Autonomously oscillating biochemical systems: parametric sensitivity of extrema and period

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Abstract: This work addresses sensitivity analysis of autonomously oscillating biochemical systems. Building on results from the engineering literature, a general analysis is presented which addresses key features of oscillatory trajectories, namely the period and local maximum or minimum values of species concentrations and reaction rates. A discussion of sensitivity invariants generalises results from steady-state sensitivity analysis to this context. The results are illustrated by the application to a model of a circadian oscillator.

1 Introduction

Sensitivity analysis plays an important role in the study of biochemical systems. Mathematical approaches to such analysis have been introduced through the fields of metabolic control analysis (MCA) [1, 2, 3, 4] and biochemical systems theory (BST) [5, 6]. These analyses have proven useful both by providing insight into the nature and function of biochemical systems as well as by serving to predict the results of interventions, e.g. for pharmaceutic or metabolic engineering purposes. In addition, issues of sensitivity often arise in model validation, since models of biological systems are expected to reflect the robustness observed in nature.

In the study of biochemical networks, it is often the case that steady-state behaviour is of primary interest (not least because this is the simplest behaviour to investigate experimentally). As a consequence, the sensitivity analysis of systems at steady state has proven adequate for many investigations and continues to be the primary tool from the field which is in use today. However, the dynamic behaviour of biochemical systems is increasingly coming under investigation. This is due in part to advances in experimental techniques, and in part to an increasing awareness that a complete understanding of many of the mechanisms within the cell will only be reached once their dynamic behaviour has been described. As a result, a number of approaches to the extension of classical (steady-state) sensitivity analysis have appeared.

General definitions of time-varying sensitivity functions were first given in this context in [7] (see also [8, 9, 10]). This extension of the standard analysis allows treatment of systems whose behaviour is primarily dynamic. While these results have proven useful, there is one form of dynamic behaviour for which they do not provide satisfactory descriptions, namely autonomously oscillating systems. Such systems underly many of the periodic phenomena which have been identified in biology (e.g. glycolytic oscillations, the cell cycle, circadian rhythms, periodic neuronal signals). See [11, 12] for reviews.

It has long been recognised (e.g. [13] and references therein) that the sensitivity of various features of oscillating systems (e.g. period and amplitude) could be estimated through simulation, even if there was no general theory to provide a means of analytic computation. A first step towards such a theory was presented in [14], in which a satisfactory treatment of systems exhibiting *forced* oscillations is given. This theory was complemented by the results in [15] which address sensitivity of Fourier coefficients (see also [16, 17]). Autonomously oscillating systems were treated by this approach in [18]. Despite these excellent contributions, there has yet to appear a general treatment of sensitivity of autonomously oscillating biochemical systems. This paper complements the existing work by providing such an exposition.

Sensitivity analysis has a long history in the context of automatic control systems, and has been extensively treated by the engineering community. In [19], Buré and Rozenvasser present a method for deriving the sensitivity of features of limit cycle trajectories to changes in parameters. The same results were derived independently in [16, 20]. As the current paper will demonstrate, this method can be readily applied to biochemical systems and the resulting analytic description can be used to provide additional insight in this setting. In addition, an analysis of sensitivity invariants is presented which provides generalisations of the summation and connectivity theorems of MCA to this context.

2 Preliminaries

The analysis will treat a general network of n chemical species $(\mathbf{s} = (s_1, s_2, \ldots, s_n))$ involved in m reactions in a fixed volume. The system will be modelled as depending on a single scalar parameter p. (While sensitivity analysis is often carried out with respect to a vector of parameters, this simply amounts to a notational convenience.) The system is described by the n by m stoichiometry matrix $\mathbf{N}$ and the m-vector valued reaction rate function $\mathbf{v} = \mathbf{v}(\mathbf{s}, p)$. The dynamics are given by

$$\frac{d}{dt}\mathbf{s}(t) = \mathbf{N}\mathbf{v}(\mathbf{s}(t), p) \quad \text{for all } t \geq 0 \tag{1}$$

The parameter p describes a particular direction in parameter space. As such, it could represent any of the parameters of

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the model (e.g. a $V_{\max}$ or K_m value) or any coordinated change in multiple parameters (e.g. a simultaneous increase in all enzyme concentrations). The function $\mathbf{v}$ is assumed continuously differentiable.

Stoichiometric systems may contain redundant state variables (e.g. due to conserved moieties), which correspond to linear dependencies among the rows of $\mathbf{N}$. In such cases, the number of state variables can be reduced for the purposes of analysis and computation, as discussed in [21] (see also [22]). In order to simplify the presentation in what follows, we will assume that no such reduction is called for. The Appendix includes statements of the main results in the general case.

We make the standing assumption that for each value of the parameter p in the range of interest the system (1) exhibits a periodic trajectory $\mathbf{s}_{\text{per}}(t,p)$, with period $T(p)$, i.e.

$$\mathbf{s}_{\text{per}}(t+T(p),p) = \mathbf{s}_{\text{per}}(t,p) \quad \text{for all } t \geq 0$$

Moreover, it will be assumed that each such trajectory is a stable limit cycle.

3 Sensitivity analysis

Of primary interest in the context of oscillatory behaviour is the asymptotic sensitivity, which we now define.

Definition 3.1: Given a nominal parameter value p_0 and a corresponding periodic trajectory $\mathbf{s}_{\text{per}}(t,p_0)$, define the *asymptotic concentration response coefficient* (or *asymptotic sensitivity function*) as the n-vector valued function $\mathbf{R}^{\mathbf{s}}_*(t)$ given by

$$\mathbf{R}^{\mathbf{s}}_*(t) = \left.\frac{\partial \mathbf{s}_{\text{per}}(t,p)}{\partial p}\right|_{p=p_0} = \lim_{\Delta p \to 0} \frac{\mathbf{s}_{\text{per}}(t,p_0+\Delta p) - \mathbf{s}_{\text{per}}(t,p_0)}{\Delta p} \qquad (2)$$

for each $0 \leq t < T(p_0)$ for which the limit exists.

Unfortunately, direct computation of this asymptotic sensitivity requires an analytic description of the dependence of $\mathbf{s}_{\text{per}}(t,p)$ on the parameter p, which is generally not available. As will be shown below, certain values of this asymptotic sensitivity can be derived in terms of the general transient response coefficient, defined as follows [7, 9].

Definition 3.2: Given a nominal parameter value p_0 and an initial state $\mathbf{s}(0) = \mathbf{s}^0$, define the (transient) *response coefficient* (or *sensitivity function*) as the n-vector valued function $\mathbf{R}^{\mathbf{s}}(t)$ given by

$$\mathbf{R}^{\mathbf{s}}(t) = \left.\frac{\partial \mathbf{s}(t,p)}{\partial p}\right|_{p=p_0} = \lim_{\Delta p \to 0} \frac{\mathbf{s}(t,p_0+\Delta p) - \mathbf{s}(t,p_0)}{\Delta p} \quad \text{for all } t \geq 0 \qquad (3)$$

where $\mathbf{s}(t,p_0)$ is the trajectory resulting from (1).

More precisely, these are called *unscaled* response coefficients. When reporting sensitivities, it is typically more useful to use coefficients which have been scaled to give measures of the relative effects of parameter changes.

