
Modeling the effects of augmentation strategies on the control of dengue fever

Xianghong Zhang · Sanyi Tang · Robert A. Cheke · Huaiping Zhu

Abstract Dengue fever has rapidly become the world's most common vector-borne viral disease. Use of endosymbiotic *Wolbachia* is an innovative technology to prevent vector mosquitoes from reproducing and so break the cycle of dengue transmission. Note that the success of different strategies, such as population eradication and replacement, needs to choose different augmentations of *Wolbachia* infected mosquitoes, and not all field trials of mosquito releases can succeed. So a four dimensional pulse differential system, incorporating the effects of cytoplasmic incompatibility (CI) and the augmentation of *Wolbachia* infected mosquitoes with different sex, is proposed to describe the spread of *Wolbachia* in mosquito populations. We evaluated a series of relevant issues regarding the system, including tackling questions such as (a) how each parameter value contributes to the success of population replacement? (b) how different release quantities of infected mosquitoes with different sex affect the success of the two strategies? And (c) how the success of population suppression or replacement can be fulfilled to block the transmission of dengue fever? The analysis of stability, bifurcation and sensitivity reveals the existence of forward and backward bifurcations, multiple attractors and the contribution of each parameter on the success of the strategies. The results indicate that initial

Xianghong Zhang · Sanyi Tang
School of Mathematics and Information Science, Shaanxi Normal University, Xi'an, 710062,
P.R. China
Tel.: +86-29-85310232
E-mail: zhangxh3764@snnu.edu.cn & sytang@snnu.edu.cn

Robert A. Cheke
Natural Resources Institute, University of Greenwich at Medway, Central Avenue,
Chatham Maritime, Chatham, Kent, ME4 4TB, UK
E-mail: r.a.cheke@greenwich.ac.uk

Huaiping Zhu
LAMPS, Department of Mathematics and Statistics, York University, Toronto, ON, M3J
1p3, Canada
E-mail: huaiping@mathstat.yorku.ca

density of mosquitoes and the quantity of mosquito augmentation on different sex have impacts on whether or not the strategies of population suppression or replacement can be achieved. Therefore, successful strategies rely on selecting suitable strains of *Wolbachia* and carefully designing the programme of mosquito augmentation.

Keywords Dengue fever · *Wolbachia* · Backward bifurcation · Mosquito augmentation

1 Introduction

Dengue fever is one of the major public health problems in tropical and subtropical regions, where almost 400 million infections are estimated to occur each year (Bhatt et al. , 2013; Lambrechts et al. , 2015). People may be infected with dengue more than once because it is caused by any one of four related viruses (DENV1-4) which is transmitted by mosquitoes, especially *Aedes aegypti* and *Aedes albopictus* (Rigau-Pérez , 2006). Dengue fever itself is rarely fatal, but more severe forms, dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS), cause 22,000 deaths annually (WHO , 2012), and mostly among children. In the absence of licensed vaccines or therapeutic drugs, valid prevention of dengue diseases is restricted to the control of the main mosquito vectors. However, traditional control measures have some drawbacks more or less. For example, the extensive use of insecticides may lead to insecticide resistance and major toxicological effects on human health and environment (Ritter et al. , 1995; Zhang et al. , 2015c). Another common approach is source reduction of mosquito populations which involves the destruction of mosquito oviposition sites. But it is hard to sustain such interventions successfully over large areas (Lambrechts et al. , 2015; Burattini et al. , 2008).

The augmentation (release) of mosquito populations carrying particular strains of endosymbiotic *Wolbachia* bacteria is proposed to be a promising novel approach for dengue control that has been advanced in field trials (James et al. , 2011; McGraw et al. , 2013; Hoffmann et al. , 2011). It has been estimated that *Wolbachia* can infect up to 65% of insect species and approximately 28% of the surveyed mosquito species (Werren et al. , 2008; Kittayapong et al. , 2000). They mainly live in the reproductive organs of their hosts, and they can interfere with the insects' reproductive mechanisms, through the processes such as maternal transmission, cytoplasmic incompatibility (CI), male killing, feminization and parthenogenesis, to facilitate their own survival. CI occurs when *Wolbachia* infected male insects mate with *Wolbachia* uninfected females which will produce non-viable offsprings, while other mates cannot be affected. These female biased reproductive manipulations can drive a high frequency of *Wolbachia* infections in wild populations (Hoffmann et al. , 2011).

In practice, two strategies have been proposed: population suppression (eradication) and population replacement (*Wolbachia* infected insects established and replacing *Wolbachia* uninfected ones). The success of the two strate-

gies depends on the selection of *Wolbachia* strains and the design of the augmentation of *Wolbachia* infected mosquitoes (Bull and Turelli , 2013). Based on CI, the strategy of population suppression can be realized if only *Wolbachia* infected males are inundatively released, as the successful suppression of *Culex pipiens* populations in field tests (Laven , 1967). Some *Wolbachia* cannot only successfully spread within mosquito populations but also may act as a 'vaccine' to stop mosquitoes from replicating and transmitting dengue virus (Walker et al. , 2011; Hoffmann et al. , 2011). So based on the mechanisms of CI and maternal inheritance, the strategy of population replacement can be achieved by inoculative releases of *Wolbachia* infected mosquitoes (Hoffmann et al. , 2011; Walker et al. , 2011; Turelli , 2010; Yeap et al. , 2011).

The potential of *Wolbachia* strains as agents for the biological control of pathogen transmission has drawn attention from both biological and mathematical researchers. Recently, researchers in some countries have released or intend to release mosquitoes infected with different strains of *Wolbachia* bacteria to block the spread of dengue virus. The first open releases of *wMel Wolbachia* infected mosquitoes were carried out in Yorkeys Knob and Gordonvale in north-eastern Australia in 2011 (Hoffmann et al. , 2011). Hoffmann et al. (2011) described how the *wMel Wolbachia* infection successfully invaded into two natural *A. aegypti* populations, reaching near-fixation in a few months. They also demonstrated that the frequency of *Wolbachia* infected mosquitoes needs to exceed 30% for invasion. Based on the success of these field trials in Australia, mosquitoes infected with *wMelPop* and *wMel Wolbachia* were also released in Tri Nguyen Island, Vietnam, in April 2013 (failure) and May 2014 (success)(VED , 2014). Moreover, there have been other open releases of *wMel* mosquitoes in Yogyakarta in Indonesia, Rio de Janeiro in Brazil and elsewhere.

Note that mosquito augmentation does not always occur as 1:1 sex ratio of male to female. In 2014, the worst ever dengue outbreak is occurred in Guangdong province, China, with more than 47,000 cases (The Guardian , 2015). Compared with the open field trials in other countries, there are two procedures for the releases of *Wolbachia* infected mosquitoes in Shazai Island, in Guangzhou. At first, since Mar. 12, 2015, only *Wolbachia* male (sterile) mosquitoes were openly released with three times a week, and every time about 70,000–100,000 sterile mosquitoes released, to significantly reduce the density of *Wolbachia* uninfected female mosquitoes (population suppression). In order to fulfill a better suppression, the ratio of sterile male mosquitoes to wild males was kept as 5 : 1 so that the mating rate among uninfected females and sterile males was over 80%, and then their offsprings would not been hatched. Next, *Wolbachia* infected female mosquitoes will be released for the success of population replacement. Moreover, not all open field trials of mosquito augmentation can be successful in the end. **In this study, it is necessary to develop a four dimensional impulsive model to investigate the effect of the augmentation of *Wolbachia* infected mosquitoes with different sex on the success of population eradication or replacement for the control of dengue transmission. Based on the model, we evaluate a series of relevant issues, including (a) how each parameter**

value contributes to the success of population replacement? (b) how different release quantities of infected mosquitoes with different sex affect the success of the two strategies? And (c) how the success of population suppression or replacement can be fulfilled to block the transmission of dengue fever?

The paper is organized as follows. In Section 2, based on previous works, we introduce the augmentation of *Wolbachia* infected mosquitoes with different sex at regular pulse moments as a four dimensional impulsive system. The stability and permanence of periodic solutions of the system with perfect transmission rate is proved in Section 3. In Section 4, the existence of forward and backward bifurcations is investigated by employing the bifurcation theory of [Lakmeche and Aarino \(2000\)](#). In Section 5, we employ sensitivity analysis to evaluate the contribution of each parameter on the two output variables, then we study the effects of initial density, pulse period and the quantity of mosquito augmentation with different sex on the success of the two strategies by numerical simulations. Some discussion is given in the last section.

2 Model formulation

In recent years, different mathematical models have been proposed to analyze the spread of *Wolbachia* in populations, including discrete time models ([Turelli , 1994](#); [Vautrin et al. , 2007](#); [Haygood and Turelli , 2009](#)), continuous time models ([Farkas and Hinow , 2010](#); [Keeling et al. , 2003](#); [Schofield , 2002](#); [Hughes and Britton , 2013](#); [Zheng et al. , 2014](#)), stochastic models ([Jansen et al. , 2008](#)) and impulsive models ([Zhang et al. , 2015b,c](#)). From [Keeling et al. \(2003\)](#), the total density of mosquito populations P is subdivided into four classes, uninfected females F_U , infected females F_I , uninfected males M_U and infected males M_I . We first assume that uninfected and infected individuals have different natural birth rates, denoted as b_1 and b_2 , respectively, and the same natural death rate d . But infected individuals have an additional fitness cost D ($d + D > 0$), either disadvantageous ($D > 0$) or advantageous ($D < 0$) depending on mosquito species and *Wolbachia* strains ([Zhang et al. , 2015b](#)). The bacterium is mostly passed from infected females to their offsprings with a probability $\tau \in (0, 1]$. The effect of the CI mechanism results in zygotic death of potential offsprings with a probability $q \in [0, 1]$ when an infected male mates with a uninfected female. Then we have the following model.

$$\begin{cases} \frac{dF_U(t)}{dt} = (1 - \tau)b_2F_I + b_1F_U\left(1 - \frac{qM_I}{M_U + M_I}\right) - dPF_U, \\ \frac{dM_U(t)}{dt} = (1 - \tau)b_2F_I + b_1F_U\left(1 - \frac{qM_I}{M_U + M_I}\right) - dPM_U, \\ \frac{dF_I(t)}{dt} = \tau b_2F_I - (d + D)PF_I, \\ \frac{dM_I(t)}{dt} = \tau b_2F_I - (d + D)PM_I. \end{cases} \quad (1)$$

In order to study the effect of mosquito augmentation on the control of dengue transmission, the dynamics of mosquito populations with continuous

augmentation of *Wolbachia* infected mosquitoes was developed by [Zheng et al. \(2014\)](#) using delay differential equations. They denoted F_R and M_R as the densities of *Wolbachia* infected female and male mosquitoes released, respectively. Then the dynamics of the released populations was modeled by the following equations.

$$\begin{cases} \frac{dF_R(t)}{dt} = -(d + D)PF_R, \\ \frac{dM_R(t)}{dt} = -(d + D)PM_R, \end{cases} \quad (2)$$

with $P = F_U + M_U + F_I + M_I + F_R + M_R$. The dynamics of the other four cases of mosquito populations was the same as in model (1). Similarly, [Zheng et al. \(2014\)](#) proved that there existed a threshold for the success of *Wolbachia* invasion. They also investigated the relationships of the minimal releasing of infected mosquitoes and the waiting time with the time delay, and the waiting time in relation to the sex ratio of released populations. Recently, impulsive differential equation has been widely introduced in modelling the control of epidemic diseases, integrated pest management and biological resource management ([Hu et al. , 2009](#); [Gourley et al. , 2007](#); [Simons and Gourley , 2006](#); [Tang et al. , 2015](#); [Xu et al. , 2014](#)). Note that the releases of *Wolbachia* infected mosquitoes are carried out at regular pulse moments, instead of continuous time interval, in the field trials ([Hoffmann et al. , 2011](#)). So a more realistically impulsive differential equation was proposed in our previous work ([Zhang et al. , 2015b](#)). Because nearly an identical sex ratio of *Wolbachia* infected mosquitoes were augmented in some field trails ([Hoffmann et al. , 2011](#)), we assumed $M_I/F_I = M_U/F_U$, and let $I = F_I + M_I, U = F_U + M_U$. Denote that *Wolbachia* infected mosquitoes were augmented as the quantity series $\varphi_n > 0$ at the corresponding impulsive point series ($\lambda_n > 0, n = 1, 2, \dots$). Then the dynamics of augmented mosquitoes was described as follows.

$$\left. \begin{aligned} I(\lambda_n^+) &= I(\lambda_n) + \varphi_n \\ U(\lambda_n^+) &= U(\lambda_n), \end{aligned} \right\} t = \lambda_n, \quad (3)$$

where the items $I(\lambda_n^+)$ and $U(\lambda_n^+)$ denoted the densities of infected and uninfected mosquitoes after pulse releases at the time λ_n , respectively. We revealed that it was impossible for the success of population suppression. The effects of the initial densities of mosquitoes, augmentation timings, augmentation quantities and numbers of augmentations on the success of population replacement were also investigated.

However, as mentioned in the introduction, the augmentation of *Wolbachia* infected mosquito is often carried out with a fix pulse period, and it does not always occur as identical sex ratio of male to female in some open field trials, for example the open field releases in Shazai Island, in Guangzhou ([The Guardian , 2015](#)). So in order to investigate how mosquito augmentation with different sex affects the success of population suppression or replacement for the control of dengue transmission, based on model (1), we assume that the quantities of *Wolbachia* infected female and male mosquitoes are augmented as $\theta_1 > 0$ and $\theta_2 > 0$, respectively at pulse moments $\lambda_n (n = 1, 2, \dots)$. Denote

the augmentation period as a constant T and $\lambda_{n+1} - \lambda_n = T$ for all n ($n \in \mathcal{N}$). Then the **release** actions can be described as follows.

$$\left. \begin{aligned} F_U(nT^+) &= F_U(nT), \\ M_U(nT^+) &= M_U(nT), \\ F_I(nT^+) &= I(nT) + \theta_1, \\ M_I(nT^+) &= M(nT) + \theta_2, \end{aligned} \right\} t = nT, n \in \mathcal{N}. \quad (4)$$

Based on the above, in order to investigate how the mosquito augmentation with different sex affect the success of population suppression or replacement for the control of dengue fever, in this paper we propose a four dimensional impulsive equations model to describe the dynamics of mosquito populations as follows.

$$\left. \begin{aligned} \left. \begin{aligned} \frac{dF_U(t)}{dt} &= (1 - \tau)b_2F_I + b_1F_U\left(1 - \frac{qM_I}{M_U + M_I}\right) - dPF_U, \\ \frac{dM_U(t)}{dt} &= (1 - \tau)b_2F_I + b_1F_U\left(1 - \frac{qM_I}{M_U + M_I}\right) - dPM_U, \\ \frac{dF_I(t)}{dt} &= \tau b_2F_I - (d + D)PF_I, \\ \frac{dM_I(t)}{dt} &= \tau b_2F_I - (d + D)PM_I, \end{aligned} \right\} t \neq nT, n \in \mathcal{N}, \\ \left. \begin{aligned} F_U(t^+) &= F_U(t), \\ M_U(t^+) &= M_U(t), \\ F_I(t^+) &= F_I(t) + \theta_1, \\ M_I(t^+) &= M_I(t) + \theta_2, \end{aligned} \right\} t = nT, n \in \mathcal{N}, \end{aligned} \right\} \quad (5)$$

where parameter meanings are the same as in models (1) and (4), and more details about parameter definitions are shown in Tables 1 and 2.

3 The stability and permanence of periodic solutions

In order to realize the success of population suppression or replacement for the control of dengue transmission, it is necessary to analyze the existence and stability of periodic solutions of system (5). Note that system (5) with perfect transmission rate may lead to the success of complete population replacement. So in this section, based on system (5) with perfect transmission rate, we first study the threshold conditions for the existence and stability of *Wolbachia* mosquito established periodic solution (WEPS), then we obtain the condition for the permanence of *Wolbachia* uninfected mosquitoes. Before obtaining these key results, we first need to give the proof of Lemma 1 as follows.

Let $\tau = 1$ for system (5), then its subsystem in the *Wolbachia* mosquito established subspace $X_I = \{(F_I, M_I, F_U, M_U) : F_I \geq 0, M_I \geq 0, F_U = 0, M_U = 0\}$ is as follows:

$$\left. \begin{aligned} \left. \begin{aligned} \frac{dF_I(t)}{dt} &= b_2F_I - (d + D)(F_I + M_I)F_I, \\ \frac{dM_I(t)}{dt} &= b_2F_I - (d + D)(F_I + M_I)M_I, \end{aligned} \right\} t \neq nT, n \in \mathcal{N}, \\ \left. \begin{aligned} F_I(t^+) &= F_I(t) + \theta_1, \\ M_I(t^+) &= M_I(t) + \theta_2, \end{aligned} \right\} t = nT, n \in \mathcal{N}. \end{aligned} \right\} \quad (6)$$

For $t \in (nT, (n+1)T]$, the solution of the above subsystem is solved as follows

$$\begin{cases} F_I(t) = \frac{b_2 e^{b_2(t-nT)} F_I(nT^+)}{2(d+D)(e^{b_2(t-nT)}-1)F_I(nT^+)-b_2(d+D)(F_I(nT^+)-M_I(nT^+))(t-nT)+b_2} + \theta_1, \\ M_I(t) = \frac{b_2((e^{b_2(t-nT)}-1)F_I(nT^+)+M_I(nT^+))}{2(d+D)(e^{b_2(t-nT)}-1)F_I(nT^+)-b_2(d+D)(F_I(nT^+)-M_I(nT^+))(t-nT)+b_2} + \theta_2. \end{cases} \quad (7)$$

Denote $X_n = F_I(nT^+)$, $Y_n = M_I(nT^+)$, then we have

$$\begin{cases} X_{n+1} = \frac{b_2 e^{b_2 T} X_n}{2(d+D)(e^{b_2 T}-1)X_n - b_2(d+D)T(X_n - Y_n) + b_2} + \theta_1, \\ Y_{n+1} = \frac{b_2((e^{b_2 T}-1)X_n + Y_n)}{2(d+D)(e^{b_2 T}-1)X_n - b_2(d+D)T(X_n - Y_n) + b_2} + \theta_2. \end{cases} \quad (8)$$

It follows from (8), denote (X, Y) as one of its equilibria, then X and Y satisfy the following quadratic equations

$$\begin{cases} L_1 : A_1 X^2 + 2B_1 XY + C_1 Y^2 + 2D_1 X + 2E_1 Y + F_1 = 0, \\ L_2 : A_2 X^2 + 2B_2 XY + C_2 Y^2 + 2D_2 X + 2E_2 Y + F_2 = 0, \end{cases} \quad (9)$$

where

$$\begin{aligned} A_0 &= e^{b_2 T}, \quad A_1 = (d+D)(b_2 T - 2(A_0 - 1)) < 0, \quad B_1 = \frac{-b_2(d+D)}{2} < 0, \quad C_1 = 0, \\ D_1 &= \frac{(2\theta_1(d+D)+b_2)(A_0-1)-b_2\theta_1(d+D)T}{2}, \quad E_1 = \frac{b_2\theta_1(d+D)T}{2} > 0, \quad F_1 = b_2\theta_1 > 0, \\ A_2 &= (A_0 - 1) > 0, \quad B_2 = \frac{-(A_0-1)}{2} < 0, \quad C_2 = 0, \\ D_2 &= \frac{A_0(\theta_2-\theta_1)+\theta_1}{2}, \quad E_2 = -\frac{\theta_1}{2} < 0, \quad F_2 = 0. \end{aligned}$$

Denote the invariants of the two conic curves as

$$I_1^{(i)} = A_i + C_i, \quad I_2^{(i)} = \begin{vmatrix} A_i & B_i \\ B_i & C_i \end{vmatrix}, \quad I_3^{(i)} = \begin{vmatrix} A_i & B_i & D_i \\ B_i & C_i & E_i \\ D_i & E_i & F_i \end{vmatrix}, \quad i = 1, 2.$$

For the curve L_1 , by simple calculation, we obtain $I_1^{(1)} < 0$, $I_2^{(1)} < 0$ and $I_3^{(1)} = 2B_1 D_1 E_1 - A_1 E_1^2 - B_1^2 F_1$. So it is a hyperbola provided $I_2^{(1)} < 0$, $I_3^{(1)} \neq 0$, and it degenerates as two intersectant straight lines provided $I_2^{(1)} < 0$, $I_3^{(1)} = 0$. Based on the first equation of (9), the curve L_1 passes through three fixed points $P_1(x_1, 0)$, $P_2(x_2, 0)$ and $P_3(0, y_1)$, and one of its asymptote is $X = x_3$ with

$$\begin{aligned} x_1 &= \frac{-D_1 + \sqrt{D_1^2 - A_1 F_1}}{A_1} < 0, \quad x_2 = \frac{-D_1 - \sqrt{D_1^2 - A_1 F_1}}{A_1} > 0, \\ y_1 &= -\frac{1}{(d+D)T} < 0, \quad x_3 = \theta_1 > 0. \end{aligned}$$

Similarly for the curve L_2 , we have $I_1^{(2)} > 0$, $I_2^{(2)} < 0$ and $I_3^{(2)} = 2B_2 D_2 E_2 - A_2 E_2^2$. It is also a hyperbola provided $I_2^{(2)} < 0$, $I_3^{(2)} \neq 0$, and it degenerates as two intersectant straight lines provided $I_2^{(2)} < 0$, $I_3^{(2)} = 0$. Based on the second equation of (9), the curve L_2 passes through two fixed points $P_0(0, 0)$ and $P_4(x_4, 0)$, and one of its asymptote is $X = x_5$ with

$$x_4 = -\frac{A_0(\theta_2 - \theta_1) + \theta_1}{A_0 - 1}, \quad x_5 = -\frac{\theta_1}{A_0 - 1} < 0.$$

Note that if $\theta_1 > \theta_2$, then $I_3^{(2)} < 0$, $x_4 > x_5$; if $\theta_1 = \theta_2$, then $I_3^{(2)} = 0$, $x_4 = x_5$; if $\theta_1 < \theta_2$, then $I_3^{(2)} > 0$, $x_4 < x_5$.

