Time delay and protein modulation analysis in a model of RNA silencing

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Appendix

Abstract

RNA silencing is a recently discovered mechanism for posttranscriptional regulation of gene expression. Precisely, in RNA interference, RNAi, endogenous expressed or exogenously promoted small RNAs promote and modulate the degradation of complementary messenger RNA involved in the synthesis of targeted proteins. In this paper we investigated the role of time delay and protein regulation in the posttranslational protein regulation through RNA interference. Towards this end, we used and modified a simple model accounting for RNAi and used qualitative bifurcation analysis, sensitivity analysis and predictive simulations to analyze it. Our results suggest that some processes in the system, like Dicer-mediated FD_{SRNA} mRNA degradation or non specific mRNA degradation, play an important role in the modulation of RNA silencing, whereas silencing seems virtually independent of modulation in other processes.

Keywords: delay differential equations; RNA silencing; Andronov-Hopf bifurcation; sensitivity analysis

Model calibration

Parameter	Calculated value[1]	Original value[2]
a	4	10
b	0.002	0.001
h	1000	1000
g	0.4	1
$\boldsymbol{d_M}$	1	1
$\boldsymbol{d_R}$	0.1	0.1
$\boldsymbol{d_C}$	2	1
n	5	5

1. Values estimated using model calibration in the way discussed in the text.
2. Values used in Bergstrom et al. 2003.

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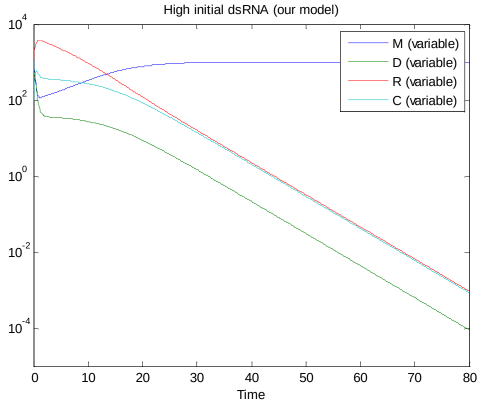
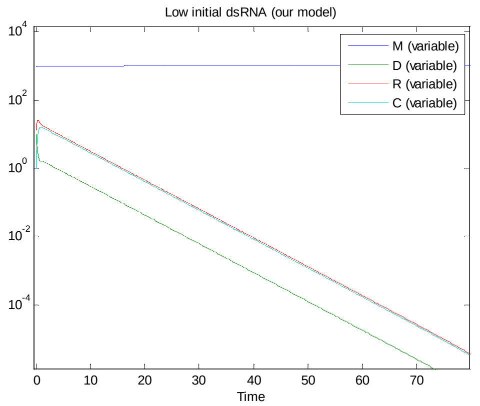
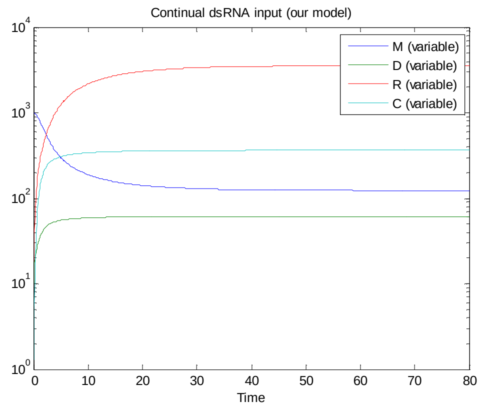
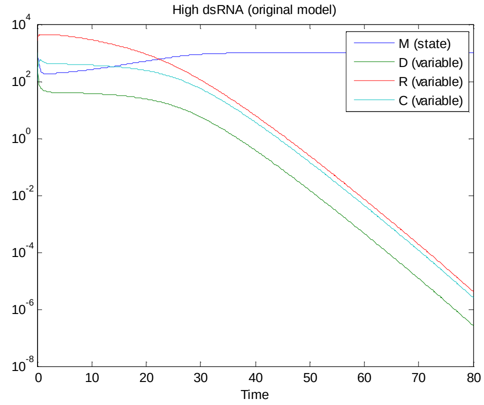
Calculated parameters