The evolution of the transient response coefficients is described by the first order linear differential equation

$$\frac{d}{dt}\mathbf{R}^{\mathbf{s}}(t) = \left(\mathbf{N}\frac{\partial \mathbf{v}(t)}{\partial \mathbf{s}}\right)\mathbf{R}^{\mathbf{s}}(t) + \mathbf{N}\frac{\partial \mathbf{v}(t)}{\partial p} \quad \text{for all } t \geq 0 \qquad (4)$$

which follows from taking the derivative of (1) with respect to p and switching the order of differentiation. The partial derivatives $\frac{\partial \mathbf{v}(t)}{\partial \mathbf{s}}$ and $\frac{\partial \mathbf{v}(t)}{\partial p}$ are evaluated along the trajectory $\mathbf{s}(t,p_0)$. The remainder of this Section is dedicated to showing how values of the asymptotic sensitivity $\mathbf{R}^{\mathbf{s}}_*$ can be expressed in terms of the response coefficients $\mathbf{R}^{\mathbf{s}}$. To that end, given a parameter value p_0 we will consider transient sensitivities arising from initial state $\mathbf{s}^0 = \mathbf{s}_{\text{per}}(0,p_0)$ (so that the resulting trajectory is $\mathbf{s}(t,p_0) \equiv \mathbf{s}_{\text{per}}(t,p_0)$).

The response of the system (1) to perturbations in a parameter which affects only the initial conditions is easily dealt with, as was discussed in [9]. Henceforth we will assume that a change in the parameter p has no effect at time $t = 0$, and so we will take the initial condition $\mathbf{R}^{\mathbf{s}}(0) = \mathbf{0}$ in (4). It is a consequence of this choice of initial condition that $\mathbf{R}^{\mathbf{s}}$ is not equivalent to $\mathbf{R}^{\mathbf{s}}_*$. The asymptotic sensitivity $\mathbf{R}^{\mathbf{s}}_*(t)$ is the solution of (4) with initial condition $\mathbf{R}^{\mathbf{s}}_*(0) = \frac{d}{dp}\mathbf{s}_{\text{per}}(0,p)$. This value is not typically known and is generally non-zero for any parameter p which has an effect on the periodic trajectory.

In standard sensitivity analysis, the response coefficients are used directly to determine the system behaviour under perturbations. However, for systems exhibiting autonomous oscillations, the transient sensitivity functions $\mathbf{R}^{\mathbf{s}}$ grow unbounded in time and hence are of little direct use in addressing asymptotic behaviour (as shown in [14]). (The reason for this divergent behaviour is that a change in the parameter p typically results in a change in the period of oscillation. If this is the case, then no matter how small the change, eventually the nominal and perturbed trajectory are very far apart, as they grow out of phase.) Nonetheless, these divergent sensitivity functions can be used to describe the asymptotic response of certain properties of the oscillations, as shown in [19] and outlined below (see also [23]).

Before addressing sensitivity, we make a remark regarding (4). Having chosen an initial condition $\mathbf{s}^0$ which lies on a periodic orbit $\mathbf{s}_{\text{per}}(t,p_0)$, we see that both $\mathbf{N}\frac{\partial \mathbf{v}(t)}{\partial \mathbf{s}}$ and $\mathbf{N}\frac{\partial \mathbf{v}(t)}{\partial p}$ are $T(p_0)$-periodic when evaluated along that trajectory. Thus, the homogeneous part of (4), whose fundamental matrix $\mathbf{H}(t)$ is the solution of

$$\frac{d}{dt}\mathbf{H}(t) = \left(\mathbf{N}\frac{\partial \mathbf{v}(t)}{\partial \mathbf{s}}\right)\mathbf{H}(t) \quad \mathbf{H}(0) = \mathbf{I}_n \qquad (5)$$

can be addressed by Floquet Theory (see, e.g. [24]). The result is a solution of the form

$$\mathbf{H}(t) = \mathbf{D}(t)e^{\mathbf{B}t} \qquad (6)$$

where the matrix $\mathbf{D}(t)$ is $T(p_0)$-periodic. The eigenvalues of the matrix $e^{\mathbf{B}T(p_0)}$ are called the *characteristic multipliers* (or *characteristic roots*) of the system. Under our assumption that the system (1) admits the $T(p_0)$-periodic solution $\mathbf{s}_{\text{per}}(t,p_0)$, it follows that the differential equation in (5) likewise admits a $T(p_0)$-periodic solution, namely $\frac{d}{dt}\mathbf{s}_{\text{per}}(t,p_0)$. Comparing with (6), one concludes that the matrix $e^{\mathbf{B}T(p_0)}$ must have at least one eigenvalue equal to one.

We now make the assumption that all of the other characteristic multipliers are inside the unit circle, which is consistent with the fact that the periodic trajectory $\mathbf{s}_{\text{per}}(t,p_0)$ is a stable limit cycle. Under this assumption, the fundamental matrix $\mathbf{H}(t)$ can be expressed as

$$\mathbf{H}(t) = \mathbf{H}_{\text{per}}(t) + \mathbf{H}_{\text{trans}}(t)$$

where $\mathbf{H}_{\text{per}}(t)$ is $T(p_0)$-periodic and $\mathbf{H}_{\text{trans}}(t)$ converges exponentially to $\mathbf{0}$ with time.

Lastly, we note that, as shown in [19], the general solution to (4) takes the form

$$\mathbf{R}^{\mathbf{s}}(t) = t\mathbf{W}(t) + \mathbf{Z}(t)$$

where $\mathbf{W}(t)$ and $\mathbf{Z}(t)$ are $T(p_0)$-periodic vector functions. (This is consistent with the linear growth of $\mathbf{R}^{\mathbf{s}}(t)$ demonstrated by alternate means in [14].) This description reveals the unbounded nature of the transient sensitivity function. As mentioned earlier, this unwieldy behaviour means that $\mathbf{R}^{\mathbf{s}}(t)$ cannot be used directly to describe the asymptotic system response. However, the periodic nature of the components $\mathbf{W}(t)$ and $\mathbf{Z}(t)$ suggest that the *variation* in the sensitivity function over the period $T(p_0)$ may be better behaved, and hence prove useful. This is indeed the case, as described below.

3.1 Sensitivity of oscillatory characteristics

In addressing the sensitivity of stable or periodic systems, it is the asymptotic response which is of primary interest. In the case of asymptotically stable systems, as time moves on, the transient response coefficients as defined above tend towards the steady-state sensitivity coefficients. For autonomously oscillating systems, this interpretation cannot be exploited since the response coefficients diverge. However, their values do converge to a description of the asymptotic behaviour of the *extreme points* on the periodic trajectory, as shown next.

3.1.1 Sensitivity of local extrema:

The oscillatory behaviour of systems is often described in terms of local extrema, e g. maximal or minimal levels of chemical species or of flux through pathways. Having identified such extrema as being of interest, one can then ask how they may be affected by a parameter variation. A satisfactory answer can be given provided one characterises the *time* at which the extreme value occurs. That is, having fixed a time t^0 of interest on the periodic trajectory $\mathbf{s}_{\text{per}}(t, p_0)$, one can describe the response of the system at time t^0 to a change in p, provided this time represents a local maximum or minimum value for the variable of interest, as we now demonstrate. (The following is a straightforward extension of results in [16, 19]).