According to above analysis, there exists one intersection for the two curves in the first quadrant as show in Fig 1 if and only if $x_2 > x_4$ holds true, i.e.,

$$(A_0 - 1)(-D_1 + \sqrt{D_1^2 - A_1 F_1}) + A_1(A_0(\theta_2 - \theta_1) + \theta_1) < 0. \quad (10)$$

So when (10) holds true, there exists only one positive equilibrium $E_1^* = (X_1^*, Y_1^*)$ for system (8), which indicates its global stability. Based on the relationship between systems (6) and (8), there exists a unique interior periodic solution for system (6), denoted as $(\tilde{F}_I(t), \tilde{M}_I(t))$ with

$$\begin{aligned} \tilde{F}_I(t) &= \frac{b_2 e^{b_2(t-nT)} X_1^*}{2(d+D)(e^{b_2(t-nT)} - 1)X_1^* - b_2(d+D)(X_1^* - Y_1^*)(t-nT) + b_2}, \\ \tilde{M}_I(t) &= \frac{b_2((e^{b_2(t-nT)} - 1)X_1^* + Y_1^*)}{2(d+D)(e^{b_2(t-nT)} - 1)X_1^* - b_2(d+D)(X_1^* - Y_1^*)(t-nT) + b_2}. \end{aligned} \quad (11)$$

When (10) is not formed, then there is no positive equilibrium for system (8), which indicates that the solutions of subsystem (6) may tend to infinity and it is not an expected result for mosquito releases in field trials. So in the work, we focus on analyzing system (5) when (10) holds true. Then we have the following Lemma.

Lemma 1. *When (10) holds true, for subsystem (6), there exists a unique positive T -periodic solution $(\tilde{F}_I(t), \tilde{M}_I(t))$, and for any solution $(F_I(t), M_I(t))$, we have $F_I(t) \rightarrow \tilde{F}_I(t)$ and $M_I(t) \rightarrow \tilde{M}_I(t)$ as $t \rightarrow \infty$.*

Based on Lemma 1, in the following, we mainly study the stability of the WEPS $(\tilde{F}_I(t), \tilde{M}_I(t), 0, 0)$ and the permanence of *Wolbachia* uninfected mosquitoes for system (5) with perfect transmission rate. For convenience, we first admit $(\tilde{F}_I(t), \tilde{M}_I(t), 0, 0)$ on every impulsive interval $(nT, (n+1)T]$. Then some notations are given, for example, $A(t)$ be $n \times n$ matrix and $\Phi_{A(\cdot)}(t)$ be the fundamental solution matrix of linear ordinary differential system $x'(t) = A(t)x(t)$, then denote $r(\Phi_{A(\cdot)}(\bar{t}))$ as the spectral radius of $\Phi_{A(\cdot)}(\bar{t})$.

Now we utilize the theory of Floquet multiplier to prove the stability of the WEPS. Let $F_I(t) = f_i(t) + \tilde{F}_I(t)$, $M_I(t) = m_i(t) + \tilde{M}_I(t)$, $F_U(t) = f_u(t)$, $M_U(t) = m_u(t)$, $y(t) = (f_i(t), m_i(t), f_u(t), m_u(t))$, then we have the linear system of (5) at the WEPS as follows.

$$\begin{cases} y'(t) = Q(t)y(t), t \neq nT, n \in \mathcal{N}, \\ y(t) = \mathcal{P}y(t), t = nT, n \in \mathcal{N}, \end{cases} \quad (12)$$

where $Q(t) = \begin{pmatrix} V_1 & V_2 \\ \mathcal{O} & V_3 \end{pmatrix}$ and $\mathcal{P} = \begin{pmatrix} P & \mathcal{O} \\ \mathcal{O} & P \end{pmatrix}$ with

$$\begin{aligned} V_1 &= \begin{pmatrix} b_2 - (d+D)(2\tilde{F}_I + \tilde{M}_I) & -(d+D)\tilde{F}_I \\ b_2 - (d+D)\tilde{M}_I & -(d+D)(\tilde{F}_I + 2\tilde{M}_I) \end{pmatrix}, \\ V_2 &= \begin{pmatrix} -(d+D)\tilde{F}_I & -(d+D)\tilde{F}_I \\ -(d+D)\tilde{M}_I & -(d+D)\tilde{M}_I \end{pmatrix}, V_3 = \begin{pmatrix} b_1(1-q) - d(\tilde{F}_I + \tilde{M}_I) & 0 \\ b_1(1-q) & -d(\tilde{F}_I + \tilde{M}_I) \end{pmatrix}, \end{aligned}$$

$$\mathcal{O} = \begin{pmatrix} 0 & 0 \\ 0 & 0 \end{pmatrix} \text{ and } P = \begin{pmatrix} 1 & 0 \\ 0 & 1 \end{pmatrix}.$$

Let $\Phi_Q(t) = (\Phi_{ij})_{1 \leq i, j \leq 2}$ be the fundamental solution matrix of $y'(t) = Q(t)y(t)$, so $\Phi'_Q(t) = Q(t)\Phi_Q(t)$ with $\Phi_Q(0) = E_4$. Solving the equation yields

$$\Phi_Q(t) = \begin{pmatrix} e^{\int_0^t V_1(\nu) d\nu} & \Phi_{12}(t) \\ 0 & e^{\int_0^t V_3(\nu) d\nu} \end{pmatrix}, \quad (13)$$

then we have

$$\mathcal{P}\Phi_Q(t) = \begin{pmatrix} P e^{\int_0^t V_1(\nu) d\nu} & \Phi_{12}(t) \\ 0 & P e^{\int_0^t V_3(\nu) d\nu} \end{pmatrix}. \quad (14)$$

From the stability of $(\tilde{F}_I(t), \tilde{M}_I(t))$ for system (6), we have $r(P e^{\int_0^T V_1(\nu) d\nu}) < 1$. Denote

$$R_0 \triangleq r(P e^{\int_0^T V_3(\nu) d\nu}),$$

so based on Floquet theory, the WEPS of system (5) is locally stable provided that $R_0 < 1$.

Next we aim to prove that if $R_0 > 1$, then the permanence of *Wolbachia* uninfected mosquitoes. It means there exists $\eta > 0$ such that $\liminf_{t \rightarrow \infty} R_U(t) \geq \eta > 0$, $R = F, M$.

First we prove the following claim: there exists a positive constant η such that $\limsup_{t \rightarrow \infty} R_U(t) \geq \eta > 0$, $R = F, M$. Otherwise, there exists a $t_1 > 0$ such that $0 \leq R_U(t) < \eta$ for all $t > t_1$.

By the last two equations of system (5), we have

$$\left\{ \begin{array}{l} \frac{dF_I(t)}{dt} \leq b_2 F_I - (d + D)(F_I + M_I)F_I, \\ \frac{dM_I(t)}{dt} \leq b_2 F_I - (d + D)(F_I + M_I)M_I, \end{array} \right\} t \neq nT, n \in \mathcal{N}, \quad (15)$$

$$\left\{ \begin{array}{l} F_I(t^+) = F_I(t) + \theta_1, \\ M_I(t^+) = M_I(t) + \theta_2, \end{array} \right\} t = nT, n \in \mathcal{N}.$$

Consider the following comparison system

$$\left\{ \begin{array}{l} \frac{dx_1}{dt} = b_2 x_1 - (d + D)(x_1 + x_2)x_1, \\ \frac{dx_2}{dt} = b_2 x_1 - (d + D)(x_1 + x_2)x_2, \end{array} \right\} t \neq nT, n \in \mathcal{N}, \quad (16)$$

$$\left\{ \begin{array}{l} x_1(t^+) = x_1(t) + \theta_1, \\ x_2(t^+) = x_2(t) + \theta_2, \end{array} \right\} t = nT, n \in \mathcal{N}.$$

It is the same with system (6), system (16) exists a globally asymptotically stable positive periodic solution $\tilde{x} = (\tilde{x}_1, \tilde{x}_2) = (\tilde{F}_I(t), \tilde{M}_I(t))$. By the comparison theorem, there exists $t_2 > t_1$ and $\epsilon_1 > 0$, such that $F_I(t) \leq x_1(t) \leq \tilde{x}_1(t) + \epsilon_1 = \tilde{F}_I(t) + \epsilon_1$ and $M_I(t) \leq x_2(t) \leq \tilde{x}_2(t) + \epsilon_1 = \tilde{M}_I(t) + \epsilon_1$ for $t > t_2$. By the first two equations of system (5), we have

$$\left\{ \begin{array}{l} \frac{dF_U(t)}{dt} \geq b_1 F_U \left(1 - \frac{q(\tilde{M}_I + \epsilon_1)}{M_U + \tilde{M}_I + \epsilon_1} \right) - d(\tilde{F}_I + \tilde{M}_I + 2\epsilon_1 + 2\eta)F_U, \\ \frac{dM_U(t)}{dt} \geq b_1 F_U \left(1 - \frac{q(\tilde{M}_I + \epsilon_1)}{M_U + \tilde{M}_I + \epsilon_1} \right) - d(\tilde{F}_I + \tilde{M}_I + 2\epsilon_1 + 2\eta)M_U. \end{array} \right. \quad (17)$$

If ϵ_1 and η are sufficiently small, consider the following comparison system

$$\frac{du(t)}{dt} = V_3 u(t), \quad (18)$$

where $u = (u_1, u_2)$, then $u(t) = u(0)\Phi_{V_3}(t) = u(0)e^{\int_0^t V_3(\nu)d\nu}$. When $R_0 > 1$, there exists $t_3 > t_2$ such that $u_1 \rightarrow \infty$ and $u_2 \rightarrow \infty$ for all $t > t_3$. Thus $\lim_{t \rightarrow \infty} R_U = \infty$ ($R = F, M$), which contradicts to the boundedness of R_U . So the claim is proved, i.e., $\limsup_{t \rightarrow \infty} R_U(t) \geq \eta > 0, R = F, M$.

From the claim, there are two options for discussion.

- (a) $R_U(t) > \eta$ ($R = F, M$) for all large enough t ;
- (b) $R_U(t)$ ($R = F, M$) oscillates about η for all large t .

If case (a) occurs, then we complete the proof. In the following, we consider option (b). Since $\limsup_{t \rightarrow \infty} R_U(t) \geq \eta > 0, R = F, M$, there exists $\tau_1 \in (n_1 T, (n_1 + 1)T]$ such that $R_U(\tau_1) \geq \eta$. Similarly, there exists another $\tau_2 \in (n_2 T, (n_2 + 1)T]$ such that $R_U(\tau_2) \geq \eta$, where $n_2 - n_1 \geq 0$ is finite. Based on system (5) in the time interval $[\tau_1, \tau_2]$, we have

$$\begin{cases} \frac{dF_U(t)}{dt} \geq -d(F_U + M_U + F_I + M_I)F_U, \\ \frac{dM_U(t)}{dt} \geq -d(F_U + M_U + F_I + M_I)M_U. \end{cases} \quad (19)$$

For system (5), when $R_0 > 1$ holds true, then its solution $R_I(t)$ ($R = F, M$) is either finite or infinite. It means that there exist positive constants $\rho_i, i = 1, 2$ such that $\rho_1 \leq R_I(t) \leq \rho_2$ for any $t > t_1$ or $R_I(t) \rightarrow \infty$ ($R = F, M$) as $t \rightarrow \infty$.

If $\rho_1 \leq R_I(t) \leq \rho_2$ ($R = F, M$), then system (19) becomes as

$$\begin{cases} \frac{dF_U(t)}{dt} \geq -d(2\rho_2 + F_U + M_U)F_U, \\ \frac{dM_U(t)}{dt} \geq -d(2\rho_2 + F_U + M_U)M_U. \end{cases} \quad (20)$$

So we have

$$\begin{aligned} F_U(t) &\geq \frac{2\rho_2 F_U(\tau_1)}{(2\rho_2 + F_U(\tau_1) + M_U(\tau_1))e^{2\rho_2 d(t-\tau_1)} - F_U(\tau_1) - M_U(\tau_1)} \\ &\geq \frac{2\rho_2 F_U(\tau_1) e^{-2\rho_2 d(\tau_2 - \tau_1)}}{2\rho_2 + F_U(\tau_1) + M_U(\tau_1)} \\ &\geq \frac{\rho_2 \eta e^{-2\rho_2 d(n_2 - n_1 + 1)T}}{\rho_2 + \eta + M_U(\tau_1^*)} \\ &\triangleq F_U^{(1)}, \end{aligned} \quad (21)$$

where $\tau_1^* \in [\tau_1, \tau_2]$ such that $M_U(t)$ being largest. Similarly,

$$M_U(t) \geq \frac{\rho_2 \eta e^{-2\rho_2 d(n_2 - n_1 + 1)T}}{\rho_2 + \eta + F_U(\tau_2^*)} \triangleq M_U^{(1)}, \quad (22)$$

where $\tau_2^* \in [\tau_1, \tau_2]$ such that $F_U(t)$ being largest. Denote $\eta_1 = \min\{F_U^{(1)}, M_U^{(1)}\}$, then $\eta_1 > 0$ cannot be infinitely small provided that $n_2 - n_1 \geq 0$ is finite. So $R_U(t) \geq \eta_1 > 0$ ($R = F, M$). By the same method, for $t > t_2$, we can get a finite positive η_2 . In sequence, we can obtain the series $\eta_i, i = 1, 2, \dots, k, \dots$, with $\eta_k = \min\{F_U^{(k)}, M_U^{(k)}\}$. For any η_k , it is not be infinitely small provided that

$n_{k+1} - n_k \geq 0$ is finite. The solution of system (5) $R_U(t) \geq \eta_k > 0, R = F, M$ holds true in the time interval $[t_k, t_{k+1}]$, $t_k \in (n_k T, (n_k + 1)T]$, $t_{k+1} \in (n_{k+1} T, (n_{k+1} + 1)T]$. Denote $\eta^* \triangleq \min\{\eta_i\} > 0, i = 1, 2, \dots$, hence $R_U(t) \geq \eta^* > 0, R = F, M$ for all $t > t_1$.

Based on system (5), if one of the solutions $R_I(t) \rightarrow \infty$ ($R = F, M$), then there exists a large enough constant $G > 0$ such that

$$\frac{dR_U(t)}{dt} \geq -G > -\infty.$$

Similarly, we can obtain the series $\eta'_k, k = 1, 2, \dots, k, \dots$, where

$$\eta'_k = \min\{\eta - G(n'_{k+1} - n'_k)T, \eta - G(n'_{k+1} - n'_k)T\} < 0$$

and $R_U(t) \geq \eta'_k, R = F, M$ holds true in the time interval $[t'_k, t'_{k+1}]$, $t'_k \in (n'_k T, (n'_k + 1)T]$, $t'_{k+1} \in (n'_{k+1} T, (n'_{k+1} + 1)T]$. Denote $\eta_* \triangleq \min\{\eta'_i\} < 0, i = 1, 2, \dots$, hence for biological meaning $R_U(t) \geq 0 \geq \eta_*, R = F, M$ for all $t > t_1$. If $R_U(t) = 0$, which contradicts with case (b). If $R_U(t) > 0$, there exists a small enough constant $\epsilon_2 > 0$ such that $R_U(t) \geq \epsilon_2$ for $t > t_1$. Therefore, there exists $\eta = \min\{\eta^*, \epsilon_2\} > 0$ such that $\liminf_{t \rightarrow \infty} R_U(t) \geq \eta, R = F, M$.

Therefore, based on above analysis, we have the following main results.

Theorem 1 *When (10) holds true, for system (5) with perfect transmission rate (i.e., $\tau = 1$), if $R_0 < 1$, then the WEPS $(\tilde{F}_I(t), \tilde{M}_I(t), 0, 0)$ is locally asymptotically stable; if $R_0 > 1$, then *Wolbachia* uninfected mosquitoes are permanent, namely, there exists a constant $\eta > 0$ such that $\liminf_{t \rightarrow \infty} R_U(t) \geq \eta, R = F, M$.*

Remark For system (5) with perfect transmission rate, when $R_0 < 1$, then the local stability of the WEPS indicates that it is possible to realize a complete (here also called high level) population replacement from some initial values. While when $R_0 > 1$, then the permanence of *Wolbachia* uninfected mosquitoes implies the failure of population replacement (here also called a low level of replacement) from any initial values.

4 Forward and backward bifurcations of system (5)

In epidemiological model, when forward bifurcation occurs, the threshold for the stability of disease free equilibrium can describe the elimination or permanence of epidemic diseases. However, when backward bifurcation occurs, the system exhibits a unstable and a stable endemic equilibria along with a stable disease free equilibrium, so the threshold of the stability for disease free equilibrium cannot describe the necessary elimination of epidemic diseases, so a more accurate threshold for the elimination is determined by the coincidence of the two endemic equilibria (Wang, 2006). Similarly, in

this section, in order to realize a complete population replacement for the control of dengue transmission, it is necessary to investigate the existence of forward and backward bifurcations for system (5) following the scheme of Lakmeche and Aarino (2000).

The release quantity of infected female mosquitoes θ_1 is chosen as bifurcation parameter instead of pulse period T in Lakmeche and Aarino (2000). For convenience, we first introduce the following notations. Let

$$X(t) := (F_I(t), M_I(t), F_U(t), F_V(t))$$

be a solution vector of system (5). The right side of the first four equations of system (5) is denoted as

$$H(X(t)) := (H_1(X(t)), H_2(X(t)), H_3(X(t)), H_4(X(t))),$$

and the impulsive effect is the mapping

$$\begin{aligned} I(\theta_1, X(t)) &= (I_1(\theta_1, X(t)), I_2(\theta_1, X(t)), I_3(\theta_1, X(t)), I_4(\theta_1, X(t))) \\ &= (X_1(t) + \theta_1, X_2(t) + \theta_2, X_3(t), X_4(t)). \end{aligned} \quad (23)$$

Denote $\Phi(t, X_0) = (\Phi_1(t, X_0), \Phi_2(t, X_0), \Phi_3(t, X_0), \Phi_4(t, X_0))$ as the flow of the system associated to the first four equations of system (5), then $X(t) = \Phi(t, X_0)$, $0 < t \leq T$, $X(T) = \Phi(T, X_0) := \Phi(X_0)$ and $X(T^+) = I(\theta_1, X(t))$ with $X(0) = X_0$. Define the operate Ψ as

$$\Psi(\theta_1, X) := (\Psi_1(\theta_1, X), \Psi_2(\theta_1, X), \Psi_3(\theta_1, X), \Psi_4(\theta_1, X)) = I(\theta_1, \Phi(X))$$

and denote $D_X \Psi$ as the derivation of Ψ with respect to X .

We fix all the parameters except for θ_1 and denote θ_0 as a critical release quantity of *Wolbachia* infected females, which corresponds to $R_0 = 1$. In the following, we focus on the bifurcation of nontrivial periodic solutions near to the WEPS $X^* = (\tilde{F}_I, \tilde{M}_I, 0, 0)$, which starts from X_0 with release quantity θ_0 , then $\Phi_3(X_0) = \Phi_4(X_0) = 0$.