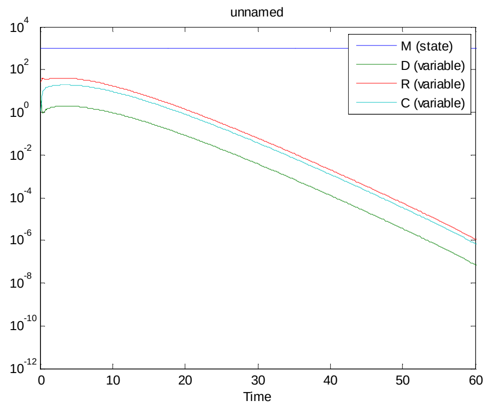


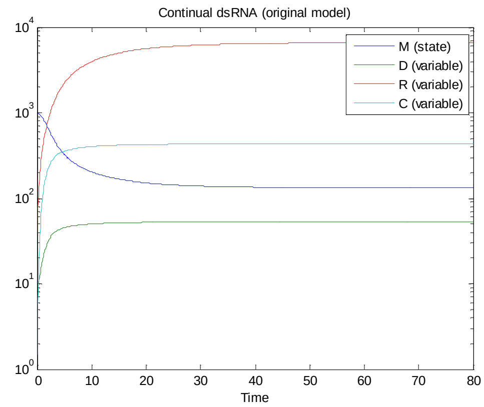




Original simulations (Bergstrom et al. 2003)







Complete derivation used in our qualitative bifurcation analysis

In Nikolov and Petrov [6] we investigated the bifurcation behavior of a model of RNA silencing with one time delay, where the delay function $C(t-\tau)$ express the assumption that the net rate of dsRNA degradation by Dicer and background process as well as the net rate of dsRNA loss are proportional, thus triggering the process of mRNA binding to form the RISC-mRNA complex at the moment $(t-\tau)$. In [6], in order to make the analytical investigation of time delay system easier, we assume that the two times –of the regeneration and degradation of the RISC-mRNA are equal. Of course, the finite time τ_1 of regeneration can be different from that of degeneration τ_2 [12, 22, 23]. Hence, we obtain a system with two delays in the form:

$$\begin{aligned}
\frac{dD}{dt} &= -a.D + g.C(t-\tau_1), \\
\frac{dR}{dt} &= an.D - d_R.R - b.RM, \\
\frac{dC}{dt} &= b.RM - (g+d_c).C(t-\tau_2), \\
\frac{dM}{dt} &= h - d_M M - b.RM,
\end{aligned} \tag{4}$$

where the state variables D, R, C, M represent the concentrations of the dsRNA, RISC, RISC-mRNA complex, and mRNA, respectively, at time *t*. With $a, b, d_C, d_M, d_R, g, h$ and n are noted the kinetic rate constants. Hence, system (4) has two steady states: the trivial $\left(\bar{D}=\bar{C}=\bar{R}=0,\ \bar{M}={h}/{d_M}\right)$ and $\left(\bar{D}=\frac{g}{a}\bar{C},\ \bar{R}=\frac{\varsigma}{d_R}\bar{C},\ \bar{C}=\frac{h}{g+d_C}-\frac{d_M d_R}{b\varsigma},\ \bar{M}=\frac{(g+d_C)d_R}{b\varsigma}\right)$, where $\varsigma=[g(n-1)-d_C]$. Here we note that the original ODE system has the same fixed points which are always stable.

Furthermore, we investigate the bifurcation structure- particularly the Andronov-Hopf bifurcation- for system (4), using time delays τ_1 or τ_2 as bifurcation parameters. First, we obtain the characteristic equation for the linearization of system (4) near the equilibrium $\bar{E}\left(\bar{D}>0,\ \bar{C}>0,\ \bar{R}>0,\ \bar{M}>0\right)$, i.e. all are positive and the silencing reaction controls the level of mRNA below its normal level. Next, we consider a small perturbation about the equilibrium level, i.e. $D=\bar{D}+x,\ R=\bar{R}+y,\ C=\bar{C}+z,\ M=\bar{M}+w$. Substituting these into the differential equations in system (4), we have

$$\begin{aligned}
\frac{dx}{dt} &= -ax + g\ell^{-\tau_1\chi} z, \\
\frac{dy}{dt} &= anx - a_1 y - a_2 w - byw, \\
\frac{dz}{dt} &= a_3 y - a_4 \ell^{-\tau_2\chi} z + a_2 w + byw, \\
\frac{dw}{dt} &= -a_3 y - a_5 w - byw,
\end{aligned} \tag{5}$$

