Appendix

1 Experimental and Simulation Methods

One liter of yeast strain BY4743 was grown to mid-log phase, $OD_{600} = 0.5$, in a 3.8-liter fernbot flask (Sigma) in yeast nitrogen base YNB (Difco) medium without amino acids (save the auxotrophic supplements Leu and Trp) and without ammonium sulfate, supplemented with 2% glucose and 0.1% glutamine in a shaking incubator at 300 rpm at 30°C. A 50 ml aliquot was taken and quenched, and total RNA was harvested as described below. This sample was labeled $t = -\infty$. The cells were pelleted by centrifugation and resuspended in fresh, prewarmed, preaerated, 2% glucose / 0.1% proline medium by rapidly pipeting up and down for 20 seconds. A second 50 ml aliquot was then taken and quenched, and total RNA was harvested as described below. This sample was labeled $t = 0$. The fernbot was returned to the shaking incubator and subsequent aliquots were taken every 5 minutes.

Each 50 ml sample, containing $3.0\,x\,10^8$ cells, was withdrawn from the fernbot in $<$ 10 seconds and immediately mixed with 100 ml of medium prechilled at 4°C and mixed with -20°C ice. The sample was then rapidly transferred to a 4°C cold room in an ice bath, and drawn through a vacuum filter apparatus with a cellulose acetate filter. Cells were scraped from the filter into an Eppendorf tube and placed in an ethanol dry-ice bath and then placed in a -70°C freezer.

Probes for ribonuclease protection assays were prepared for each of the five nitrogen catabolite repression (NCR) circuit genes and ACT1 as an internal control. PCR was performed with the following primers.

- DAL80 primers: 5'-GCTTGAAGACGGACACCATT-3', 5'-ACTCCTTTGTGTTGGGAACG-3'
- GLN3 primers: 5'-AAGACGGTCAAGGACACCAC-3', 5'-TCACCATTTTGTTGGTTGGA-3'
- URE2 primers: 5'-TCAACGCATGGTTGTTCTTC-3', 5'-CCGCATTTTCCGTGTCTAAT-3'
- DEH1 primers: 5'-ATGTCGGGAGATTGTTCGAG-3', 5'-GTTGAATTGCGACCGTTCTT-3'
- GAT1 primers: 5'-CAGCGTCCAAGAGGAAGAAC-3', 5'-CAACCAATCCAGGCTCAGAT-3'
- ACT1 primers: 5'-TGTCACCAACTGGGACGATA-3' 5'-AACCAGCGTAAATTGGAACG-3'

PCR products were ligated into pCR2.0 TopoTA vector (Invitrogen), and their sequences were verified. Vectors were linearized, and antisense RNA probes to each of the five NCR genes and ACT1 were synthesized by *in vitro* transcription using Ambion MAXIscript kit (Ambion 1308).

Total RNA was harvested from each sample using the Ambion RiboPure-Yeast kit (Ambion 1926). Specific amounts of the relevant mRNA's were determined by RNAse protection assay using the Ambion RPAIII kit (Ambion 1415). Equal amounts (10 μg) of total RNA were hybridized at each time point. The data were quantitated by phosphorimager using a Molecular Dynamics GP storage phosphor cassette and Molecular Dynamics Typhoon 9200 phosphorimager scanner. The data were extracted with the ImageQuant5.2 (Molecular Dynamics) software, and ACT1 normalized ratios are reported in Figure 2.

1.1 Full system definition and simulation

Numerical simulation of System **8** was performed using a Runge-Kutta 4th order scheme with a time step of 0.06 minutes, implemented in the freely available software package xppaut5.41.

The GLN3 and URE2 variables are as before. Let $\{y, Y, \psi\}$ be the Dal80 variables, $\{z, Z, \zeta\}$ the GAT1 variables, and finally $\{w, W, \omega\}$ corresponds to DEH1. The functions H_i represent transcriptional reaction terms.

Transcriptional time delays, ς, are incorporated into the nuclear protein variables to account for mRNA elongation and transport. Therefore, the transcriptional reaction terms have the form

$$H_i(x(t-\varsigma_1), y(t-\varsigma_1), z(t-\varsigma_1), w(t-\varsigma_1)).$$

These time delays do not appear notationally in the equations of the full System **8** to improve readability. Further, simulations performed for this paper were performed without time delays.

$$
\begin{aligned}
\dot{x} &= K_{imp}(\xi(t-\tau)) - K_{exp}(x(t)) - \alpha(N)x(t) \\
\dot{X} &= r_g S(t,s_0) - \beta(N)X(t) \\
\dot{\xi} &= T(X(t-\delta_1)) - \gamma(N)\xi(t) - k_f(N)\xi(t)\mu(t) + k_r(N)C(t) + \\
&\quad K_{exp}(x(t-\tau)) - K_{imp}(\xi(t)) \\
\dot{U} &= r_u S(t,s_1) - \theta(N)U(t) \\
\dot{\mu} &= T(U(t-\delta_2)) - \kappa(N)\mu(t) - k_f(N)\xi(t)\mu(t) + k_r(N)C(t) \\
\dot{C} &= k_f(N)\xi(t)\mu(t) - k_r(N)C(t) \\
\dot{y} &= K_{imp}(\psi(t-\tau)) - K_{exp}(y(t)) - \pi(N)y(t) \\
\dot{Y} &= S(t,s_2)H_1(x,y,z,w) - \phi(N)Y(t) \\
\dot{\psi} &= T(Y(t-\delta_3)) - \eta(N)\psi(t) + K_{exp}(y(t-\tau)) - K_{imp}(\psi(t)) \\
\dot{z} &= K_{imp}(\zeta(t-\tau)) - K_{exp}(z(t)) - \rho(N)z(t) \\
\dot{Z} &= S(t,s_3)H_2(x,y,z) - \varpi(N)Z(t) \\
\dot{\zeta} &= T(Z(t-\delta_4)) - \varrho(N)\zeta(t) + K_{exp}(z(t-\tau)) - K_{imp}(\zeta(t)) \\
\dot{w} &= K_{imp}(\omega(t-\tau)) - K_{exp}(w(t)) - \varphi(N)w(t) \\
\dot{W} &= S(t,s_4)H_3(x,y,z) - \chi(N)W(t) \\
\dot{\omega} &= T(W(t-\delta_5)) - \nu(N)\omega(t) + K_{exp}(w(t-\tau)) - K_{imp}(\omega(t))
\end{aligned} \qquad [8]
$$