Let $Y = Y(\mathbf{s}, p)$ be some (scalar-valued) function of the state and the parameter, and identify a time $t^0 = t^0(p_0)$ in the interval $[0, T(p_0))$ at which the function $Y(\mathbf{s}_{\text{per}}(t, p_0), p_0)$ achieves a local maximum or minimum. Then, for integers m

$$\left.\frac{d}{dp} Y(\mathbf{s}_{\text{per}}(t^0(p), p), p)\right|_{p=p_0} = \lim_{m\to\infty} \frac{\partial Y}{\partial \mathbf{s}} \mathbf{R}^{\mathbf{s}}(t^0 + mT(p_0)) + \frac{\partial Y}{\partial p} \tag{7}$$

where the partial derivatives of Y are evaluated at $(\mathbf{s}_{\text{per}}(t^0, p_0), p_0), p_0)$. Moreover, the convergence is exponential, and so moderate values of m will typically yield accurate estimates. The proof of this result appears in the Appendix.

In addressing biochemical networks, the most common choices for the function Y are specific species concentrations or reaction rates. This latter choice leads to sensitivity functions known as *flux response coefficients*, denoted by the vector $\mathbf{R}^{\mathbf{v}}$. Using $\mathbf{R}^{\mathbf{v}}_*$ to denote the asymptotic response of the reaction rates, we have the following: if a species concentration s_j achieves an extreme value at time t^0, then

$$\mathbf{R}^{s_j}_*(t^0) = \lim_{m\to\infty} \mathbf{R}^{s_j}(t^0 + mT(p_0))$$

and if a reaction rate v_k achieves an extreme value at time t^0, then

$$\mathbf{R}^{v_k}_*(t^0) = \lim_{m\to\infty} \frac{\partial v_k}{\partial \mathbf{s}} \mathbf{R}^{\mathbf{s}}(t^0 + mT(p_0)) + \frac{\partial v_k}{\partial p}$$

where the partial derivatives are evaluated at $(\mathbf{s}_{\text{per}}(t^0, p_0), p_0)$.

3.1.2 Sensitivity of the period:

In addition to maximal and minimal values, the asymptotic response of the oscillation can also be characterised by the change in the period. An auxiliary definition will be needed.

Definition 3.3: Given a solution $\mathbf{R}^{\mathbf{s}}(t)$ of (4), define the *variation of the sensitivity* over the period of the nominal oscillation by:

$$\Delta\mathbf{R}^{\mathbf{s}}(t) = \mathbf{R}^{\mathbf{s}}(t + T(p_0)) - \mathbf{R}^{\mathbf{s}}(t) \quad \text{for all } t \geq 0$$

Assuming $T(p)$ is differentiable, the sensitivity of the period can be expressed as follows ([19, 20]): for any $j = 1, 2, \ldots, n$, let $\Delta\mathbf{R}^{s_j}(t)$ denote the jth element of $\Delta\mathbf{R}^{\mathbf{s}}(t)$, let $\mathbf{N}_j$ be the jth row of $\mathbf{N}$, and choose any unbounded increasing sequence of times $\{t_k\}_{k=1}^{\infty}$ such that $\mathbf{N}_j\mathbf{v}(\mathbf{s}_{\text{per}}(t_k, p_0), p_0) \neq 0$ for each k. Then:

$$\left.\frac{d}{dp} T(p)\right|_{p=p_0} = \lim_{k\to\infty} -\frac{\Delta\mathbf{R}^{s_j}(t_k)}{\mathbf{N}_j\mathbf{v}(\mathbf{s}_{\text{per}}(t_k, p_0), p_0)} \tag{8}$$

Again, convergence is exponential and so simulations of moderate length provide accurate estimates of the limit. The sequence $\{t_k\}$ is introduced only to avoid division by zero. The proof is included in the Appendix.

3.2 A remark on computation

Previously, the sensitivity coefficients derived above have only been treated analytically in special cases (e.g. [7]). More commonly, they have been approximated through simulation i.e. by changing a parameter by a few percent and observing the resulting behaviour (e.g. [13, 25]). Such investigations can provide accurate estimates provided the perturbation is sufficiently small, but may lead to numerical difficulties since the sensitivity coefficient is derived as a ratio of small numbers (resulting in errors introduced by limits in precision). The derivation presented here does not suffer from this numerical handicap.

Nevertheless, it must be observed that calculation of the sensitivity of extrema in the manner shown above could prove numerically difficult. The sensitivity function $\mathbf{R}^{\mathbf{s}}(t)$ oscillates more and more widely as time moves on, making resolution of particular values problematic. Fortunately, an alternative, as presented in [26], is to calculate the (well-behaved) variation $\Delta\mathbf{R}^{\mathbf{s}}(t)$ and use that to determine the values of $\mathbf{R}^{\mathbf{s}}(t)$ through:

$$\mathbf{R}^{\mathbf{s}}(t + mT(p)) = \mathbf{R}^{\mathbf{s}}(t) + \sum_{k=0}^{m-1} \Delta\mathbf{R}^{\mathbf{s}}(t + kT(p)) \quad \text{for any } 0 \leq t \leq T(p) \tag{9}$$

The variation $\Delta\mathbf{R}^{\mathbf{s}}(t)$ can be easily calculated as the solution of (5) with initial condition $\Delta\mathbf{R}^{\mathbf{s}}(0) = \mathbf{R}^{\mathbf{s}}(T(p))$. In practise, this computational improvement will only be required when a high degree of accuracy is needed.

4 Sensitivity invariants

When systems are subjected to sensitivity analysis, their particular form often imposes certain restrictions, known as *sensitivity invariants*, on the resulting sensitivity functions [26]. In the case of biochemical networks, the stoichiometric

nature of the system leads to such interconnections between the response coefficients. Within the MCA community, the standard descriptions of sensitivity invariants are the *summation* and *connectivity* theorems. In the classical case of steady-state analysis, these theorems describe algebraic constraints on the sensitivity coefficients [21]. An equivalent statement of these results can be given as a description of the response of the system to particular perturbations. In this latter form, the theorems have been generalised to statements regarding transient sensitivity functions [9]. In the current context, a consideration invariants leads to the following statements regarding the asymptotic response coefficients. Recall that p represents an arbitrary direction in parameter space, which could correspond to a particular parameter or to a coordinated change in several parameters (e.g. a coordinated change in all enzyme levels).

Summation theorem: If the parameter p is chosen so that $\frac{\partial \mathbf{v}(t)}{\partial p}$ lies in the null-space of $\mathbf{N}$ for each time t during the oscillation $\mathbf{s}_{\text{per}}(t,p)$, then

$$\mathbf{R}^{\mathbf{s}}_{*}(t) = \mathbf{0}$$
$$\mathbf{R}^{\mathbf{v}}_{*}(t) = \frac{\partial \mathbf{v}(t)}{\partial p}$$

for each t satisfying $0 \le t < T(p)$. Moreover, $\frac{dT}{dp} = 0$.

Connectivity theorem: If the parameter p is chosen so that there is some n-vector $\mathbf{m}$ for which $\frac{\partial \mathbf{v}(t)}{\partial p} = \frac{\partial \mathbf{v}(t)}{\partial \mathbf{s}}\mathbf{m}$ for each time t during the oscillation $\mathbf{s}_{\text{per}}(t,p)$, then

$$\mathbf{R}^{\mathbf{s}}_{*}(t) = (\mathbf{H}_{\text{per}}(t) - \mathbf{I}_n)\mathbf{m}$$
$$\mathbf{R}^{\mathbf{v}}_{*}(t) = \frac{\partial \mathbf{v}(t)}{\partial \mathbf{s}}\mathbf{H}_{\text{per}}(t)\mathbf{m}$$

for each t satisfying $0 \le t < T(p)$. Moreover, $\frac{dT}{dp} = 0$.

