria vectors in their resting locations.

Previous studies on the impact of fogging as a malaria vector control intervention were reviewed in Pryce et al (2018) and suggest that the outdoor mortality of adult anopheline mosquitoes, produced by this technique, fluctuates between 50%-100%. Consequently, in our model, we evaluate the impact of space spraying by assuming outdoor mortality percentages of 50%, 70% and 90%.

4.3 Comparison of target scenarios for the rainy season

The model parameters are defined in table 3 and depend on if the simulation is for the rainy or dry season and the pre-release mitigation strategy being used. We release an equal number of infected males and females on day 30 of the simulation. This ratio is based on the assumption that the birth rates of males and females are equal. We then compare the number of days needed to reach the endemic state (90% of adult infection) for the following vector control approaches:

- Larviciding (kill rate of 90% larvae)
- Thermal fogging (kill adult mosquitoes at 50%, 70% and 90% levels)
- Hybrid intervention (larviciding with 90% kill rate and thermal fogging with 90% kill rate)

The results for both rainy seasons are summarized in table 4.

4.3.1 Answer Q1: Rainy Season

According to the results presented in table 4, we observe that for both rainy seasons the achievement of a stable endemic state had the shortest time when we simulated thermal fogging with 90% kill rate.

Table 4 Comparison of pre-release mitigation strategies during rainy season: Fractional values are number of mosquitoes relative to DFE of the corresponding mosquito life stage. ♣ *Kill is relative to DFE, but release is absolute number* ♣

Pre-release Mitigation	Eggs	Larvae	Adults	Threshold release number	Release number to reach 90% by one month
No mitigation (DFE)	1.0	1.0	1.0	1.13	25.1
Thermal fogging	1.0	1.0	0.8	1.03	23.8
	1.0	1.0	0.6	0.93	22.5
	1.0	1.0	0.4	0.82	21.3
Larviciding	1.0	0.8	1.0	1.04	21.5
	1.0	0.6	1.0	0.96	18.1
	1.0	0.4	1.0	0.88	15.1
Fogging + Larviciding	1.0	0.4	0.8	0.79	14
	1.0	0.4	0.6	0.69	12.9
	1.0	0.4	0.4	0.60	11.8

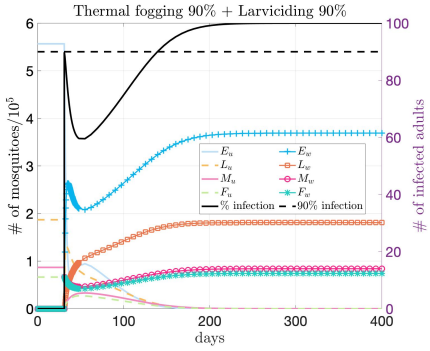

Fig. 3 Rainy season, April-June: At the beginning of the rainy season, the mosquito populations are reduced to X% of their original populations using larvicide and thermal fogging. On day 30, the infected female and male mosquitoes are released. The infected mosquito population is then maintained and reaches the endemic equilibrium about 6 months after the release. ♣ *It seems we need two or three side-by-side plots to show the difference between different pre-release strategies. MH* ♣

4.3.2 Answer Q2: Rainy Season

The model predicts that releasing both the pregnant infected females F_w and infected males M_w was more effective than releasing just the males or females. When we applied ITN in the dry season, September-November, releasing just pregnant females did not reduce the time to achieve a stable endemic state within 90 days.

4.3.3 Preliminary findings

Without interventions, the endemic equilibrium of *Wolbachia* infection among *Anopheles* mosquitoes is reached, suggesting the need to consider other vector control tools in elimination settings (Figure 2a). The model indicates that thermal fogging is an effective pre-release strategy for reducing the time to achieve an endemically infected mosquito population.

Findings from the rainy season indicate the release scenario of infected pregnant females and infected males, the most aggressive form of introducing *Wolbachia* infection, is the combination resulting in the least amount of days to endemic infection. Consequently, acquiring endemic *wAlbB*infection can reduce malaria transmission in *Anopheles* mosquitoes and thereby can be a potential supplementary vector control strategy in elimination settings.