- **Compound variables and noise**

$$
\begin{aligned}
\text{noise}(x) &= \max(0.0, normal(x, x*x/12)) \\
q1 &= x + z + (y/2) \\
q2 &= x + z + (y/2) + (w/2) \\
f1 &= (x+z)/q1 \\
f2 &= (x+z)/q2 \\
pf1 &= \text{noise}(f1) \\
pf2 &= \text{noise}(f2)
\end{aligned}
$$

- **Definition of volume and reaction functions**

$$
\begin{aligned}
J(t,a) &= \lfloor (t-a)/143.0 \rfloor \\
N(t,a) &= \text{heav}(t-a) + \max(J(t,a), 0) \\
V(t) &= 35exp(0.00485*t) \\
S(t,a) &= \frac{2^{N(t,a)}}{V(t)} \\
h(A,B,x) &= \frac{Ax}{(B+x)} \\
r(A,x) &= \frac{A^2}{A^2+x^2} \\
k(A,B,C,x) &= \frac{Ax(1+Bx)}{C+x}
\end{aligned}
$$

- **Correspondence to fullsystem**

$$
\begin{aligned}
K_{imp}(\cdot) &= k(60.0, 0.03, 4.0, \cdot) \\
K_{exp}(\cdot) &= k(60.0, 0.03, 4.0, \cdot) \\
H_1(x, y, z, w) &= 0.22h(30.0, 0.0001, pf2)r(0.2, 1 - pf2) \\
H_2(x, y, z) &= 0.41h(10.0, 0.001, pf1)r(0.2, 1 - pf1) \\
H_3(x, y, z) &= 0.15h(2.0, 0.05, pf1)r(0.2, 1 - pf1) \\
T(p) &= h(0.1, 0.26, p)p.
\end{aligned}
$$

- **Degradation parameters** $\alpha = \pi = \rho = \varphi = 0.00485$, $\beta = \theta = \chi = 0.022$, $\gamma = \kappa = \eta = \varrho = \nu = 0.009$, $\phi = \varpi = 0.095$.
- **Cell cycle parameters** $s0 = 110$, $s1 = 115$, $s2 = 120$, $s3 = 130$, $s4 = 140$.
- **Miscellaneous parameters** $r_g = 0.2$, $r_\mu = 0.05$, $kf = 0.00001$, $kr = 0.13$.
- **Initial conditions** $x(0) = 0$, $X(0) = 0.2$, $\xi(0) = 0.001$, $U(0) = 0.05$, $\mu(0) = 0.001$, $C(0) = 0.5$, $z(0) = 0$, $Z(0) = 0.41$, $\zeta(0) = 0.001$, $y(0) = 0.001$, $Y(0) = 0.22$, $\psi(0) = 0.001$, $w(0) = 0.001$, $W(0) = 0.15$, $\omega(0) = 0.001$.

2 Mathematical background

We first review some mathematical background. We introduce mathematical concepts in five subsections devoted to the description of the phase space, complete continuity, dissipativeness, injectivity of the solution map, and monotonicity respectively.

2.1 Phase space

In order to formulate our results precisely we need to introduce the concept of a phase space for System **5**. We write $w = (x, \xi) \in \mathbf{R}^2$. Even though w is a two-dimensional vector, $\mathbf{R}^2$ is not a proper phase space for the System **5**. The reason is that in order to determine future behavior of the system, we need to know not only value of w at a particular time t, but values of $w(t + \theta)$ for all $\theta \in [-\tau, 0]$. This leads to the following definition. Let $C([-\tau, 0], \mathbf{R}^n)$ be a Banach space of continuous functions on $[-\tau, 0]$. If we set

$$y_t(\theta) := y(t + \theta) \quad -\tau \le \theta \le 0,$$

then y_t belongs to the space $C([-\tau, 0], \mathbf{R}^n)$.

In the case of System **5** we define

$$w_t(\theta) = (x_t(\theta), \xi_t(\theta))$$

and $w_t(\theta) \in C([-\tau, 0], \mathbf{R}^2)$. In the case of System **7** we define

$$w_t(\theta) = (x_t(\theta), \xi_t(\theta), \mu_t(\theta))$$

and $w_t(\theta) \in C([-\tau, 0], \mathbf{R}^3)$.

2.2 Complete continuity

Our main source in first two parts is the monograph on delay-differential equations by Hale and Verduyn-Lunel (1).

Definition 2.1 If X, Y are metric spaces, a map $A : X \to Y$ is *completely continuous* if it maps any bounded set to a subset of a compact set.

