A MATHEMATICAL ANALYSIS OF AN ACTIVATOR-INHIBITOR RHO GTPASE MODEL

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Abstract. Recent experimental observations reveal that local cellular con-
traction pulses emerge via a combination of fast positive and slow negative 
feedbacks based on a signal network composed of Rho, GEF and Myosin inter-
actions [22]. As an examplary, we propose to study a plausible, hypothetical 
temporal model that mirrors general principles of fast positive and slow neg-
ative feedback, a hallmark for activator-inhibitor models. The methodology 
involves (i) a qualitative analysis to unravel system switching between differ-
ent states (stable, excitable, oscillatory and bistable) through model parameter 
variations; (ii) a numerical bifurcation analysis using the positive feedback me-
diator concentration as a bifurcation parameter, (iii) a sensitivity analysis to 
quantify the effect of parameter uncertainty on the model output for different 
dynamic regimes of the model system; and (iv) numerical simulations of the 
model system for model predictions. Our methodological approach supports 
the role of mathematical and computational models in unravelling mechanisms 
for molecular and developmental processes and provides tools for analysis of 
temporal models of this nature.

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1. Introduction. Cell motility is an important process in the development and maintenance of multicellular organisms [29]. It is a cyclic multi-step process, which consists of actin polymerization-dependent pseudopod protrusion at the leading edge of the cell; integrin-mediated adhesion to extracellular matrix (ECM); contact-dependent ECM cleavage by cell surface proteases; and actomyosin-mediated contraction of the cell body and rear retraction and translocation of the cell body [12, 37, 38, 46]. Cells can therefore sense both physical and chemical signals from their environment to guide their migratory pathways [23, 39]. This mechanosensing process of chemical signals (such as growth factor concentration) and mechanical cues such as stiffness plays an important role in processes such as wound healing, germ cell migration during embryonic development and angiogenesis [10, 16, 38]. It is known that the mechanosensing process largely depends on myosin motors, in association with F-actin, that are responsible for producing contractile forces during cell migration [11]. One key molecular driver of cell contraction, adhesion and protrusion during cell migration are Rho family GTPases [37, 38, 46]. Rho family GTPases are molecular switches that regulate cytoskeletal dynamics and cell movement through a complex spatiotemporal organisation of their activity [17]. They cycle between active (GTP-bound) and inactive (GDP-bound) conformal states. The activation and inhibition of Rho GTPases are mediated respectively by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs) [36]. The inactive Rho GTPases are sequestered in the cytosol by guanine nucleotide dissociation inhibitors (GDIs), that prevent the association of Rho GTPases with the plasma membrane [6, 17, 32]. Rho GTPases’ based signal networks are thought to control cellular dynamics by coordinating protrusions and retractions during the process of cell migration [15]. The most studied forms of Rho GTPases are Rac, Cdc42 and Rho [3].

Recent advances by theoretical biologists have demonstrated that molecular regulatory networks can be described accurately in mathematical terms [43]. Mathematical and computational modelling have become more universally accepted and are increasingly becoming more closely integrated with experimental research thereby offering a more advanced theoretical and computational methodology for quantitative analysis. These mathematical and computational models shed light on the mechanistic basis of biological control systems and make predictions that can be verified experimentally.

The mathematical model we propose to study is inspired by previous studies, with the first example being the work of Tyson and co-workers [43, 44, 45]. In this previous work, mathematical modelling was used effectively as a tool for investigating alternative hypotheses about the molecular mechanisms controlling cellular processes (e.g. cell division cycle). In the present work, these models were adapted to study cell contraction dynamics. For instance, the equations for Figure 2b in [43] can be written in a non-reduced form with the notation that $E_P(t) = G(t)$, which denotes the concentration of GEF that amplifies Rho GTPase activity, $R(t)$ denotes Rho GTPase concentration, $S$ is an external signal and $X(t) = M(t)$ denotes the concentration of Myosin that acts as a Rho inhibitor. The three-component model is then given by

$$\frac{dG}{dt} = \frac{k_3 R(G_{Total} - G)}{K_{m3} + G_{Total} - G} - \frac{k_4 G}{K_{m4} + G},$$

(1a)
\[
\begin{align*}
\frac{dR}{dt} &= k_0 G + k_1 S - (k_2 + k'_2 M) R, \quad \text{(1b)} \\
\frac{dM}{dt} &= k_5 R - k_6 M. \quad \text{(1c)}
\end{align*}
\]

In this model, it is assumed that the activation of \(G(t)\) and its constitutive inactivation result from enzymatic activities, hence can be described by Michaelis-Menten kinetics. A first order reaction is used to describe the activation of \(R(t)\) by \(G(t)\). The signal \(S\) enhances \(R(t)\) activity. The removal of \(R\) is via constitutive degradation and through the direct interaction with \(M(t)\), which is implemented via the law of mass action. The activation and degradation of \(M(t)\) is described by linear terms. This model predicts different dynamics, dependent on the signal \(S\). With increasing signal, the system signal response, \(R\) shifts from stable to oscillatory and then back to stable.

A second example comes from the recent work by Kamps and co-workers whereby a three-species model for a cell contraction signalling pathway was formulated based on detailed experimental observations \[22\]. Adopting the same notation for the variables as in Model (1), we have the following three-molecular species model

\[
\begin{align*}
\frac{dG}{dt} &= k_3 R (G_T - G) - k_4 MG, \quad \text{(2a)} \\
\frac{dR}{dt} &= k_1 G(R_T - R) - \frac{k_2 R}{K_{m1} + (R_T - R)} - \frac{k_2 R}{K_{m2} + R}, \quad \text{(2b)} \\
\frac{dM}{dt} &= k_5 R (M_T - M) - \frac{k_6 M}{K_{m5} + (M_T - M)} - \frac{k_6 M}{K_{m6} + M}, \quad \text{(2c)}
\end{align*}
\]

In contrast to Model (1), this model assumes the conservation of the molecular species between their active and inactive forms, neglects the effect of external signal and was formulated based on the following biological assumptions; GEF activation and its inhibition by Rho and Myosin respectively are based on molecule interactions, hence mathematically translated via mass action kinetics. The activation and inhibition of Rho are considered enzymatic activities and Michaelis-Menten kinetics are used; and lastly the activation and inactivation of Myosin are mediated by multi-step enzymatic activities, these are however simplified and also implemented via a Michaelis-Menten function. This model predicted total GEF concentration \(G_T\) dependent switching between distinct dynamic regimes. Hence, the model predicts oscillatory dynamics at the intermediate values of \(G_T\) and stable dynamics at very low and high \(G_T\) values.

In order for mathematical models to be useful to biologists and other applied scientists, it is critical that methodological tools are developed to support model prediction and validation. Currently, tools that apply for specific cases can be found in some of these previous works, however, a holistic approach to model analysis and validation is lacking. This is precisely our motivation, to present a methodological structure that can be taken as a recipe for rigorous mathematical analysis of models derived experimentally, hypothetically or otherwise.