Note that in these special cases the sensitivity at arbitrary times t is derived, not just at the extremal points. The proofs are provided in the Appendix.

These results are direct generalisations of the corresponding steady-state theorems [21, 22], since for asymptotically stable steady states, the fundamental matrix which corresponds to $\mathbf{H}$ tends to zero as time increases. Moreover, these statements are immediately recognisable as specialisations of the general time-varying theorems presented in [9]. Thus, as often happens in the analysis of dynamical systems, the periodic case presents itself as intermediate between steady-state and general time-varying behaviour. In each of these cases the theorems serve to describe families of parameter perturbations which elicit particular responses in the system.

While the theorems presented here may find fewer applications than their steady-state counterparts, the hypotheses are less restrictive than they may at first appear. For instance, while the connectivity theorem demands equality of two functions of time, one must keep in mind that the two functions (namely $\frac{\partial \mathbf{v}(t)}{\partial \mathbf{s}}\mathbf{m}$ and $\frac{\partial \mathbf{v}(t)}{\partial p}$) are being forced by the same oscillatory dynamics. The hypothesis of the summation theorem is even less restrictive. Recall that the null-space of $\mathbf{N}$ is a sub-space of the m-dimensional space in which $\frac{\partial \mathbf{v}(t)}{\partial p}$ resides. Thus the condition does not demand that $\frac{\partial \mathbf{v}(t)}{\partial p}$ is constant, but rather that it is restricted to a particular sub-space as it evolves in time.

A straightforward application of this summation theorem is to a system comprised of an autonomous oscillator forcing the reactions in a metabolic chain. If the effect of the forcing is symmetric along the reactions in the chain, then the flux through the chain varies periodically and the metabolite levels remain at a fixed steady state. If a perturbation p were chosen which had a uniform effect on the forcing, then the summation theorem stated above would apply to describe the periodic response in the pathway flux and the null response in the metabolite concentrations. Such a system can be realised by an oscillating genetic network which includes an operon. If the operon is responsible for production of a series of enzymes in a pathway, then the summation theorem applies to any parameter which has a uniform effect on the activities of the enzymes, e.g. the rate of RNA translation.

An alternative generalisation of the classical summation theorem to oscillating systems was given in [7, 27]. This alternative statement addresses the behaviour of the system under changes in a parameter direction p which appears linearly in the vector of reaction rates $\mathbf{v}(\mathbf{s}, p)$. The motivation for addressing this form of perturbation comes from consideration of a simultaneous change in all enzyme levels, which are (very reasonably) assumed to enter linearly in the reaction rates.

In the current context, this assumption of linearity is equivalent to a choice of p so that $\frac{\partial \mathbf{v}}{\partial p} = \frac{1}{p}\mathbf{v}$. It can be observed immediately that in this case the hypothesis of the summation theorem presented above is not satisfied, since

$$\mathbf{N}\frac{\partial \mathbf{v}(t)}{\partial p} = \mathbf{N}\frac{1}{p}\mathbf{v}(t) = \frac{1}{p}\frac{d}{dt}\mathbf{s}(t)$$

which cannot be zero at any point along a limit cycle. Thus, the results derived in [7, 27] are distinct from those stated here.

Nevertheless, the case of p appearing linearly in $\mathbf{v}(\mathbf{s}, p)$ can be addressed within the current framework, as follows. In this case, the transient response $\mathbf{R}^{\mathbf{s}}(t)$ is defined as the solution to the initial value problem

$$\frac{d}{dt}\mathbf{R}^{\mathbf{s}}(t) = \left(\mathbf{N}\frac{\partial \mathbf{v}(t)}{\partial \mathbf{s}}\right)\mathbf{R}^{\mathbf{s}}(t) + \mathbf{N}\frac{1}{p_0}\mathbf{v}(\mathbf{s}_{\text{per}}(t), p_0)$$
$$\mathbf{R}^{\mathbf{s}}(0) = \mathbf{0}$$

which is (4) with $\frac{1}{p_0}\mathbf{v}$ in the place of $\frac{\partial \mathbf{v}}{\partial p}$. The solution to this equation is

$$\mathbf{R}^{\mathbf{s}}(t) = \frac{t}{p_0}\mathbf{N}\mathbf{v}(\mathbf{s}_{\text{per}}(t), p_0) \quad \text{for all } t \ge 0$$

as can be verified by direct substitution. Applying the results described in Section 3.1 one concludes that the asymptotic response in extreme values of species concentrations is zero, since the corresponding component of $\mathbf{R}^{\mathbf{s}}(t)$ is zero at each point an extremum is reached. Likewise there is only the direct response in extremes of flux. The sensitivity of the period is also directly computable: in this case $\Delta\mathbf{R}^{\mathbf{s}}(t) = \frac{T(p_0)}{p_0}\mathbf{R}^{\mathbf{s}}(t)$, so the (absolute) sensitivity of the period is $\frac{T(p_0)}{p_0}$, yielding a relative sensitivity of unity. These concur with the results in [7, 27] and indeed can be verified without calculation, since this parameter perturbation is equivalent to a scaling of time (and consequently this description of the system response is accurate for arbitrarily large perturbations in this parameter, as described in [28].

5 Application: circadian oscillator

The analysis presented above will next be illustrated by its application to a model describing the circadian oscillations of the *per* gene product in *Drosophila*. The *per* gene was shown to play a role in circadian rhythms in 1971 when Konopka and Benzer [29] obtained mutants which exhibited

rhythms with periods significantly different from 24 hours. See [11] for a review of their results and of later work.

A first attempt at providing a mathematical model of the mechanism underlying these oscillations was presented by Goldbeter in [25]. This model was built in the absence of detailed molecular descriptions of the reactions involved, and was proposed as a minimal model which was able to reproduce experimental observations of wild type and mutant behaviour. Since its publication, the model has been extended and refined (e.g. [30, 31]) to provide an improved description of the mechanism. Nevertheless, the original model suffices for investigations of the core behaviour of the system, and its simplicity recommends it for an illustrative example.

Figure 1 shows the model architecture [25]. The mechanism can be described as follows. The protein PER (P_0) is produced in the cytosol at a rate determined by the concentration of *per* mRNA (M). It is then reversibly phosphorylated at two sites (producing species P_1 and P_2). The fully phosphorylated protein can then be degraded or can migrate across the nuclear membrane. The variable P_N describes the concentration of nuclear PER, which inhibits transcription of *per* mRNA. This mRNA is subsequently degraded. The reaction rates (with nominal parameter values) [25] are given by:

$$
\begin{aligned}
\mathbf{v}_1 &= k_s M \\
\mathbf{v}_2 &= V_1 \frac{P_0}{K_1 + P_0} - V_2 \frac{P_1}{K_2 + P_1} \\
\mathbf{v}_3 &= V_3 \frac{P_1}{K_3 + P_1} - V_4 \frac{P_2}{K_4 + P_2} \\
\mathbf{v}_4 &= v_d \frac{P_2}{K_d + P_2} \\
\mathbf{v}_5 &= k_1 P_2 - k_2 P_N \\
\mathbf{v}_6 &= v_s \frac{K_I^n}{K_I^n + P_N^n} \\
\mathbf{v}_7 &= v_m \frac{M}{K_m + M} \\
k_s &= 0.38 \\
V_1 &= 3.2,\ K_1 = 2,\ V_2 = 1.58,\ K_2 = 2 \\
V_3 &= 5,\ K_3 = 2,\ V_4 = 2.5,\ K_4 = 2 \\
v_d &= 0.95,\ K_d = 0.2 \\
k_1 &= 1.9,\ k_2 = 1.3 \\
v_s &= 0.76,\ K_I = 1,\ n = 4 \\
v_m &= 0.65,\ K_m = 0.5.
\end{aligned}
$$