Definition 2.2 *[1, Definition 3.6.2]* If X, Y are metric spaces, then a mapping $A : X \to Y$ is *bounded* if A takes bounded sets of X to bounded sets of Y. If $A(\lambda) : X \to Y$ depends on a parameter in a metric space Λ, then $A(\lambda)$ is said to be *bounded uniformly on compact sets* of Λ if for any compact set $\Lambda_0 \subset \Lambda$ and any bounded set $X_0 \subset X$, there is a bounded set $Y_0 \subset Y$ such that $A(\lambda)X_0 \subset Y_0$ for all $\lambda \in \Lambda_0$.

Let $C = C([-\tau, 0], \mathbf{R}^n)$ be a set of continuous maps from $[-\tau, 0]$ to $\mathbf{R}^n$.

Theorem 2.3 *[1, Corollary 3.6.2] Consider $x' = f(t, x_t)$ where $f : \mathbf{R} \times C \to \mathbf{R}^n$. If f is a bounded continuous map and the solution map $T(t_0, t, \cdot) : C \to C$ is bounded uniformly on compact sets of $[t_0, \infty)$, then $T(t_0, t, \cdot)$ is completely continuous for all $t \geq t_0 + \tau$.*

2.3 Dissipativeness

Definition 2.4 A map $F : C \to C$ is *dissipative*, if every solution enters a bounded region of C and stays there.

2.4 Injectivity of the solution map for delay equations

For Banach spaces X and Y, $\mathcal{L}(X, Y)$ is the Banach space of bounded linear mappings from X to Y with the operator topology. If $L \in \mathcal{L}(C, \mathbf{R}^n)$ then the Riesz representation theorem implies that there is $n \times n$ matrix function η on $[-\tau, 0]$ of bounded variation such that

$$L\varphi = \int_{-\tau}^{0} d[\eta(\theta)]\varphi(\theta). \qquad [9]$$

For any such η it is always understood that we have extended the definition to $\mathbf{R}$ so that $\eta(\theta) = \eta(-\tau)$ for $\theta \leq -\tau$, $\eta(\theta) = \eta(0)$ for $\theta \geq 0$.

Definition 2.5 *[1, Definition 2.5.2]* If $\beta \in \mathbf{R}$, function L is given by Equation **9**, and the matrix

$$A(\beta, L) := \eta(\beta^+) - \eta(\beta^-) = \lim_{h \to 0} \int_{\beta}^{\beta+h} d[\eta(\theta)] - \int_{\beta-H}^{\beta} d[\eta(\theta)]$$

is non singular, we say that L is *atomic* at β.

Definition 2.6 *[1, Definition 2.5.3]* Let Ω be an open subset of C. A function $f : \Omega \to \mathbf{R}^n$ is *atomic at β on Ω* if f is continuous together with its first and second Fréchet derivatives with respect to φ; and f_φ, the derivative with respect to φ, is atomic at β on Ω.

The importance of these concepts lies in the following theorem.

Theorem 2.7 *[1, Corollary 2.5.1] Consider a system*

$$w' = f(t, w_t),$$

and suppose Ω is an open set in $\mathbf{R} \times C$, $f : \Omega \to \mathbf{R}^n$ is continuous and $T(t, t_0, \varphi) : \Omega \to C$ is the solution map for this system. If f is atomic at $-\tau$ on Ω, then $T(t, t_0, \varphi)$ is one-to-one for all t in the interval of existence of the solution.

2.5 Monotonicity and strong monotonicity

The main reference for this section is the monograph by H. Smith (2).

A closed convex cone $D \subset C$, where $C = C([-\tau, 0], \mathbf{R}^n)$ is a Banach space, is an *order cone* if $D \cap (-D) = \{0\}$. Given an order cone $D \subset C$ we define an order on C by writing $\varphi \geq \psi$ if, and only if, $\varphi - \psi \in D$. We will write $\varphi > \psi$ if $\varphi \geq \psi$ and $\varphi \neq \psi$. If the cone D has nonempty interior, then we write $\varphi >> \psi$ if, and only if, $\varphi - \psi \in \text{int}D$.

To illustrate these concepts, take D the cone of nonnegative functions in C. Then the order is defined by $\varphi \geq \psi$ if, and only if, $\varphi - \psi \geq 0$, and $\varphi >> \psi$ if, and only if, $\varphi_i - \psi_i > 0$ for all i.

It is important to note that although, as will be shown in Lemma 3.1 and Lemma 3.2, the phase spaces of Systems **5** and **7** are the positive cones C^1_+ and C^0_+, the order cone D does not have to be a subset of either C^1_+ or C^0_+. Monotonicity of the solution map implies that two solutions starting with a particular order in the phase determined by a cone D maintain that order for all time. What phase space these solutions belong to is a different issue. This becomes important in System **7**, where the order cone D is not the positive cone C^0_+.

Definition 2.8 The map $F : C \to C$ is *monotone* if $\varphi \leq \psi$ implies $F(\varphi) \leq F(\psi)$. The differential $d_x F$ of the period map $F(\varphi) = T(r, 0, \varphi)$ is strongly monotone for all $\varphi \in C$ if $\psi > 0$ implies $d_x F(\varphi)\psi >> 0$.

A set X is called *order convex* if $[\varphi, \psi] \subset X$ whenever $\varphi, \psi \in X$ satisfy $\varphi < \psi$.