Hence, this article is organised as follows. In Section 2 we present a plausible hypothetical mathematical model, an activator-inhibitor system, in the context of Rho-Myosin-GEF temporal dynamics in the absence of spatial variations on which our methodological tools will be developed and applied. The model consists of a system of three ordinary differential equations (ODEs). We invoke a quasi-steady state assumption to reduce the model to a system of two ODEs. Section 3 encodes
the key methodological tools, it focuses on the mathematical analysis of the reduced model using the sign pattern and phase-plane analysis. The section concludes with numerical bifurcation analysis that summarizes results of the theoretical analysis of the model. Thereafter, Section 4 focuses on quantifying the effect of uncertainty of parameters on the limit cycle of the oscillatory dynamics using sensitivity analysis. Finally, we conclude and discuss our findings in Section 5.

2. An illustrative activator-inhibitor model. In order to motivate our model, we consider the context of Rho-Myosin-GEF signalling pathways (see for example [15, 21, 22, 43]). Hence, we consider three species $R(t), M(t)$ and $G(t)$, with their interactions depicted in Figure 1 A and these take the form of an activator-inhibitor system. For this network, the activator is $R(t)$, while the inhibitor is $M(t)$. In Figure 1 B, an explicit reaction network is considered, in which we incorporate both active and inactive forms of the three species and their conservation is assumed. Denoting

\begin{align*}
G_i(t) + G(t) &= G_T, \\
R_i(t) + R(t) &= R_T, \\
M_i(t) + M(t) &= M_T,
\end{align*}

where $G_T, R_T$ and $M_T$ are the total concentrations of $G−$, $R−$ and $M−$ specie, respectively.

![Figure 1](image_url)

**Figure 1.** Schematic representation of an activator-inhibitor system. A: An illustrative activator-inhibitor network model. In this set-up $R$ activates $M$; which in turn inhibits both $R$ and $G$. $R$ and $G$ form a positive feedback loop [15, 22]. B: Flow diagram representing the interactions between active and inactive forms of species. The active species are denoted respectively by the variables; $R(t), M(t)$ and $G(t)$. Their total concentrations are respectively denoted, $R_T, M_T$ and $G_T$ and these are conserved. Dotted arrows represent the catalytic activity while full arrows represent chemical reactions.
Using the conservation of mass, instead of considering a six species system, the general interaction model reduces to a system of three ODEs in $G(t)$, $R(t)$ and $M(t)$ of the form

$$\frac{dG}{dt} = f_1(G, R, M),$$
$$\frac{dR}{dt} = f_2(G, R, M),$$
$$\frac{dM}{dt} = f_3(G, R, M),$$

where the functions $f_1$, $f_2$ and $f_3$ describe the kinetic interactions between $G(t)$, $R(t)$ and $M(t)$. The ODE system is then closed with appropriate positive initial conditions.

Depending on the biological observations, different assumptions or mathematical translations of these observations may result in different mathematical representations. Since we are interested in presenting a general methodological approach, we will adopt one plausible model for illustrative purposes.

One possible and plausible mathematical interpretation of the activator-inhibitor network illustrated in Figure 1 can be postulated by the following set of three ODEs

$$\frac{dG}{dt} = \frac{k_3 R (G_T - G)}{K_{g3} + (G_T - G)} - \frac{k_1 M G}{K_{g4} + G},$$
$$\frac{dR}{dt} = \alpha G \frac{(R_T - R)}{K_{r0} + (R_T - R)} + \frac{k_3 (R_T - R)}{K_{r2} + R} - \frac{k_5 R M}{K_{r5} + M_T - M} - \frac{k_7 (M_T - M)}{M_T - M},$$
$$\frac{dM}{dt} = \frac{k_5 R (M_T - M)}{K_{m5} + M_T - M} + \frac{k_7 (M_T - M)}{M_T - M} - \frac{k_6 M}{K_{m6} + M}.$$

System (3) is defined with positive constant parameters listed in Table 1 and non-negative initial conditions are prescribed as $G(t_0) = G_0$, $R(t_0) = R_0$ and $M(t_0) = M_0$.

The following assumptions are considered in (3); $G$ activation by $R$ and inhibition by $M$ are modelled using Michaelis-Menten kinetics; the activation of $R$ by $G$ and its baseline inhibition are modelled by Michaelis-Menten kinetics; $R$ baseline activation and inhibition by $M$ are modelled by the law of mass action; the activation of $M$ by $R$ is modelled by Michaelis-Menten kinetics, whereas $M$ baseline activation and its decay are modelled by the law of mass action.

For instance, in the Rho-Myosin-GEF signalling pathway context, experimental results show that the activities of Rho and GEF change much faster than those of Myosin activities [15, 22]. To translate this biological observation, the system of ODEs can be formulated to account for different time scales. Alternatively, a quasi-steady state approximation on $G$ can be applied, that reduces System (3) to a system of two ODEs. A similar approach can be done on $R$ (see Juma [21] for
Therefore, System (3) reduces to a system of two ODEs given by

\[ 0 = \frac{k_3 R (G_T - G)}{K_{g3} + (G_T - G)} - \frac{k_4 M G}{K_{g4} + G}. \]  

(4)

This implies that \( G(t) \) equilibrates very fast. To simplify calculations, let \( u = k_3 R, \ v = k_4 M, \ J = K_{g3}/G_T, \ K = K_{g4}/G_T \) and \( g^* = G/G_T \). Substituting this in Equation (4) and rearranging we have:

\[ (v - u)g^{*2} - (v - u + vJ + uK)g^{*} + uK = 0. \]  

(5)

This quadratic equation has two roots. As \( g^* \) represents the adimensional concentration of \( G \), only roots satisfying \( 0 < g^* < 1 \) are considered; hence, there is a unique feasible root

\[ g^* = \frac{2uK}{v - u + vJ + uK + \sqrt{(v - u + vJ + uK)^2 - 4(v - u)uK}}. \]  

(6)

Equation (6) is known as the Goldbeter-Koshland function \([14]\) that represents the steady state fraction of \( G \) namely \( G^*/G_T \) and is denoted \( E_p(\cdot) \) which is given by

\[ E_p(k_3 R, k_4 M, K_{g3}/G_T, K_{g4}/G_T) = \frac{G^*}{G_T} = \frac{2k_3 \left( \frac{K_{g4}}{G_T} \right) R}{Q + \sqrt{Q^2 - 4(k_4 M - k_3 R)k_3 \left( \frac{K_{g4}}{G_T} \right) R}}, \]  

(7)

where

\[ Q = k_4 M - k_3 R + k_4 \left( \frac{K_{g4}}{G_T} \right) M + k_3 \left( \frac{K_{g4}}{G_T} R \right). \]

Therefore, System (3) reduces to a system of two ODEs given by

\[
\frac{dR}{dt} = k_0 E_p (k_3 R, k_4 M, K_{g3}/G_T, K_{g4}/G_T) \frac{(R_T - R)}{K_{r0} + (R_T - R)} + k_1 (R_T - R) - \frac{k_2 R}{K_{r2} + R} - k_2 MR := f(R, M),
\]

\[
\frac{dM}{dt} = \frac{k_3 R (M_T - M)}{K_{m5} + (M_T - M)} - k_6 M + k_7 (M_T - M) := g(R, M),
\]

(8)

where \( k_0 = \alpha G_T \) and \( E_p(\cdot) \) is Equation (7). System (8) is considered with initial conditions defined by

\[ R(t_0) = R_0 \ \text{and} \ M(t_0) = M_0, \]  

(9)

where \( 0 \leq R_0 \leq R_T \) and \( 0 \leq M_0 \leq M_T \), and \( R_T \) and \( M_T \) represent, respectively, the total concentrations of \( R \) and \( M \). Model parameters are listed in Table 1.