4.4 Sensitivity Analysis

The baseline values stated in table 3 represent our best estimates of the model parameters. We quantify their significance in the model predictions using local sensitivity analysis to get a better insight into the model response under changes to parameters. In particular, we focus on how sensitive the estimate of $\mathbb{R}_0$ is too small changes in the parameters defining $\mathbb{R}_0$ (eq. (11)). The respective local relative sensitivity indexes are described by table 5.

Table 5 Sensitivity analysis for the quantity of interest $\mathbb{R}_0$. We present the local relative sensitivity index (*LRSI*) for each parameter (POI) involved in formula (eq. (11)). ♣ *This would work better as a horizontal table.* ♣

POI	***LRSI***
v_w	1
b_f	.5
ψ	0.229
π	0
μ_{au}	0.270
μ_{fu}	1
ϕ_u	-1
ϕ_w	1
μ_{aw}	-0.5
μ_{fw}	-1

Let us recall that the normalized relative sensitivity index of a quantity of interest (QOI), $q(p)$, with respect to a parameter of interest POI, p, is defined as

$$\mathcal{S}_p^q := \frac{p}{q} \times \frac{\partial q}{\partial p}$$

on a plausible range for the parameter p. This index measures the percentage change in the QOI given the percentage change in the input POI. In other words, if parameter p changes by $\alpha\%$, then q will change by $\mathcal{S}_p^q \times \alpha\%$. The sign determines the decreasing or increasing behavior of the quantity. If we

evaluate this index at the baseline parameter value $p^* = p$, then

$$\mathcal{S}^q_{p^*} := \mathcal{S}^q_p \Big\|_{p=p^*} = \frac{p^*}{q^*} \times \frac{\partial q}{\partial p} \Big\|_{p=p^*} \tag{14}$$

is called the local relative sensitivity index of q at p^*. Here q^* represents the baseline value for the quantity of interest. In our particular case, the results of table 5, should be interpreted as follows: if we increase any of the parameters in column **POI** by 1%, then the basic reproductive number $\mathbb{R}_0$ will change by the quantity given in column **LSRI**.

5 Discussion

To be written in the coming months.

6 Acknowledgements

The authors would like to thank Mac and Zhuolin for their constant support and guidancce throughout the semester.

Appendices

A.1 Proof of Theorem 1 (Stability of DFE)

Let us consider the variable vector containing the compartments (re-arranged by the infection status), $\mathbf{Y} = (E_u, L_u, F_u, M_u, E_w, L_w, F_w, M_w)$, then the corresponding Jacobian, denoted by J, of the system $\frac{d\mathbf{Y}}{dt} = J\mathbf{Y}$, is given by

$$\mathbf{J} = \left(\begin{array}{cccc:cccc} -\delta-\mu_{eu} & 0 & \mathbb{M}_u\phi_u & 0 & 0 & 0 & v_u\phi_w & 0 \\ \delta\mathbb{K} & -\frac{\delta E_u + K_l(\mu_l+\psi)}{K_l} & 0 & 0 & 0 & -\frac{\delta}{K_l}E_u & 0 & 0 \\ 0 & b_f\psi & -\mu_{fu} & 0 & 0 & 0 & 0 & 0 \\ 0 & b_m\psi & 0 & -\mu_{mu} & 0 & 0 & 0 & 0 \\ \hdashline 0 & 0 & 0 & 0 & -\delta-\mu_{ew} & 0 & v_w\phi_w & 0 \\ 0 & -\frac{\delta}{K_l}E_w & 0 & 0 & \delta\mathbb{K} & -\frac{\delta E_w + K_l(\mu_l+\psi)}{K_l} & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & b_f\psi & -\mu_{fw} & 0 \\ 0 & 0 & 0 & 0 & 0 & b_m\psi & 0 & -\mu_{mw} \end{array}\right).$$