Definition 2.9 Consider a system

$$w' = f(t, w_t), \qquad [\mathbf{10}]$$

where $f : \mathbf{R} \times \Omega \to \mathbf{R}^n$, where Ω is an open subset of C, is continuously differentiable. The System **10** is *cooperative* if Ω is order convex and if $df(w)$ satisfies

$$df_i(t, w)\varphi \geq 0 \text{ whenever } \varphi \geq 0 \text{ and } \varphi_i(0) = 0 \qquad [\mathbf{11}]$$

for every $w \in \Omega$ and all t. If the system is cooperative, then by Lemma 5.1.2 in ref (2), the derivative $df(t, w)$ can be represented as

$$df_i(t, w)\varphi = a_i(t, w)\varphi_i(0) + \sum_{j=1}^{n} \int_{-\tau}^{0} \varphi_j(\theta) d_\theta \eta_{ij}(t, w, \theta)$$

where $a_i(t, w)$ and $\eta_{ij}(t, w, \theta)$ are continuous functions of $w \in \Omega$ and $t \in \mathbf{R}$. System **10** is *cooperative and irreducible* if it is cooperative and the following hold:

(i) the matrix

$$A(t, w) := col(df(t, w)\hat{e}_1, \dots df(t, w)\hat{e}_n) \qquad [\mathbf{12}]$$

is irreducible for every $w \in \Omega$ and $t \in \mathbf{R}$. Here $\hat{e}_j \in C$ is the obvious embedding of the standard unit vector $e_j \in \mathbf{R}^n$.

(ii) for every j such that the jth variable is delayed in the ith equation, we have

$$\eta_{ij}(t, w)([-\tau, -\tau + \epsilon)) > 0 \qquad [\mathbf{13}]$$

for all small $\epsilon > 0$ and all $t \in \mathbf{R}$.

For a constant $a \in \mathbf{R}^n$ we will denote by $\hat{a}$ the obvious embedding of a into C. Let $C^+ \subset C$ be the set of all $\varphi \in C$ such that $\varphi(\theta) > 0$ for all $\theta \in [-\tau, 0]$. This set is the *positive cone* of C.

Definition 2.10 The set of all $\varphi \in C^+$ such that $0 \leq \varphi \leq \hat{k}$, is called *an interval* and will be denoted by $[\hat{0}, \hat{k}]$. The inequality here refers to the regular ordering with respect to the cone C^+.

Lemma 2.11 *[2, Theorem 5.2.1 and Remark 5.2.1] Consider System* **10** *on* $C = C([-\tau, 0], \mathbf{R}^n)$. *The interval* $[\hat{0}, \hat{k}]$, *for some* $k \in \mathbf{R}^n$, *is positively invariant provided that the following condition holds. Whenever* $w \in [\hat{0}, \hat{k}]$ *and* $w_i(0) = 0$ $(w_i(0) = k_i)$ *for some* i, *then* $f_i(t, w) \geq 0$ $(f_i(t, w) \leq 0)$ *for all* t.

Theorem 2.12 *Consider an* r*-periodic system defined on* $C([-\tau, 0]), \mathbf{R}^n)$

$$w' = f(t, w_t)$$

and assume that it is cooperative and irreducible. Let $T(t, t_0, w_0)$ *be a solution with the initial condition* $w_0 \in C([-\tau, 0]), \mathbf{R}^n)$ *at time* t. *Then the differential* $d_x F$ *of the period map*

$$F(w_0) = T(r, 0, w_0)$$

is strongly monotone for all $w_0 \in C([-\tau, 0]), \mathbf{R}^n)$, *provided* $r \geq n\tau$.

Proof. Let $\psi \geq 0$ such that $\psi_i = 0$. Let $y(t, \psi)$ be a solution of the variational equation

$$y'(t) = df(w(t, 0, w_0))y_t, \quad y_0 = \psi.$$

Then

$$d_x F(w_0)\psi = y(r, \psi).$$

It is easy to see that $L(t) = df(w(t, 0, w_0))$ satisfies Conditions **11-13** on $t \geq 0$. By [2, Lemma 3.2] if $\psi > 0$, then $y(t, \beta) >> 0$ for $t \geq n\tau$. Therefore $d_x F(w_0)$ is strongly monotone, if $r \geq n\tau$. □

3 Proofs of the main results

Systems **5** and **7** posses an important invariance property that we formulate and verify in a pair of related lemmas.

3.1 Invariance properties

Lemma 3.1 *Assume that the initial condition $w_0 = (x_0, \xi_0)$ is nonnegative, i.e. $x_0(\theta) \geq 0$ and $\xi_0(\theta) \geq 0$ for all $\theta \in [-\tau, 0]$. Then the solution w_t starting at w_0 stays nonnegative for all $t \geq 0$. In other words $x_t(\theta) \geq 0$ and $\xi_t(\theta) \geq 0$ for all $\tau \geq 0$ and the non-negative cone*

$$C^1_+ := \{(x_t, \xi_t) \in C([-\tau, 0], \mathbf{R}^2), x_t \geq 0, \xi_t \geq 0\}$$

is positively invariant under the semiflow generated by System **5**.

Proof. Consider a first time t when $x_t(0) = 0$. Then

$$\dot{x}(t) = K_{imp}(\xi(t - \tau)) \geq 0$$

and so $x(s) \geq 0$ for all $s \geq t$, $s - t$ sufficiently small. Similarly, if $\xi_t(0) = 0$ then

$$\dot{\xi}(t) = T(\bar{X}(t - \delta_1)) + K_{exp}(x(t - \tau)) \geq 0$$

and so $\xi(s) \geq 0$ for all $s \geq t$, $s - t$ sufficiently small. Hence, C^1_+ is positively invariant. □

Lemma 3.2 *Assume that the initial condition $w_0 = (x_0, \xi_0, \mu_0)$ is nonnegative, i.e. $x_0(\theta) \geq 0$, $\xi_0(\theta) \geq 0$ and $\mu_0(\theta) \geq 0$ for all $\theta \in [-\tau, 0]$. Then the solution w_t starting at w_0 stays nonnegative for all $t \geq 0$. In other words the non-negative cone*

$$C^0_+ := \{(x_t, \xi_t, \mu_t) \in C([-\tau, 0], \mathbf{R}^3), x_t \geq 0, \xi_t \geq 0, \mu_t \geq 0\}$$

is positively invariant under the semiflow generated by System **6**.