where $a_1 = d_R + b\bar{M}$, $a_2 = b\bar{R}$, $a_3 = b\bar{M}$, $a_4 = g + d_C$, $a_5 = d_M + b\bar{R}$. The associated characteristic equation of (5) has the following form

$$\chi^4 + K_1\chi^3 + K_2\chi^2 + K_3\chi = \ell^{-\tau_1\chi}(T_5\chi + T_6) + \ell^{-\tau_2\chi}(T_1\chi^3 + T_2\chi^2 + T_3\chi + T_4), \tag{6}$$

where

$$\begin{aligned} &K_1 = a + a_1 + a_5, K_2 = a(a_1 + a_5) + a_1a_5 - a_2a_3, K_3 = a(a_1a_5 - a_2a_3), \\ &T_1 = -a_4, T_2 = -K_1a_4, T_3 = -a_4[a(a_1 + a_5) + a_1a_5 - a_2a_3], \\ &T_4 = aa_4(a_2a_3 - a_1a_5), T_5 = aa_3ng, T_6 = aa_3ng(a_5 - a_2). \end{aligned} \tag{7}$$

Because of the presence of two different delays in (4) the analysis of the sign of the real parts of eigenvalues is very complicated and a direct approach cannot be considered [10]. Thus, in our analysis we will use a method consisting of determining the stability of steady state when one delay is equal to zero similar as [24, 25].

2.1. The case $\tau_1 = 0$ and $\tau_2 > 0$.

Hence, we assume that the finite time delay τ_2 of degeneration is longer than the time of regeneration of RISC-mRNA complex, τ_1.

Setting $\tau_1 = 0$ in (6), the characteristic equation becomes

$$\chi^4 + K_1\chi^3 + K_2\chi^2 + K_{31}\chi - T_6 = \ell^{-\tau_2\chi}(T_1\chi^3 + T_2\chi^2 + T_3\chi + T_4) \tag{8}$$

where $K_{31} = K_3 - T_5$. For small delay $\tau_2 < 1$, we use linear stability analysis. Thus, let $\ell^{-\chi\tau_2} \approx 1 - \chi\tau_2$; then, the eigenvalue equation becomes

$$\chi^4 + p\chi^3 + q\chi^2 + r\chi + s = 0. \tag{9}$$

By the Hopf bifurcation theorem and Routh-Hurwitz criteria [30], an Andronov-Hopf bifurcation occurs at a value $\tau = \tau_b$ where

$$\begin{aligned} &p = \frac{K_1 + T_2\tau_2 - T_1}{\delta} > 0, \quad q = \frac{K_2 + T_3\tau_2 - T_2}{\delta}, \quad s = -\frac{T_4 + T_6}{\delta} > 0, \\ &r = \frac{K_{31} + T_4\tau_2 - T_3}{\delta}, \quad l = pqr - sp^2 - r^2 = 0, \end{aligned} \tag{10}$$

where $\delta = 1 + T_1\tau_2$ and the condition $T_1\tau_2 \neq -1$ is valid. Let

$$h(\chi, \tau_2) = \chi^4 + p\chi^3 + q\chi^2 + r\chi + s. \tag{11}$$

Evaluating h at $\tau_2 = \tau_b$ yields

$$h(\tau_b, \chi(\tau_b)) = \chi^4 + p\chi^3 + q\chi^2 + k^2p\chi + k^2(q - k^2), \tag{12}$$

where $k^2 = \dfrac{r}{p}$. The eigenvalues of (9) at τ_b are

$$\chi_{1,2} = \pm ik = \pm\sqrt{\frac{r}{p}}, \tag{13}$$

and the type of the other root pair depends on the sign of the equality $\Delta_1 = \frac{sp}{r} - \frac{p}{4}$. Here i is an imaginary unit. If $\Delta_1 > 0$, then

$$\chi_{3,4} = -\frac{p}{2} \pm \Delta_2 i, \tag{14}$$

where $\Delta_2^2 = \frac{sp}{r} - \frac{p^2}{4} (\Delta_2 > 0)$; if $\Delta_1 < 0$, then

$$\chi_{3,4} = -\frac{p}{2} \pm \Delta_2, \tag{15}$$

where now $\Delta_2 = \sqrt{-\Delta_1}$. Implicitly differentiating $h(\tau_b, \chi(\tau_b))$ yields