3. Mathematical analysis and numerical simulations. Now we proceed to present mathematical tools for the analysis of such models. First the well-posedness of model is verified.

It can be easily verified that the set

\[ S = \{(R, M) : 0 \leq R \leq R_T, \ 0 \leq M \leq M_T\}, \]  

(10)

is positively invariant under the flow of System (8); thus (8) is well-posed with bounded non-negative solutions. We now embark on investigating the asymptotic behaviour of System (8).
Parameter | Description | Base value
---|---|---
$k_0$ | $R$ activation by $G$ per unit time | 4
$k_1$ | Rate of $R$ baseline activation | 0.6
$k_2$ | $R$ baseline inhibition per unit time | 1
$k_2'$ | $R$ inhibition by $M$ per unit time | 1
$k_3$ | Rate of $G$ activation by $R$ | 1
$k_4$ | Rate of $G$ inhibition by $M$ | 1
$k_5$ | $M$ activation rate | 0.035
$k_6$ | $M$ decay rate | 0.01
$k_7$ | $M$ baseline recruitment rate | 0.001
$K_{r0}$ | Michaelis-Menten constant for $R$ activation | 1
$K_{r2}$ | Michaelis-Menten constant for $R$ self inhibition | 1
$K_{m5}$ | Michaelis-Menten constant for $M$ activation | 1
$K_{g3}$ | Michaelis-Menten constant for $G$ activation | 0.12
$K_{g4}$ | Michaelis-Menten constant for $G$ inhibition | 0.075
$R_T$ | $R$ total concentration | 1
$M_T$ | $M$ total concentration | 1
$G_T$ | $G$ total concentration | variable

Table 1. Parameters, their descriptions and values used for simulations and bifurcation analysis. Base line parameter values are taken from [43]. Some of them were adjusted to illustrate the qualitative dynamics hypothesised. These values could be estimated when considering experimental data through a parameter inference approach, this forms part of our current work (see for example [4]). The parameters $k_i$, $i = 0, \cdots, 7$, represent the reaction constants, while $K_i$, $i = r0, r2, m5, g3, g4$, represent Michaelis-Menten constants.

3.1. Qualitative analysis. The curves defined by

$$f(R, M) = 0,$$  (11a)
$$g(R, M) = 0,$$  (11b)

are respectively called the $R$- and $M$-nullclines of System (8). A steady state $(R^*, M^*)$ of System (8) satisfies $f(R^*, M^*) = g(R^*, M^*) = 0$.

Note that in System (8) the $M$–nullcline remains fixed as $G_T$ varies, while $R$–nullcline evolves. In Figure 2, $R$– and $M$– nullclines are plotted for different values of $G_T$; at small values of $G_T$, there is only one intersection of nullclines and as $G_T$ increases, we obtain up to three distinct intersections. This result forms Theorem 3.1.

**Theorem 3.1.** System (8) has at least one positive steady state solution and at most three.

**Proof.** Proof of Theorem 3.1 can be found in the appendix, see Proof A.

We are now in a position to describe the nature of the steady states. To analyse the stability of the equilibrium points of System (8), we linearise the system around the steady state $(R^*, M^*)$. With the linearisation, we obtain the Jacobian matrix
In the expressions above, \( H \) is varied. If a sign pattern allows part. It is used to investigate the existence of periodic solutions as a parameter pair of them with negative real part crosses the imaginary axis to have positive real part. It is known that the sign of the eigenvalues of its Jacobian matrix evaluated at the steady state. The solution may approach this steady state (when all eigenvalues have negative real parts) or move away from it (when some eigenvalues have positive real parts) \[5\].

Furthermore, we use the sign pattern of the Jacobian matrix to analyse the stability of the steady states of System (8), since the method does not involve quantitative analysis of the Jacobian matrix, but only signs of the corresponding entries. For an \( n \times n \) real matrix \( J \), its sign pattern \( \mathcal{J} \) is the matrix having four entries the signs of the corresponding entries in \( J \). It can be easily seen from the Jacobian matrix (12), that \( g_M < 0 \) and \( g_R > 0 \) but \( f_R \) and \( f_M \) are not sign-definite. Thus, the Jacobian matrix \( J \) has the sign pattern given by

\[
\mathcal{J} = \left[ \begin{array}{cc} \oplus & \oplus \\ + & - \end{array} \right].
\]

where \( \oplus \) represents \( -, + \) or 0 depending on the sign of the corresponding entry term. Sign pattern analysis allows to explore the possibility of having periodic solutions arising from Hopf bifurcation, just by analysing if the sign matrix of the Jacobian matrix admits some sets of refined inertia; the refined inertia of an \( n \times n \) real matrix is an ordered 4-tuple \( ri(J) = (n_+, n_-, n_z, 2n_p) \) where \( n_i, \ i = +, -, z, p \), refers to the number of eigenvalues with positive and negative real parts, zero eigenvalues and purely imaginary eigenvalues, respectively \[24\]. To aid analysis, the following set of three refined inertia will be used:

\[
\mathbb{H}_n = \{(0, n, 0, 0), (0, n - 2, 0, 2), (2, n - 2, 0, 0)\}
\]

\( \mathbb{H}_n \) was introduced in \[1\] and it corresponds to the transition of eigenvalues as the pair of them with negative real part crosses the imaginary axis to have positive real part. It is used to investigate the existence of periodic solutions as a parameter is varied. If a sign pattern allows \( \mathbb{H}_n \) when a particular parameter varies \[9\], then
there is a Hopf bifurcation at some value of the parameter and hence the possibility of linearly stable periodic solutions. The only $2 \times 2$ sign pattern that allows $H_2$ is

$$
\begin{pmatrix}
  + & - \\
  + & -
\end{pmatrix},
$$

(14)

and the equivalent sign patterns obtained from (14) by any combination of transposition, permutation or signature similarity [5]. Such sign patterns may admit periodic solutions [1, 9]. The sign pattern shown in Equation (13) admits $H_2$ if $f_R > 0$ and $f_M < 0$. With numerical computations, we verified that for parameter values from Table 1, $f_M$ is always negative; then, the stability of the eigenvalues will depend upon the sign of $f_R$. We therefore state the following particular result on conditions for the existence of oscillatory regime.