Proof. The proof of this fact is similar to proof of Lemma 3.1. In fact if either $x_t(0) = 0$ or $\xi_t(0) = 0$ the proof is identical. Assume now that at time t we have $\mu_t(0) = 0$. Then

$$\dot{\mu}(t) = T(\bar{U}(t - \delta_2)) \geq 0,$$

which implies that $\mu(s) \geq 0$ for all $s \geq t$ with $s - t$ small enough. Hence C^0_+ is positively invariant. □

In view of this result we write System **6** as

$$w'(t) = f^0(t, w_t) \quad \text{with } w_t = (x_t, \xi_t, \mu_t) \in C^0_+ \qquad [\mathbf{14}].$$

Let $U(t, t_0, w_0)$ be the solution of the System **5** with initial condition $U(t_0, t_0, w_0) = w_0$, $w_0 \in C^0_+$. Let $G : C^0_+ \to C^0_+$ a time r solution map of the System **5**, i.e.

$$G(w_0) := U(r, 0, w_0), \quad w_0 \in C^0_+.$$

3.2 Proof of Theorem 1.

Proof of this theorem is based on two abstract results.

Theorem 3.3 *[3, Theorem 5.1] Assume F is a C^2, monotone, one-to-one, completely continuous dissipative mapping such that its differential d_xF is a strongly monotone operator on C^1_+. Then the set of points $w_0 \in C^1_+$ such that $T(t,t_0,w_0)$ converges to a single periodic orbit with period equal to some multiple of r, contains an open and dense set for any t_0.*

The second part of the Theorem **1** is based on the following result.

Theorem 3.4 *[4, Corollary 4] Assume F is C^2, monotone, one-to-one, completely continuous dissipative mapping such that its differential d_xF is a strongly monotone operator. Assume further that $\mathcal{G}$ is an open, bounded positively invariant subset of C^1_+. Then there is an m such that the set*

$$\{w_0 \in \mathcal{G} \; : \; T(w_0,t_0,t) \text{ converges to a single periodic orbit of period at most } mr\}$$

contains an open and dense subset of $\mathcal{G}$.

In light of Theorems 3.3 and 3.4 we need to verify that F is C^2, one-to-one, completely continuous, monotone and that dF is strongly monotone on C^+. Further, we will show that all solutions enter a fixed bounded region $\mathcal{U} \subset C^1_+$ and that this region $\mathcal{U}$ gives rise to a positively invariant region $\mathcal{G} \subset C^1_+$. We apply Theorem 3.4 to this region to conclude that there is an integer m such that all solutions converge to solution of period sr with $s \leq m$.

Since all functions in the System **5** are smooth, the solution map $T(t,t_0,w_0)$ is smooth and hence the mapping F is smooth.

We first compute the derivative df^1, where f^1 is the right hand side of System **5**

$$df^1(x,\xi) = \begin{bmatrix} -\frac{dK_{exp}}{dz} - \alpha & \frac{dK_{imp}}{dz} \\ \frac{dK_{exp}}{dz} & -\gamma - \frac{dK_{imp}}{dz} \end{bmatrix} \qquad [\mathbf{15}]$$

1. The map F is one-to-one We show that the System **5** is atomic at $-\tau$ by showing that the derivative is atomic at $-\tau$. We evaluate Derivative **15** at an arbitrary $w_0 = (x_0,\xi_0)$ and apply this matrix to $\psi := (\psi_x,\psi_\xi)$:

$$df^1(w_0)\psi = \begin{bmatrix} -\frac{dK_{exp}}{dz}(x_0(0)) - \alpha & \frac{dK_{imp}}{dz}(\xi_0(-\tau)) \\ \frac{dK_{exp}}{dz}(x_0(-\tau) & -\gamma - \frac{dK_{imp}}{dz}(\xi_0(0)) \end{bmatrix} \cdot \begin{pmatrix} \psi_x \\ \psi_\xi \end{pmatrix}$$

We can separate the action of the derivative matrix into a part that acts on $\psi_0(0)$ and the part that acts $\psi_0(-\tau)$ as

$$\begin{aligned} df^1(w_0)\psi &= \begin{bmatrix} -\frac{dK_{exp}}{dz}(x_0(0)) - \alpha & 0 \\ 0 & -\gamma - \frac{dK_{imp}}{dz}(\xi_0(0)) \end{bmatrix} \cdot \begin{pmatrix} \psi_x(0) \\ \psi_\xi(0) \end{pmatrix} \\ &+ \begin{bmatrix} 0 & \frac{dK_{imp}}{dz}(\xi_0(-\tau)) \\ \frac{dK_{exp}}{dz}(x_0(-\tau) & 0 \end{bmatrix} \cdot \begin{pmatrix} \psi_x(-\tau) \\ \psi_\xi(-\tau) \end{pmatrix}. \end{aligned}$$

The matrix $A(w_0,-\tau)$ is the part that acts on $\psi_0(-\tau)$

$$A(w_0,-\tau) = \begin{bmatrix} 0 & \frac{dK_{imp}}{dz}(\xi_0(-\tau)) \\ \frac{dK_{exp}}{dz}(x_0(-\tau)) & 0 \end{bmatrix}$$

and

$$detA(w_0,-\tau) = \frac{dK_{imp}}{dz}(\xi_0(-\tau))\frac{dK_{exp}}{dz}(x_0(-\tau)) > 0,$$

for all w_0. Therefore the System **5** is atomic and solution mapping $T(t,t_0,w_0)$ is one-to-one for all t in the interval of existence. It follows that F is one-to-one as well.