$$\frac{d\chi}{d\tau} = -\frac{\partial h}{\partial \tau} \Big/ \frac{\partial h}{\partial \chi} = -\frac{p_1\chi^3 + q_1\chi^2 + r_1\chi + s_1}{4\chi^3 + 3p\chi^2 + 2q\chi + k^2 p}, \tag{16}$$

where

$$\begin{aligned} p_1 &= \frac{T_2\delta - T_1(K_1 - T_1 + T_2\tau_2)}{\delta^2}, \quad q_1 = \frac{T_3\delta - T_1(K_2 - T_2 + T_3\tau_2)}{\delta^2}, \\ r_1 &= \frac{T_4\delta - T_1(K_{31} - T_3 + T_4\tau_2)}{\delta^2}, \quad s_1 = \frac{T_1(T_4 + T_6)}{\delta^2}. \end{aligned} \tag{17}$$

Evaluating the required derivatives of h at τ_b, we obtain

$$\frac{d\chi_1(\tau_b)}{d\tau} = \frac{2k^2 N + 2k[(s_1 - q_1k^2)(q - 2k^2) + pk^2(r_1 - p_1k^2)]i}{L^2 + I^2}, \tag{18}$$

where $L = -2pk^2$, $I = 2k(q - 2k^2)i$, and $N = (p_1k^2 - r_1)(q - 2k^2) + p(s_1 - q_1k^2)$. The real part of (18) has the form

$$\mathrm{Re}\left(\frac{d\chi_1(\tau_b)}{d\tau}\right) = \frac{2k^2 N}{L^2 + I^2}. \tag{19}$$

and is always positive if $N > 0$, i.e. if the following conditions are valid:

$$\left| \begin{array}{l} p_1k^2 > r_1 \\ q > 2k^2 \\ s_1 > q_1k^2 \end{array} \right. \quad or \quad \left| \begin{array}{l} p_1k^2 < r_1 \\ q < 2k^2 \\ s_1 > q_1k^2 \end{array} \right. \tag{20}$$

It is well known that for a larger time delay τ_2, linear stability analysis is no longer effective and we need to use another approach [8, 10, 24-27]. The stability of equilibrium state depends on the sign of the real parts of the roots of (8). We let $\chi = m + in$ $(m, n \in R)$, and rewrite (9) in terms of its real and imaginary parts as

$$\left|\begin{array}{l} m^4+n^4-6m^2n^2+K_1m\left(m^2-3n^2\right)+K_2\left(m^2-n^2\right)+K_{31}m-T_6=\ell^{-m\tau_2}\left\{T_1\left[m\left(m^2-3n^2\right)\cos n\tau_2+\right.\right.\\ \quad \left.+n\left(3m^2-n^2\right)\sin n\tau_2\right]+T_2\left[\left(m^2-n^2\right)\cos n\tau_2+2mn\sin n\tau_2\right]+T_3\left(m\cos n\tau_2+n\sin n\tau_2\right)+T_4\cos n\tau_2\big\},\\ 4mn\left(m^2-n^2\right)+K_1\left(3m^2-n^2\right)n+2K_2mn+K_{31}n=\ell^{-m\tau_2}\left\{T_1\left[n\left(3m^2-n^2\right)\cos n\tau_2+m\left(3n^2-m^2\right)\sin n\tau_2\right]+\right.\\ \quad +T_2\left[2mn\cos n\tau_2+\left(n^2-m^2\right)\sin n\tau_2\right]+T_3\left(n\cos n\tau_2-m\sin n\tau_2\right)-T_4\sin n\tau_2\big\}. \end{array}\right.$$

(21)

To find the first bifurcation point we look for purely imaginary roots $\chi=\pm in, n\in R$, of (8), i.e. we set $m=0$. Then, the above two equations reduce to

$$\left|\begin{array}{l} n^4-K_2n^2-T_6=\left(-T_1n^3+T_3n\right)\sin n\tau_2+\left(-T_2n^2+T_4\right)\cos n\tau_2,\\ -K_1n^3+K_{31}n=\left(-T_1n^3+T_3n\right)\cos n\tau_2+\left(T_2n^2-T_4\right)\sin n\tau_2, \end{array}\right. \tag{22}$$

or another

$$\begin{array}{l} \cos n\tau_2=\dfrac{\left(n^4-K_2n^2-T_6\right)\left(T_2n^2-T_4\right)-\left(-K_1n^3+K_{31}n\right)\left(-T_1n^3+T_3n\right)}{\left(T_2n^2-T_4\right)^2+\left(-T_1n^3+T_3n\right)^2},\\ \sin n\tau_2=\dfrac{\left(-K_1n^3+K_{31}n\right)\left(T_2n^2-T_4\right)+\left(n^4-K_2n^2-T_6\right)\left(-T_1n^3+T_3n\right)}{\left(T_2n^2-T_4\right)^2+\left(-T_1n^3+T_3n\right)^2}. \end{array} \tag{23}$$