Based on the above notations, X is a T -periodic solution of system (5) if and only if its initial value is a fixed point for $\Psi(\theta_1, X)$, i.e., $\Psi(\theta_1, X) = X$. For easy computation, we denote $\theta_1 = \theta_0 + \bar{\theta}_1$ and $X = X_0 + \bar{X}$, then the fixed point problem is transformed as

$$N(\bar{\theta}_1, \bar{X}) = 0, \quad (24)$$

where

$$\begin{aligned} N(\bar{\theta}_1, \bar{X}) &= (N_1(\bar{\theta}_1, \bar{X}), N_2(\bar{\theta}_1, \bar{X}), N_3(\bar{\theta}_1, \bar{X}), N_4(\bar{\theta}_1, \bar{X})) \\ &= X_0 + \bar{X} - \Psi(\theta_0 + \bar{\theta}_1, X_0 + \bar{X}). \end{aligned} \quad (25)$$

Consider the variational equation associated with the first four equations of system (5), which is obtained by a formal derivation with respect to initial value X_0 as

$$\begin{aligned} \frac{d}{dt}(D_X \Phi(t, X_0)) &= D_X F(\Phi(t, X_0))(D_X \Phi(t, X_0)) \\ &= Q(t)(D_X \Phi(t, X_0)) \end{aligned} \quad (26)$$

where the initial condition

$$D_X \Phi(0, X_0) = E_4 \text{ and } \Phi(0, X_0) = (\Phi_1(0, X_0), \Phi_2(0, X_0), 0, 0),$$

with E_4 and later E_2 denoting identity matrixes with fourth and second orders, respectively. Let the derivation of N be given by the following matrix

$$D_X N(0, O) = \begin{pmatrix} E_2 - P e^{\int_0^T V_1(\nu) d\nu} & -P \Phi_{12}(T) \\ \mathcal{O} & E_2 - P e^{\int_0^T V_3(\nu) d\nu} \end{pmatrix} \quad (27)$$

with $O = (0, 0, 0, 0)$. A necessary condition for the bifurcation of a non-trivial periodic solution near to $X^* = (\tilde{F}_I, \tilde{M}_I, 0, 0)$ is $\det[D_X N(0, O)] = 0$. Based on the stability of period solution $(\tilde{F}_I, \tilde{M}_I)$ of subsystem (6), we have $\det[E_2 - P e^{\int_0^T V_1(\nu) d\nu}] \neq 0$. So $\det[D_X N(0, O)] = 0$ is reduced to $\det[E_2 - P e^{\int_0^T V_3(\nu) d\nu}] = 0$. Note that we have $\det[E_2 - P e^{\int_0^T V_3(\nu) d\nu}] = 0$ provided $R_0 = 1$. In the following, when $R_0 = 1$ holds true, we investigate the sufficient conditions for the existence of nontrivial T-period solutions arising from bifurcation.

Denote

$$D_X N(\bar{\theta}_1, \bar{X}) = \begin{pmatrix} a_{11} & a_{12} & a_{13} & a_{14} \\ a_{21} & a_{22} & a_{23} & a_{24} \\ a_{31} & a_{32} & a_{33} & a_{34} \\ a_{41} & a_{42} & a_{43} & a_{44} \end{pmatrix}, \quad (28)$$

with

$$D_X N(0, O) = \begin{pmatrix} a'_{11} & a'_{12} & a'_{13} & a'_{14} \\ a'_{21} & a'_{22} & a'_{23} & a'_{24} \\ 0 & 0 & a'_{33} & 0 \\ 0 & 0 & a'_{43} & a'_{44} \end{pmatrix} \text{ and } \mathcal{H} = \begin{pmatrix} a'_{11} & a'_{12} & a'_{13} \\ a'_{21} & a'_{22} & a'_{23} \\ 0 & 0 & a'_{33} \end{pmatrix}, \quad (29)$$

with $a_{ij} = a'_{ij}$, $i, j = 1, 2, 3, 4$ for $(\bar{\theta}_1, \bar{X}) = (0, 0)$. The expression of each element in the above matrices is shown in Appendix 1. Since $\det[D_X N(0, O)] = 0$, it implies that there exists a constant such that $a'_{43} = k a'_{33}$ and $a'_{44} = 0$, so we fail to use implicit function theory to obtain variable X as a function of θ_1 . Lyapunov-Schmidt reduction is considered as the following three steps so that we can utilize the implicit function theory (Golubisky and Schaeffer, 1985). For convenience, denote $D_X N(0, O) = G$ as the matrix of a linear map.

Step 1: Decompose the \mathcal{R}^4 space and utilize it into (24).

From (29), we have $\dim \ker G = 1$ and $\text{codim}(\text{Im}(G)) = 3$. Denote G_1 and G_2 as the projects onto $\ker G$ and $\text{Im}(G)$ respectively such that $G_1 + G_2 = \text{Id}_{\mathcal{R}^4}$, $G_1(\mathcal{R}^4) = \text{span}\{Y_1\} = \ker G$, $G_2(\mathcal{R}^4) = \text{span}\{Y_2, Y_3, Y_4\} = \text{Im}(G)$, where

$$Y_1 = \left(\frac{a'_{12}(a'_{11}a'_{24} - a'_{14}a'_{21}) - a'_{14}(a'_{11}a'_{22} - a'_{12}a'_{21})}{a'_{11}(a'_{11}a'_{22} - a'_{12}a'_{21})}, \frac{a'_{14}a'_{21} - a'_{11}a'_{24}}{a'_{11}a'_{22} - a'_{12}a'_{21}}, 0, 1 \right), \quad (30)$$

$Y_2 = (1, 0, 0, 0)$, $Y_3 = (0, 1, 0, 0)$, $Y_4 = (0, 0, 1, 0)$ and $Y_1 \triangleq (Y_{11}, Y_{12}, Y_{13}, Y_{14})$. From the decomposition $\mathcal{R}^4 = \ker G \oplus \text{Im}(G)$, there exist unique $\alpha_i \in \mathcal{R}$ ($i = 1, 2, 3, 4$) such that $\bar{X} = \alpha_1 Y_1 + \alpha_2 Y_2 + \alpha_3 Y_3 + \alpha_4 Y_4$. So (24) is equivalent to

$$N_i(\bar{\theta}_1, \alpha_1, \alpha_2, \alpha_3, \alpha_4) = N_i(\bar{\theta}_1, \alpha_1 Y_1 + \alpha_2 Y_2 + \alpha_3 Y_3 + \alpha_4 Y_4) = 0, i = 1, 2, 3, 4. \quad (31)$$

Step 2: Employ the implicit function theorem on (31).

From (31), we have

$$\begin{aligned} \frac{D(N_1, N_2, N_3)(0, 0)}{D(\alpha_2, \alpha_3, \alpha_4)} &= \frac{D(N_1, N_2, N_3)(0, 0)}{D(\bar{X}_1, \bar{X}_2, \bar{X}_3)} \frac{D(\bar{X}_1, \bar{X}_2, \bar{X}_3)}{D(\alpha_2, \alpha_3, \alpha_4)} \\ &= |\mathcal{H}E_3| = |\mathcal{H}| \neq 0. \end{aligned} \quad (32)$$

Based on the implicit function theory, there exists $\delta > 0$ sufficiently small and unique continuous functions $\tilde{\alpha}_i$ ($i = 2, 3, 4$) with respect to variables $\bar{\theta}_1$ and α_1 , i.e., $\tilde{\alpha}_i = \tilde{\alpha}_i(\bar{\theta}_1, \alpha_1)$ such that $\tilde{\alpha}_i(0, 0) = 0$ and

$$N_j(\bar{\theta}_1, \alpha_1) = N_j(\bar{\theta}_1, \alpha_1 Y_1 + \tilde{\alpha}_2 Y_2 + \tilde{\alpha}_3 Y_3 + \tilde{\alpha}_4 Y_4) = 0, j = 1, 2, 3, \quad (33)$$

for every $(\bar{\theta}_1, \alpha_1)$ with $|\bar{\theta}_1| < \delta$ and $|\alpha_1| < \delta$. Moreover we have

$$\frac{\partial \tilde{\alpha}_i(\bar{\theta}_1, \alpha_1)}{\partial \alpha_1} = 0, i = 2, 3, 4, \quad (34)$$

as shown in Appendix 3.

Step 3: Determine the Taylor expansion of $N_4(\bar{\theta}_1, \alpha_1)$ around $(0, 0)$.

Based on Step 2, $N(\bar{\theta}_1, \bar{X}) = 0$ if and only if

$$N_4(\bar{\theta}_1, \alpha_1) = N_4(\bar{\theta}_1, \bar{X}(\bar{\theta}_1, \alpha_1)) = 0 \quad (35)$$

with $\bar{X}(\bar{\theta}_1, \alpha_1) = (Y_{11}\alpha_1 + \tilde{\alpha}_2, Y_{12}\alpha_1 + \tilde{\alpha}_3, Y_{13}\alpha_1 + \tilde{\alpha}_4, Y_{14}\alpha_1)$. So the number of periodic solutions of system (5) is equal to that of solutions of (35). It is obvious that $N_4(\bar{\theta}_1, \alpha_1)$ vanishes at $(0, 0)$, so we need to determine the Taylor expansion of $N_4(\bar{\theta}_1, \alpha_1)$ around $(0, 0)$. It is necessary to compute higher order derivatives of $N_4(\bar{\theta}_1, \alpha_1)$ up to the order k for which $D^k N_4(0, 0) \neq 0$, then it gives

$$N_4(\bar{\theta}_1, \alpha_1) = \frac{1}{k!} \left(\bar{\theta}_1 \frac{\partial}{\partial \bar{\theta}_1} + \alpha_1 \frac{\partial}{\partial \alpha_1} \right)^k N_4(0, 0) + o(\bar{\theta}_1, \alpha_1)((|\bar{\theta}_1| + |\alpha_1|)^k) \quad (36)$$

with $k \geq 1$.

First, we compute the first order partial derivations of $N_4(\bar{\theta}_1, \alpha_1)$, and we obtain

$$\frac{\partial N_4(0, 0)}{\partial \alpha_1} = \frac{\partial N_4(0, 0)}{\partial \bar{\theta}_1} = 0$$

as shown in **Appendix 4**. So next, it is necessary to compute the second order partial derivations of $N_4(\bar{\theta}_1, \alpha_1)$, and define

$$A = \frac{\partial^2 N_4(0, 0)}{\partial \bar{\theta}_1^2}, B = \frac{\partial^2 N_4(0, 0)}{\partial \bar{\theta}_1 \partial \alpha_1}, C = \frac{\partial^2 N_4(0, 0)}{\partial \alpha_1^2}.$$

From Appendix 5, we obtain $A = 0$,

$$\begin{aligned} B &= - \left[\frac{a'_{22}}{a'_{11}a'_{22} - a'_{12}a'_{21}} \left(\frac{\partial^2 \Phi_4(X_0)}{\partial X_1 \partial X_4} - \frac{a'_{43}}{a'_{33}} \frac{\partial^2 \Phi_3(X_0)}{\partial X_1 \partial X_4} \right) - \frac{a'_{21}}{a'_{11}a'_{22} - a'_{12}a'_{21}} \left(\frac{\partial^2 \Phi_4(X_0)}{\partial X_2 \partial X_4} - \frac{a'_{43}}{a'_{33}} \frac{\partial^2 \Phi_3(X_0)}{\partial X_2 \partial X_4} \right) \right], \\ C &= \sum_{i=1}^4 \sum_{j=1}^4 \left(\frac{a'_{43}}{a'_{33}} \frac{\partial^2 \Phi_3(\theta_0, X_0)}{\partial X_i \partial X_j} - \frac{\partial^2 \Phi_4(\theta_0, X_0)}{\partial X_i \partial X_j} \right) Y_{1i} Y_{1j}. \end{aligned} \quad (37)$$

Therefore, we have

$$N_4(\bar{\theta}_1, \alpha_1) = B\alpha_1\bar{\theta}_1 + \frac{C}{2}\alpha_1^2 + o(\bar{\theta}_1, \alpha_1)((|\bar{\theta}_1| + |\alpha_1|)^2) = \frac{\alpha_1}{2}\tilde{N}_4(\bar{\theta}_1, \alpha_1) \quad (38)$$

with

$$\tilde{N}_4(\bar{\theta}_1, \alpha_1) = 2B\bar{\theta}_1 + C\alpha_1 + o(\bar{\theta}_1, \alpha_1)((|\bar{\theta}_1| + |\alpha_1|)^2).$$

Note that

$$\frac{\partial \tilde{N}_4(0, 0)}{\partial \bar{\theta}_1} = 2B \quad \text{and} \quad \frac{\partial \tilde{N}_4(0, 0)}{\partial \alpha_1} = C,$$

so the study of the equation $N_4(\bar{\theta}_1, \alpha_1) = 0$ near $(0, 0)$ depends on whether $B \neq 0$ or $C \neq 0$ holds true. If $B \neq 0$ (or $C \neq 0$), we can use the implicit function theorem and solve the equation $\tilde{N}_4(\bar{\theta}_1, \alpha_1) = 0$ near $(0, 0)$ with respect to $\bar{\theta}_1$ (or α_1) as a function of α_1 (or $\bar{\theta}_1$), which gives $\bar{\theta}_1 = \sigma(\alpha_1)$ (or $\alpha_1 = \gamma(\bar{\theta}_1)$). We deduce it as follows: for any α_1 (or $\bar{\theta}_1$) near 0, there exists $\sigma(\alpha_1)$ (or $\gamma(\bar{\theta}_1)$), such that $\tilde{N}_4(\sigma(\alpha_1), \alpha_1) = 0$ (or $\tilde{N}_4(\bar{\theta}_1, \gamma(\bar{\theta}_1)) = 0$) and $\sigma(0) = 0$ (or $\gamma(0) = 0$). If $BC \neq 0$, then the solutions of $N_4(\bar{\theta}_1, \alpha_1) = 0$ imply $\alpha_1/\bar{\theta}_1 \simeq -2B/C$, which allows us to determine the sign of $\bar{\theta}_1\alpha_1$. While if $BC = 0$, then we cannot determine the solutions of $N_4(\bar{\theta}_1, \alpha_1) = 0$ with respect to $\bar{\theta}_1$ and α_1 . So in this case, it is necessary to expand the third order partial derivation of $N_4(0, 0)$. Finally, we have the following conclusions.

Theorem 2 *When (10) holds true, for system (5) with perfect transmission rate ($\tau = 1$), if $R_0 = 1$ are satisfied, then as parameter θ_1 passes through the critical value θ_0 , a nontrivial periodic solution appears near the fixed point X_0 . The bifurcation is supercritical provided that $BC < 0$, while it is subcritical provided that $BC > 0$.*

Note that the threshold R_0 decreases as θ_1 increases, so a supercritical bifurcation means a backward bifurcation in system (5), while a subcritical bifurcation corresponds to a forward bifurcation in the $\theta_1 - \alpha_1$ plane. Hence we have the following results.

Theorem 3 *When (10) holds true, for system (5) with perfect transmission rate ($\tau = 1$), if $R_0 = 1$ are satisfied, then as parameter θ_1 passes through the critical value θ_0 , a backward bifurcation occurs provided that $BC < 0$, while a forward bifurcation occurs provided that $BC > 0$.*

5 Simulations

In this section, we first explore the uncertainty and sensitivity analysis of each parameter value on the two critical output variables (here R_0 and FI_m defined in (39)), using a Latin hypercube sampling (LHS) method (Blower and Dowlatabadi, 1994; Marino et al., 2008), to understand the effects of different parameters on the success of population replacement (see Figs 2 and 3). Second we compare the regions and threshold values of backward bifurcations of system (5) with and without mosquito augmentation (see Fig 4). Further, we carry out some numerical simulations to illustrate the effects of augmentation period and different tactics of mosquito augmentation on the success of population suppression or replacement (see Figs 5-12). Numerical results are obtained by using parameter values given in Tables 1 and 2.

5.1 Uncertainty and sensitivity analysis

Sensitivity analysis is performed by evaluating partial rank correlation coefficients (PRCCs) between each input parameter and output variable, which can determine the importance of parameters contributed to the value of outcome variable. Absolute values of PRCCs which belong to (0.4, 1), (0.2, 0.4), and (0, 0.2) indicate very important, moderate correlations and not significantly different from zero between input parameters and output variables, respectively. We perform the uncertainty and sensitivity analysis of each parameter in system (5) using LHS with 3000 samples. In the absence of available data on the distribution functions of input parameters, we choose uniform distributions for parameters with pulse control (i.e., θ_1, θ_2) due to the lack of further information; while we choose normal distributions for parameters without pulse control (i.e., except for θ_1, θ_2). The mean values and possible ranges of parameters are given in Tables 1 and 2.

Note that the threshold value R_0 determines the stability of the WEPS and the existence of forward or backward bifurcation, which greatly impacts the success of population replacement. Define average net reproductive rate of infected females within one pulse period FI_m as follows

$$FI_m := \frac{1}{T} \int_{nT}^{(n+1)T} b_2 F_I(t) - (d + D)N(t)F_I(t), \text{ for system (5),} \quad (39)$$

where n is large enough to make sure the stability of the WEPS and FI_m keeping as a constant. FI_m affects the success and speed of population replacement. Therefore, we mainly study the contribution of each parameter of **system (5)** on the two outcome variables R_0 and FI_m . Note that the explicit values of R_0 and FI_m cannot be calculated in theory, we can numerically obtain them.

Fig 2(A) and Table 3 show the influence of each parameter of system (5) on the outcome variables R_0 . The first four parameters with most impact on R_0 are fitness cost D , the natural birth rate of *Wolbachia* uninfected mosquitoes b_1 , pulse period T and the strength of CI q . The ratio of natural birth rate of *Wolbachia* infected mosquitoes to uninfected ones α has a moderate impact on R_0 , while other parameters have slightly impact. Especially, decreasing D or increasing $b_1, \alpha, d, q, T, \theta_1, \theta_2$ can lead to the decrease of R_0 , which is benefit to fulfill complete population replacement. Similarly, the contribution of each input parameter on the outcome variable FI_m is shown in Fig 2(B) and Table 3. FI_m is most sensitive to parameters D and θ_1 , while others slightly contribute to it.

In order to analysis the sample results of the two outcome variables R_0 and FI_m , we show the frequency and cumulative distributions of them with 3000 samples of LHS (see Fig 3). From Fig 3(A) and (B), the means for the two outcome variables are $MR_0 = 0.1361$, $MFI_m = -0.0104$ with standard deviations $SR_0 = 2.7462 \times 10^{-5}$ and $SFI_m = 1.9049 \times 10^{-4}$, respectively. The coefficient of variation (CV) is calculated from the ratio of the standard deviation of a variable to its mean, which is applied to estimate the degree of concentration of the variable. We consider the frequency distribution for a variable to be dispersed if CV is greater than 10%, or to be concentrated otherwise. So Fig 3(A) and (C) indicate that the derived frequency of R_0 is quite concentrated ($CVR_0 = 0.02\% < 10\%$), with the minimum and maximum estimates being 0.0158 and 0.3175, respectively. Similarly, the frequency distribution for FI_m ranges from -6.6834 to 0.0132 and is concentrated ($|CVFI_m| = 1.83\% < 10\%$) as shown in Fig 3(B) and (D). The probability of FI_m less than zero is larger than 90%, which indicates that for system (5) without pulse control (i.e., system (1)) in the given parameter regions, **it is hard to realize the strategy of population replacement. Based on the uncertainty technique**, the degree of prediction imprecision can be quantified by comparing the expected results of the two output variables with the observed results.

5.2 Backward bifurcation

Based on Theorem 3, when $R_0 = 1$ holds true for system (5) with perfect transmission rate, there exists a backward (or forward) bifurcation if θ_1 passes through the critical value θ_0 such that $BC < 0$ (or $BC > 0$). When $R_0 > 1$ holds true for system (5), there exists a forward bifurcation, and the solutions of system (5) may tend to an interior period solution (low level of population replacement) from any initial values. In order to study the region and the threshold value of backward bifurcation of system (5), it follows from Fig 4, we compare the change of the upper and lower bounds of the period solutions of system (5) to the change of the equilibria of system (1), with respect to $R_0(\alpha)$ and $R_0^*(\alpha)$, respectively, (here $R_0^*(\alpha) \triangleq (\alpha d \tau - D)/d$ denotes the threshold condition of the local stability of the boundary equilibrium of system (1), we omit the calculation). From Fig 4, when backward bifurcation of system (1)

occurs (i.e., $R_0^{*c} < R_0^*(\alpha) < 1$), the solutions of the system from different initial values have different stable states, which means that some solutions can stabilize at the *Wolbachia* mosquito eradicated equilibrium (i.e., the failure of population replacement), while others will stabilize at the *Wolbachia* mosquito established equilibrium (i.e., the success of population replacement). When backward bifurcation of system (5) occurs (i.e., $R_0^c < R_0(\alpha) < 1$), its solutions may tend to the WEPS (high level of population replacement) or interior period solution (low level of population replacement) depending on different initial values as shown in Fig 5. Compared with system (1), mosquito augmentation in system (5) not only reduces the quantity of *Wolbachia* uninfected mosquitoes, which is necessary for the control of dengue transmission; but also increases the threshold values of backward bifurcations from -0.05 to 0.75 and shrinks the region of backward bifurcation, which indicates the possibility of backward bifurcation is reduced by pulse mosquito augmentation to some extent. Note that the existence of backward bifurcation is not desired for the success of population replacement. Therefore, mosquito augmentation contributes to the effective control of dengue transmission.

When backward bifurcation occurs for system (5), we first can increase parameter α large enough such that R_0 less than a threshold value ($R_0^c \approx 0.75$ as shown in Fig 4), then the WEPS of system (5) is globally stable. Next we investigate the effects of initial densities of infected females and males with the same initial density of females, males or the same sex ratio on the success of population replacement, respectively, as shown in Fig 6(A)-(C). If initial densities $F_I(0)$, $M_I(0)$ or $M_I(0)/F_I(0)$ are too low, the high level of population replacement cannot be achieved at all (see green and black curves). However, if initial densities increase enough, the solutions of system (5) will stabilize at the WEPS, which indicates that population replacement is achieved completely. Moreover, the more initial densities of the three indexes increase, the easier it is to realize complete population replacement (i.e. it is faster to stabilize at the WEPS), as shown the magenta, blue to red solution curves in sequence in Fig 6.