2. The differential d_xF is strongly monotone.
In light of Theorem 2.12 we need to show that the System **5** is cooperative and irreducible.

A. System 5 is cooperative.
We choose cone $D^1 = C^1_+$ the positive cone of C. This D^1 is clearly order convex.
We computed derivative $df(w,t)$ in Equation **15**. By assumption

$$\frac{dK_{imp}}{d\xi} > 0 \text{ and } \frac{dK_{exp}}{dx} > 0 \text{ for } \xi \geq 0, x \geq 0. \qquad [\mathbf{16}]$$

Take $\psi = (\psi_x, \psi_\xi) \in D^1$ i.e both components are nonnegative. We want to verify Condition **11**. Take $i = 1$. Then

$$df_1^1(w)(0,\psi_\xi)^T = \frac{dK_{imp}}{d\xi}\psi_\xi \geq 0$$

by Inequalities **16** and $\psi_\xi \geq 0$. For $i = 2$ we get

$$df_2^1(w)(\psi_x, 0)^T = \frac{dK_{exp}}{dx}\psi_x \geq 0.$$

Therefore the System **5** is cooperative.

B. System 5 is irreducible.
Recall that a 2×2 matrix A is irreducible if the off-diagonal entries are not zero. The Equation **15** shows that the matrix A satisfies this property and hence is irreducible. This verifies (i) of the definition of irreducibility. To verify (ii) it is enough to observe that $\eta_{12} = \eta_{21} = \delta_{-\tau}$ a delta function at $-\tau$. This shows that System **5** is cooperative and irreducible. By Theorem 2.12 the differential d_xF is strongly monotone for $t \geq 2\tau$.

3. The map F is completely continuous and dissipative
We need to verify for our system assumptions of Theorem 2.3. The map f^1 is clearly bounded and continuous so we need to show that the solution map is bounded uniformly on compact subsets of $[t_0, \infty)$. Recall that r is a period of the function $\bar{X}(t)$. We set

$$\begin{aligned} U_0 &:= \max_u K_{imp}(u), \\ U_1 &:= \max_{t\in[0,r]} T(X(s)) \\ U_2 &:= \max_u K_{exp}(u). \end{aligned} \qquad [\mathbf{17}]$$

Set

$$R_x := \frac{U_0}{\alpha}, \qquad R_\xi := \frac{U_1 + U_2}{\gamma}, \qquad R := (R_x, R_\xi) \qquad [\mathbf{18}]$$

Now we verify conditions of Lemma 2.11 for an interval $[\hat{0}, \hat{R}]$. We take an initial condition $w_0 = (x_0, \xi_0)$. Assume first that $x_0(0) = 0$ and both $x_0(\theta) \geq 0$ and $\xi_0(\theta) \geq 0$ for all $\theta \in [-\tau, 0]$. Then $f_1^1(w_0) = K_{imp}(\xi_0(-\tau)) \geq 0$. Let now $x_0(0) = R_x$ and $0 \leq \xi_0(\theta) \leq R_\xi$ for all $\theta \in [-\tau, 0]$. Then,

$$\begin{aligned} f_1^1(w_0) &= K_{imp}(\xi_0(-\tau)) - K_{exp}(x_0(0)) - ax_0(0) \\ &\leq U_0 - \alpha R_x - K_{exp}(R_x) \\ &\leq -K_{exp}(R_x) \\ &< 0. \end{aligned}$$

For the second coordinate we let $\xi_0(0) = 0$ and $0 \leq x_0(\theta) \leq R_\xi$ for all $\theta \in [-\tau, 0]$. Then $f_2^1(w_0) = T(\bar{X}(t - \delta_1)) + K_{exp}(x_0(-\tau)) > 0$. Finally, if $\xi_0(0) = R_\xi$ and $0 \leq x_0(\theta) \leq R_x$ for all $\theta \in [-\tau, 0]$, then

$$\begin{aligned} f_2^1(w_0) &= T(\bar{X}) - \gamma\xi_0(0) - K_{imp}(\xi_0(-\tau)) + K_{exp}(x_0(-\tau)) \\ &\leq U_1 - pR_\xi - K_{imp}(R_\xi) + K_{exp}(x_0(0)) \\ &< U_1 + U_2 - \gamma R_\xi \\ &\leq 0. \end{aligned}$$

By Lemma 2.11 the interval $[0, \hat{R}]$ is a positively invariant set in C^1_+. Since the above argument is true for any $R >> \hat{R}$ every solution starting at $w_0 \in C^1_+$ exists for $t \in [0, \infty]$. It also shows that all solutions enter the closed and bounded region $[\hat{0}, \hat{R}_x] \times [\hat{0}, \hat{R}_\xi] \subset C^1_+$. Hence, both the semiflow $T(w_0, t_0, t)$ and the map $F : C^1_+ \to C^1_+$ are dissipative. This also implies that the solution map is uniformly bounded on $[t_0, \infty)$ and thus by Theorem 2.3 F is completely continuous.