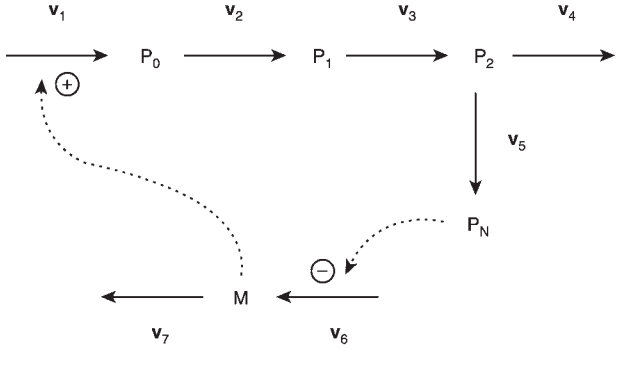

Fig. 1 *Circadian oscillations of period protein (PER)*

The limit cycle behaviour of the system is dependent on the non-linearity of the negative feedback (i.e. the transcriptional inhibition) and the multiple phosphorylation steps which act to delay the effect of the negative regulation [25].

The model can be used to investigate mutant phenotypes by addressing the response of the system to changes in parameter values. This analysis can be carried out globally, e.g. through bifurcation analysis (which describes the range of parameter values over which oscillatory behaviour persists) or locally, through sensitivity analysis (which describes the effect of small parameter changes on features of the oscillation).

In [25], sensitivity analysis was carried out through simulation, by modulating parameters by 5% and observing the outcome. It was discovered that the period increases with v_d (the rate of PER degradation) and decreases as k_1 (the rate of PER transport into the nucleus) increases. These results suggest possible effects of the *per* mutations which yield altered periods. Further studies, presented in [11], represent the effects of increased gene dosage by an increase in v_s (the rate of transcription of *per* mRNA). In addition, the effect of these perturbations on the amplitude of the oscillations in various chemical species was addressed.

Using the results described in Section 3, the analysis can be refined by precise calculation of the sensitivities. To begin with, the period of the limit cycle was found (by beginning a simulation in the basin of attraction and running for a sufficiently long time), and an arbitrary point on the cycle was chosen as an initial condition. In this case, the period was found to be 23.66. The initial point $\mathbf{s}^0 = (P_0(0), P_1(0), P_2(0), P_N(0), M(0)) = (1.44, 0.72, 0.48, 0.63, 2.81)$ was chosen. Next, a particular parameter was chosen, $p = v_d$. (Recall that the analysis applies equally well to coordinated perturbations in multiple parameters.) Then, starting with $\mathbf{s}(0) = \mathbf{s}^0$ and $\mathbf{R}^{\mathbf{s}}(0) = \mathbf{0}$, the differential equations (1) and (4) were solved simultaneously, with the value of the state variables $\mathbf{s}(t)$ being used to calculate the right-hand side of (4). (Since the state trajectory $\mathbf{s}(t)$ lies on the limit cycle, the computational cost of this procedure could be reduced by storing the values of $\mathbf{s}(t)$ is a look-up table rather than repeatedly calculating them by simulation. However, this improvement comes at the cost of setting up the solution of (4) to coordinate with the look-up table, which may be more trouble than it is worth.) The partial results of one such simulation are shown in Fig. 2, where we

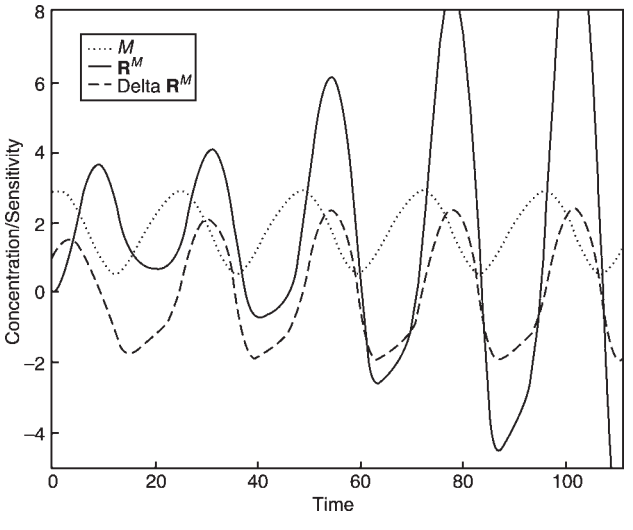

Fig. 2 *M, the sensitivity function* $\boldsymbol{R}^M$ *and its variation* $\Delta \boldsymbol{R}^M$

Table 1: Selected sensitivities of the circadian oscillator

Parameter	Relative sensitivity period	M^{max}	M^{min}	$\mathbf{v}_5^{max}$	$\mathbf{v}_5^{min}$
v_d	0.29	0.59	2.1	−0.58	0.46
k_1	−0.37	−0.34	0.76	0.16	1.1
k_2	0.29	0.30	−0.37	−0.45	−0.45
v_s	0.016	1.4	−6.5	3.2	−3.5

see the oscillatory behavior of $M(t)$ and its (divergent) sensitivity function $\mathbf{R}^M(t)$. The other four components of $\mathbf{s}$ and $\mathbf{R}^{\mathbf{s}}$ are not shown, but exhibit similar behaviour. Also shown in Fig. 2 is the variation in $\mathbf{R}^M$ over a single period, which is calculated as $\Delta\mathbf{R}^M(t) = \mathbf{R}^M(t+T) - \mathbf{R}^M(t)$.

Sensitivities were found as follows. The sensitivity of the period was found as the asymptotic value of the ratio $-\frac{\Delta\mathbf{R}^M(t)}{\frac{d}{dt}M(t)}$ (avoiding times when $\frac{d}{dt}M(t) = 0$). The choice of M here is arbitrary; calculation of the corresponding ratio for any other state variable leads to the same value. This absolute sensitivity was found to be 7.22. Scaling by the ratio of the nominal parameter value to the nominal period $\left(\frac{v_d}{T} = \frac{0.95}{23.66}\right)$ led to a relative sensitivity of 0.29.

Sensitivities of the extrema were determined from (7). Times at which the maxima and minima in $M(t)$ occur were found. The corresponding values of $\mathbf{R}^M(t)$ converge to the absolute sensitivities of the extrema. Sensitivity of extreme levels of fluxes are found similarly: by examining values of $\frac{\partial \mathbf{v}_k}{\partial \mathbf{s}}\mathbf{R}^{\mathbf{s}}(t) + \frac{\partial \mathbf{v}_k}{\partial p}$ at times in which the extrema in flux $\mathbf{v}_k$ occur. Since convergence is guaranteed, the asymptotic values of these sequences were determined by sampling at sufficiently large times. The system was solved for about ten periods, and the sensitivity was taken from the response at the last extremum in the simulation. Again, scaling led to relative measures of sensitivity.

These results, along with parallel analysis for some other choices of parameters are shown in Table 1, where attention has been restricted to the period, the mRNA concentration M, and the rate of PER migration into the nucleus $\mathbf{v}_5$. The Matlab code which produced these results is available from the author.