Note that $n=0$ can be a solution of (23) if $T_4=T_6$. If the first bifurcation point is $\left(n_b^0,\tau_b^0\right)$, then the other bifurcation points $\left(n_b,\tau_b\right)$ satisfy $n_b\tau_b=n_b^0\tau_b^0+2\nu\pi,\quad \nu=1,2,...,\infty$.

One can notice that if n is a solution of (22) (or (23)), then so $-n$. Hence, in the following we only investigate for positive solutions n of (22), or (23) respectively. By squaring the two equations into system (22) and then adding them, it follows that

$$\begin{array}{l} n^8+\left(K_1-2K_2-T_1^2\right)n^6+\left[K_2^2-T_2^2+2\left(T_1T_3-K_1K_{31}-T_6\right)\right]n^4+\\ \quad +\left[K_{31}^2-T_3^2+2\left(K_2T_6+T_2T_4\right)\right]n^2-T_4^2+T_6^2=0. \end{array} \tag{24}$$

Here, we note that this is a quartic equation on n^2 and that the left side is positive for large values of n^2 and negative for $n=0$ if and only if $T_4^2>T_6^2$, i.e Eq. (24) has at least one positive real root. Moreover, to apply the Hopf bifurcation theorem, according to [28], the following theorem in this situation applies:

Theorem 1. *Suppose that n_b is the least positive simple root of (24). Then, $in\left(\tau_b\right)=in_b$ is a simple root of (8) and $m\left(\tau_2\right)+in\left(\tau_2\right)$ is differentiable with respect to τ_2 in a neighborhood of $\tau_2=\tau_b$.*

To establish Andronov-Hopf bifurcation at $\tau_2=\tau_b$, we need to show that the following transversality condition $\left.\dfrac{dm}{d\tau_2}\right|_{\tau=\tau_b}\neq 0$ is satisfied.

Hence, we if denote

$$H\left(\chi,\tau_2\right)=\chi^4+K_1\chi^3+K_2\chi^2+K_3\chi-\ell^{-\tau_2\chi}\left(T_1\chi^3+T_2\chi^2+T_3\chi+T_4\right), \tag{25}$$

then

$$\frac{d\chi}{d\tau_2} = -\frac{\partial H}{\partial \tau_2} \Big/ \frac{\partial H}{\partial \chi} = \tag{26}$$

$$= \frac{-\chi \ell^{-\chi\tau_2}\left(T_1\chi^3 + T_2\chi^2 + T_3\chi + T_4\right)}{4\chi^3 + 3K_1\chi^2 + 2K_2\chi + K_{31} + \tau_2 \ell^{-\tau_2\chi}\left(T_1\chi^3 + T_2\chi^2 + T_3\chi\right) + T_4 - \ell^{-\tau_2\chi}\left(3T_1\chi^2 + 2T_2\chi + T_3\right)}$$

Evaluating the real part of this equation at $\tau_2 = \tau_b$ and setting $\chi = in_b$ yield

$$\left.\frac{dm}{d\tau_2}\right|_{\tau_2=\tau_b} = \mathrm{Re}\left.\left(\frac{d\chi}{d\tau_2}\right)\right|_{\tau_2=\tau_b} =$$

$$= \frac{n_b^2\left\{4n_b^6 + 3\left(K_1^2 - 2K_2 - T_1^2\right)n_b^4 + 2\left[K_2^2 - T_2^2 + 2\left(T_1T_3 - K_1K_{31} - T_6\right)\right]n_b^2 + K_{31}^2 - T_3^2 + 2\left(T_2T_4 + K_2T_6\right)\right\}}{L_1^2 + I_1^2}$$

(27)

where $L_1 = -3K_1n_b^2 + K_{31} + \tau_2\left(n_b^4 - K_2n_b^2 - T_6\right) - \left(-3T_1n_b^2 + T_3\right)\cos n_b\tau_2 - 2T_2n_b\sin n_b\tau_2$ and $I_1 = 4n_b^3 - 2K_2n_b - \tau_2\left(-K_1n_b^3 + K_{31}n_b\right) + 2T_2n_b\cos n_b\tau_2 - \left(-3T_1n_b^2 + T_3\right)\sin n_b\tau_2$.