**Theorem 3.2.** Provided that $f_M < 0$, for the System (8), we have the following:

i) If $f_R \leq 0$, there exists an equilibrium point that is locally asymptotically stable and there is no possibility of periodic solutions.

ii) Otherwise if $f_R > 0$ then periodic solutions are possible and Hopf bifurcation occurs for some parameter values.

**Proof.** The proof of Theorem 3.2 can be found in the appendix, see Proof B.

![Figure 2](image.png)

**Figure 2.** Typical shapes for nullclines corresponding to System (8) using different values of $G_T$. As the parameter $G_T$ varies, we obtain up to three different nullcline intersections. The blue curve indicates the M-nullcline, while the brownish to redish curves indicate R-nullclines at different values of $G_T$.

Moreover, as it can be observed in Figure 2, the local configurations of both nullclines at a point of intersection differ and that can be used to characterise and classify steady states. As $G_T$ increases, possible equilibria are labelled $E_i$, $i =$
1, \cdots, 6, in Figure 3, and there exist three different types of local configurations grouped as

\[ E_1 \sim E_3 \sim E_4 \sim E_6, \]
\[ E_2 \text{ and } E_5. \]

The nature of the possible steady states catalogued in Figure 3 is summarised by

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{nullclines.png}
\caption{Qualitative forms of nullcline intersections corresponding to System (8) as the parameter $G_T$ varies. (a) For $G_T = 0.5$, the steady state $E_1$ is globally asymptotic stable (G.A.S.). (b) With $G_T = 1.2$, there is possibility of $H_2$ and hence periodic solutions might occur around $E_2$, or $E_2$ is asymptotically stable. (c) For $G_T = 7$, the steady state in the form of $E_3$ is G.A.S. (d) For $G_T = 20$, there exists a bistable behaviour ($E_4$ and $E_6$ are locally asymptotically stable (L.A.S.) and $E_5$ is a saddle point).}
\end{figure}

Theorem 3.3.

**Theorem 3.3.** The stability of steady states of System (8), $E_i$, for $i = 1, \ldots, 6$ defined in Figure 3 when they exist can be classified as follows.
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Case 1: When there exists an unique equilibrium point,

(i): Any equilibrium in the form of $E_1$ (see Figure 3(a)), or $E_3$ (see Figure 3(c)) is globally asymptotically stable (G.A.S.).

(ii): For the case of an equilibrium in the form of $E_2$ (see Figure 3(b)) with $\det(J) > 0$, the following possibilities arise:

- If $\text{tr}(J) < 0$, $E_2$ is G.A.S. when there exists no limit cycle or $E_2$ is locally asymptotically stable (L.A.S) when there exists a limit cycle.
- If $\text{tr}(J) > 0$, we have an unstable node for $\text{tr}(J)^2 > 4\det(J)$, or an unstable spiral for $\text{tr}(J)^2 < 4\det(J)$. In both cases, there exits a stable limit cycle.

Case 2: When there exist three equilibrium points, $E_4$, $E_5$ and $E_6$ (see Figure 3(d)), the equilibrium points are L.A.S., unstable (a saddle point) and L.A.S., respectively.

Proof. The proof of Theorem 3.3 can be found in the appendix, see Proof C.

3.2. Numerical simulations. The system of ODEs is solved numerically by using ode45 in MATLAB [30, 40]. Figure 4 shows the numerical simulations corresponding to System (8), illustrating various dynamic regimes at different values of $G_T$. For small values of $G_T$, the system has a unique steady state which is globally asymptotically stable (see numerical simulation in Figure 4(a)), this is the case shown in Figure 3(a). As the value of $G_T$ increases, the steady state becomes unstable and hence we observe periodic solutions as shown by the numerical simulation in Figure 4(b), a case shown in Figure 3(b). Further increase in the value of $G_T$ makes the system exhibit excitable dynamics (case of Figure 3(c) and numerical simulation shown in Figure 4(c)). At higher values of $G_T$, the system exhibits bistable dynamics as shown in Figure 4(d) and case shown in Figure 3(d).

In the next section, we demonstrate using numerical bifurcation analysis, how the system transitions between these regimes as the total $G$ concentration $G_T$ is varied.

3.3. Numerical bifurcation analysis. The numerical bifurcation analysis was carried out using Matcont [7, 8, 18, 41]. Matcont is a MATLAB based software for interactive study of dynamical systems. This software allows the computation of equilibrium solutions and their continuation with respect to parameters. We used the parameter values listed in Table 1 for numerical bifurcation analysis.

Numerical bifurcation analysis provides a summary of the effect $G_T$, on the value, the number and nature of the equilibrium points. In Figure 5, we show bifurcation diagrams when $G_T$ is varied. For some parameter values just by varying $G_T$, System (8) exhibits up to three dynamical regimes, corresponding to stable, oscillatory and bistable solutions as shown in Figure 5(a). As $G_T$ increases, $R^*$ slowly decreases and then at high values of $G_T$, the model has three steady states, two of which are stable and separated by a saddle point. The one-parameter bifurcation diagram Figure 5(a) is extended in the $G_T$–$k_1$ plane resulting in a two-parameter bifurcation diagram as shown in Figure 5(b). Figure 5(b) gives regions of the $G_T$–$k_1$ plane with distinct dynamical behaviours where, the colourless regions define a parameter space characterised by stable steady state dynamics, red region defines a parameter space with oscillatory behavior, while the yellow region defines a parameter space with bistable dynamics.
Figure 4. Numerical simulations illustrating time-series dynamics of \( R(t) \) and \( M(t) \) for System (8) corresponding to different dynamic regimes. As the value of \( G_T \) increases, the system transitions from stable ((a), \( G_T = 0.5 \)), oscillatory ((b), \( G_T = 2 \)), excitable ((c), \( G_T = 7 \)) and then to bistable ((d), \( G_T = 15 \)). A minimum perturbation of 0.09 from the steady state has to be taken to exhibit excitable dynamics. In the bistable regime, we used initial conditions \((0.4, 0.7)\) and \((0.6, 0.3)\) to approach both L.A.S. equilibria. Base values for parameters are shown Table 1.

4. Sensitivity analysis. To close the array of mathematical tools for model analysis, we present sensitivity analysis. Due to the uncertainty in model parameters, we carry out sensitivity analysis to quantify the effect of parameter variations or perturbations on the model output in different dynamic regimes (stable and oscillatory). We use local sensitivity analysis to characterise the limit cycle of an oscillatory dynamical system in terms of parameter variations. This allows us to characterise amplitude and period sensitivity to parameter variations. We use the direct differential method (DDM) to compute the local sensitivity, since it provides.
a complete information on each sensitivity index as a function of the independent variable [35, 47].