The results concur with those in [11] and [25], and provide an improved description of the local response of the system. We see, for example, that while the period increases with v_s and v_d, the sensitivity to the rate of degradation is roughly twenty-times that with respect to the rate of synthesis. Likewise, while k_1 and k_2 have direct effects on the extreme values of $\mathbf{v}_5$, the effect of perturbations in v_s on this reaction is appreciably stronger. Similar observations can be made regarding the effects of parameter variations on the amplitude and extreme values of the oscillation in M.

6 Discussion

Analysis of the dependence of system behaviour on parameter values often plays a crucial role in understanding the nature of the system and predicting the consequence of intervention. Parametric dependence is typically addressed at two separate scales: global analysis treats the effects of large changes in parameter values, while local analysis is confined to approximating the effects of small changes.

In the case of systems exhibiting limit cycle behaviour, the primary tool for global analysis is bifurcation theory (e.g. [32]) which can be used to determine the range of parameters over which the oscillatory behaviour persists. A recent development in this context, pioneered in [33], is the application of tools from robust control theory to address the global effect of simultaneous changes in multiple parameters.

Complementing these global approaches is local sensitivity analysis, in which the linearisation of the system is addressed. This analysis cannot reveal the effects of large deviations in parameter values, but provides an elegant description of the effect of small perturbations, since these are well approximated by the corresponding linearised response.

The parametric sensitivities addressed in this paper have been used as effective tools for the study of oscillatory biochemical systems. Their values have been well approximated by direct simulation. Indeed, in terms of computation, the current contribution is relatively minor. More important is the general analytic description of these sensitivities, which had been previously presented only in the special case of parameters appearing linearly in the reaction rates [7, 27] (as discussed in Section 4).

Such an analytic description allows precise computation of the sensitivity, so that results can be reported without the preface that "parameter values were modulated by $n\%$." Moreover, this analytic representation allows sensitivities of autonomously oscillating systems to be placed within a broader context. In many ways, periodic behaviour can be understood as intermediate between steady-state and arbitrary time-varying activity. As such, one would expect that the seamless framework previously outlined for sensitivity analysis of steady-state [21, 22] and time-varying trajectories [7, 8, 9] would incorporate autonomous oscillations as well. This seemed not to be the case [9, 14]. However, in the light of the results of [19] as presented here, it becomes clear that the general time-varying sensitivities defined in [7, 9] do indeed specialise to sensitivities of systems undergoing limit cycles, but only at points of extreme behaviour. This insight is a step toward bridging the conceptual gap which was exposed when oscillating systems failed to fit into the general framework. This state of affairs is mirrored in the treatment of sensitivity invariants described by the summation and connectivity theorems. As discussed in Section 4, the theorems presented here provide direct generalisations of the standard steady-state results [21, 22], and are direct specialisations of the general time-varying results in [9].

In addition to providing theoretical insight, an analytic description of sensitivity for autonomously oscillating systems also allows the application of existing analytical tools to this case. As an example, an extensive theory of the computation of system sensitivities has been developed, as reviewed in [34] (including a number of different computational approaches, e.g. the Green's function method). Such tools cannot be applied unless an analytic description of the sensitivity is available.

7 Conclusion

A general sensitivity analysis of autonomously oscillating biochemical systems has been provided. The sensitivity functions derived here had previously been addressed only through approximation by simulation. The analysis presented here provides accurate derivations of these sensitivities, which can then be used for quantitative studies of system response (e.g. comparison of strengths of the effect of parameter perturbations). In addition, the analytic description of responses outlined here allows autonomously oscillating systems to be understood within the existing framework of sensitivity analysis of biochemical systems.

When coupled with global techniques such as bifurcation analysis, these local sensitivity techniques provide an invaluable tool for probing the behaviour of oscillatory biochemical systems, leading to insight into their internal nature and predictions of the effect of external perturbations.

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10 Appendix

10.1 Structural conservations

As mentioned in Section 2, any structural conservations (e.g. conserved moieties) inherent in the system should be addressed before analysis is carried out. These can be treated as follows [21].

Given a system as in (1), let n_0 denote the row rank of $\mathbf{N}$. If $n_0 = n$, then no structural conservations are present. Otherwise, there are $n - n_0$ linearly dependent rows which can be removed to arrive at a reduced stoichiometry matrix $\mathbf{N_R}$. The original stoichiometry matrix can be recovered from $\mathbf{N_R}$ through the n by n_0 *link matrix* $\mathbf{L}$:

$$\mathbf{N} = \mathbf{L}\mathbf{N_R}$$

Those species which correspond to the rows of $\mathbf{N}$ which were removed are dependent on the others, and are described by the vector $\mathbf{s^d}$. The others form the vector of independent species $\mathbf{s^i}$. The dependent species concentrations can be derived from the independent concentrations through:

$$\mathbf{s}(t) = \mathbf{L}\mathbf{s^i}(t) + \sigma \quad \text{for all } t \geq 0$$

where the constant vector σ is fixed by the initial conditions. Attention can then be restricted to the reduced system:

$$\frac{d}{dt}\mathbf{s^i}(t) = \mathbf{N_R}\mathbf{v}(\mathbf{L}\mathbf{s^i}(t) + \sigma, p) \tag{10}$$

Let $\mathbf{s^i_{per}}(t, p)$ denote the $T(p)$-periodic trajectory of independent species concentrations, so that:

$$\mathbf{s^i_{per}}(t + T(p), p) = \mathbf{s^i_{per}}(t, p) \quad \text{for all } t \geq 0 \tag{11}$$

10.2 Sensitivity

Focusing on the sensitivity coefficients for the independent species (denoted $\mathbf{R^{s^i}}$), we see that they satisfy

$$\frac{d}{dt}\mathbf{R^{s^i}}(t) = \left(\mathbf{N_R}\frac{\partial \mathbf{v}(t)}{\partial \mathbf{s}}\mathbf{L}\right)\mathbf{R^{s^i}}(t) + \mathbf{N_R}\frac{\partial \mathbf{v}(t)}{\partial p} \quad \text{for all } t \geq 0 \tag{12}$$

as shown in [9].

The fundamental solution $\mathbf{H^i}(t)$ of the homogeneous part of equation (12), i.e. the solution of

$$\frac{d}{dt}\mathbf{H^i}(t) = \left(\mathbf{N_R}\frac{\partial \mathbf{v}(t)}{\partial \mathbf{s}}\mathbf{L}\right)\mathbf{H^i}(t) \quad \mathbf{H^i}(0) = \mathbf{I}_{n_0} \tag{13}$$

is related to the solution $\mathbf{H}(t)$ of (5) by $\mathbf{H}(t) = \mathbf{L}\mathbf{H^i}(t)$, and so we can write

$$\mathbf{H^i}(t) = \mathbf{H^i_{per}}(t) + \mathbf{H^i_{trans}}(t) \quad \text{for all } t \geq 0 \tag{14}$$

where $\mathbf{H^i_{per}}(t)$ is $T(p)$-periodic and $\mathbf{H^i_{trans}}(t)$ tends to zero exponentially.