Further note that nearly all the estimates of R_0 are less than one as shown in Fig 3, which indicates that when the samples of parameter values lie in the given parameter regions (see Tables 1 and 2), there is a great chance for the success of high level of population replacement when there are enough mosquitoes augmented in a long enough time. However, in reality, it is impractical to implement pulse augmentation at infinite time intervals. So based on Fig 4, if the initial densities of *Wolbachia* uninfected mosquitoes are fixed, then we can obtain the basin of attraction of the two stable states with respect to the initial densities of infected females and males as shown in Fig 7. Therefore, mosquito augmentation is considered to alter their dynamic behavior such that the densities of *Wolbachia* infected female and male mosquitoes lie in the desired zone (i.e., the white areas in Fig 7) after finite pulse control. If so, the control of dengue diseases can be realized.

5.3 Effectiveness of mosquito augmentation with different strategies

To assess the effectiveness of mosquito augmentation on the success of population replacement for the control of dengue transmission, assuming that initial infected mosquitoes are zero because of dengue mosquitoes not naturally carrying *Wolbachia* in practice, and the number of pulse times are 14 or 20 with four pulses in one week (i.e., 3.5 or 5 months) based on some field trials (Hoffmann et al., 2011), we numerically display the effects of different pulse periods and the quantities of mosquito augmentation with different sex on the prevalence of mosquito populations in Figs 8-12. In generally, the curves with circles represent the corresponding solutions with the number of pulses increasing from 14 to 20. For comparison, the black dotted lines denote the solutions of system (5) without pulse control.

From Fig 8, although the pulse period is too large to realize the strategy of high level of population replacement after 14 pulses (see black and purple curves), with decreasing pulse period, increasing release amount or the number of pulses, high level of population replacement can be realized (see magenta curves or purple curves with circles). By continuing to decrease the pulse period, it is easier and faster to realize the high level of population replacement (see magenta, blue and red curves).

It follows from Fig 9 that uninfected female mosquitoes can die out when only *Wolbachia* infected males are released. Because only female mosquitoes contribute to the spread of dengue virus, it can be regarded as a special type of mosquito eradication compared with all classes of mosquitoes dying out. If the quantity of *Wolbachia* infected males released is too low, then the strategy of eradication will fail after 14 pulses. However, it can be realized by increasing either the number of pulses (20 pulses) or the quantity of infected males such that the densities of infected female mosquitoes tend to zero. When only infected female mosquitoes are released, Fig 10 shows the solutions of system (5) with different quantities of *Wolbachia* infected females released. If the quantity of infected females released is too low, then the strategy of high level of population replacement will fail after 14 pulses (see black and magenta curves). With increasing the number of pulses (20 pulses), it can be realized (magenta curves with circles). By continuing to increase the quantity of infected females released, it is easier and faster to realize the high level of population replacement (see blue and red curves). For Fig 11, when both infected females and males are released, with increasing the quantities of infected mosquitoes released θ_1 (or θ_2), there are similar results as in Fig 10. Especially, comparing Fig 9 with Fig 11, although there are low quantity of *Wolbachia* infected females released, it can lead to the strategies of dengue control changing from population eradication to population replacement. Assuming total mosquito augmentation fixed as a constant 2.2, Fig 12 shows the effect of different sex ratios of mosquito releases on the strategies of dengue control. Note that the ratio of *Wolbachia* infected females released is too small to realize the strategy of high level of population replacement after 14 pulses (see black and purple curves). However, with increasing the number of pulses to 20, it can be re-

alized (purple curve with circles). If we continue to increase the ratio of the females released, it is easier and faster to realize the high level of population replacement (see magenta, blue and red curves).

6 Discussion

This paper develops a four dimensional impulsive system and explores how mosquito augmentation with different sex affects the success of population suppression or replacement for the control of dengue transmission. At first, we study the stability and permanence of periodic solutions of the system with perfect transmission rate. Considering the effects of forward and backward bifurcations on the success of population replacement, we obtain the conditions for the existence of the two bifurcations for system (5) by employing the bifurcation theory of Lakmeche and Arino. Moreover we compare the bifurcation diagrams of system (5) with and without pulse mosquito augmentation, as shown in Fig 4. Further uncertainty and sensitivity analysis is performed using a LHS method to show the influence of each parameter of system (5) on the outcome variables R_0 and FI_m . In addition, we study the effects of initial density, pulse period and the quantity of mosquito augmentation with different sex on the success of the two strategies by numerical simulations.

Note that *Aedes* mosquitoes can not naturally carry *Wolbachia*, so before the tactics of mosquito augmentation, the initial density of *Wolbachia* infected mosquitoes are assumed to be zero. Based on Theorem 3, when $BC > 0$ holds true for the regions of parameter values, there exists a forward bifurcation for system (5). So the high level of population replacement can be achieved for any initial values if $R_0 < 1$. When $BC < 0$ holds true for the regions of parameter values, there exists a backward bifurcation for system (5). If the threshold R_0 is less than the critical value R_0^c , then the high level of population replacement can be realized for any initial values. If the threshold R_0 lies in the region of backward bifurcation (i.e., $R_0^c < R_0 < 1$), then high or low level of population replacement (the WEPS or interior period solution) for system (5) can be realized depending on initial values. On the one hand, we can decrease the fitness cost D or increase other parameters such that R_0 is less than the critical value R_0^c , then the backward bifurcation may vanish and the high level of population replacement can be realized for any initial values (Fig 4). On the other hand, nearly all the estimates of R_0 are less than one in the given parameter regions, as shown in Fig 3, so the high level of population replacement can always be achieved when enough quantity of *Wolbachia* infected mosquitoes are augmented in a long enough time. However, in practice, it is impossible to implement pulse mosquito augmentation in the open field trials at infinite time intervals. For example, the strategy of mosquito augmentation is usually carried out in finite time intervals with finite number of pulse times, such as 14 or 20 pulses during 3.5 or 5 months in some open

field trials (Hoffmann et al. , 2011; The Guardian , 2015). Therefore, when the initial densities of *Wolbachia* uninfected mosquitoes are fixed, it is necessary to increase enough the quantities of *Wolbachia* infected females or males, the proportions of *Wolbachia* infected females or the number of pulse releases in finite time intervals such that the densities of infected females and males can lie in the basin of attraction of *Wolbachia* mosquito established equilibrium (i.e., the white areas in Fig 7) after a finite number of mosquito augmentations (Figs 10-12)). If so, we can realize a complete or high level of population replacement depending on whether or not the transmission rate is perfect. If $R_0 > 1$, we just realize a low level of population replacement and fail to realize a high level for any initial values. So if the release of infected females θ_1 or the ratio parameter α is increased such that $R_0 < 1$ (Fig 4), then the high level of population replacement may be realized for some initial values.

Moreover, when only *Wolbachia* infected males are released with a large enough quantity, then the density of uninfected females tends to zero (see Fig 9), which indicates the success of a special type of population suppression. However, although there are a little quantity of infected females released, it can dramatically change the strategy from population eradication to a high level of population replacement (see Figs 9 and 11), which indicates infected females act as seeds for the spread of *Wolbachia* strains, while infected males can reduce the density of uninfected females so that it is easier to realize a high level of population replacement.

Our model investigate the effects of different sex of mosquito augmentation on control strategies of dengue fever. In practice, the life cycle of mosquitoes undergoes four different stages, such as egg, larva, pupa and adult. Different age stages possess different fertility and mortality rates, in particular the density-dependent competition in the larva stage. Moreover dengue virus is spread between human and mosquito populations by mosquito bites. Therefore, in the future work, it is reasonable to develop an epidemic model with pulse control, including human, mosquito populations with stage structure and *Wolbachia* bacteria. In addition, considering that mosquitoes have a proliferate in huge numbers under suitable temperature and humidity conditions in tropical and subtropical regions, alone pulsing mosquito augmentation cannot fight against the spread of dengue diseases. So it is necessary to study how to carry out an integrated pest management approach, i.e., mosquito augmentation together with pulsing insecticide treatments to block the transmission of dengue virus.

Acknowledgements Tang is partially supported by the National Natural Science Foundation of China (NSFC, 11171199,11471201) and by the Fundamental Research Funds for the Central Universities (GK201305010,GK201401004). Zhu is partially supported by NSERC

and CIHR of Canada. **Zhang** is partially supported by Excellent Doctoral Dissertation of Shaanxi Normal University (S2014YB01).

Appendix 1. The expression of each element of $D_X N(\bar{\theta}_1, \bar{X})$

First from (25) and derivative of a multivariate compound function, we have the expression of each element of $D_X N(\bar{\theta}_1, \bar{X})$ as follows

$$\begin{cases} a_{ij} = - \sum_{k=1}^4 \frac{\partial I_i(\theta_0 + \bar{\theta}_1, X_0 + \bar{X})}{\partial X_k} \frac{\Phi_k(X_0 + \bar{X})}{\partial X_j}, i \neq j, \\ a_{ii} = 1 - \sum_{k=1}^4 \frac{\partial I_i(\theta_0 + \bar{\theta}_1, X_0 + \bar{X})}{\partial X_k} \frac{\Phi_k(X_0 + \bar{X})}{\partial X_i}, i = j. \end{cases}$$

According to (26), we have the differential equations of derivations of $\Phi = (\Phi_1, \Phi_2, \Phi_3, \Phi_4)$ for $(\bar{\theta}_1, \bar{X}) = (0, 0)$ with respect to $X = (X_1, X_2, X_3, X_4)$. Note that

$$\begin{cases} \frac{d}{dt} \frac{\partial \Phi_3(t, X_0)}{\partial X_1} = (b_1(1-q) - d(\tilde{F}_I + \tilde{M}_I)) \frac{\partial \Phi_3(t, X_0)}{\partial X_1}, \frac{\partial \Phi_3}{\partial X_1}(0, X_0) = 0, \\ \frac{d}{dt} \frac{\partial \Phi_4(t, X_0)}{\partial X_1} = b_1(1-q) \frac{\partial \Phi_3(t, X_0)}{\partial X_1} - d(\tilde{F}_I + \tilde{M}_I) \frac{\partial \Phi_4(t, X_0)}{\partial X_1}, \frac{\partial \Phi_4}{\partial X_1}(0, X_0) = 0, \\ \frac{d}{dt} \frac{\partial \Phi_3(t, X_0)}{\partial X_2} = (b_1(1-q) - d(\tilde{F}_I + \tilde{M}_I)) \frac{\partial \Phi_3(t, X_0)}{\partial X_2}, \frac{\partial \Phi_3}{\partial X_2}(0, X_0) = 0, \\ \frac{d}{dt} \frac{\partial \Phi_4(t, X_0)}{\partial X_2} = b_1(1-q) \frac{\partial \Phi_3(t, X_0)}{\partial X_2} - d(\tilde{F}_I + \tilde{M}_I) \frac{\partial \Phi_4(t, X_0)}{\partial X_2}, \frac{\partial \Phi_4}{\partial X_2}(0, X_0) = 0. \end{cases}$$

So we obtain

$$\frac{\partial \Phi_i}{\partial X_j}(t, X_0) \equiv 0, i = 3, 4, j = 1, 2, \quad (40)$$

for $0 \leq t < T$. Then we have

$$\begin{cases} \frac{d}{dt} \frac{\partial \Phi_1(t, X_0)}{\partial X_1} = (b_2 - (d+D)(2\tilde{F}_I + \tilde{M}_I)) \frac{\partial \Phi_1(t, X_0)}{\partial X_1} - (d+D)\tilde{F}_I \frac{\partial \Phi_2(t, X_0)}{\partial X_1}, \frac{\partial \Phi_1}{\partial X_1}(0, X_0) = 1, \\ \frac{d}{dt} \frac{\partial \Phi_1(t, X_0)}{\partial X_2} = (b_2 - (d+D)(2\tilde{F}_I + \tilde{M}_I)) \frac{\partial \Phi_1(t, X_0)}{\partial X_2} - (d+D)\tilde{F}_I \frac{\partial \Phi_2(t, X_0)}{\partial X_2}, \frac{\partial \Phi_1}{\partial X_2}(0, X_0) = 0, \\ \frac{d}{dt} \frac{\partial \Phi_2(t, X_0)}{\partial X_1} = (b_2 - (d+D)\tilde{M}_I) \frac{\partial \Phi_1(t, X_0)}{\partial X_1} - (d+D)(\tilde{F}_I + 2\tilde{M}_I) \frac{\partial \Phi_2(t, X_0)}{\partial X_1}, \frac{\partial \Phi_2}{\partial X_1}(0, X_0) = 0, \\ \frac{d}{dt} \frac{\partial \Phi_2(t, X_0)}{\partial X_2} = (b_2 - (d+D)\tilde{M}_I) \frac{\partial \Phi_1(t, X_0)}{\partial X_2} - (d+D)(\tilde{F}_I + 2\tilde{M}_I) \frac{\partial \Phi_2(t, X_0)}{\partial X_2}, \frac{\partial \Phi_2}{\partial X_2}(0, X_0) = 1. \end{cases}$$

So the solution $\frac{\partial \Phi_i}{\partial X_j}(t, X_0)$ ($i, j = 1, 2$) of the above system for $0 \leq t < T$ can be solved which is not identically equal to zero. Further, we have

$$\begin{cases} \frac{d}{dt} \frac{\partial \Phi_1(t, X_0)}{\partial X_3} = (b_2 - (d+D)(2\tilde{F}_I + \tilde{M}_I)) \frac{\partial \Phi_1(t, X_0)}{\partial X_3} - (d+D)\tilde{F}_I \frac{\partial \Phi_2(t, X_0)}{\partial X_3} \\ \quad - (d+D)\tilde{F}_I \frac{\partial \Phi_3(t, X_0)}{\partial X_3} - (d+D)\tilde{F}_I \frac{\partial \Phi_4(t, X_0)}{\partial X_3}, \frac{\partial \Phi_1}{\partial X_3}(0, X_0) = 0, \\ \frac{d}{dt} \frac{\partial \Phi_1(t, X_0)}{\partial X_4} = (b_2 - (d+D)(2\tilde{F}_I + \tilde{M}_I)) \frac{\partial \Phi_1(t, X_0)}{\partial X_4} - (d+D)\tilde{F}_I \frac{\partial \Phi_2(t, X_0)}{\partial X_4} \\ \quad - (d+D)\tilde{F}_I \frac{\partial \Phi_3(t, X_0)}{\partial X_4} - (d+D)\tilde{F}_I \frac{\partial \Phi_4(t, X_0)}{\partial X_4}, \frac{\partial \Phi_1}{\partial X_4}(0, X_0) = 0, \\ \frac{d}{dt} \frac{\partial \Phi_2(t, X_0)}{\partial X_3} = (b_2 - (d+D)\tilde{M}_I) \frac{\partial \Phi_1(t, X_0)}{\partial X_3} - (d+D)(\tilde{F}_I + 2\tilde{M}_I) \frac{\partial \Phi_2(t, X_0)}{\partial X_3} \\ \quad - (d+D)\tilde{M}_I \frac{\partial \Phi_3(t, X_0)}{\partial X_3} - (d+D)\tilde{M}_I \frac{\partial \Phi_4(t, X_0)}{\partial X_3}, \frac{\partial \Phi_2}{\partial X_3}(0, X_0) = 0, \\ \frac{d}{dt} \frac{\partial \Phi_2(t, X_0)}{\partial X_4} = (b_2 - (d+D)\tilde{M}_I) \frac{\partial \Phi_1(t, X_0)}{\partial X_4} - (d+D)(\tilde{F}_I + 2\tilde{M}_I) \frac{\partial \Phi_2(t, X_0)}{\partial X_4} \\ \quad - (d+D)\tilde{M}_I \frac{\partial \Phi_3(t, X_0)}{\partial X_4} - (d+D)\tilde{M}_I \frac{\partial \Phi_4(t, X_0)}{\partial X_4}, \frac{\partial \Phi_2}{\partial X_4}(0, X_0) = 0, \\ \frac{d}{dt} \frac{\partial \Phi_3(t, X_0)}{\partial X_3} = (b_1(1-q) - d(\tilde{F}_I + \tilde{M}_I)) \frac{\partial \Phi_3(t, X_0)}{\partial X_3}, \frac{\partial \Phi_3}{\partial X_3}(0, X_0) = 1, \\ \frac{d}{dt} \frac{\partial \Phi_3(t, X_0)}{\partial X_4} = (b_1(1-q) - d(\tilde{F}_I + \tilde{M}_I)) \frac{\partial \Phi_3(t, X_0)}{\partial X_4}, \frac{\partial \Phi_3}{\partial X_4}(0, X_0) = 0, \\ \frac{d}{dt} \frac{\partial \Phi_4(t, X_0)}{\partial X_3} = b_1(1-q) \frac{\partial \Phi_3(t, X_0)}{\partial X_3} - d(\tilde{F}_I + \tilde{M}_I) \frac{\partial \Phi_4(t, X_0)}{\partial X_3}, \frac{\partial \Phi_4}{\partial X_3}(0, X_0) = 0, \\ \frac{d}{dt} \frac{\partial \Phi_4(t, X_0)}{\partial X_4} = b_1(1-q) \frac{\partial \Phi_3(t, X_0)}{\partial X_4} - d(\tilde{F}_I + \tilde{M}_I) \frac{\partial \Phi_4(t, X_0)}{\partial X_4}, \frac{\partial \Phi_4}{\partial X_4}(0, X_0) = 1. \end{cases}$$

We can solve the above equations and give the expression of solutions for the last four equations as follows

$$\begin{aligned} \frac{\partial \Phi_3}{\partial X_3}(t, X_0) &= e^{\int_0^t (b_1(1-q) - d(\tilde{F}_I + \tilde{M}_I)) d\eta} \triangleq \beta(t), \quad \frac{\partial \Phi_3}{\partial X_4}(t, X_0) \equiv 0, \\ \frac{\partial \Phi_4}{\partial X_3}(t, X_0) &= \int_0^t (\beta(s) e^{(b_1(1-p)s - d(\tilde{F}_I + \tilde{M}_I)) ds}) d\eta, \quad \frac{\partial \Phi_4}{\partial X_4}(t, X_0) = e^{\int_0^t (-d(\tilde{F}_I + \tilde{M}_I)) d\eta}. \end{aligned} \quad (41)$$

Thus the expression of each element of $D_X N(0, O)$ is as follows

$$\begin{aligned}
a'_{11} &= 1 - \frac{\partial \Phi_1}{\partial X_1}(T, X_0), & a'_{21} &= -\frac{\partial \Phi_2}{\partial X_1}(T, X_0), \\
a'_{12} &= -\frac{\partial \Phi_1}{\partial X_2}(T, X_0), & a'_{22} &= 1 - \frac{\partial \Phi_2}{\partial X_2}(T, X_0), \\
a'_{13} &= -\frac{\partial \Phi_1}{\partial X_3}(T, X_0), & a'_{23} &= -\frac{\partial \Phi_2}{\partial X_3}(T, X_0), \\
a'_{14} &= -\frac{\partial \Phi_1}{\partial X_4}(T, X_0), & a'_{24} &= -\frac{\partial \Phi_2}{\partial X_4}(T, X_0), \\
a'_{31} &= -\frac{\partial \Phi_3}{\partial X_1}(T, X_0) = 0, & a'_{41} &= -\frac{\partial \Phi_4}{\partial X_1}(T, X_0) = 0, \\
a'_{32} &= -\frac{\partial \Phi_3}{\partial X_2}(T, X_0) = 0, & a'_{42} &= -\frac{\partial \Phi_4}{\partial X_2}(T, X_0) = 0, \\
a'_{33} &= 1 - \frac{\partial \Phi_3}{\partial X_3}(T, X_0), & a'_{43} &= -\frac{\partial \Phi_4}{\partial X_3}(T, X_0), \\
a'_{34} &= -\frac{\partial \Phi_3}{\partial X_4}(T, X_0) = 0, & a'_{44} &= 1 - \frac{\partial \Phi_4}{\partial X_4}(T, X_0).
\end{aligned} \tag{42}$$