For completeness’ sake, we present a brief description of local sensitivity technique next. The interested reader can find further detailed descriptions in [25, 27, 28, 42, 47]. Consider a general ordinary differential equation system given by

$$\dot{x}(t) = f(x(t), p), \quad x(t_0) = x_0, \quad (15)$$

where $x \in \mathbb{R}^m$ is the vector of dependent or state variables and $p \in \mathbb{R}^m$ are the parameters of the model. If the solution of System (15) exists, then the state sensitivity matrix is defined by

$$S(t) = \frac{\partial x}{\partial p} \bigg|_{(x(t, p_0), p_0)} , \quad (16)$$

where $p_0$ defines the base parameter values. To find the state sensitivity matrix (16), Equation (15) is differentiated with respect to parameter, $p$, to get

$$\dot{S} = J(t, p_0)S + B(t, p_0), \quad S(t_0, p_0) = S_0, \quad (17)$$

where $J$ is the Jacobian of the model, $B(t, p_0) = \frac{\partial f}{\partial p} \bigg|_{p_0}$ is the Jacobian with respect to parameters and $S(t_0, p_0) = S_0$ is the initial sensitivity to parameters, it is generally taken as zero for each parameter.
Equations (15) and (17) are then solved simultaneously to obtain the sensitivity matrix \( S \) given initial conditions \( x(t_0) = x_0 \), baseline parameter values \( p_0 \) and initial sensitivity to parameter \( S_0 \). Here, we assume that System (15) can exhibit a stable or oscillatory regime as parameters are varied as our system of interest. We subdivide the local sensitivity analysis into two parts; in the first part, the baseline parameter values are selected to have the dynamics in the stable regime and in the second part, they are selected to place the system in the oscillatory regime. When the steady state is stable, then the DDM solution to the system given by Equations (15) and (17) also converges [27, 47], and hence the sensitivity to each parameter can be extracted. This is then used to interpret the sensitivity analysis results in the stable regime.

For the local sensitivity analysis in the oscillatory regime, suppose that the solution of the system of differential equations is periodic in time with period \( \tau \), then

\[
x(t + \tau) = x(t).
\]

From (18), it is then possible to expand each of the state variables \( x_i(t) \) of \( x(t) \) as Fourier series, [25, 27, 42, 47] as

\[
x_i(t) = \sum_{n=0}^{\infty} \left[ a_{ni} \cos \left( \frac{2n\pi t}{\tau} \right) + b_{ni} \sin \left( \frac{2n\pi t}{\tau} \right) \right].
\]

(19)

Fourier coefficients \( a_{ni} \) and \( b_{ni} \) are functions of the parameters. Assuming that \( \tau \), depends on at least one parameter and suppose \( S_\tau \) is the period sensitivity, then

\[
S_\tau = \left[ \frac{\partial \tau}{\partial p_1}, \ldots, \frac{\partial \tau}{\partial p_m} \right].
\]

(20)

\( S_\tau \) is a constant vector that contains sensitivity of the period to individual parameters. Since \( \tau \) depends on \( p \) and using Equation (19), the state sensitivity matrix (17) in the oscillatory regime can be decomposed into two parts such that

\[
S = S_{ub} + S_c,
\]

where \( S_{ub} = -\frac{t}{\tau} f S_\tau \) and \( S_c = \left[ \frac{\partial x_i}{\partial p_j} \right]_{\tau} \).

In the oscillatory regime, since \( f \neq 0 \) and \( S_\tau \neq 0 \), it is clear that \( S_{ub} \) grows linearly with \( t \), hence it becomes the dominant term at very large points. Therefore, as \( t \to \infty \), Equation (21) can be approximated by

\[
S \approx S_{ub} = -\frac{t}{\tau} f S_\tau.
\]

(21)

Furthermore, \( S_c \), which is an \( m_x \times m_p \) matrix, is the cleaned-out sensitivity matrix, evaluated at a constant period [42]. It is temporal periodic and captures the effect of parameters on the shape of the trajectory at a constant period [27, 28, 47]. To calculate the period sensitivity, a singular value decomposition (SVD) method (see [28, 47] for the derivation) is applied to the state sensitivity matrix (21), leading to the period sensitivity formula

\[
S_\tau \approx -\frac{\tau}{\phi^2 t^2} f^T \tilde{S}_1,
\]

(22)

where \( \phi^2 = f^T f \) and the term \( \tilde{S}_1 = \sigma_1 u_1 v_1^T \) is the largest SVD term of the state sensitivity matrix \( S \), with \( \sigma_1 \) the largest singular value of \( S \) while \( u_1 \) and \( v_1 \) are the respective output and input vectors corresponding to \( \sigma_1 \). It is shown that \( S_{ub} \) and \( S_c \) form orthogonal components of the SVD matrix, and \( S_{ub} = \tilde{S}_1 \) at higher time.
points \[27\]. Therefore, \( S \) is approximated as the sum of all the remaining SVD terms and is given by

\[
S_c \approx \sum_{i=2}^{r} \tilde{S}_i, \quad \text{where} \quad \tilde{S}_i = \sigma_i u_i v_i^T \quad \text{and} \quad r = \text{rank}(S).
\]  

Having calculated the cleaned-out sensitivity matrix, we calculate the amplitude sensitivity by first defining the amplitude for the variable \( x_i \) as

\[
A_{mi} = x_i(t_{max,i}) - x_i(t_{min,i}),
\]

where \( t_{max,i} \) and \( t_{min,i} \) are the time points where the local maximum and minimum occur within the period. At the local extrema of \( x_i \), \( f_i = 0 \), hence we can clearly see from Equation (21) that \( S_i = S_{ci} \), therefore, the amplitude sensitivity \( S_{A_{mi}} \) corresponding to the variable \( x_i \) is calculated from (see [42] for further details)

\[
S_{A_{mi}} = S_{ci}(t_{max,i}) - S_{ci}(t_{min,i}).
\]

Now we are in a position to apply the derived sensitivity approach to our ODE system.

In Figure 6, we illustrate the local sensitivity results for the ODE System (8) around the baseline parameter values given in Table 1. We use parameters \( G_T = 0.25 \) and \( G_T = 0.4 \) to calculate local sensitivity in the stable and oscillatory regimes, respectively. In the stable regime, the sensitivity matrix is bounded as shown in Figures 6(a) and 6(b). The steady state sensitivity, calculated from this, results after the convergence has been achieved, (results are shown in Figure 6(c)). The sensitivity values are normalised to enable comparison between results. Both \( R^* \) and \( M^* \) are highly sensitive to parameters \( k_3, k_4 \) and \( k_0 \). Some parameters (for example, \( k_5, k_6 \) and \( K_{m5} \)) have an opposite effect to \( R^* \) and \( M^* \) steady states. These parameters have direct influence on the \( M(t) \) dynamics. Small variations in these parameters, produce opposite effects to \( R(t) \) and \( M(t) \) activities, since \( M(t) \) is an inhibitor of \( R(t) \). Other parameters like \( k_1, k_7 \) and \( G_T \) have very little influence on both values of \( R^* \) and \( M^* \). From the sensitivity analysis results, the steady state values are highly sensitive to variations in parameters related to the positive feedback mechanism.