4. The map F is monotone.

We have shown above that the System **5** is cooperative. By [2, Lemma 5.3.3] this implies that f^1 is *quasi-monotone*, that is

$$\text{whenever } \varphi \leq \psi \text{ and } \varphi_i(0) \leq \psi_i(0) \text{ holds for some } i, \text{ then } f^1_i(t, \varphi) \leq f^1_i(t, \psi).$$

By [2, Theorem 5.1.1] quasi-monotonicity of f^1 implies that whenever $\varphi \leq \psi$ then $T(t, 0, \varphi) \leq T(t, 0, \psi)$ for any $t \geq 0$. Since $F(\cdot) = T(r, 0, \cdot)$ this proves the monotonicity of F.

This verifies all assumptions of Theorem 3.3. Take the region $\mathcal{U} := (\hat{0}, \hat{R}) \subset C^1_+$. By the argument for complete continuity the set

$$\mathcal{G}_\mathcal{U} := \bigcup_{t>0} T(t, 0, \mathcal{U})$$

is open, bounded, and positive invariant subset of C^1_+. Theorem 3.4 applied to the set $\mathcal{G}_\mathcal{U}$ produces an integer m such that all solutions starting in $\mathcal{G}_\mathcal{U}$ converge to periodic solutions with periods sr with $s \leq m$. Since $T(t, t_0, w_0)$ is dissipative and all solutions eventually enter $[\hat{0}, \hat{R}]$ this statement is valid for any initial data in C^1_+. This concludes the proof of Theorem **1**. □

3.3 Proof of Theorem 2

We first observe that convergence of $X(t) \to \bar{X}(t)$, $U(t) \to \bar{U}(t)$ and $C(0)e^{-k_r t} \to 0$ is exponentially fast. Following the ideas outlined in ref (5), we now embed the semiflows of Systems **4** and **3** into one autonomous semiflow. Given a non-autonomous semiflow Φ defined by System **4** and a limiting semiflow Ψ defined by System **3** we choose the state space $Z = [t_0, \infty] \times C^1_+$ where $[t_0, \infty]$ is compactified in the usual way. Z is then a metric space. We define an autonomous semiflow Θ on Z by

$$\Theta(t, (s, w)) = \left\{ \begin{array}{cc} (t + s, \Phi(t + s, s, w)) & t_0 \leq s < \infty \\ (\infty, \Psi(t, w) & s = \infty \end{array} \right\},$$

into the full System **4** as described in ref (5). Since we assume hyperbolicity in the limiting System **3**, there is only finitely many invariant sets in the System **3**. Let $\mathcal{S}$ be an unstable invariant set of the limit System **3** and assume that the unstable manifold has dimension k. Then the stable manifold of $\mathcal{S}$ in the full System **4** has codimension at least k and hence is closed and nowhere dense in $\mathbf{R} \times C^1_+$. Since there is only finitely many such unstable invariant sets, the union of their stable manifolds is closed and nowhere dense in $\mathbf{R} \times C^1_+$. This implies that for (t_0, w_0) in an open and dense subset of $\mathbf{R} \times C^1_+$ the solution $T(t, t_0, w_0)$ converges to a periodic trajectory, which corresponds to a period sr point of the map F with $s \leq m$. □

3.4 Proof of Theorem 3

The proof of this theorem is analogous to Theorem **1** with one notable exception. Theorem 3.3 requires that the flow defined map G is one-to-one. Delay differential equations do, in general, admit solutions which merge in finite time. This means that the solution map may not be one-to-one. While we are able to prove that the solution map of System **4** is one-to-one, it can be shown easily that this is not the case for the System **7**. Therefore we are forced to use an unpublished result of I. Tereščák (personal communication P. Poláčik) where the injectivity assumption is not required.

Theorem 3.5 (Tereščák,Poláčik) *Assume G is a C^2, monotone, completely continuous and dissipative mapping such that its differential $d_x G$ is strongly monotone operator on C^0_+. Then the set of points $w_0 \in C^0_+$*

such that $U(t, t_0, w_0)$ converges to a single periodic orbit with period equal to some multiple of r, contains an open and dense set.

We now verify the assumptions of this Theorem. Let

$$D^0 := \{(w_1, w_2, w_3) \in C([-\tau, 0], \mathbf{R}^3) \mid w_1(\theta) \geq 0, w_2(\theta) \geq 0, w_3(\theta) \leq 0\}$$

be a cone in C. Notice that the cone D^0 is not a subspace of the positive cone C_+^0. We will show that the map G is a C^2, compact, monotone (with respect to the cone D^0) mapping on its domain of definition. Furthermore, for any $w_0 \in C_+^0$, the differential $d_w G$ is a strongly monotone operator with respect to the cone D^0. We compute the derivative df^0, where f^0 is the right hand side of System **7**

$$df^0(w,t) = \begin{bmatrix} -\frac{dK_{exp}}{dz} - \alpha & \frac{dK_{imp}}{dz} & 0 \\ \frac{dK_{exp}}{dz} & -\gamma - \frac{dK_{imp}}{dz} - k_f \mu & -k_f \xi \\ 0 & -\kappa - k_f \mu & -k_f \xi \end{bmatrix}. \qquad [19]$$

1. The differential $d_w G$ is strongly monotone.

In light of Theorem 2.12 we need to show that the System **7** is cooperative with respect to the cone D^0 and irreducible.