At the disease free equilibrium, the Jacobian becomes

$$\mathbf{J}_{DFE} = \left(\begin{array}{c:c} \mathbf{A}_{DFE} & * \\ \hdashline \mathbf{0} & \mathbf{D}_{DFE} \end{array}\right), \quad \text{where} \tag{A.1}$$

$$\mathbf{A}_{DFE} = \left(\begin{array}{ccc:c} -\delta-\mu_{eu} & 0 & \phi_u & 0 \\ \frac{\delta}{\mathbb{G}_{0u}} & -\mathbb{G}_{0u}(\mu_l+\psi) & 0 & 0 \\ 0 & b_f\psi & -\mu_{fu} & 0 \\ \hdashline 0 & b_m\psi & 0 & -\mu_{mu} \end{array}\right) = \left(\begin{array}{cc:c} & & \\ & A_{s1} & \mathbf{0} \\ & & \\ \hdashline * & & -\mu_{mu} \end{array}\right), \quad \text{(A.2)}$$

and

$$\mathbf{D}_{DFE} = \left(\begin{array}{ccc:c} -\delta-\mu_{ew} & 0 & v_w\phi_w & 0 \\ \frac{\delta}{\mathbb{G}_{0u}} & -\mu_l-\psi & 0 & 0 \\ 0 & b_f\psi & -\mu_{fw} & 0 \\ \hdashline 0 & b_m\psi & 0 & -\mu_{mw} \end{array}\right) = \left(\begin{array}{cc:c} & & \\ & D_{s1} & \mathbf{0} \\ & & \\ \hdashline * & & -\mu_{mw} \end{array}\right). \quad \text{(A.3)}$$

Observe first that the matrix $\mathbf{J}_{DFE}$ is an upper triangular block matrix, and the two 4×4 diagonal elements $\mathbf{A}_{DFE}$ and $\mathbf{D}_{DFE}$ (eq. (A.1)). Thus, the stability of the matrix $\mathbf{J}_{DFE}$ is equivalent to showing the stability for both matrices.

To show the stability of $\mathbf{A}_{DFE}$, notice that it's a lower triangular block matrix with $-\mu_{mu} < 0$ (eq. (A.2)), thus we just need to consider the 3×3 leading principal submatrix A_{s1}, which can be further partitioned as follows,

$$A_{s1} = \left(\begin{array}{cc:c} -\delta-\mu_{eu} & 0 & \phi_u \\ \delta/\mathbb{G}_{0u} & -\mathbb{G}_{0u}(\mu_l+\psi) & 0 \\ \hdashline 0 & b_f\psi & -\mu_{fu} \end{array}\right) = \left(\begin{array}{c:c} A_1 & B_1 \\ \hdashline C_1 & D_1 \end{array}\right).$$

To prove the stability of the matrix A_{s1}, we use a result on Metzler matrices stated in Proposition 3.1 in Kamgang and Sallet (2008). Since A_{s1} is a Metzler matrix, A_{s1} is Metzler stable if and only if A_1 and $D_1 - C_1A_1^{-1}B_1$ are Metzler stable. With this in mind, observe that since the matrix A_1 is lower triangular, its eigenvalues are its corresponding diagonal entries $-(\delta+\mu_{ew})$ and $-\mathbb{G}_{0u}(\mu_l+\psi)$, which are both negative. This implies that A_1 is Metzler stable. Meanwhile, the matrix $D_1 - C_1A_1^{-1}B_1$ satisfies the following property:

$$D_1 - C_1A_1^{-1}B_1 = \mu_{fu}\left(\frac{1}{\mathbb{G}_{0u}} - 1\right) < 0, \quad \text{if } \mathbb{G}_{0u} > 1. \quad \text{(A.4)}$$

Thus, when $\mathbb{G}_{0u} > 1$, A_{s1} is Meltzer stable, so as the matrix $\mathbf{A}_{DFE}$.

Similarly, we derive the condition for the stability of $\mathbf{D}_{DFE}$. Given it is a lower triangular block matrix with $-\mu_{mw} < 0$ (eq. (A.3)), we are left with the 3×3 leading submatrix

$$D_{s1} = \left(\begin{array}{cc:c} -\delta-\mu_{ew} & 0 & v_w\phi_w \\ \delta/\mathbb{G}_{0u} & -(\mu_l+\psi) & 0 \\ \hdashline 0 & b_f\psi & -\mu_{fw} \end{array}\right) = \left(\begin{array}{c:c} A_2 & B_2 \\ \hdashline C_2 & D_2 \end{array}\right).$$

By the same argument used previously, A_2 is Metzler stable and $D_2 - C_2 A_2^{-1} B_2$ satisfies the inequality:

$$D_2 - C_2 A_2^{-1} B_2 = \mu_{fw}(\mathbb{R}_0 - 1) < 0, \quad \text{if } \mathbb{R}_0 < 1. \tag{A.5}$$

In consequence, when $\mathbb{R}_0 < 1$, D_{s1} is Metzler stable, so as the matrix $\mathbf{D}_{DFE}$.