In the oscillatory regime, the sensitivity matrix is unbounded and grows linearly with time as shown in Figures 6(d) and 6(e). Therefore, at large time point, the cleaned-out sensitivity \( S_c \) becomes negligible. Hence to find the sensitivity in the oscillatory regime, a singular value decomposition approach is applied to the state sensitivity matrix [28, 47]. This enables the determination of period sensitivity from Equation (22). The period sensitivity time series is convergent, and therefore at higher time values, the sensitivity of the period to parameters is calculated and results are as shown in Figure 7. It turns out that the period is highly sensitive to \( k_3, k_4, G_T \) and \( k_5 \). An infinitesimal increase in \( k_3 \) increases the period of oscillation while \( k_4 \) has the opposite effect. Similarly an infinitesimal increase in \( G_T \) increases the period. The parameter \( G_T \) has little effect on the steady state values of \( R(t) \) and \( M(t) \), but it greatly influences its oscillatory behaviour. \( k_7 \) has very little influence on the period. The amplitude sensitivity is the difference between the cleaned-out sensitivities evaluated at the time points where the local maximum and minimum of \( R(t) \) and \( M(t) \) occur within the period. The cleaned-out sensitivity is bounded and periodic, while its corresponding state sensitivity is unbounded. To illustrate this behaviour, we show in Figure 8(a) the cleaned-out sensitivity for only two parameters. Figure 8(b) shows amplitude sensitivity to parameters, the top panel.
Local sensitivity profiles for System (8) with $G_T = 0.25$ and $G_T = 0.4$ selected to represent stable and oscillatory regimes respectively. Other parameters are fixed as shown in Table 1. The sensitivity matrix is bounded in the stable regime, but unbounded in the oscillatory regime. The colour codes used in (d) and (e) are the same as those used in (a) and (b).
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Figure 7. Period sensitivity results corresponding to System (8). (a) shows convergent time series of period sensitivity calculated from Equation (22) while (b) shows a bar graph of normalised period sensitivities extracted from (a) after convergence.

shows $R(t)$ amplitude sensitivity while the bottom panel shows $M(t)$ amplitude sensitivity.

To summarise, in both stable and oscillatory regimes, $k_3$ and $k_4$ are among the parameters which the system is highly sensitive to their variations. Therefore, we remark that the system is sensitive to dynamics associated to the positive feedback mediator, G. This is in agreement with the experimental results in [15, 22], which reveal that the positive feedback mediator is essential for oscillatory Rho activity dynamics. We also notice that small variations in the parameters which directly affect $M(t)$ dynamics ($k_5$, $K_{M5}$ and $k_6$) induce opposite responses to $R(t)$ and $M(t)$ activities. Furthermore, we note that small changes to the parameters associated with $R(t)$ inhibition ($k_2$, $K_{r2}$ and $K_{g4}$), induce similar responses to the period of oscillations and $M(t)$ amplitude. However, they induce an opposite response to $R(t)$ amplitude.

5. Conclusion and discussions. Mathematical and computational models have the capacity and ability to assist theoreticians and experimentalists in gaining an understanding of the mechanisms that drive biological processes. Given the increasing availability of detailed experimental datasets, new mathematical models are being formulated and these require detailed mathematical analysis to unravel the model dynamics when parameters are varied. In this study, we presented a hypothetical activator-inhibitor model that describes a GEF, Rho and Myosin interaction network. We then used this model to expose mathematical tools for its analysis. The methodological toolkits presented include nullcline and sign pattern analysis, numerical bifurcation analysis, and sensitivity analysis. Theoretical results were illustrated by use of numerical simulations of the model system. The mathematical tools presented here can be applied to analyse model systems of this nature. See for example [2, 21, 22, 31].

The mathematical analysis of the model’s steady state has allowed us to characterise regimes where the model exhibits different dynamical behaviour dependent
Figure 8. Cleaned-out and amplitude sensitivity results for System (8). (a) The blue curve is the cleaned-out sensitivity obtained from Equation (23). The red curve is the corresponding unbounded state sensitivity before SVD was applied as shown in Figures 6 (d) and (e). (b) $R(t)$ and $M(t)$ amplitude sensitivities to parameters calculated with Equation (25).
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Numerical bifurcation analysis provided a summary of the effect of the total concentration of the positive feedback mediator, $G_T$, on the value, number and nature of the steady state solutions. For small values of $G_T$, the model has only one steady state which is stable. As the value of $G_T$ increases, the steady state loses its stability via Hopf bifurcation, giving rise to stable periodic solutions. Further increases in $G_T$ transforms the steady state back to stable (excitable) via another Hopf bifurcation and further increase pushes the system to the region characterised by three steady states, two are stable separated by a saddle point. Therefore, the activator-inhibitor system exhibits up to four distinct regimes depending on the value of $G_T$, corresponding to stable, oscillatory, excitable and bistable. We note that results of the sensitivity analysis show that the system is more sensitive to parameter values associated with the positive feedback module.

In our model, we used assumptions based on the work of Tyson et al. [43], combined with insights inspired by the recent work of Graessl et al. [15] and Kamps et al. [22] to describe species interactions. The variations of these mathematical assumptions or translations of the biological observations are deferred to future studies, in which we will consider specific mechanisms of different reactions and their mathematical translations based on specific biological assumptions. The interested reader is referred to the work by Juma [21] in which a series of different mathematical models were derived and presented depending on the mathematical interpretations of the molecular pathways. We reiterate again, that in this work, we restrict ourselves to one plausible model describing the temporal dynamics of the three species.

It must be noted, however, that the current model predicts the qualitative dynamics and switching of different states of the biological network of GEF, Rho and Myosin interactions. The switching is mediated by expression levels of the positive feedback mediator, GEF. Furthermore, experimental results showed that the activity dynamics of Rho are dependent on positive feedback mediator’s expression levels [15, 22].

In future studies, we will carry out model comparisons to explore the best model that best-fits experimental observations in a quantitative approach. The variations of the mathematical assumptions and interpretations give rise to different models (see for example [21]). Such models can be analysed using the tools presented here. Results obtained will form a basis for model comparison, which provides a powerful tool to identify mechanisms that drive dynamics (see for example [19, 26, 34, 33]). With a given set of experimental data, such models can be fitted, for example via a Bayesian approach [4]. The best model to fit experimental data can then be selected among the many formulated.

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**Data Management and Sharing:** All data generated computationally is included. MATLAB algorithms are available on request.

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Appendix A. Proof of Theorem 3.1.

Proof. Since System (8) is planar and has bounded solutions in $\mathcal{S}$, it therefore follows that it has at least one positive equilibrium solution in $\mathcal{S}$ by Poincaré theory [20]. It is easy to verify that there is no boundary equilibrium.

To prove that we have at most three steady state solutions, we consider $R$–nullcline and $M$–nullcline, $f(R, M) = 0$ and $g(R, M) = 0$, and show that $R$–nullcline is a cubic curve in $R$ while $M$–nullcline is monotonic increasing in $\mathcal{S}$.

