Version CRIB

EGT2
ENGINEERING TRIPOS PART IIB

CRIB

Module 4G7

CONTROL & COMPUTATION IN LIVING SYSTEMS

*Answer **both** questions in section A. Answer **one** question in section B.*

All questions carry the same number of marks.

*The **approximate** percentage of marks allocated to each part of a question is indicated in the right margin.*

Manuscripts referenced in section B are attached.

STATIONERY REQUIREMENTS
Single-sided script paper

SPECIAL REQUIREMENTS TO BE SUPPLIED FOR THIS EXAM
Engineering Data Book
CUED approved calculator allowed

10 minutes reading time is allowed for this paper at the start of the exam.

You may not start to read the questions printed on the subsequent pages of this question paper until instructed to do so.

You may not remove any stationery from the Examination Room.

SECTION A

Answer **both** questions.

1 Consider the case of a species X governed by the antithetic integral feedback Z, represented by the dynamics

$$\begin{aligned}\dot{x} &= z_1 - \gamma x + w \\ \dot{z}_1 &= \mu - \eta z_1 z_2 \\ \dot{z}_2 &= \theta x - \eta z_1 z_2 \,.\end{aligned}$$

z_1 and z_2 are the concentrations of the species used for implementing the antithetic integral feedback, x is the concentration of the controlled species, and w is an additional exogenous perturbation. Take $w = 1$, $\theta = 1$, $\gamma = 1$, $\mu = 10$, $\eta = 10$.

(a) Explain how the antithetical integral feedback implements integral action. Why is integral action important for homeostasis? [20%]

An integrator is realised by the difference $z_1 - z_2$, which integrates the mismatch between the production rate μ and the measured output θx. For instance,

$$\dot{z_1} - \dot{z}_2 = \mu - \theta x \quad \Rightarrow \quad z_1(t) - z_2(t) = \int_0^t \mu - \theta x(\tau) d\tau \,.$$

Integral action is needed to achieve $x = \mu/\theta$ at steady state. The integral $\int_0^t \mu - \theta x(\tau) d\tau$ converges to a constant, i.e. to an equilibrium, only when $\mu = \theta x$. It is used to estimate the control action needed to bring the mismatch $\mu - \theta x$ to zero, at steady state.

(b) Compute the equilibrium of the system and discuss why closed-loop stability is needed to guarantee homeostasis. [20%]

From second and third equations, $x_e = \dfrac{\mu}{\theta} = 10$. Substituting into the first equation, $z_{1,e} = \gamma x_e - w = 10 - 1 = 9$. Substituting into the third equation, $z_{2,e} = \dfrac{\theta x_e}{\eta z_{1,e}} = \frac{1}{9}$.

The equilibrium of the system is attractive, i.e. system trajectories converge asymptotically to it, only if the closed-loop system is stable. In other words, if the state of the system is temporarily perturbed by exogenous perturbations, stability guarantees that the system state returns to the equilibrium.

(c) The Nyquist plot below is obtained by "opening the loop" at x, as illustrated by the block diagram below. Derive the range of $\theta \geq 0$ that guarantees homeostasis. Explain your answer. [20%]

The Nyquist locus intersects the real axis at about 0.01. From the Nyquist criterion, closed-loop stability is thus guaranteed for $0 \leq \theta < 100$ (no encirclements of the point -1). Since $x_e = \frac{\mu}{\theta}$, we need $\theta > 0$. Likewise, $z_{1,e} = \gamma x_e - w = \frac{\mu}{\theta} - 1$, which is greater than or equal to zero only if $\theta \leq 10$. So, for homeostasis, θ must satisfy $0 < \theta \leq 10$.

(d) Discuss how delays on the controlled species X affect the behaviour of the system. [20%]

A large delay $T > 0$ can make the closed-loop system unstable. With a delay, the feedback information stored by the integrator has a time lag $\int_0^t \mu - \theta x(\tau - T) d\tau$. This may induce under/over-corrections, leading to instability in the form of undamped oscillations. The destabilising effect of a delay can also be observed on the Nyquist diagram, where a delay induces a (frequency-dependent) phase shift. This may lead to the Nyquist locus crossing the point -1 even if $\theta < 10$.

(e) Briefly discuss how the parameter γ affects the closed-loop behaviour. [20%]

A larger γ has a stabilising effect on the closed-loop behaviour (by reducing the gain in the feedback loop). Looking at the equilibrium, $z_{1,e} = \gamma x_e - w = \gamma \frac{10}{\theta} - 1$, which is greater than or equal to zero only if $\theta \leq 10\gamma$. This extends the range of θ that guarantees homeostasis. Likewise, $z_{2,e} = \frac{\theta x_e}{\eta z_{1,e}} = \frac{1}{\gamma \frac{10}{\theta} - 1}$, which shows how z_2 is subject to a larger depletion to compensate for the larger degradation rate γ.