Appendix 2. The second order partial derivations of $\Phi_i, i = 3, 4$

From (26), we have

$$\begin{aligned}
\frac{d}{dt} \frac{\partial \Phi_i(t, X_0)}{\partial X_j} &= \frac{\partial F_i(\tilde{X}(t))}{\partial X_1} \frac{\partial \Phi_1(t, X_0)}{\partial X_j} + \frac{\partial F_i(\tilde{X}(t))}{\partial X_2} \frac{\partial \Phi_2(t, X_0)}{\partial X_j} \\
&\quad + \frac{\partial F_i(\tilde{X}(t))}{\partial X_3} \frac{\partial \Phi_3(t, X_0)}{\partial X_j} + \frac{\partial F_i(\tilde{X}(t))}{\partial X_4} \frac{\partial \Phi_4(t, X_0)}{\partial X_j},
\end{aligned}$$

with $i, j = 1, 2, 3, 4$. Then

$$\begin{aligned}
\frac{d}{dt} \frac{\partial^2 \Phi_i(t, X_0)}{\partial X_j \partial X_k} &= \frac{\partial^2 F_i(\tilde{X}(t))}{\partial X_1 \partial X_k} \frac{\partial \Phi_1(t, X_0)}{\partial X_j} + \frac{\partial F_i(\tilde{X}(t))}{\partial X_1} \frac{\partial^2 \Phi_1(t, X_0)}{\partial X_j \partial X_k} \\
&\quad + \frac{\partial^2 F_i(\tilde{X}(t))}{\partial X_2 \partial X_k} \frac{\partial \Phi_2(t, X_0)}{\partial X_j} + \frac{\partial F_i(\tilde{X}(t))}{\partial X_2} \frac{\partial^2 \Phi_2(t, X_0)}{\partial X_j \partial X_k} \\
&\quad + \frac{\partial^2 F_i(\tilde{X}(t))}{\partial X_3 \partial X_k} \frac{\partial \Phi_3(t, X_0)}{\partial X_j} + \frac{\partial F_i(\tilde{X}(t))}{\partial X_3} \frac{\partial^2 \Phi_3(t, X_0)}{\partial X_j \partial X_k} \\
&\quad + \frac{\partial^2 F_i(\tilde{X}(t))}{\partial X_4 \partial X_k} \frac{\partial \Phi_4(t, X_0)}{\partial X_j} + \frac{\partial F_i(\tilde{X}(t))}{\partial X_4} \frac{\partial^2 \Phi_4(t, X_0)}{\partial X_j \partial X_k},
\end{aligned}$$

with $i, j, k = 1, 2, 3, 4$. By simple calculation, it follows that

$$\begin{aligned}
\frac{\partial F_i(\tilde{X}(t))}{\partial X_j} &= \frac{\partial \Phi_i(t, X_0)}{\partial X_j} = 0, \quad i = 3, 4, j = 1, 2, \\
\frac{\partial F_i^2(\tilde{X}(t))}{\partial X_1^2} &= \frac{\partial F_i^2(\tilde{X}(t))}{\partial X_1 \partial X_2} = \frac{\partial F_i^2(\tilde{X}(t))}{\partial X_j \partial X_k} = 0, \quad i = 3, 4, j, k = 1, 2, \\
\frac{\partial F_3(\tilde{X}(t))}{\partial X_4} &= 0.
\end{aligned}$$

Thus

$$\begin{aligned}
\frac{d}{dt} \frac{\partial^2 \Phi_3(t, X_0)}{\partial X_1^2} &= \frac{\partial F_3(\tilde{X}(t))}{\partial X_3} \frac{\partial^2 \Phi_3(t, X_0)}{\partial X_1^2}, \\
\frac{d}{dt} \frac{\partial^2 \Phi_4(t, X_0)}{\partial X_1^2} &= \frac{\partial F_4(\tilde{X}(t))}{\partial X_3} \frac{\partial^2 \Phi_3(t, X_0)}{\partial X_1^2} + \frac{\partial F_4(\tilde{X}(t))}{\partial X_4} \frac{\partial^2 \Phi_4(t, X_0)}{\partial X_1^2}, \\
\frac{d}{dt} \frac{\partial^2 \Phi_3(t, X_0)}{\partial X_2^2} &= \frac{\partial F_3(\tilde{X}(t))}{\partial X_3} \frac{\partial^2 \Phi_3(t, X_0)}{\partial X_2^2}, \\
\frac{d}{dt} \frac{\partial^2 \Phi_4(t, X_0)}{\partial X_2^2} &= \frac{\partial F_4(\tilde{X}(t))}{\partial X_3} \frac{\partial^2 \Phi_3(t, X_0)}{\partial X_2^2} + \frac{\partial F_4(\tilde{X}(t))}{\partial X_4} \frac{\partial^2 \Phi_4(t, X_0)}{\partial X_2^2},
\end{aligned} \tag{43}$$

with the initial conditions

$$\frac{\partial^2 \Phi_3(0, X_0)}{\partial X_1^2} = \frac{\partial^2 \Phi_4(0, X_0)}{\partial X_1^2} = \frac{\partial^2 \Phi_3(0, X_0)}{\partial X_2^2} = \frac{\partial^2 \Phi_4(0, X_0)}{\partial X_2^2} = 0.$$

Solving (43) deduces that

$$\frac{\partial^2 \Phi_3(t, X_0)}{\partial X_1^2} = \frac{\partial^2 \Phi_4(t, X_0)}{\partial X_1^2} = \frac{\partial^2 \Phi_3(t, X_0)}{\partial X_2^2} = \frac{\partial^2 \Phi_4(t, X_0)}{\partial X_2^2} = 0. \tag{44}$$

Similarly, we can obtain that

$$\frac{\partial^2 \Phi_3(t, X_0)}{\partial X_1 \partial X_2} = \frac{\partial^2 \Phi_3(t, X_0)}{\partial X_2 \partial X_1} = \frac{\partial^2 \Phi_4(t, X_0)}{\partial X_1 \partial X_2} = \frac{\partial^2 \Phi_4(t, X_0)}{\partial X_2 \partial X_1} = 0. \tag{45}$$

Appendix 3. The partial derivation of $\tilde{\alpha}_i, i = 2, 3, 4$ at $(0, 0)$

First, we calculate the values of $\partial\tilde{\alpha}_i(0, 0)/\partial\alpha_1$ and $\partial\tilde{\alpha}_i(0, 0)/\partial\bar{\theta}_1, i = 2, 3, 4$. From (33), it follows

$$\begin{aligned} 0 &= \left. \frac{\partial N_j}{\partial \alpha_1} \right|_{(0,0)} = \left. \frac{\partial N_j}{\partial X_1} \frac{\partial X_1}{\partial \alpha_1} + \frac{\partial N_j}{\partial X_2} \frac{\partial X_2}{\partial \alpha_1} + \frac{\partial N_j}{\partial X_3} \frac{\partial X_3}{\partial \alpha_1} + \frac{\partial N_j}{\partial X_4} \frac{\partial X_4}{\partial \alpha_1} \right|_{(0,0)} \\ &= \left. \frac{\partial N_j}{\partial X_1} (Y_{11} + \frac{\partial \tilde{\alpha}_2}{\partial \alpha_1}) + \frac{\partial N_j}{\partial X_2} (Y_{12} + \frac{\partial \tilde{\alpha}_3}{\partial \alpha_1}) + \frac{\partial N_j}{\partial X_3} (Y_{13} + \frac{\partial \tilde{\alpha}_4}{\partial \alpha_1}) + \frac{\partial N_j}{\partial X_4} Y_{14} \right|_{(0,0)}. \end{aligned} \quad (46)$$

Since Y_1 is a basis of $\ker(D_X N(0, 0))$, then

$$\left. \frac{\partial N_i}{\partial X_1} Y_{11} + \frac{\partial N_i}{\partial X_1} Y_{12} + \frac{\partial N_i}{\partial X_1} Y_{13} + \frac{\partial N_i}{\partial X_1} Y_{14} \right|_{(0,0)} = 0, i = 1, 2, 3, 4. \quad (47)$$

Hence from (46) and (47), we deduce that

$$\begin{aligned} a'_{11} \frac{\partial \tilde{\alpha}_2}{\partial \alpha_1} + a'_{12} \frac{\partial \tilde{\alpha}_3}{\partial \alpha_1} + a'_{13} \frac{\partial \tilde{\alpha}_4}{\partial \alpha_1} \Big|_{(0,0)} &= 0, \\ a'_{21} \frac{\partial \tilde{\alpha}_2}{\partial \alpha_1} + a'_{22} \frac{\partial \tilde{\alpha}_3}{\partial \alpha_1} + a'_{23} \frac{\partial \tilde{\alpha}_4}{\partial \alpha_1} \Big|_{(0,0)} &= 0, \\ a'_{31} \frac{\partial \tilde{\alpha}_2}{\partial \alpha_1} + a'_{32} \frac{\partial \tilde{\alpha}_3}{\partial \alpha_1} + a'_{33} \frac{\partial \tilde{\alpha}_4}{\partial \alpha_1} \Big|_{(0,0)} &= 0. \end{aligned} \quad (48)$$

Solving (48) with respect to $\partial\tilde{\alpha}_i/\partial\alpha_1, i = 2, 3, 4$ obtains

$$\frac{\partial \tilde{\alpha}_2(0, 0)}{\partial \alpha_1} = \frac{\partial \tilde{\alpha}_3(0, 0)}{\partial \alpha_1} = \frac{\partial \tilde{\alpha}_4(0, 0)}{\partial \alpha_1} = 0. \quad (49)$$

Again considering (33), we have

$$\begin{cases} N_i(\bar{\theta}_1, \alpha_1) = X_{0i} + Y_{1i}\alpha_1 + \tilde{\alpha}_{i+1}(\bar{\theta}_1, \alpha_1) - (\Phi_i(\theta_0 + \bar{\theta}_1, X_0 + \bar{X}(\bar{\theta}_1, \alpha_1)) + \theta_0 + \bar{\theta}_1), \\ N_j(\bar{\theta}_1, \alpha_1) = X_{0j} + Y_{1j}\alpha_1 + \tilde{\alpha}_{j+1}(\bar{\theta}_1, \alpha_1) - (\Phi_j(\theta_0 + \bar{\theta}_1, X_0 + \bar{X}(\bar{\theta}_1, \alpha_1)) + \theta_2), \\ N_1(\bar{\theta}_1, \alpha_1) = N_2(\bar{\theta}_1, \alpha_1) = N_3(\bar{\theta}_1, \alpha_1) = 0, \end{cases} \quad (50)$$

with $i = 1, 3, j = 2, 4, \tilde{\alpha}_5 = 0, X_0 = (X_{01}, X_{02}, X_{03}, X_{04})$ and $\bar{X} = (\bar{X}_1, \bar{X}_2, \bar{X}_3, \bar{X}_4)$. Hence based on (42) and (50), we can deduce that

$$\begin{aligned} 0 &= \frac{\partial N_1(0, 0)}{\partial \theta_1} = \frac{\partial \tilde{\alpha}_2(0, 0)}{\partial \theta_1} - \left(1 + \sum_{i=1}^3 \left(\frac{\partial \Phi_1(\theta_0, X_0)}{\partial X_i} \frac{\partial X_i(0, 0)}{\partial \theta_1} \right)\right) \\ &= \frac{\partial \tilde{\alpha}_2(0, 0)}{\partial \theta_1} + (a'_{11} - 1) \frac{\partial \tilde{\alpha}_2(0, 0)}{\partial \theta_1} + a'_{12} \frac{\partial \tilde{\alpha}_3(0, 0)}{\partial \theta_1} + a'_{13} \frac{\partial \tilde{\alpha}_4(0, 0)}{\partial \theta_1} - 1 \\ &= a'_{11} \frac{\partial \tilde{\alpha}_2(0, 0)}{\partial \theta_1} + a'_{12} \frac{\partial \tilde{\alpha}_3(0, 0)}{\partial \theta_1} + a'_{13} \frac{\partial \tilde{\alpha}_4(0, 0)}{\partial \theta_1} - 1. \end{aligned} \quad (51)$$

Similarly, from (42) and (50), we can obtain that

$$\begin{aligned} \frac{\partial N_2(0, 0)}{\partial \theta_1} &= a'_{21} \frac{\partial \tilde{\alpha}_2(0, 0)}{\partial \theta_1} + a'_{22} \frac{\partial \tilde{\alpha}_3(0, 0)}{\partial \theta_1} + a'_{23} \frac{\partial \tilde{\alpha}_4(0, 0)}{\partial \theta_1} = 0, \\ \frac{\partial N_3(0, 0)}{\partial \theta_1} &= a'_{31} \frac{\partial \tilde{\alpha}_2(0, 0)}{\partial \theta_1} + a'_{32} \frac{\partial \tilde{\alpha}_3(0, 0)}{\partial \theta_1} + a'_{33} \frac{\partial \tilde{\alpha}_4(0, 0)}{\partial \theta_1} \\ &= a'_{33} \frac{\partial \tilde{\alpha}_4(0, 0)}{\partial \theta_1} = 0. \end{aligned} \quad (52)$$

Solving (51) and (52) with respect to $\partial\tilde{\alpha}_i(0, 0)/\partial\bar{\theta}_1, i = 2, 3, 4$ yields that

$$\begin{aligned} \frac{\partial \tilde{\alpha}_2(0, 0)}{\partial \theta_1} &= \frac{a'_{22}}{a'_{11}a'_{22} - a'_{12}a'_{21}}, \\ \frac{\partial \tilde{\alpha}_3(0, 0)}{\partial \theta_1} &= -\frac{a'_{21}}{a'_{11}a'_{22} - a'_{12}a'_{21}}, \\ \frac{\partial \tilde{\alpha}_4(0, 0)}{\partial \theta_1} &= 0. \end{aligned} \quad (53)$$

Based on the first equation of (50), we have that

$$\begin{aligned}
0 &= \frac{\partial^2 N_3(0,0)}{\partial \bar{\theta}_1^2} = \frac{\partial}{\partial \theta_1} \frac{\partial N_3(0,0)}{\partial \theta_1} \\
&= \frac{\partial^2 \tilde{\alpha}_4(0,0)}{\partial \theta_1^2} - \frac{\partial}{\partial \theta_1} \sum_{i=1}^3 \left(\frac{\partial \Phi_3(\theta_0, X_0)}{\partial X_i} \frac{\partial X_i(0,0)}{\partial \theta_1} \right) \\
&= \frac{\partial^2 \tilde{\alpha}_4(0,0)}{\partial \theta_1^2} - \left[\frac{\partial \Phi_3(\theta_0, X_0)}{\partial X_1} \frac{\partial^2 \tilde{\alpha}_2(0,0)}{\partial \theta_1^2} \right. \\
&\quad + \frac{\partial \Phi_3(\theta_0, X_0)}{\partial X_2} \frac{\partial^2 \tilde{\alpha}_3(0,0)}{\partial \theta_1^2} + \frac{\partial \Phi_3(\theta_0, X_0)}{\partial X_3} \frac{\partial^2 \tilde{\alpha}_4(0,0)}{\partial \theta_1^2} \\
&\quad + \frac{\partial \tilde{\alpha}_2(0,0)}{\partial \theta_1} \left(\frac{\partial^2 \Phi_3(\theta_0, X_0)}{\partial X_1^2} \frac{\partial \tilde{\alpha}_2(0,0)}{\partial \theta_1} + \frac{\partial^2 \Phi_3(\theta_0, X_0)}{\partial X_1 \partial X_2} \frac{\partial \tilde{\alpha}_3(0,0)}{\partial \theta_1} + \frac{\partial^2 \Phi_3(\theta_0, X_0)}{\partial X_1 \partial X_3} \frac{\partial \tilde{\alpha}_4(0,0)}{\partial \theta_1} \right) \\
&\quad + \frac{\partial \tilde{\alpha}_3(0,0)}{\partial \theta_1} \left(\frac{\partial^2 \Phi_3(\theta_0, X_0)}{\partial X_2 \partial X_1} \frac{\partial \tilde{\alpha}_2(0,0)}{\partial \theta_1} + \frac{\partial^2 \Phi_3(\theta_0, X_0)}{\partial X_2^2} \frac{\partial \tilde{\alpha}_3(0,0)}{\partial \theta_1} + \frac{\partial^2 \Phi_3(\theta_0, X_0)}{\partial X_2 \partial X_3} \frac{\partial \tilde{\alpha}_4(0,0)}{\partial \theta_1} \right) \\
&\quad \left. + \frac{\partial \tilde{\alpha}_4(0,0)}{\partial \theta_1} \left(\frac{\partial^2 \Phi_3(\theta_0, X_0)}{\partial X_3 \partial X_1} \frac{\partial \tilde{\alpha}_2(0,0)}{\partial \theta_1} + \frac{\partial^2 \Phi_3(\theta_0, X_0)}{\partial X_3 \partial X_2} \frac{\partial \tilde{\alpha}_3(0,0)}{\partial \theta_1} + \frac{\partial^2 \Phi_3(\theta_0, X_0)}{\partial X_3^2} \frac{\partial \tilde{\alpha}_4(0,0)}{\partial \theta_1} \right) \right]. \tag{54}
\end{aligned}$$

Substituting (40), (41), (44), (45) and (53) into (54) yields that

$$\frac{\partial^2 \tilde{\alpha}_4(0,0)}{\partial \bar{\theta}_1^2} = 0. \tag{55}$$

Then, we calculate the value of $\partial^2 \tilde{\alpha}_4(0,0)/\partial \bar{\theta}_1 \partial \alpha_1$. From the first equation of (50) and combination with (30), (40), (41), (44), (45), (49) and (53), we have

$$\begin{aligned}
0 &= \frac{\partial^2 N_3(0,0)}{\partial \theta_1 \partial \alpha_1} = \frac{\partial}{\partial \alpha_1} \frac{\partial N_3(0,0)}{\partial \theta_1} \\
&= \frac{\partial^2 \tilde{\alpha}_4(0,0)}{\partial \theta_1 \partial \alpha_1} - \frac{\partial}{\partial \alpha_1} \sum_{i=1}^3 \left(\frac{\partial \Phi_3(\theta_0, X_0)}{\partial X_i} \frac{\partial X_i(0,0)}{\partial \theta_1} \right) \\
&= \frac{\partial^2 \tilde{\alpha}_4(0,0)}{\partial \theta_1 \partial \alpha_1} - \left[\frac{\partial \Phi_3(\theta_0, X_0)}{\partial X_1} \frac{\partial^2 \tilde{\alpha}_2(0,0)}{\partial \theta_1 \partial \alpha_1} + \frac{\partial \Phi_3(\theta_0, X_0)}{\partial X_2} \frac{\partial^2 \tilde{\alpha}_3(0,0)}{\partial \theta_1 \partial \alpha_1} + \frac{\partial \Phi_3(\theta_0, X_0)}{\partial X_3} \frac{\partial^2 \tilde{\alpha}_4(0,0)}{\partial \theta_1 \partial \alpha_1} \right. \\
&\quad + \frac{\partial \tilde{\alpha}_2(0,0)}{\partial \theta_1} \frac{\partial}{\partial \alpha_1} \left(\frac{\partial \Phi_3(\theta_0, X_0)}{\partial X_1} \right) + \frac{\partial \tilde{\alpha}_3(0,0)}{\partial \theta_1} \frac{\partial}{\partial \alpha_1} \left(\frac{\partial \Phi_3(\theta_0, X_0)}{\partial X_2} \right) + \frac{\partial \tilde{\alpha}_4(0,0)}{\partial \theta_1} \frac{\partial}{\partial \alpha_1} \left(\frac{\partial \Phi_3(\theta_0, X_0)}{\partial X_3} \right) \left. \right] \\
&= \frac{\partial^2 \tilde{\alpha}_4(0,0)}{\partial \theta_1 \partial \alpha_1} - \left[\frac{\partial \Phi_3(\theta_0, X_0)}{\partial X_3} \frac{\partial^2 \tilde{\alpha}_4(0,0)}{\partial \theta_1 \partial \alpha_1} + \frac{\partial \tilde{\alpha}_2(0,0)}{\partial \theta_1} \frac{\partial}{\partial \alpha_1} \left(\frac{\partial \Phi_3(\theta_0, X_0)}{\partial X_1} \right) + \frac{\partial \tilde{\alpha}_3(0,0)}{\partial \theta_1} \frac{\partial}{\partial \alpha_1} \left(\frac{\partial \Phi_3(\theta_0, X_0)}{\partial X_2} \right) \right] \\
&= \frac{\partial^2 \tilde{\alpha}_4(0,0)}{\partial \theta_1 \partial \alpha_1} - \left[\frac{\partial \Phi_3(\theta_0, X_0)}{\partial X_3} \frac{\partial^2 \tilde{\alpha}_4(0,0)}{\partial \theta_1 \partial \alpha_1} \right. \\
&\quad + \frac{\partial \tilde{\alpha}_2(0,0)}{\partial \theta_1} \left(\frac{\partial^2 \Phi_3(\theta_0, X_0)}{\partial X_1^2} (Y_{11} + \frac{\partial \tilde{\alpha}_2(0,0)}{\partial \alpha_1}) + \frac{\partial^2 \Phi_3(\theta_0, X_0)}{\partial X_1 \partial X_2} (Y_{12} + \frac{\partial \tilde{\alpha}_3(0,0)}{\partial \alpha_1}) \right) \\
&\quad + \frac{\partial^2 \Phi_3(\theta_0, X_0)}{\partial X_1 \partial X_3} (Y_{13} + \frac{\partial \tilde{\alpha}_2(0,0)}{\partial \alpha_1}) + \frac{\partial^2 \Phi_3(\theta_0, X_0)}{\partial X_1 \partial X_4} Y_{14} \\
&\quad + \frac{\partial \tilde{\alpha}_3(0,0)}{\partial \theta_1} \left(\frac{\partial^2 \Phi_3(\theta_0, X_0)}{\partial X_2 \partial X_1} (Y_{11} + \frac{\partial \tilde{\alpha}_2(0,0)}{\partial \alpha_1}) + \frac{\partial^2 \Phi_3(\theta_0, X_0)}{\partial X_2^2} (Y_{12} + \frac{\partial \tilde{\alpha}_3(0,0)}{\partial \alpha_1}) \right) \\
&\quad + \left. \frac{\partial^2 \Phi_3(\theta_0, X_0)}{\partial X_2 \partial X_3} (Y_{13} + \frac{\partial \tilde{\alpha}_4(0,0)}{\partial \alpha_1}) + \frac{\partial^2 \Phi_3(\theta_0, X_0)}{\partial X_2 \partial X_4} Y_{14} \right] \\
&= \frac{\partial^2 \tilde{\alpha}_4(0,0)}{\partial \theta_1 \partial \alpha_1} - \left[\frac{\partial \Phi_3(\theta_0, X_0)}{\partial X_3} \frac{\partial^2 \tilde{\alpha}_4(0,0)}{\partial \theta_1 \partial \alpha_1} + \frac{\partial \tilde{\alpha}_2(0,0)}{\partial \theta_1} \frac{\partial^2 \Phi_3(\theta_0, X_0)}{\partial X_1 \partial X_4} + \frac{\partial \tilde{\alpha}_3(0,0)}{\partial \theta_1} \frac{\partial^2 \Phi_3(\theta_0, X_0)}{\partial X_2 \partial X_4} \right].
\end{aligned}$$