Let $\theta = n_b^2$; then, (28) reduces to

$$\begin{aligned} g(\theta) = \theta^4 + \left(K_1 - 2K_2 - T_1^2\right)\theta^3 + \left[K_2^2 - T_2^2 + 2\left(T_1T_3 - K_1K_{31} - T_6\right)\right]\theta^2 + \\ + \left[K_{31}^2 - T_3^2 + 2\left(K_2T_6 + T_2T_4\right)\right]\theta - T_4^2 + T_6^2. \end{aligned} \tag{28}$$

Then, for $g'(\theta)$ we have

$$\begin{aligned} \left.g'(\theta)\right|_{\tau_2=\tau_b} &= \left.\frac{dg}{d\theta}\right|_{\tau_2=\tau_b} = \\ &= 4\theta^3 + 3\left(K_1 - 2K_2 - T_1^2\right)\theta^2 + 2\left[K_2^2 - T_2^2 + 2\left(T_1T_3 - K_1K_{31} - T_6\right)\right]\theta + K_{31}^2 - T_3^2 + 2\left(K_2T_6 + T_2T_4\right). \end{aligned} \tag{29}$$

If n_b is the least positive simple root of (24), then

$$\left.\frac{dg}{d\tau_2}\right|_{\theta=n_b^2} > 0. \tag{30}$$

Hence,

$$\left.\frac{dm}{d\tau_2}\right|_{\tau_2=\tau_b} = \mathrm{Re}\left.\left(\frac{d\chi}{d\tau_2}\right)\right|_{\tau_2=\tau_b} = \frac{n_b^2 g'\left(n_b^2\right)}{L_1^2 + I_1^2} > 0. \tag{31}$$

According to the Hopf bifurcation theorem [29], we define the following Theorem 2:

Theorem 2. *If n_b is the least positive root of (24), then an Andronov-Hopf bifurcation occurs as τ_2 passes through τ_b.*

Corollary 2.1. *When $\tau_2 < \tau_b$, then the steady state $\bar{E}$ of system (4) is locally asymptotically stable.*

2.2. The case $\tau_1, \tau_2 > 0$. We return to the study of (6) with $\tau_1, \tau_2 > 0$. In order to investigate the local stability of the equilibrium state $\bar{E}$ of system (4), we first prove a result regarding the sign of the real parts of characteristic roots of (6) in the next Theorem.

Theorem 3. *If all roots of (8) are with negative real parts for $\tau_2 > 0$, then there exists a $\tau_1^{bif}(\tau_2) > 0$ such that all roots of characteristic equation (6) have negative real parts at $\tau_1 < \tau_1^{bif}(\tau_2)$, i.e. when $\tau_1 \in \left[0, \quad \tau_1^{bif}(\tau_2)\right)$.*

Proof. Similar to [7], let we assume that (8) has no roots with nonnegative real part when $\tau_2 > 0$. Therefore, characteristic equation (6) has no root with nonnegative real part when $\tau_1 = 0$ and $\tau_2 > 0$. Regard τ_1 as parameter, then (6) is analytic about χ and τ_1. By Theorem 2.1 of [24], when τ_1 varies, then the sum of the multiplicity of zeros of (6) in the open right half plane can only change if a zero appears on or crosses the imaginary axis. Because (6) (with $\tau_1 = 0$) has no root with nonnegative real part, there exists a $\tau_1^{bif}(\tau_2) > 0$ such that all roots of (10) with $\tau_1 < \tau_1^{bif}(\tau_2)$ have negative real part.

Corollary 3.1. *If τ_2^{bif} is defined as in Theorem 2, then for any $\tau_2 \in [0, \tau_b)$, there exists a $\tau_1^{bif}(\tau_2) > 0$ such that the steady state $\bar{E}$ of system (4) is locally asymptotically stable when $\tau_1 \in \left[0, \quad \tau_1^{bif}(\tau_2)\right)$.*