10.2.1 Sensitivity of local extrema: Let $Y = Y(\mathbf{s}, p)$ be some scalar-valued function of the independent species and the parameter, and identify a time $t^0 = t^0(p_0)$ in the interval $[0, T(p_0))$ at which the function $Y(\mathbf{s}^{\mathbf{i}}_{per}(t, p_0), p_0)$ achieves a local maximum or minimum. Then, for integers m

$$\frac{d}{dp} Y(\mathbf{s}^{\mathbf{i}}_{\text{per}}(t^0(p), p), p)\Big|_{p=p_0} = \lim_{m\to\infty} \frac{\partial Y}{\partial \mathbf{s}^{\mathbf{i}}} \mathbf{R}^{\mathbf{s}^{\mathbf{i}}}(t^0 + mT(p_0)) + \frac{\partial Y}{\partial p} \tag{15}$$

where the partial derivatives of Y are evaluated at $(\mathbf{s}^{\mathbf{i}}_{\text{per}}(t^0, p_0), p_0)$. Moreover, convergence to the limit is exponential.

Proof: (a minor extension of [17]) Since $Y(\mathbf{s}^{\mathbf{i}}_{\text{per}}(t^0, p_0), p_0)$ is a smooth function of t and achieves an extremum at t^0, it follows that

$$\frac{d}{dt} Y(\mathbf{s}^{\mathbf{i}}_{\text{per}}(t, p_0), p_0)\Big|_{t=t^0} = \frac{\partial Y}{\partial \mathbf{s}^{\mathbf{i}}} \frac{\partial \mathbf{s}^{\mathbf{i}}_{\text{per}}}{\partial t}\Big|_{t=t^0} = 0$$

We then have

$$\begin{aligned}
&\frac{\partial}{\partial p} Y(\mathbf{s}^{\mathbf{i}}_{\text{per}}(t^0(p), p), p)\Big|_{t=t^0} \\
&= \frac{\partial Y}{\partial \mathbf{s}^{\mathbf{i}}} \frac{\partial \mathbf{s}^{\mathbf{i}}_{\text{per}}}{\partial t} \frac{dt^0}{dp} + \frac{\partial Y}{\partial \mathbf{s}^{\mathbf{i}}} \frac{\partial}{\partial p} \mathbf{s}^{\mathbf{i}}_{\text{per}}(t^0, p) + \frac{\partial Y}{\partial p} \\
&= \frac{\partial Y}{\partial \mathbf{s}^{\mathbf{i}}} \mathbf{R}^{\mathbf{s}^{\mathbf{i}}}_*(t^0) + \frac{\partial Y}{\partial p} \\
&= \frac{\partial Y}{\partial \mathbf{s}^{\mathbf{i}}} \lim_{m\to\infty} \mathbf{R}^{\mathbf{s}^{\mathbf{i}}}(t^0 + mT(p_0)) + \frac{\partial Y}{\partial p}
\end{aligned}$$

The calculation shows why the sensitivity function describes asymptotic behaviour only at extrema; at any other point the (divergent) sensitivity of the phase enters through the term $\frac{dt^0}{dp}$.

Exponential convergence in (15) is a consequence of the exponential convergence in (14), as we now show. Since $\Delta\mathbf{R}^{\mathbf{s}^{\mathbf{i}}}(t)$ is the solution of (13) with initial condition $\mathbf{R}^{\mathbf{s}^{\mathbf{i}}}(T(p_0))$, it converges exponentially to $\mathbf{H}^{\mathbf{i}}_{\text{per}}(t)\mathbf{R}^{\mathbf{s}^{\mathbf{i}}}(T(p_0))$. From the stability assumptions we have that

$$\mathbf{H}^{\mathbf{i}}_{\text{per}}(t) = \left[\frac{d}{dt}\mathbf{s}^{\mathbf{i}}_{\text{per}}(t, p_0)\ \mathbf{0}_{n_0\times(n_0-1)}\right]\mathbf{P}$$

for some $n_0 \times n_0$ matrix $\mathbf{P}$ (see [19] for details). Then $\Delta\mathbf{R}^{\mathbf{s}^{\mathbf{i}}}(t)$ converges exponentially to $\left[\frac{d}{dt}\mathbf{s}^{\mathbf{i}}_{\text{per}}(t, p_0)\ \mathbf{0}_{n_0\times(n_0-1)}\right]\mathbf{P}\mathbf{R}^{\mathbf{s}^{\mathbf{i}}}(T(p_0))$. Since $\frac{\partial Y}{\partial \mathbf{s}^{\mathbf{i}}}\frac{d}{dt}\mathbf{s}^{\mathbf{i}}_{\text{per}}(t, p_0) = 0$ at time t^0, we have exponential convergence in the following limit:

$$\begin{aligned}
&\lim_{m\to\infty} \frac{\partial Y}{\partial \mathbf{s}^{\mathbf{i}}} \Delta\mathbf{R}^{\mathbf{s}^{\mathbf{i}}}(t^0 + mT(p)) \\
&= \frac{\partial Y}{\partial \mathbf{s}^{\mathbf{i}}}\left[\frac{d}{dt}\mathbf{s}^{\mathbf{i}}_{\text{per}}(t, p_0)\ \mathbf{0}_{n_0\times(n_0-1)}\right]\mathbf{P}\mathbf{R}^{\mathbf{s}^{\mathbf{i}}}(T(p_0)) \\
&= 0
\end{aligned}$$

Finally, invoking (9) gives the desired result.

10.2.2 Sensitivity of the period: For any $j = 1, 2, \ldots, n_0$, let $\Delta\mathbf{R}^{\mathbf{s}^{\mathbf{i}}}_j(t)$ denote the jth element of $\Delta\mathbf{R}^{\mathbf{s}^{\mathbf{i}}}(t)$, let $\mathbf{N}_{\mathbf{R}_j}$ be the jth row of $\mathbf{N}_{\mathbf{R}}$, and choose any unbounded increasing sequence of times $\{t_k\}_{k=1}^{\infty}$ such that $\mathbf{N}_{\mathbf{R}j}\mathbf{v}\left(\mathbf{L}\mathbf{s}^{\mathbf{i}}_{\text{per}}(t_k, p_0) + \sigma, p_0\right) \neq 0$ for all k. Then:

$$\frac{d}{dp} T(p)\Big|_{p=p_0} = \lim_{k\to\infty} -\frac{\Delta\mathbf{R}^{\mathbf{s}^{\mathbf{i}}}_j(t_k)}{\mathbf{N}_{\mathbf{R}_j}\mathbf{v}(\mathbf{L}\mathbf{s}^{\mathbf{i}}_{\text{per}}(t_k, p_0) + \sigma, p_0)} \tag{16}$$

Moreover, convergence is exponential.

Proof: (from [19]) Differentiation of (11) with respect to p yields:

$$\begin{aligned}
&\frac{\partial}{\partial t}\mathbf{s}^{\mathbf{i}}_{\text{per}}(t, p_0)\frac{d}{dp}T(p) + \frac{\partial}{\partial p}\mathbf{s}^{\mathbf{i}}_{\text{per}}(t + T(p_0), p) \\
&\quad = \frac{\partial}{\partial p}\mathbf{s}^{\mathbf{i}}_{\text{per}}(t, p).
\end{aligned}$$

With (10) and (2), this can be rewritten as:

$$\begin{aligned}
&\mathbf{N}_{\mathbf{R}}\mathbf{v}\left(\mathbf{L}\mathbf{s}^{\mathbf{i}}_{\text{per}}(t, p_0) + \sigma, p_0\right)\frac{d}{dp}T(p) \\
&\quad = -\lim_{t\to\infty}\left(\mathbf{R}^{\mathbf{s}^{\mathbf{i}}}(t + T(p_0)) - \mathbf{R}^{\mathbf{s}^{\mathbf{i}}}(t)\right) \\
&\quad = -\lim_{t\to\infty}\Delta\mathbf{R}^{\mathbf{s}^{\mathbf{i}}}(t)
\end{aligned}$$

Equating jth elements and dividing yields (16). Convergence is exponential since $\Delta\mathbf{R}^{\mathbf{s}^{\mathbf{i}}}(t)$ converges exponentially to a $T(p_0)$-periodic function as shown above.