A. System 7 is cooperative.

Our choice guarantees that D is order convex. We computed the derivative $df^0(w,t)$ in Equation **19**. Take $\psi = (\psi_x, \psi_\xi, \psi_\mu) \in D^0$, which means that $\psi_x(\theta) \geq 0, \psi_\xi(\theta) \geq 0$ and $\psi_\mu(\theta) \leq 0$ for all $\theta \in [-\tau, 0]$. Take the derivative $df^0(w_0, t)$ evaluated at point w_0 in the phase space C_+^0 with $x_0(\theta) \geq 0, \xi_0(\theta) \geq 0, \mu_0(\theta) \geq 0$ for all $\theta \in [-\tau, 0]$. We show cooperativity with respect to the cone D^0. Take $i = 1$. Then

$$df_1^0(w_0, t)(0, \psi_\xi, \psi_\mu)^T = \frac{dK_{imp}}{d\xi} \psi_\xi \geq 0$$

since the coefficient $\frac{dK_{imp}}{d\xi} > 0$. For $i = 2$ we get

$$df_2^0(w_0, t)(\psi_x, 0, \psi_\mu)^T = \frac{dK_{exp}}{dx} \psi_x - k_f \xi_0 \psi_\mu \geq 0$$

since $\xi_0 \geq 0$, $k_f > 0$ and $\psi_\mu \leq 0$. Finally, for $i = 3$ we have

$$df_3^0(w_0, t)(\psi_x, \psi_\xi, 0)^T = (-\kappa - k_f \mu_0) \psi_\xi \leq 0$$

since $\mu_0 \geq 0$ and $\psi_\xi \geq 0$. Therefore the System **7** is cooperative with respect to the cone D^0.

B. System 7 is irreducible.

Equation **19** shows that the matrix df^0 is irreducible since for every nonempty, proper subset I of $N := \{1, 2, 3\}$ there is an $i \in I$ and $j \in N \setminus I$ such that $df_{ij}^0 \neq 0$. This verifies (i) of the definition of irreducibility. To verify (ii) it is enough to observe that $\eta_{12} = \eta_{21} = \delta_{-\tau}$ a delta function at $-\tau$.

This shows that System **7** is cooperative and irreducible. By the Theorem 2.12, the differential $d_x G$ is strongly monotone with respect to D^0.

2. The map G is completely continuous and dissipative.

We need to verify for our system the assumptions of Theorem 2.3. The argument is completely analogous to the argument from proof of Theorem **1**. The map f^0 is clearly bounded and continuous so we need to show that the solution map is bounded uniformly on compact subsets of $[t_0, \infty)$. In addition to definition of U_0, U_1 and U_2 in Equation **17** we define

$$U_3 := \max_{t \in [0,r]} T(U(s)), \quad U_4 := \min_{t \in [0,r]} T(U(s)).$$

In addition to R_x and R_ξ (see Equations **18**) we define

$$R_\mu := \frac{U_3}{q}.$$

We now show that System **7** admits a positively invariant interval of the form $[\hat{0}, \hat{R}] \subset C_+^0$ with $R = (R_x, R_\xi, R_\mu)$. Notice that inequalities in this part of the proof are with respect of the cone C_+^0 and not the cone D^0. The estimates for $i = 1$ and $i = 2$ are identical to the estimates for System **5** which were performed in the proof of Theorem **1**. When $i = 3$ we let $w_0 = (x_0, \xi_0, \mu_0)$ and assume $\mu_0(0) = 0$, $0 \leq x_0(\theta) \leq R_x$ and $0 \leq \xi_0(\theta) \leq R_\xi$ for all $\theta \in [-\tau, 0]$. Then by direct inspection

$$f_3^0(x_0, \xi_0, \mu_0) \geq U_4 > 0.$$

For $\mu_0(0) = R_\mu$, $0 \leq x_0(\theta) \leq R_x$ and $0 \leq \xi_0(\theta) \leq R_\xi$ for all $\theta \in [-\tau, 0]$, we get

$$f_3^0(x_0, \xi_0, \mu_0) \leq U_3 - \kappa R_\mu - k_f R_\mu R_\xi < 0.$$

It follows from Lemma 2.11 that the interval of the form $[\hat{0}, \hat{R}]$ with $R = (R_x, R_\xi, R_\mu)$ is positively invariant in C_+^0. Since the above argument is true for any $R >> \hat{R}$ every solution starting at $w_0 \in C_+^0$ exists for $t \in [0, \infty]$. It also shows that all solutions in C_+^0 enter the closed and bounded region $[\hat{0}, \hat{R}_x] \times [\hat{0}, \hat{R}_\xi] \times [\hat{0}, \hat{R}_\mu]$. Hence, G is dissipative and thus the solution map is uniformly bounded on $[t_0, \infty]$. By Theorem 2.3 this implies that G is a completely continuous map.

3. The map G is monotone.

We have shown above that the System **7** is cooperative. By [2, Lemma 5.3.3] this implies quasi-monotonicity of the right hand side f^0 of System **7**

$$\text{whenever } \varphi \leq \psi \text{ and } \varphi_i(0) \leq \psi_i(0) \text{ holds for some } i, \text{ then } f_i^0(t, \varphi) \leq f_i^0(t, \psi).$$

By [2, Theorem 5.1.1] quasi-monotonicity of f^0 implies that whenever $\varphi \leq \psi$ then $U(t, t_0, \varphi) \leq U(t, t_0, \psi)$. Since $G(\cdot) = U(r, 0, \cdot)$ this proves monotonicity of G. This verifies the assumptions of Theorem 3.5 and thus proves the Theorem **3**. Finally, the proof of Theorem **4** is completely analagous to the proof of Theorem **2**.

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