Consider $R$–nullcline given by:

$$f(R, M) = k_0 E_p(\cdot) \frac{(R_T - R)}{K_{r_0} + (R_T - R)} + k_1(R_T - R) - \frac{k_2 R}{K_{r_2} + R} - k_2^2 M R = 0.$$  \hspace{1cm} (26)

$E_p(\cdot)$ represents the steady state fraction of the concentration of $G$, $G^*/G_T$ where $0 \leq G^* \leq G_T$ is the steady state of $G$. Hence we have that $0 < E_p(\cdot) \leq 1$. Equation (26) is simplified by taking $E_p(\cdot)$ as a parameter. Appropriate mathematical substitutions entail that Equation (26) can be written as a cubic polynomial in $R$ given by:

$$A_3 R^3 + A_2 R^2 + A_1 R + A_0 = 0,$$  \hspace{1cm} (27)

where the constants $A_i$, $i = 1, 2, 3$ depend on parameters, $E_p(\cdot)$ and $M$.

Consider also the $M$–nullcline given by:

$$g(R, M) = \frac{k_5 R (M_T - M)}{K_{m_5} + (M_T - M)} - k_6 M + k_7 (M_T - M) = 0.$$  \hspace{1cm} (28)

The total derivative of $g(R, M) = 0$ gives:

$$\frac{dM}{dR} \bigg|_{g=0} = -\frac{g_R}{g_M} > 0,$$

since $g_R = \frac{k_5 (M_T - M)}{K_{m_5} + (M_T - M)} > 0$, and $g_M = -\frac{k_5 R K_{m_5}}{(K_{m_5} + (M_T - M))^2} - k_6 - k_7 < 0$. Thus, $g(R, M)$ Equation (28) is monotonic increasing in $\mathcal{S}$. This implies that there can only be at most three distinct intersections between $R$– and $M$–nullclines, and hence, Theorem 3.1 holds. \hfill $\square$

Appendix B. Proof of Theorem 3.2.

Proof. Assume that $f_M < 0$.

Case 1: Suppose $f_R \leq 0$. The sign matrix takes the form:

$$\mathcal{J} = \begin{bmatrix} - & - \\ + & - \end{bmatrix}.$$  

It can be easily seen that $\text{tr}(J) < 0$ and $\det(J) > 0$. Therefore, the equilibrium point is locally asymptotically stable.

Case 2: Suppose $f_R > 0$. The sign matrix takes the form:

$$\mathcal{J} = \begin{bmatrix} + & - \\ + & - \end{bmatrix}.$$  

This sign pattern allows:

$$\mathcal{H}_2 = \{(0, 2, 0, 0), (0, 0, 0, 2), (2, 0, 0, 0)\}.$$
Appendix C. Proof of Theorem 3.3.

Proof. Consider the general system given by:

\[
\frac{dR}{dt} = f(R, M), \quad (29a)
\]
\[
\frac{dM}{dt} = g(R, M). \quad (29b)
\]

Define \( f_* = \frac{\partial f(R, M)}{\partial R} \) and \( g_* = \frac{\partial g(R, M)}{\partial R} \), where \(* \) represents \( R \) or \( M \). The functions \( f_R, f_M, g_R \) and \( g_M \) are evaluated at the point of interest \( E_i \) shown in Figure 3. To characterize the nature of a steady state \( E_i \), we consider the local configuration of nullclines at the intersection defining \( E_i \). At a steady state \( E_i \), the shape of the nullclines is shown in Figure 3. Furthermore, on the \( R \)-nullcline \( f(R, M) = 0 \), we have:

\[
df = f_R dR + f_M dM = 0,
\]
and therefore get;

\[
\frac{dM}{dR} \bigg|_{f=0} = -\frac{f_R}{f_M}. \quad (30)
\]

Similarly along the \( M \)-nullcline \( g(R, M) = 0 \), we have;

\[
\frac{dM}{dR} \bigg|_{g=0} = -\frac{g_R}{g_M}. \quad (31)
\]

(a): Steady state \( E_1 \)

From the local configurations of nullclines at the steady state \( E_1 \) as shown in Figure 3(a), we have that;

\[
\frac{dM}{dR} \bigg|_{f=0} < \frac{dM}{dR} \bigg|_{g=0}.
\]

Equivalently,

\[
-\frac{f_R}{f_M} < -\frac{g_R}{g_M}.
\]

This simplifies to

\[
\frac{f_R}{f_M} > \frac{g_R}{g_M}. \quad (32)
\]

We first find signs of entries of the Jacobian matrix. At \( E_1 \),

\[
\frac{dM}{dR} \bigg|_{f=0} = -\frac{f_R}{f_M} < 0,
\]

therefore, \( f_R \) and \( f_M \) have the same sign. As we move parallel to the \( R \)-axis through the point of intersection \( E_1 \), we observe that \( f(R, M) \) changes from positive to negative. This means \( f(R, M) \) decreases and therefore, \( f_R < 0 \) which implies \( f_M < 0 \).

Similarly, at \( E_1 \),

\[
\frac{dM}{dR} \bigg|_{g=0} = -\frac{g_R}{g_M} > 0,
\]
therefore, \( g_R \) and \( g_M \) have opposite signs. As we move parallel to the \( M \)-axis through the point of intersection \( E_1 \), we observe that \( g(R, M) \) changes from positive to negative. This means \( g(R, M) \) decreases and therefore, \( g_M < 0 \) which implies that \( g_R > 0 \). From the above we can write the sign pattern of the Jacobian matrix around the steady state \( E_1 \) and is given by:

\[
J_{E_1} = \begin{bmatrix}
- & + \\
+ & -
\end{bmatrix}.
\]

(33)

From (33), the trace of the Jacobian matrix is such that \( \text{tr}(J(E_1)) < 0 \).

Back to Equation (32) and since \( f_M \) and \( g_M \) are both negative, we have that

\[
f_R g_M > g_R f_M,
\]

and therefore it follows that at \( E_1 \)

\[
\det(J(E_1)) = f_R g_M - g_R f_M > 0,
\]

(34)

\[
\text{tr}(J(E_1)) = f_R + g_M < 0.
\]

(35)

Conditions (34) and (35) are sufficient for local stability, and, therefore, \( E_1 \) is locally asymptotically stable (L.A.S.). Furthermore, the sign pattern \( J_{E_1} \) from (33) does not allow \( H_2 \) [1, 5]; therefore, a Hopf bifurcation leading to periodic solutions is not possible. There is no limit cycle centred at the equilibrium \( E_1 \).

In the case of \( E_1 \), the equilibrium is unique and L.A.S. (Figure 3(a)), as there is no limit cycle, the global asymptotic stability of the equilibrium can be concluded by invoking Poincaré-Bendixson theorem [20]. Hence, \( E_1 \) is globally asymptotically stable (G.A.S.).