Thus we have

$$\frac{\partial^2 \tilde{\alpha}_4(0,0)}{\partial \bar{\theta}_1 \partial \alpha_1} = \frac{1}{a'_{33}} \left(\frac{a'_{22}}{a'_{11} a'_{22} - a'_{12} a'_{21}} \frac{\partial^2 \Phi_3(X_0)}{\partial X_1 \partial X_4} - \frac{a'_{21}}{a'_{11} a'_{22} - a'_{12} a'_{21}} \frac{\partial^2 \Phi_3(X_0)}{\partial X_2 \partial X_4} \right). \tag{56}$$

Next, we calculate the value of $\partial^2 \tilde{\alpha}_i(0,0)/\partial \alpha_1^2$, $i = 2, 3, 4$. From (50) as $i = 1$, we have

$$\begin{aligned}
0 &= \frac{\partial^2 N_1(0,0)}{\partial \alpha_1^2} = \frac{\partial}{\partial \alpha_1} \frac{\partial N_1(0,0)}{\partial \alpha_1} \\
&= \frac{\partial}{\partial \alpha_1} \left(\frac{\partial N_1(\theta_0, X_0)}{\partial X_1} (Y_{11} + \frac{\partial \tilde{\alpha}_2(0,0)}{\partial \alpha_1}) + \frac{\partial N_1(\theta_0, X_0)}{\partial X_2} (Y_{12} + \frac{\partial \tilde{\alpha}_3(0,0)}{\partial \alpha_1}) \right) \\
&+ \frac{\partial N_1(\theta_0, X_0)}{\partial X_3} (Y_{13} + \frac{\partial \tilde{\alpha}_4(0,0)}{\partial \alpha_1}) + \frac{\partial N_1(\theta_0, X_0)}{\partial X_4} Y_{14} \\
&= \frac{\partial N_1(\theta_0, X_0)}{\partial X_1} \frac{\partial^2 \tilde{\alpha}_2(0,0)}{\partial \alpha_1^2} + \frac{\partial N_1(\theta_0, X_0)}{\partial X_2} \frac{\partial^2 \tilde{\alpha}_3(0,0)}{\partial \alpha_1^2} + \frac{\partial N_1(\theta_0, X_0)}{\partial X_3} \frac{\partial^2 \tilde{\alpha}_4(0,0)}{\partial \alpha_1^2} \\
&+ (Y_{11} + \frac{\partial \tilde{\alpha}_2(0,0)}{\partial \alpha_1}) \frac{\partial}{\partial \alpha_1} \left(\frac{\partial N_1(\theta_0, X_0)}{\partial X_1} \right) + (Y_{12} + \frac{\partial \tilde{\alpha}_3(0,0)}{\partial \alpha_1}) \frac{\partial}{\partial \alpha_1} \left(\frac{\partial N_1(\theta_0, X_0)}{\partial X_2} \right) \\
&+ (Y_{13} + \frac{\partial \tilde{\alpha}_4(0,0)}{\partial \alpha_1}) \frac{\partial}{\partial \alpha_1} \left(\frac{\partial N_1(\theta_0, X_0)}{\partial X_3} \right) + Y_{14} \frac{\partial}{\partial \alpha_1} \left(\frac{\partial N_1(\theta_0, X_0)}{\partial X_4} \right) \\
&= \frac{\partial N_1(\theta_0, X_0)}{\partial X_1} \frac{\partial^2 \tilde{\alpha}_2(0,0)}{\partial \alpha_1^2} + \frac{\partial N_1(\theta_0, X_0)}{\partial X_2} \frac{\partial^2 \tilde{\alpha}_3(0,0)}{\partial \alpha_1^2} + \frac{\partial N_1(\theta_0, X_0)}{\partial X_3} \frac{\partial^2 \tilde{\alpha}_4(0,0)}{\partial \alpha_1^2} \\
&+ \frac{\partial}{\partial \alpha_1} \left(Y_{11} \frac{\partial N_1(\theta_0, X_0)}{\partial X_1} + Y_{12} \frac{\partial N_1(\theta_0, X_0)}{\partial X_2} + Y_{13} \frac{\partial N_1(\theta_0, X_0)}{\partial X_3} + Y_{14} \frac{\partial N_1(\theta_0, X_0)}{\partial X_4} \right) \\
&= \frac{\partial N_1(\theta_0, X_0)}{\partial X_1} \frac{\partial^2 \tilde{\alpha}_2(0,0)}{\partial \alpha_1^2} + \frac{\partial N_1(\theta_0, X_0)}{\partial X_2} \frac{\partial^2 \tilde{\alpha}_3(0,0)}{\partial \alpha_1^2} + \frac{\partial N_1(\theta_0, X_0)}{\partial X_3} \frac{\partial^2 \tilde{\alpha}_4(0,0)}{\partial \alpha_1^2} \\
&+ Y_{11} \left[\frac{\partial^2 N_1(\theta_0, X_0)}{\partial X_1^2} (Y_{11} + \frac{\partial \tilde{\alpha}_2(0,0)}{\partial \alpha_1}) + \frac{\partial^2 N_1(\theta_0, X_0)}{\partial X_1 \partial X_2} (Y_{12} + \frac{\partial \tilde{\alpha}_3(0,0)}{\partial \alpha_1}) \right. \\
&+ \frac{\partial^2 N_1(\theta_0, X_0)}{\partial X_1 \partial X_3} (Y_{13} + \frac{\partial \tilde{\alpha}_4(0,0)}{\partial \alpha_1}) + \frac{\partial^2 N_1(\theta_0, X_0)}{\partial X_1 \partial X_4} Y_{14} \left. \right] \\
&+ Y_{12} \left[\frac{\partial^2 N_1(\theta_0, X_0)}{\partial X_2 \partial X_1} (Y_{11} + \frac{\partial \tilde{\alpha}_2(0,0)}{\partial \alpha_1}) + \frac{\partial^2 N_1(\theta_0, X_0)}{\partial X_2^2} (Y_{12} + \frac{\partial \tilde{\alpha}_3(0,0)}{\partial \alpha_1}) \right. \\
&+ \frac{\partial^2 N_1(\theta_0, X_0)}{\partial X_2 \partial X_3} (Y_{13} + \frac{\partial \tilde{\alpha}_4(0,0)}{\partial \alpha_1}) + \frac{\partial^2 N_1(\theta_0, X_0)}{\partial X_2 \partial X_4} Y_{14} \left. \right] \\
&+ Y_{13} \left[\frac{\partial^2 N_1(\theta_0, X_0)}{\partial X_3 \partial X_1} (Y_{11} + \frac{\partial \tilde{\alpha}_2(0,0)}{\partial \alpha_1}) + \frac{\partial^2 N_1(\theta_0, X_0)}{\partial X_3 \partial X_2} (Y_{12} + \frac{\partial \tilde{\alpha}_3(0,0)}{\partial \alpha_1}) \right. \\
&+ \frac{\partial^2 N_1(\theta_0, X_0)}{\partial X_3^2} (Y_{13} + \frac{\partial \tilde{\alpha}_4(0,0)}{\partial \alpha_1}) + \frac{\partial^2 N_1(\theta_0, X_0)}{\partial X_3 \partial X_4} Y_{14} \left. \right] \\
&+ Y_{14} \left[\frac{\partial^2 N_1(\theta_0, X_0)}{\partial X_4 \partial X_1} (Y_{11} + \frac{\partial \tilde{\alpha}_2(0,0)}{\partial \alpha_1}) + \frac{\partial^2 N_1(\theta_0, X_0)}{\partial X_4 \partial X_2} (Y_{12} + \frac{\partial \tilde{\alpha}_3(0,0)}{\partial \alpha_1}) \right. \\
&+ \frac{\partial^2 N_1(\theta_0, X_0)}{\partial X_4 \partial X_3} (Y_{13} + \frac{\partial \tilde{\alpha}_4(0,0)}{\partial \alpha_1}) + \frac{\partial^2 N_1(\theta_0, X_0)}{\partial X_4^2} Y_{14} \left. \right] \\
&= \frac{\partial N_1(\theta_0, X_0)}{\partial X_1} \frac{\partial^2 \tilde{\alpha}_2(0,0)}{\partial \alpha_1^2} + \frac{\partial N_1(\theta_0, X_0)}{\partial X_2} \frac{\partial^2 \tilde{\alpha}_3(0,0)}{\partial \alpha_1^2} + \frac{\partial N_1(\theta_0, X_0)}{\partial X_3} \frac{\partial^2 \tilde{\alpha}_4(0,0)}{\partial \alpha_1^2} \\
&+ \sum_{i=1}^4 \sum_{j=1}^4 \frac{\partial^2 N_1(\theta_0, X_0)}{\partial X_i \partial X_j} Y_{1i} Y_{1j}.
\end{aligned} \tag{57}$$

Submitting (29) into (57) gives that

$$\begin{aligned}
&a'_{11} \frac{\partial^2 \tilde{\alpha}_2(0,0)}{\partial \alpha_1^2} + a'_{12} \frac{\partial^2 \tilde{\alpha}_3(0,0)}{\partial \alpha_1^2} + a'_{13} \frac{\partial^2 \tilde{\alpha}_4(0,0)}{\partial \alpha_1^2} \\
&= - \sum_{i=1}^4 \sum_{j=1}^4 \frac{\partial^2 N_1(\theta_0, X_0)}{\partial X_i \partial X_j} Y_{1i} Y_{1j} \\
&= \sum_{i=1}^4 \sum_{j=1}^4 \frac{\partial^2 \Phi_1(\theta_0, X_0)}{\partial X_i \partial X_j} Y_{1i} Y_{1j}.
\end{aligned} \tag{58}$$

Similarly, we can obtain from (50) as $i = 2, 3$ that

$$\begin{aligned}
a'_{21} \frac{\partial^2 \tilde{\alpha}_2(0,0)}{\partial \alpha_1^2} + a'_{22} \frac{\partial^2 \tilde{\alpha}_3(0,0)}{\partial \alpha_1^2} + a'_{23} \frac{\partial^2 \tilde{\alpha}_4(0,0)}{\partial \alpha_1^2} &= \sum_{i=1}^4 \sum_{j=1}^4 \frac{\partial^2 \Phi_2(\theta_0, X_0)}{\partial X_i \partial X_j} Y_{1i} Y_{1j}, \\
a'_{33} \frac{\partial^2 \tilde{\alpha}_4(0,0)}{\partial \alpha_1^2} &= \sum_{i=1}^4 \sum_{j=1}^4 \frac{\partial^2 \Phi_3(\theta_0, X_0)}{\partial X_i \partial X_j} Y_{1i} Y_{1j}.
\end{aligned} \tag{59}$$

Hence we can solve the roots of equations (58) and (59) with respect to $\partial^2 \tilde{\alpha}_i(0,0)/\partial \alpha_1^2$, $i = 2, 3, 4$, and submit them with $i = 4$ into (67) in Appendix 6.

Appendix 4. The first order partial derivations of $N_4(\bar{\theta}_1, \beta_1)$

Similar to (46), (51) and (52), we can calculate from (25) that

$$\begin{aligned}
\frac{\partial N_4}{\partial \alpha_1} \Big|_{(0,0)} &= \frac{\partial N_4}{\partial X_1} (Y_{11} + \frac{\partial \tilde{\alpha}_2}{\partial \alpha_1}) + \frac{\partial N_4}{\partial X_2} (Y_{12} + \frac{\partial \tilde{\alpha}_3}{\partial \alpha_1}) + \frac{\partial N_4}{\partial X_3} (Y_{13} + \frac{\partial \tilde{\alpha}_4}{\partial \alpha_1}) + \frac{\partial N_4}{\partial X_4} Y_{14} \Big|_{(0,0)}, \\
\frac{\partial N_4}{\partial \theta_1} \Big|_{(0,0)} &= - \frac{\partial \Phi_4(X_0)}{\partial X_1} \frac{\partial \tilde{\alpha}_2(0,0)}{\partial \theta_1} - \frac{\partial \Phi_4(X_0)}{\partial X_2} \frac{\partial \tilde{\alpha}_3(0,0)}{\partial \theta_1} - \frac{\partial \Phi_4(X_0)}{\partial X_3} \frac{\partial \tilde{\alpha}_4(0,0)}{\partial \theta_1} \Big|_{(0,0)}.
\end{aligned} \tag{60}$$

Substituting (47), (49) and (53) into (60) obtains

$$\frac{\partial N_4(0,0)}{\partial \alpha_1} = \frac{\partial N_4(0,0)}{\partial \bar{\theta}_1} = 0. \quad (61)$$

Appendix 5. The second order partial derivation of $N_4(\bar{\theta}_1, \alpha_1)$

(i) Calculation the value of A.

According to the second equation of (50) as $j = 4$, we can easily get that

$$\begin{aligned} \frac{\partial^2 N_4(0,0)}{\partial \bar{\theta}_1^2} &= \frac{\partial}{\partial \bar{\theta}_1} \frac{\partial N_4(0,0)}{\partial \bar{\theta}_1} = -\frac{\partial}{\partial \bar{\theta}_1} \sum_{i=1}^3 \left(\frac{\partial \Phi_4(\theta_0, X_0)}{\partial X_i(0,0)} \frac{\partial X_i(0,0)}{\partial \bar{\theta}_1} \right) \\ &= -\left[\frac{\partial \Phi_4(\theta_0, X_0)}{\partial X_1} \frac{\partial^2 \bar{\alpha}_2(0,0)}{\partial \bar{\theta}_1^2} + \frac{\partial \Phi_4(\theta_0, X_0)}{\partial X_2} \frac{\partial^2 \bar{\alpha}_3(0,0)}{\partial \bar{\theta}_1^2} + \frac{\partial \Phi_4(\theta_0, X_0)}{\partial X_3} \frac{\partial^2 \bar{\alpha}_4(0,0)}{\partial \bar{\theta}_1^2} \right. \\ &\quad + \frac{\partial \bar{\alpha}_2(0,0)}{\partial \bar{\theta}_1} \left(\frac{\partial^2 \Phi_4(\theta_0, X_0)}{\partial X_1^2} \frac{\partial \bar{\alpha}_2(0,0)}{\partial \bar{\theta}_1} + \frac{\partial^2 \Phi_4(\theta_0, X_0)}{\partial X_1 \partial X_2} \frac{\partial \bar{\alpha}_3(0,0)}{\partial \bar{\theta}_1} + \frac{\partial^2 \Phi_4(\theta_0, X_0)}{\partial X_1 \partial X_3} \frac{\partial \bar{\alpha}_4(0,0)}{\partial \bar{\theta}_1} \right) \\ &\quad + \frac{\partial \bar{\alpha}_3(0,0)}{\partial \bar{\theta}_1} \left(\frac{\partial^2 \Phi_4(\theta_0, X_0)}{\partial X_2 \partial X_1} \frac{\partial \bar{\alpha}_2(0,0)}{\partial \bar{\theta}_1} + \frac{\partial^2 \Phi_4(\theta_0, X_0)}{\partial X_2^2} \frac{\partial \bar{\alpha}_3(0,0)}{\partial \bar{\theta}_1} + \frac{\partial^2 \Phi_4(\theta_0, X_0)}{\partial X_2 \partial X_3} \frac{\partial \bar{\alpha}_4(0,0)}{\partial \bar{\theta}_1} \right) \\ &\quad \left. + \frac{\partial \bar{\alpha}_4(0,0)}{\partial \bar{\theta}_1} \left(\frac{\partial^2 \Phi_4(\theta_0, X_0)}{\partial X_3 \partial X_1} \frac{\partial \bar{\alpha}_2(0,0)}{\partial \bar{\theta}_1} + \frac{\partial^2 \Phi_4(\theta_0, X_0)}{\partial X_3 \partial X_2} \frac{\partial \bar{\alpha}_3(0,0)}{\partial \bar{\theta}_1} + \frac{\partial^2 \Phi_4(\theta_0, X_0)}{\partial X_3^2} \frac{\partial \bar{\alpha}_4(0,0)}{\partial \bar{\theta}_1} \right) \right]. \end{aligned} \quad (62)$$

Substituting (40), (41), (44), (45) (49), (53) and (55) into (62) obtains

$$A = \frac{\partial^2 N_4(0,0)}{\partial \bar{\theta}_1^2} = 0. \quad (63)$$

(ii) Calculation the value of B.

Similarly, based on the second equation of (50), it follows that

$$\begin{aligned} \frac{\partial^2 N_4(0,0)}{\partial \bar{\theta}_1 \partial \alpha_1} &= \frac{\partial}{\partial \alpha_1} \frac{\partial N_4(0,0)}{\partial \bar{\theta}_1} = -\frac{\partial}{\partial \alpha_1} \sum_{i=1}^3 \left(\frac{\partial \Phi_4(\theta_0, X_0)}{\partial X_i(0,0)} \frac{\partial X_i(0,0)}{\partial \bar{\theta}_1} \right) \\ &= -\left[\frac{\partial \Phi_4(\theta_0, X_0)}{\partial X_1} \frac{\partial^2 \bar{\alpha}_2(0,0)}{\partial \bar{\theta}_1 \partial \alpha_1} + \frac{\partial \Phi_4(\theta_0, X_0)}{\partial X_2} \frac{\partial^2 \bar{\alpha}_3(0,0)}{\partial \bar{\theta}_1 \partial \alpha_1} + \frac{\partial \Phi_4(\theta_0, X_0)}{\partial X_3} \frac{\partial^2 \bar{\alpha}_4(0,0)}{\partial \bar{\theta}_1 \partial \alpha_1} \right. \\ &\quad + \frac{\partial \bar{\alpha}_2(0,0)}{\partial \bar{\theta}_1} \left(\frac{\partial^2 \Phi_4(\theta_0, X_0)}{\partial X_1^2} (Y_{11} + \frac{\partial \bar{\alpha}_2(0,0)}{\partial \alpha_1}) + \frac{\partial^2 \Phi_4(\theta_0, X_0)}{\partial X_1 \partial X_2} (Y_{12} + \frac{\partial \bar{\alpha}_3(0,0)}{\partial \alpha_1}) \right) \\ &\quad + \frac{\partial^2 \Phi_4(\theta_0, X_0)}{\partial X_1 \partial X_3} (Y_{13} + \frac{\partial \bar{\alpha}_4(0,0)}{\partial \alpha_1}) + \frac{\partial^2 \Phi_4(\theta_0, X_0)}{\partial X_1 \partial X_4} Y_{14} \\ &\quad + \frac{\partial \bar{\alpha}_3(0,0)}{\partial \bar{\theta}_1} \left(\frac{\partial^2 \Phi_4(\theta_0, X_0)}{\partial X_2 \partial X_1} (Y_{11} + \frac{\partial \bar{\alpha}_2(0,0)}{\partial \alpha_1}) + \frac{\partial^2 \Phi_4(\theta_0, X_0)}{\partial X_2^2} (Y_{12} + \frac{\partial \bar{\alpha}_3(0,0)}{\partial \alpha_1}) \right) \\ &\quad + \frac{\partial^2 \Phi_4(\theta_0, X_0)}{\partial X_2 \partial X_3} (Y_{13} + \frac{\partial \bar{\alpha}_4(0,0)}{\partial \alpha_1}) + \frac{\partial^2 \Phi_4(\theta_0, X_0)}{\partial X_2 \partial X_4} Y_{14} \\ &\quad + \frac{\partial \bar{\alpha}_4(0,0)}{\partial \bar{\theta}_1} \left(\frac{\partial^2 \Phi_4(\theta_0, X_0)}{\partial X_3 \partial X_1} (Y_{11} + \frac{\partial \bar{\alpha}_2(0,0)}{\partial \alpha_1}) + \frac{\partial^2 \Phi_4(\theta_0, X_0)}{\partial X_3 \partial X_2} (Y_{12} + \frac{\partial \bar{\alpha}_3(0,0)}{\partial \alpha_1}) \right) \\ &\quad \left. + \frac{\partial^2 \Phi_4(\theta_0, X_0)}{\partial X_3^2} (Y_{13} + \frac{\partial \bar{\alpha}_4(0,0)}{\partial \alpha_1}) + \frac{\partial^2 \Phi_4(\theta_0, X_0)}{\partial X_3 \partial X_4} Y_{14} \right] \\ &= -\left(\frac{\partial \bar{\alpha}_2(0,0)}{\partial \bar{\theta}_1} \frac{\partial^2 \Phi_4(\theta_0, X_0)}{\partial X_1 \partial X_4} + \frac{\partial \bar{\alpha}_3(0,0)}{\partial \bar{\theta}_1} \frac{\partial^2 \Phi_4(\theta_0, X_0)}{\partial X_2 \partial X_4} + \frac{\partial \Phi_4(\theta_0, X_0)}{\partial X_3} \frac{\partial^2 \bar{\alpha}_4(0,0)}{\partial \bar{\theta}_1 \partial \alpha_1} \right). \end{aligned} \quad (64)$$

Then substituting (53) and (56) into the above equation yields that

$$\begin{aligned} B &= \frac{\partial^2 N_4(0,0)}{\partial \bar{\theta}_1 \partial \alpha_1} \\ &= -\left[\frac{a_{22}}{a'_{11} a'_{22} - a'_{12} a'_{21}} \left(\frac{\partial^2 \Phi_4(X_0)}{\partial X_1 \partial X_4} - \frac{a'_{43}}{a'_{33}} \frac{\partial^2 \Phi_3(X_0)}{\partial X_1 \partial X_4} \right) - \frac{a'_{21}}{a'_{11} a'_{22} - a'_{12} a'_{21}} \left(\frac{\partial^2 \Phi_4(X_0)}{\partial X_2 \partial X_4} - \frac{a'_{43}}{a'_{33}} \frac{\partial^2 \Phi_3(X_0)}{\partial X_2 \partial X_4} \right) \right]. \end{aligned} \quad (65)$$

(ii) Calculation of the value of C.