Finally, combining the two conditions eqs. (A.4) and (A.5) for the stability of $\mathbf{A}_{DFE}$ and $\mathbf{D}_{DFE}$, we conclude that $\mathbf{J}_{DFE}$ is stable provided that $\mathbb{G}_{0u} > 1$ and $\mathbb{R}_0 < 1$. □

A.2 Proof of Theorem 2 (Stability of CIE)

At the complete infection equilibrium, the Jacobian is of the following form:

$$\mathbf{J_{CIE}} = \left(\begin{array}{cccc:cccc} -\delta-\mu_{eu} & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ \frac{\delta}{\mathbb{G}_{0w}} & -(\mu_l+\psi) & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & b_f\psi & -\mu_{fu} & 0 & 0 & 0 & 0 & 0 \\ 0 & b_m\psi & 0 & -\mu_{mu} & 0 & 0 & 0 & 0 \\ \hdashline 0 & 0 & 0 & 0 & -\delta-\mu_{ew} & 0 & \phi_w & 0 \\ 0 & -\frac{G_{0w}}{\delta}(\psi+\mu_l) & 0 & 0 & \frac{\delta}{\mathbb{G}_{0w}} & -\mathbb{G}_{0w}(\mu_l+\psi) & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & b_f\psi & -\mu_{fw} & 0 \\ 0 & 0 & 0 & 0 & 0 & b_m\psi & 0 & -\mu_{mw} \end{array}\right).$$

By applying the same argument we used in the previous proof, we consider the following 4×4 diagonal elements:

$$\mathbf{A_{CIE}} = \begin{pmatrix} -\delta-\mu_{eu} & 0 & 0 & 0 \\ \frac{\delta}{\mathbb{G}_{0u}} & -(\mu_l+\psi) & 0 & 0 \\ 0 & b_f\psi & -\mu_{fu} & 0 \\ 0 & b_m\psi & 0 & -\mu_{mu} \end{pmatrix}$$

and

$$\mathbf{D_{CIE}} = \begin{pmatrix} -\delta-\mu_{ew} & 0 & \phi_w & 0 \\ \frac{\delta}{\mathbb{G}_{0w}} & -\mathbb{G}_{0w}(\mu_l+\psi) & 0 & 0 \\ 0 & b_f\psi & -\mu_{fw} & 0 \\ 0 & b_m\psi & 0 & -\mu_{mw} \end{pmatrix}.$$

Observe that $\mathbf{A}_{CIE}$ is a Metlzer matrix (negative diagonal elements) and it is lower triangular, which means that its eigenvalues are the same negative diagonal elements. Thus, $\mathbf{A}_{CIE}$ is Metlzer stable. Now, we need to analise the stability conditions of $\mathbf{D}_{CIE}$. For this purpose, consider the 3×3 leading submatrix of $\mathbf{D}_{CIE}$:

$$D_{s2} = \left(\begin{array}{cc:c} -\delta-\mu_{ew} & 0 & \phi_w \\ \delta/\mathbb{G}_{0w} & -\mathbb{G}_{0w}(\mu_l+\psi) & 0 \\ \hdashline 0 & b_f\psi & -\mu_{fw} \end{array}\right) = \left(\begin{array}{c:c} A_3 & B_3 \\ \hdashline C_3 & D_3 \end{array}\right).$$

By applying a similar argument as before, A_3 is Metzler stable and the number $D_3 - C_2 A_2^{-1} B_2$ satisfies the inequality:

$$D_3 - C_3 A_3^{-1} B_3 = \mu_{fw}\left(\frac{1}{\mathbb{G}_{0w}} - 1\right) < 0, \quad \text{if } \mathbb{G}_{0w} > 1. \tag{A.6}$$

Hence, when $\mathbb{G}_{0w} > 1$, D_{s2} is Metzler stable, so as the matrix $\mathbf{D}_{CIE}$. Therefore, we conclude that eq. (A.6) ensures the stability of $\mathbf{J}_{CIE}$.

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