At \( E_3 \), nullclines exhibit the same local configuration as at \( E_1 \) (Figure 3(c)). Then a similar analysis can be carried out for equilibrium \( E_3 \). When the equilibrium \( E_3 \) exists, it is unique and L.A.S. and there is no limit cycle. Therefore, \( E_3 \) is G.A.S.

At \( E_4 \) and \( E_6 \), the nullclines exhibit the same local configuration as that at \( E_1 \) (see Figure 3(d)). Hence, some conclusions drawn for \( E_1 \) hold for \( E_4 \) and \( E_6 \); \( E_4 \) and \( E_6 \) are L.A.S. and there is no limit cycle neither centred at \( E_4 \) nor at \( E_6 \). However, when \( E_4 \) and \( E_6 \) exist, they are not unique, the equilibria \( E_4 \) and \( E_6 \) co-exist with \( E_5 \). Hence, \( E_4 \) and \( E_6 \) are L.A.S and there is no limit cycle centered at them. The nature of the stability of \( E_5 \) is investigated later.

(b): Steady state \( E_2 \)

To analyse the steady state \( E_2 \) we will consider the local configuration of nullclines shown in Figure 3(b). At the point \( E_2 \) we have that:

\[
\frac{dM}{dR}\bigg|_{f=0} < \frac{dM}{dR}\bigg|_{g=0} \Rightarrow \frac{-f_R}{f_M} < \frac{-g_R}{g_M}.
\]

This simplifies to

\[
\frac{f_R}{f_M} > \frac{g_R}{g_M}.
\]

(36)

We also find the signs of entries of the corresponding Jacobian matrix. At \( E_2 \),

\[
\frac{dM}{dR}\bigg|_{f=0} = -\frac{f_R}{f_M} > 0,
\]

therefore, \( f_R \) and \( f_M \) have opposite signs. As we move parallel to the \( R \)-axis through the point of intersection \( E_2 \), we observe that \( f(R, M) \) changes from
negative to positive. This means $f(R, M)$ increases and therefore, $f_R > 0$, this implies that $f_M < 0$. Similarly at $E_2$,
\[
\left. \frac{dM}{dR} \right|_{g=0} = -\frac{g_R}{g_M} > 0,
\]
therefore $g_R$ and $g_M$ have opposite signs. As we move parallel to the $M$-axis through the point of intersection $E_2$, we observe that $g(R, M)$ changes from positive to negative. This means $g(R, M)$ decreases and therefore $g_M < 0$, which implies that $g_R > 0$. From the above we can write the sign pattern of the Jacobian matrix at the steady state $E_2$ which is given by
\[
\mathcal{J}_{E_2} = \begin{bmatrix} + & - \\ + & - \end{bmatrix}.
\]
From (37), we find that the trace of the Jacobian matrix is such that $\text{tr}(\mathcal{J}(E_2))$ is not sign definite. Back to Equation (36) and since $f_M$ and $g_M$ are both negative, we have that
\[
\frac{f_R g_M}{f_M} > g_R f_M,
\]
and therefore it follows that at $E_2$
\[
\text{det}(\mathcal{J}(E_2)) = f_R g_M - g_R f_M > 0.
\]
From the sign pattern analysis, the sign matrix (37) allows $\mathcal{R}_2$ and therefore, it is possible to have a limit cycle and therefore, there is a possibility of linearly stable periodic solutions arising from a Hopf bifurcation \cite{1} as a parameter is varied. Summing up, for $E_2$, we can have:

(i) if $\text{tr}(\mathcal{J}(E_2)) = f_R + g_M > 0$, $E_2$ is an unstable node ($\text{tr}(\mathcal{J}(E_2))^2 - 4 \text{det}(\mathcal{J}(E_2)) > 0$) or spiral ($\text{tr}(\mathcal{J}(E_2))^2 - 4 \text{det}(\mathcal{J}(E_2)) < 0$). By the Poincaré-Bendixon criterion, as $E_2$ is a unique equilibrium point, there exists a stable limit cycle \cite{20}.

(ii) if $\text{tr}(\mathcal{J}(E_2)) = f_R + g_M < 0$, and a limit cycle centered at $E_2$ exists, $E_2$ is L.A.S. If there is no limit cycle, as $E_2$ is unique, by the Poincaré-Bendixon criterion, $E_2$ is G.A.S.

(c): Steady state $E_5$

To analyse the nature of steady state $E_5$ we will consider Figure 3(d) and what happens locally around the intersection point $E_5$. At the point $E_5$ we have that
\[
\left. \frac{dM}{dR} \right|_{f=0} > \left. \frac{dM}{dR} \right|_{g=0} \Rightarrow \frac{f_R}{f_M} > -\frac{g_R}{g_M}.
\]
This simplifies to
\[
\frac{f_R}{f_M} < \frac{g_R}{g_M}.
\]
At $E_5$,
\[
\left. \frac{dM}{dR} \right|_{f=0} = -\frac{f_R}{f_M} > 0,
\]
therefore $f_R$ and $f_M$ have opposite signs. As we move parallel to the $R$-axis through the point of intersection $E_5$, observe that $f(R, M)$ changes from negative to positive. This means $f(R, M)$ increases and therefore $f_R > 0$. This implies that $f_M < 0$. Similarly at $E_5$,
\[
\left. \frac{dM}{dR} \right|_{g=0} = -\frac{g_R}{g_M} > 0,
\]
therefore $g_R$ and $g_M$ have opposite signs. As we move parallel to the $M$-axis through the point of intersection $E_5$, we observe that $g(R, M)$ changes from positive to negative. This means $g(R, M)$ decreases and therefore $g_M < 0$. This implies also that $g_R > 0$. From the above we can write the sign pattern of the Jacobian matrix at the steady state $E_5$ which is given by

$$J_{E_5} = \begin{bmatrix} + & - \\ + & - \end{bmatrix}. \quad (40)$$

Back to Equation (39) and since $f_M$ and $g_M$ are both negative, we have that

$$f_R g_M < g_R f_M,$$

and therefore, at $E_5$

$$\det(J(E_5)) = f_R g_M - g_R f_M < 0. \quad (41)$$

The steady state $E_5$ is a saddle point and unstable. A closed path cannot surround a region containing only a saddle point [20], and therefore no possibility for existence of a limit cycle centered at $E_5$.

In situations as shown in Figures 3(a)-3(c), the System (8) has a unique equilibrium solution $E_1$, $E_2$ or $E_3$. In Figures 3(a) or 3(c), the unique equilibrium, $E_1$ or $E_3$, is G.A.S. In Figure 3(b), if $f_R > -g_M$ (trace is positive), then there exists a linearly stable limit cycle. If $f_R < -g_M$ (trace is negative), the unique equilibrium $E_2$ is G.A.S when there is no limit cycle or $E_2$ is L.A.S. when there exists a limit cycle.

In Figure 3(d), when the three steady states $E_4$, $E_5$ and $E_6$ exist: $E_4$ and $E_6$ are always L.A.S. (and no limit cycle exists) and $E_5$ is always a saddle point; there is a bistability (two stable steady states).