Similarly to (57), we have that

$$\begin{aligned} \frac{\partial^2 N_4(0,0)}{\partial \alpha_1^2} &= \frac{\partial N_4(p_0, X_0)}{\partial X_3} \frac{\partial^2 \bar{\alpha}_4(0,0)}{\partial \alpha_1^2} + \sum_{i=1}^4 \sum_{j=1}^4 \frac{\partial^2 N_4(p_0, X_0)}{\partial X_i \partial X_j} Y_{1i} Y_{1j} \\ &= a'_{43} \frac{\partial^2 \bar{\alpha}_4(0,0)}{\partial \alpha_1^2} - \sum_{i=1}^4 \sum_{j=1}^4 \frac{\partial^2 \Phi_4(p_0, X_0)}{\partial X_i \partial X_j} Y_{1i} Y_{1j}. \end{aligned} \quad (66)$$

Submitting the roots of equations (58) and (59) with $i = 4$ into (67), we can obtain that

$$C = \frac{\partial^2 N_4(0,0)}{\partial \alpha_1^2} = \sum_{i=1}^4 \sum_{j=1}^4 \left(\frac{a'_{43}}{a_{33}} \frac{\partial^2 \Phi_3(p_0, X_0)}{\partial X_i \partial X_j} - \frac{\partial^2 \Phi_4(p_0, X_0)}{\partial X_i \partial X_j} \right) Y_{1i} Y_{1j}. \quad (67)$$

References

- Bhatt S, Gething PW, Brady OJ et al (2013) The global distribution and burden of dengue. *Nature* 496:504-507
- Bian GW, Xu Y, Lu P et al (2010) The endosymbiotic bacterium *Wolbachia* induces resistance to dengue virus in *Aedes aegypti*. *PLoS Pathog* 6(4):e1000833
- Blower SM, Dowlatabadi H (1994) Sensitivity and uncertainty analysis of complex models of disease transmission: an HIV model, as an example. *Int Stat Rev* 62:229-243
- Bull JJ, Turelli M (2013) *Wolbachia* versus dengue Evolutionary forecasts. *Evol Med Public Health* 2013:197-207
- Burattini MN, Chen M, Chow A et al (2008) Modelling the control strategies against dengue in Singapore. *Epidemiol Infect* 136:309-319
- Farkas JZ, Hinow P (2010) Structured and Unstructured Continuous Models for *Wolbachia* Infections. *Bull Math Biol* 72:2067-2088
- Golubitsky M, Schaeffer DG (1985) *Singularities and Groups in Bifurcation Theory*. Springer-Verlag, New York
- Gourley SA, Liu R, Wu J (2007) Eradicating vector-borne diseases via age-structured culling. *J Math Biol* 54:309-335.
- Haygood R, Turelli M (2009) Evolution of incompatibility inducing microbes in subdivided host populations. *Evolution* 63:432-447
- Hoffmann AA, Montgomery BL, Popovici J, et al (2011) Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. *Nature* 476:454-457
- Hughes H, Britton NF (2013) Modelling the use of *Wolbachia* to control dengue fever transmission. *Bull Math Biol* 75:796-818
- Hu X, Liu Y, Wu J (2009) Culling structured hosts to eradicate vector-borne diseases. *Math Bios Eng* 325:496-516
- Iturbe-Ormaetxe I, Walker T, O'Neill SL (2011) *Wolbachia* and the biological control of mosquito-borne disease. *EMBO reports* 12:508-518
- James S, Simmons CP, James AA (2011) Ecology: Mosquito trials. *Science* 334:771-772
- Jansen VAA, Turelli M, Godfray HCJ (2008) Stochastic spread of *Wolbachia*. *Proc R Soc B* 275:2769-2776
- Keeling MJ, Jiggins FM, Read JM (2003) The invasion and coexistence of competing *Wolbachia* strains. *Heredity* 91:382-388
- Kittayapong P, Baisley KJ, Baimai V et al (2000) Distribution and diversity of *Wolbachia* infections in Southeast Asian mosquitoes (Diptera: Culicidae). *J Med Entomol* 37:340-345
- Kittayapong P, Baisley KJ, Sharpe RG et al (2002) Maternal transmission efficiency of *Wolbachia* superinfections in *Aedes albopictus* populations in Thailand. *Am J Trop Med Hyg* 66:103-107
- Lakmeche A, Aarino O (2000) Bifurcation of non-trivial periodic solutions of impulsive differential equations arising chemotherapeutic treatment. *Dyn Contin Discrete Impuls Syst* 7:265-287
- Lambrechts L, Ferguson NM, Harris E et al (2015) Assessing the epidemiological effect of *Wolbachia* for dengue control. *Personal View* 15:862-866
- Laven H (1967) Eradication of *Culex pipiens fatigans* through cytoplasmic incompatibility. *Nature* 216:383-384
- Marino S, Ian B, Hogue IB et al (2008) A methodology for performing global uncertainty and sensitivity analysis in systems biology. *J Theor Biol* 254:178-196
- McGraw EA, O'Neill SL (2013) Beyond insecticides: new thinking on an ancient problem. *Nat Rev Microbiol* 11:181-93
- McMeniman CJ, Lane RV, Cass BN et al (2009) Stable introduction of a life-shortening *Wolbachia* infection into the mosquito *Aedes aegypti*. *Science* 323:141-144
- Mehta N (2014) Brazil releases *Wolbachia* infected mosquitoes to fight dengue. <http://www.livemint.com/Consumer/T8Ok070nJy1O4zlW5C93aP/Brazil-releases-Wolbachia-infected-mosquitoes-to-fight-dengu.html>
- Ndii MZ, Hickson RI, Allingham D et al (2015) Modelling the transmission dynamics of dengue in the presence of *Wolbachia*. *Math Biosci* 262:157-166

- Rigau-Pérez JG (2006) Severe dengue: the need for new case definitions. *Lancet Infect Dis* 6:297-302
- Ritter L, Solomon KR, Forget J et al (1995) A review of selected persistent organic pollutants. World Health Organization, Geneva
- Schofield PG (2002) Spatially explicit models of Turelli-Hoffmann *Wolbachia* invasive wave fronts. *J Theor Biol* 215:121-131
- Simons RRL, Gourley SA (2006) Extinction criteria in stage-structured population models with impulsive culling. *SIAM J Appl Math* 66:1853-1870
- Tang SY, Tang B, Wang AL et al (2015) Holling II predator-prey impulsive semi-dynamic model with complex Poincaré-map. *Nonlinear Dyn* 81:1575-1596
- The Guardian (2015) Sterile mosquitoes released in China to fight dengue fever. <http://www.theguardian.com/world/2015/may/24/sterile-mosquitoes-released-in-china-to-fight-dengue-fever>**
- Turelli M (1994) Evolution of incompatibility inducing microbes and their hosts. *Evolution* 48:1500-1513
- Turelli M (2010) Cytoplasmic incompatibility in population with overlapping generations. *Evolution* 64:232-241
- Vautrin E, Charles S, Genieys S et al (2007) Evolution and invasion dynamics of multiple infections with *Wolbachia* investigated using matrix based models. *J Theor Biol* 245:197-209
- VED (2014) Vietnam-Eliminate Dengue. <http://www.eliminatedengue.com/vietnam/faqs>
- Walker T, Johnson PH, Moreira LA et al (2011) The *wMel* *Wolbachia* strain blocks dengue and invades caged *Aedes aegypti* populations. *Nature* 476:450-453
- Wang WD (2006) Backward bifurcation of an epidemic model with treatment. *Math Bioci* 201: 58-71**
- Werren JH, Baldo L, Clark ME (2008) *Wolbachia*: master manipulators of invertebrate biology. *Nat Rev Microbiol* 6:741-751
- World Health Organisation (2012) Impact of Dengue. <http://www.who.int/csr/disease/dengue/impact/en/>
- Xi ZY, Khoo CCH, Dobson SL (2005) *Wolbachia* establishment and invasion in an *Aedes aegypti* laboratory population. *Science* 14:326-328
- Xu XX, Xiao YN, Cheke RA (2014) Models of impulsive culling of mosquitoes to interrupt transmission of West Nile virus to birds. *Appl Math Model* 39:3549-3568
- Yang HM, Macoris MLG, Galvani KC et al (2009) Assessin he effects of temperature on the population of *Aedes aegypti*, the vector of dengue. *Epidemiol Infect* 137: 1188-1202**
- Yeap HL, Mee P, Walker T et al (2011) Dynamics of the 'popcorn' *Wolbachia* infection in *Aedes aegypti* in an outbred background. *Genetics* 187:583-595
- Zhang XH, Tang SY, Cheke RA (2015) Birth-pulse models of *Wolbachia*-induced cytoplasmic incompatibility in mosquitoes for dengue virus control. *Nonlinear Anal-Real* 35:236-258
- Zhang XH, Tang SY, Cheke RA (2015) Models to assess how best to replace dengue virus vectors with *Wolbachia*-infected mosquito populations. *Math Bioci* 269:164-177
- Zhang DJ, Zheng XY, Xi ZY et al (2015) Combining the Sterile Insect Technique with the Incompatible Insect Technique: I-Impact of *Wolbachia* Infection on the Fitness of Triple- and Double-Infected Strains of *Aedes albopictus*. *PLoS ONE* 10(4):e0121126
- Zheng B, Tang MX, Yu JS (2014) Modeling *Wolbachia* spread in mosquitoes through delay differential equations. *SIAM J Appl Math* 74:743-770

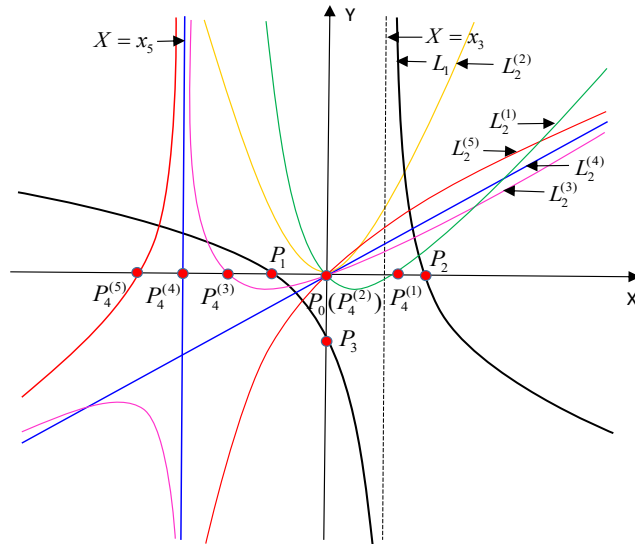


Fig. 1 Curves L_1 , L_2 illustrating the existence of positive equilibrium of system (11). With the increase of θ_2 , all the possibilities of L_2 provided $x_2 > x_4$ are shown as green, yellow, magenta, blue and red curves, respectively.

Table 1 Parameter descriptions, values and sources for the systems

Para.	Description	Value(Range)	Unit	Source
f	The proportion of mosquitoes born female	0.5(0.4,0.6)	/	(Keeling et al. , 2003; Zheng et al. , 2014)
b_1	The natural birth rate of W uninfected mosquitoes	$\frac{0.3976}{(0.2518,0.7554)}$ See Table 2	$(CD)^{-1}$	(Zheng et al. , 2014; Walker et al. , 2011)
b_2	The natural birth rate of W infected mosquitoes, $b_2 = \alpha b_1$, $\alpha \in [0.5,1]$	See Table 2	$(CD)^{-1}$	(Zheng et al. , 2014; Hughes and Britton , 2013)
τ	Probability of the offsprings infected with $Wolbachia$ from W infected females	See Table 2 (0.85,1)	/	(Ndii et al. , 2015; Walker et al. , 2011)
q	Probability of a W infected male and a W uninfected female producing inviable offsprings	See Table 2 (0.5,1)	/	(Keeling et al. , 2003)
d	The natural death rate of mosquitoes	0.0714(0.033,0.1)	$(CD)^{-1}$	(Ndii et al. , 2015; Yang et al. , 2009)
D	Fitness cost of W infected mosquitoes $D = (\beta - 1)d$	See Table 2 (-0.033, 0.1518)	$(CD)^{-1}$	Assumed
T	Pulse period	7(5,15)	D	(Hoffmann et al. , 2011; Burattini et al. , 2008)
θ_1	The quantity of W infected females released as a constant	(0.1, 30)	C	Assumed
θ_2	The quantity of W infected males released as a constant	(0.1, 30)	C	Assumed

W infected; $Wolbachia$ infected; W uninfected; $Wolbachia$ uninfected; /: N/A; C: capita; D: day.

Table 2 Phenotypes and parameters for different *Wolbachia* stains

<i>Wolbachia</i> strain	Phenotype	α	β	τ	q
<i>wAlbB</i>	CI, DenV interf. ^[1]	0.85 ^[2,3]	1 ^[2,4]	0.967 ^[5]	1 ^[3,5]
<i>wMelPop</i>	CI, Life-shortening, Embryo mortality, DenV interf. ^[1]	0.55 ^[6,7]	1.7 ^[2,7]	0.9945 ^[5]	1 ^[8]
<i>wMel</i>	CI, DenV interf. ^[1]	0.9 ^[2,9]	1.1 ^[2,9]	1 ^[7]	1 ^[7]

CI: cytoplasmic incompatibility; DenV interf.: dengue virus interference;

¹: (Iturbe–Ormaetxe et al. , 2011); ²: (Hughes and Britton , 2013);

³: (Xi et al. , 2005); ⁴: (Bian et al. , 2010);

⁵: (Kittayapong et al. , 2002); ⁶: (Zheng et al. , 2014);

⁷: (Walker et al. , 2011); ⁸: (McMeniman et al. , 2009);

⁹: (Hoffmann et al. , 2011).

Table 3 Partial rank correlation coefficients illustrating the dependence of the two variables (i.e., R_0 , FI_m) with respect to each parameter. Parameter values of *wMel* (see Tables 1 and 2) are used in the simulations of PRCCs and p -values of R_0 and FI_m . Here denote p -value as zero if it is smaller than 0.0001, and it is significant when $p < 0.01$.

Para.	R_0		FI_m	
	PRCCs	p -values	PRCCs	p -values
b_1	-0.6829	0	0.0575	0
α	-0.3982	0	0.0071	0.5278
d	-0.0669	0.0003	-0.0181	0.0418
D	0.9835	0	0.3080	0
q	-0.4491	0	0.0910	0
T	-0.4613	0	0.0384	0.0895
θ_1	-0.0897	0	-0.3111	0
θ_2	-0.0102	0.5782	-0.0085	0.1127

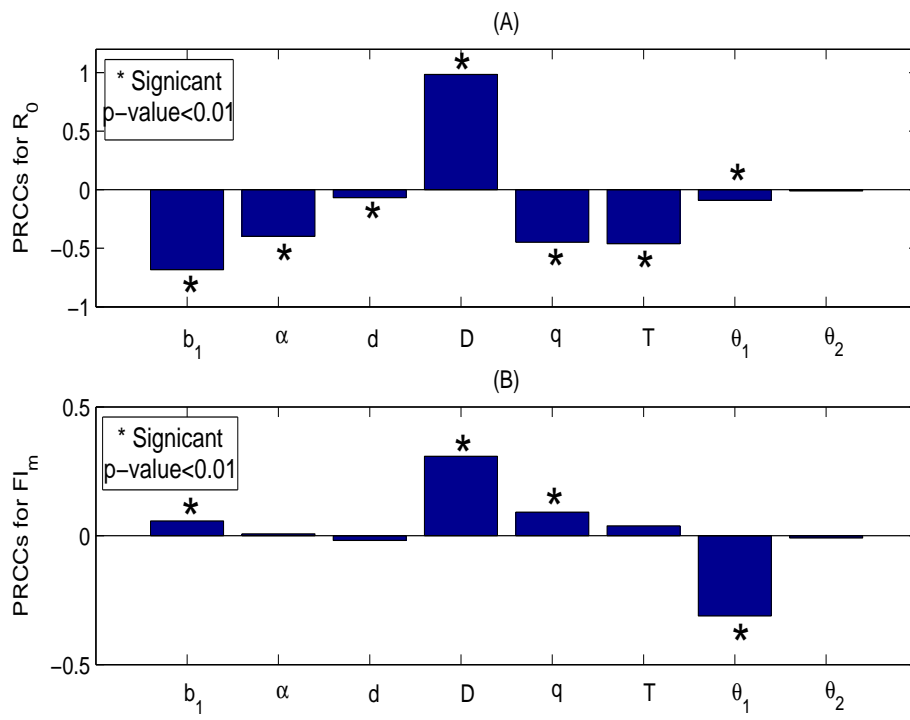


Fig. 2 PRCCs illustrating the dependence of the two outcome variables R_0 and FI_m on the input parameters. All the parameter values came from Latin Hypercube sampling.

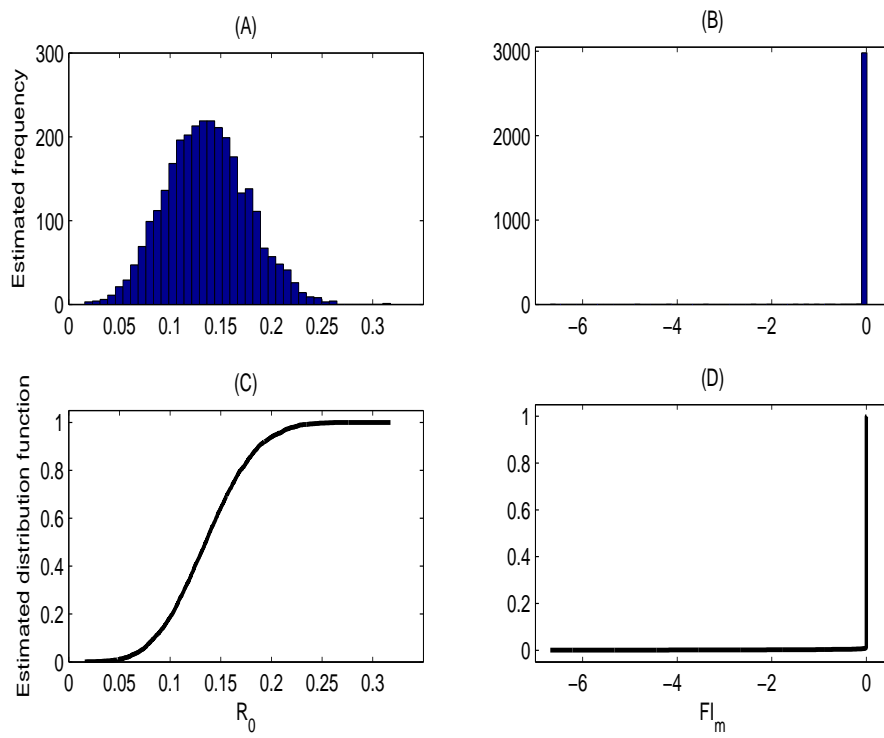


Fig. 3 Uncertainty results based on Latin hypercube sampling. The first row consists of the frequency diagrams of (A) R_0 and (B) FI_m . The second row (C) and (D) show the estimates of CDFs of the two variables, respectively.