10.3 Sensitivity invariants

Summation theorem: If the parameter p is chosen so that $\frac{\partial \mathbf{v}(t)}{\partial p}$ lies in the nullspace of $\mathbf{N}_{\mathbf{R}}$ for each time t during the oscillation $\mathbf{s}^{\mathbf{i}}_{\text{per}}(t, p)$, then

$$\begin{aligned}
\mathbf{R}^{\mathbf{s}}_*(t) &= \mathbf{0} \\
\mathbf{R}^{\mathbf{v}}_*(t) &= \frac{\partial \mathbf{v}(t)}{\partial p}
\end{aligned}$$

for each t satisfying $0 \le t < T(p)$. Moreover, $\frac{dT}{dp} = 0$.

Proof: In this case, the sensitivity function $\mathbf{R}^{\mathbf{s}^{\mathbf{i}}}$ is the solution of the initial value problem

$$\frac{d}{dt}\mathbf{R}^{\mathbf{s}^{\mathbf{i}}}(t) = \left(\mathbf{N}_{\mathbf{R}}\frac{\partial \mathbf{v}(t)}{\partial \mathbf{s}}\mathbf{L}\right)\mathbf{R}^{\mathbf{s}^{\mathbf{i}}}(t) \quad \mathbf{R}^{\mathbf{s}^{\mathbf{i}}}(0) = \mathbf{0}$$

whose unique solution is $\mathbf{R}^{\mathbf{s}^{\mathbf{i}}}(t) \equiv \mathbf{0}$. Since this is constant, $\Delta\mathbf{R}^{\mathbf{s}^{\mathbf{i}}}(t) \equiv \mathbf{0}$, and from (16) we conclude $\frac{dT}{dp} = 0$. Since

$$\begin{aligned}
\mathbf{R}^{\mathbf{s}}_*(t) &= \mathbf{L}\mathbf{R}^{\mathbf{s}^{\mathbf{i}}}_*(t) \\
\mathbf{R}^{\mathbf{v}}_*(t) &= \frac{\partial \mathbf{v}(t)}{\partial \mathbf{s}}\mathbf{L}\mathbf{R}^{\mathbf{s}^{\mathbf{i}}}_*(t) + \frac{\partial \mathbf{v}(t)}{\partial p}
\end{aligned}$$

the result follows. As there is no change in the period, the result holds for all times t in [0, $T(p)$) (since for *any* time $t^0(p)$, $\frac{dt^0}{dp} = 0$).

Connectivity theorem: If the parameter p is chosen so that there is some n_0-vector $\mathbf{m}$ for which $\frac{\partial \mathbf{v}(t)}{\partial p} = \frac{\partial \mathbf{v}(t)}{\partial \mathbf{s}}\mathbf{L}\mathbf{m}$ for each time t during the oscillation $\mathbf{s}^{\mathbf{i}}_{\text{per}}(t, p)$, then

$$\begin{aligned}
\mathbf{R}^{\mathbf{s}}_*(t) &= \mathbf{L}(\mathbf{H}^{\mathbf{i}}_{\text{per}}(t) - \mathbf{I}_{n_0})\mathbf{m} \\
\mathbf{R}^{\mathbf{v}}_*(t) &= \frac{\partial \mathbf{v}(t)}{\partial \mathbf{s}}\mathbf{L}\mathbf{H}^{\mathbf{i}}_{\text{per}}(t)\mathbf{m}
\end{aligned}$$

for each t satisfying $0 \le t < T(p)$. Moreover, $\frac{dT}{dp} = 0$.

Proof: In this case, the sensitivity function $\mathbf{R}^{\mathbf{s}^{\mathbf{i}}}$ is the solution of the initial value problem

$$\frac{d}{dt}\mathbf{R}^{\mathbf{s}^{\mathbf{i}}}(t) = \left(\mathbf{N}_{\mathbf{R}}\frac{\partial \mathbf{v}(t)}{\partial \mathbf{s}}\mathbf{L}\right)\left[\mathbf{R}^{\mathbf{s}^{\mathbf{i}}}(t) + \mathbf{m}\right] \quad \mathbf{R}^{\mathbf{s}^{\mathbf{i}}}(0) = \mathbf{0}$$

which means that $\mathbf{R}^{\mathbf{s}^{\mathbf{i}}}(t) + \mathbf{m}$ is the solution of the homogeneous equation in (13) with initial value $\mathbf{m}$. Thus

$$\mathbf{R}^{\mathbf{s}^{\mathbf{i}}}(t) + \mathbf{m} = \mathbf{H}^{\mathbf{i}}(t)\mathbf{m} \quad \text{for all } t \geq 0$$

and so

$$\mathbf{R}^{\mathbf{s}^{\mathbf{i}}}(t) = (\mathbf{H}^{\mathbf{i}}(t) - \mathbf{I}_{n_0})\mathbf{m} \quad \text{for all } t \geq 0$$

Then, from (14), we have that asymptotically

$$\mathbf{R}_*^{\mathbf{s}^{\mathbf{i}}}(t) = \left(\mathbf{H}_{\text{per}}^{\mathbf{i}}(t) - \mathbf{I}_{n_0}\right)\mathbf{m} \tag{17}$$

from which

$$\mathbf{R}_*^{\mathbf{s}}(t) = \mathbf{L}\mathbf{R}_*^{\mathbf{s}^{\mathbf{i}}}(t) = \mathbf{L}\left(\mathbf{H}_{\mathbf{per}}^{\mathbf{i}}(t) - \mathbf{I}_{n_0}\right)\mathbf{m}$$

$$\begin{aligned}\mathbf{R}_*^{\mathbf{v}}(t) &= \frac{\partial \mathbf{v}(t)}{\partial \mathbf{s}}\mathbf{L}\mathbf{R}_*^{\mathbf{s}^{\mathbf{i}}}(t) + \frac{\partial \mathbf{v}(t)}{\partial p} \\ &= \frac{\partial \mathbf{v}(t)}{\partial \mathbf{s}}\mathbf{L}\left(\mathbf{H}_{\text{per}}^{\mathbf{i}}(t) - \mathbf{I}_{n_0}\right)\mathbf{m} + \frac{\partial \mathbf{v}(t)}{\partial \mathbf{s}}\mathbf{L}\mathbf{m} \\ &= \frac{\partial \mathbf{v}(t)}{\partial \mathbf{s}}\mathbf{L}\mathbf{H}_{\text{per}}^{\mathbf{i}}(t)\mathbf{m}\end{aligned}$$

Also from (17), we have that $\mathbf{R}^{\mathbf{s}^{\mathbf{i}}}(t)$ asymptotes to a $T(p)$-periodic function, so that $\Delta\mathbf{R}^{\mathbf{s}^{\mathbf{i}}}(t)$ tends to $\mathbf{0}$ for large t. Then, from (16), the sensitivity of the period is zero, and so these coefficients describe the response at each point along the periodic trajectory.