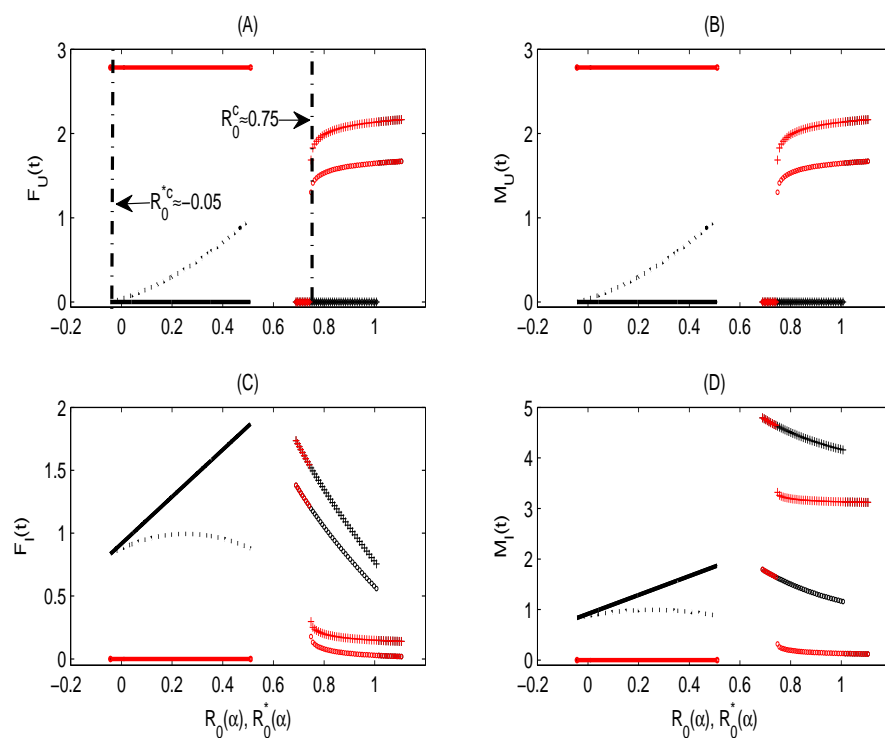


Fig. 4 The bifurcation diagrams of system (5) with and without mosquito augmentation. The black solid, red solid and black dotted curves denote the successful, unsuccessful replacement equilibria (stable) and the unstable equilibria versus $R_0^*(\alpha)$, respectively. The curves with black (or red) circles and plus signs are the upper and lower bounds of period solutions with the high (or low) level of population replacement versus $R_0(\alpha)$, respectively. The parameter values are fixed as follows: $b_1 = 0.3976$, $d = 0.0714$, $D = 0.035$, $q = 0.7$, $\tau = 1$, $T = 7$, $\theta_1 = 0.12$, $\theta_2 = 3$.

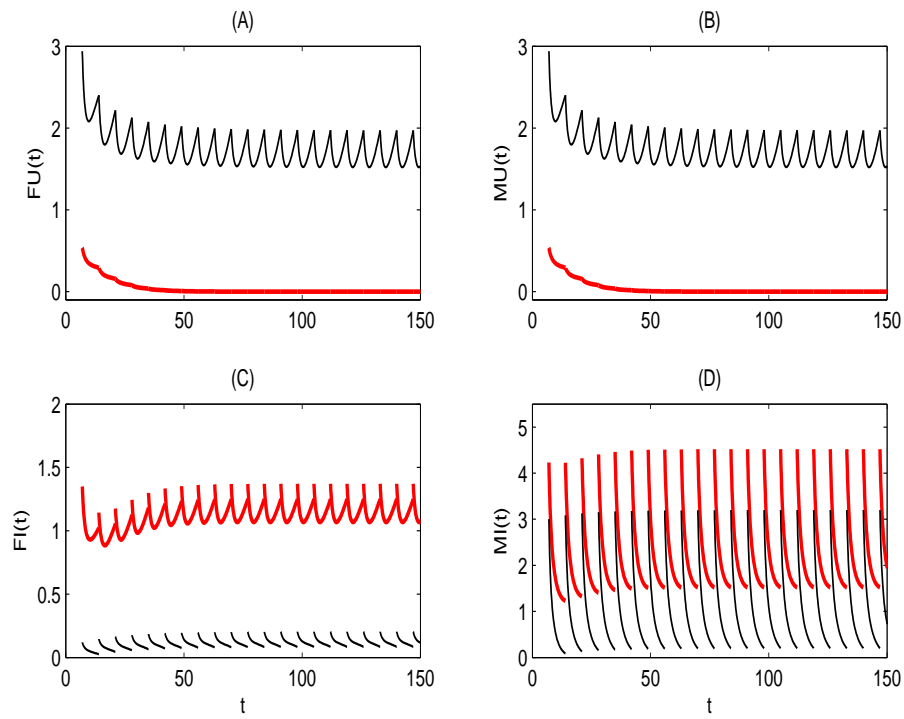


Fig. 5 The solutions of system (5) from different initial values. The red bold and black curves indicate the boundary and interior period solutions (i.e., the high and low levels of population replacement), respectively. The baseline parameter values are the same as in Fig 4, except for $\alpha = 0.85$ chosen from the region of backward bifurcation.

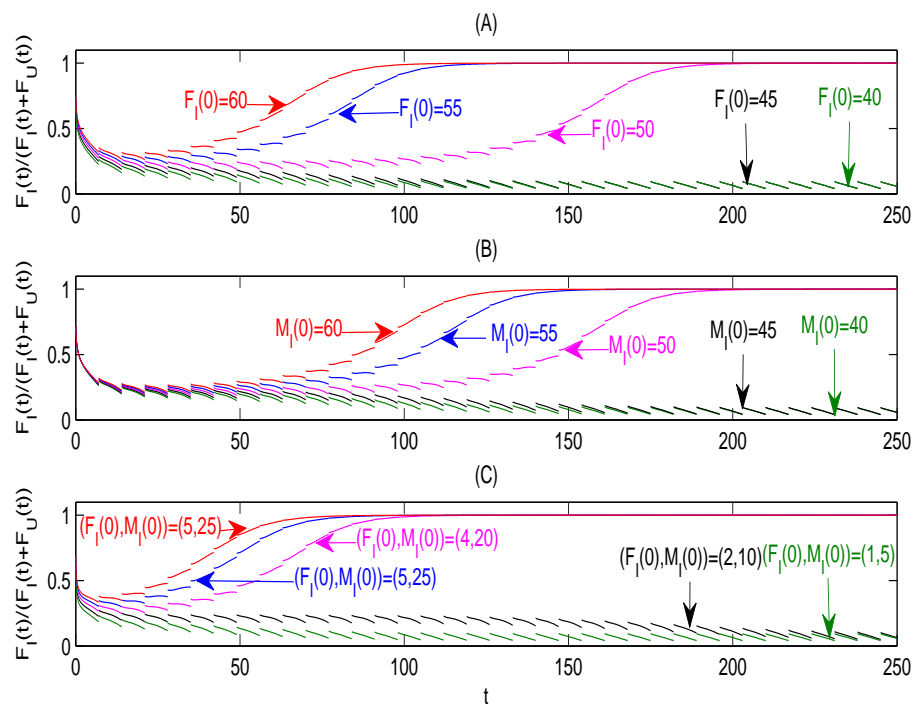


Fig. 6 The effect of different initial values, with the same initial density of (A) *Wolbachia* infected males, (B) *Wolbachia* infected females and (C) the same initial ratio of *Wolbachia* infected males to females, respectively, on the ratios of *Wolbachia* infected females to the total females for system (5). (A) $M_I(0) = 10$; (B) $F_I(0) = 5$; (C) $M_I(0)/F_I(0) = 5$. The parameter values are the same as in Fig 5.

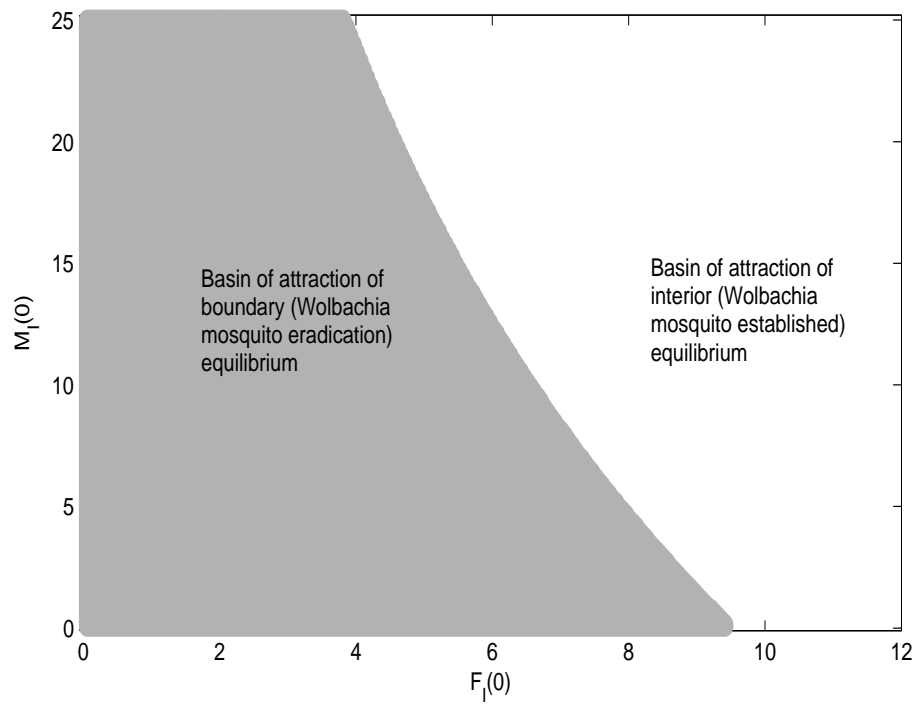


Fig. 7 Basins of attraction of equilibria for system (5) without pulse control. The initial densities of *Wolbachia* uninfected mosquitoes are $F_U(0) = M_U(0) = 20$. The baseline parameters are fixed as $b_1 = 0.75, b_2 = 0.6375, d = 0.035, D = 0.0, q = 1, \tau = 0.9$.

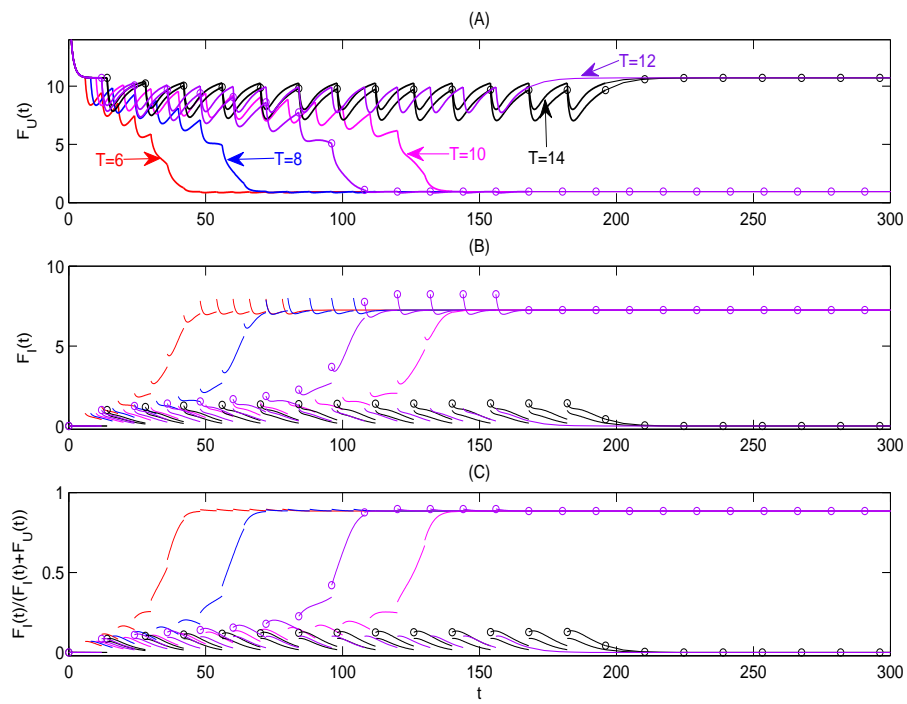


Fig. 8 The effect of different pulse periods on the solutions of female mosquitoes for system (5). The baseline parameter values are the same as in Fig 7, $\theta_1 = 0.8$ and $\theta_2 = 4$. The curves with circles represent the corresponding solutions with the quantities of Wolbachia infected mosquitoes released increasing to $(\theta_1, \theta_2) = (1, 5)$.

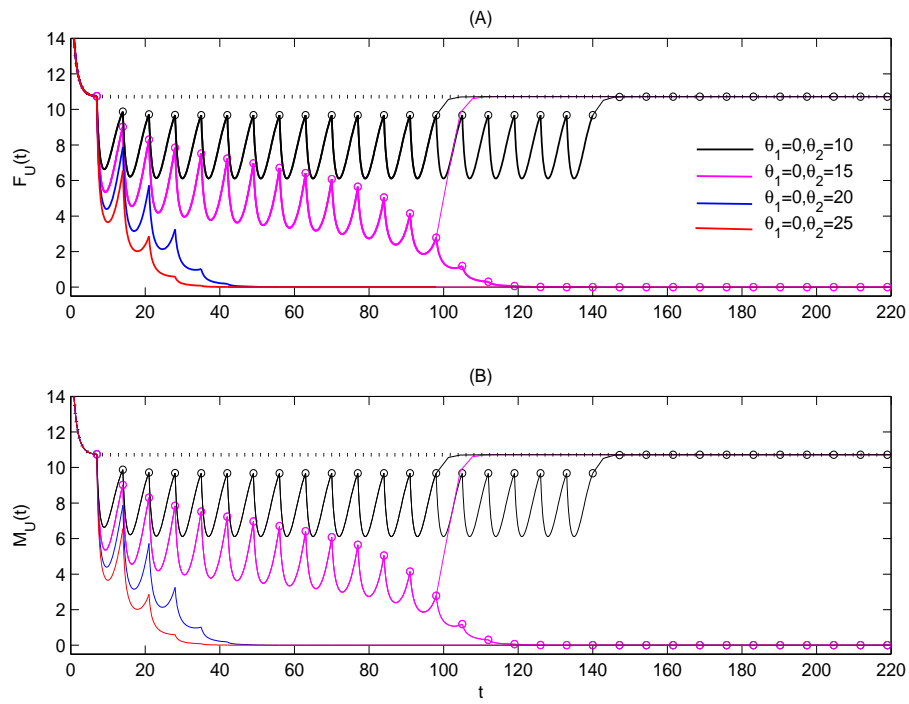


Fig. 9 The effect of releasing only *Wolbachia* infected male mosquitoes on the solutions of *Wolbachia* uninfected ones for system (5). The baseline parameter values are the same as in Fig 8, except for $T = 7$.

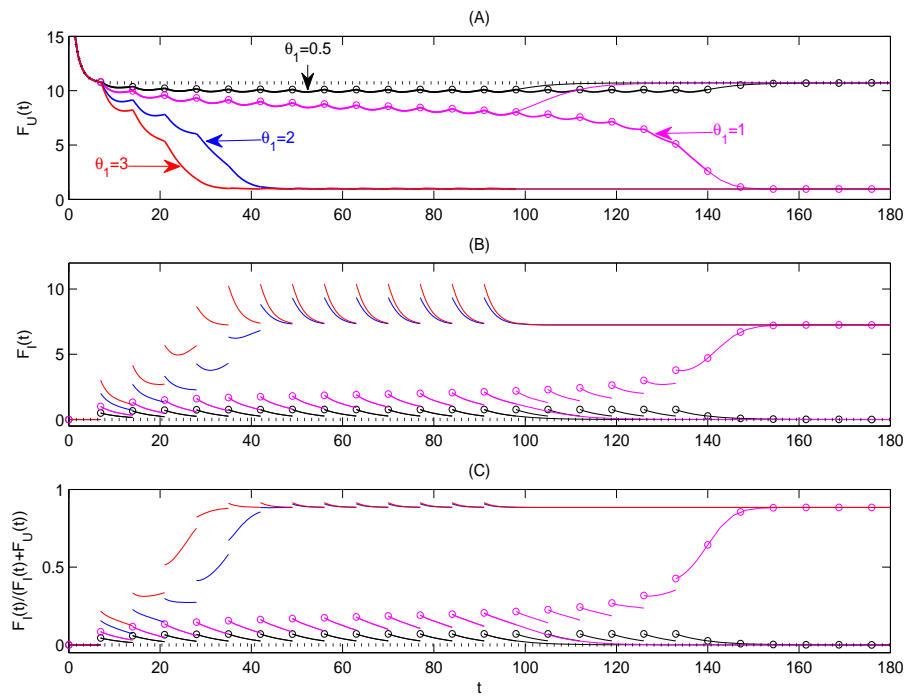


Fig. 10 The effect of releasing only *Wolbachia* infected female mosquitoes on the solutions of female ones for system (5). The baseline parameter values are the same as in Fig 9.

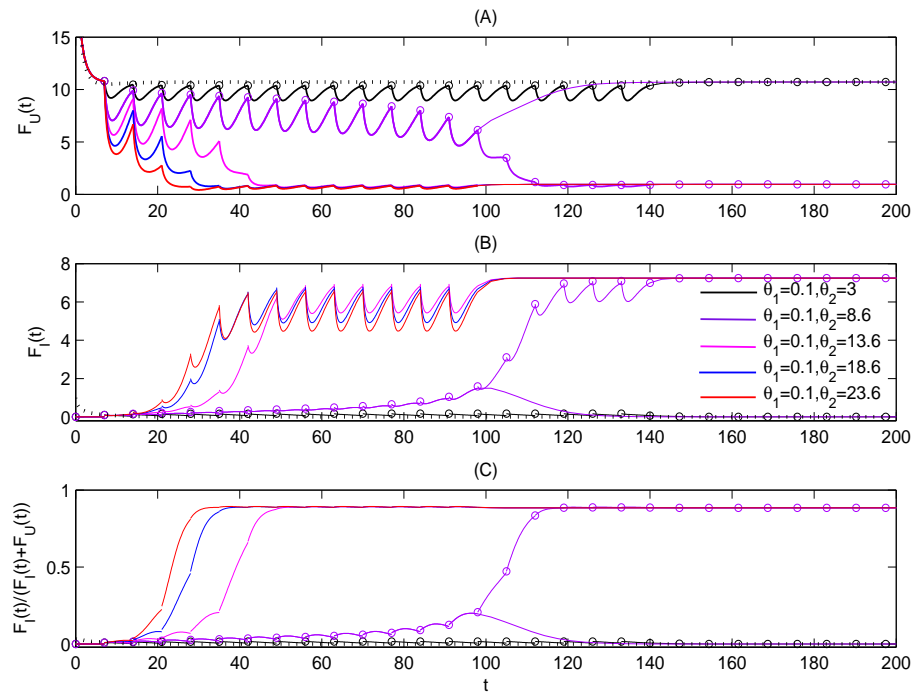


Fig. 11 The effect of releasing *Wolbachia* infected mosquitoes on the solutions of female ones for system (5). The baseline parameter values are the same as in Fig 9.

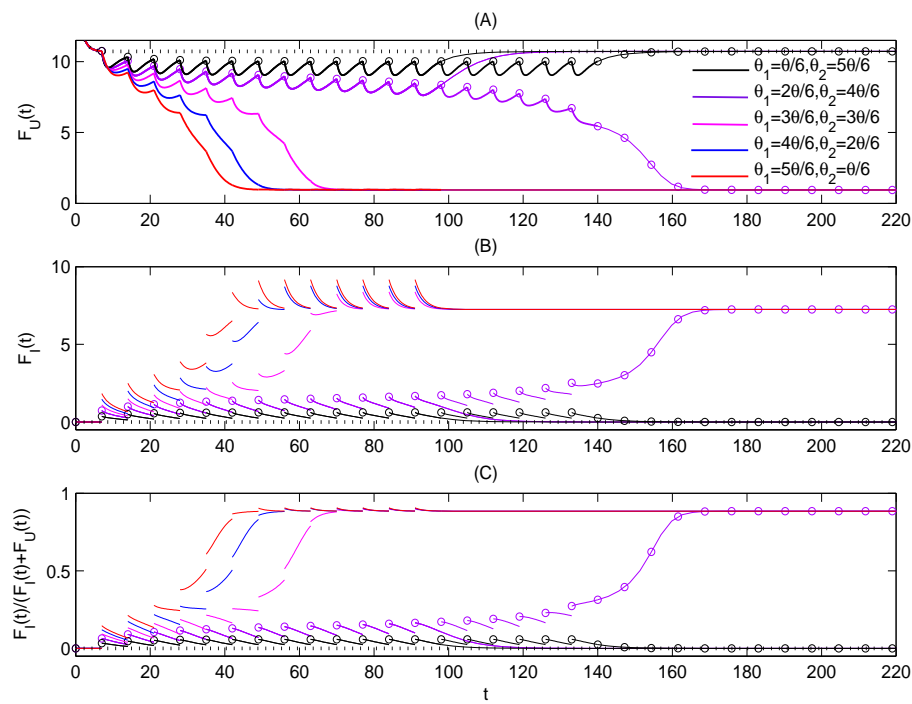


Fig. 12 The effect of releasing different sex ratios of *Wolbachia* infected mosquitoes on the solutions of female ones and their ratios for system (5). The total quantity of *Wolbachia* infected mosquitoes are fixed as a constant $\theta = 2.2$, and the baseline parameter values are the same as in Fig 9.