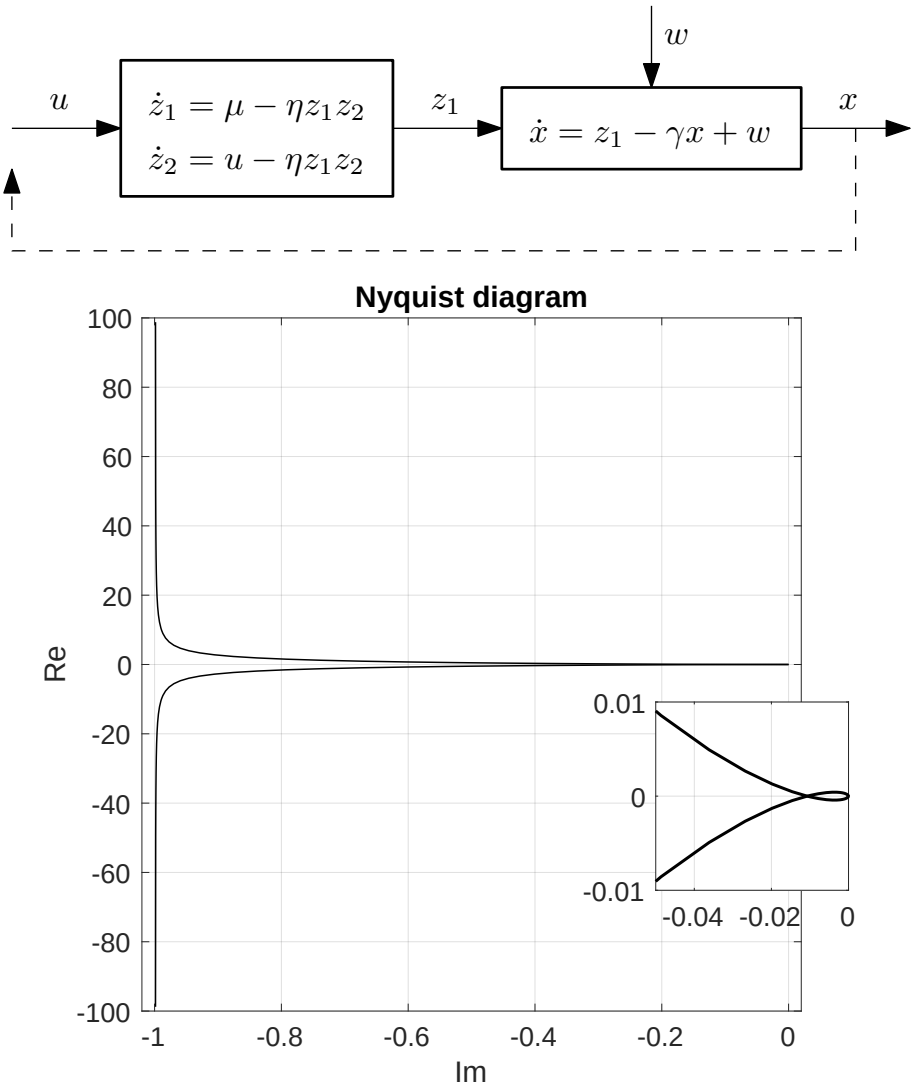

w
u
$\dot{z}_1 = \mu - \eta z_1 z_2$
$\dot{z}_2 = u - \eta z_1 z_2$
z_1
$\dot{x} = z_1 - \gamma x + w$
x
Nyquist diagram
Re
Im
100
80
60
40
20
0
-20
-40
-60
-80
-100
-1
-0.8
-0.6
-0.4
-0.2
0
0.01
0
-0.01
-0.04
-0.02
0

Fig. 1

2 (a) Consider an enzymatic reaction with Michaelis-Menten kinetics,

$$S + E \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} P + E$$

and let s, e, x and p denote the concentrations of substrate (S), enzyme (E), enzyme-substrate complex (ES) and product (P) respectively.

(i) Write down a system of ordinary differential equations describing the concentration dynamics of x and s in terms of total enzyme, $e_0 = e + x$. [10%]

$\dot{x} = k_1 e_0 s - (k_1 s + k_2 + k_3)x$

$\dot{s} = -k_1 e_0 s + (k_1 s + k_2)x$

(ii) How is the absence of a reverse reaction from $P + E$ to ES justified? [10%]

concentration of P is assumed to be very low and reverse rate less energetically favourable, so total reverse rate is negligibly small

(iii) By making the quasi-steady state assumption, $\dot{x} \approx 0$, obtain an expression for the dynamics of the substrate concentration $s(t)$ in terms of the reaction rate constants and e_0. [30%]

setting $\dot{x} = 0$ in the above ODE, we get:

$$x = \frac{k_1 e_0 s}{k_1 s + k_2 + k_3}$$

Substituting this into the expression for $\dot{s}$ we obtain,

$$\dot{s} = -k_1 e_0 s + \frac{(k_1 s - k_2) k_1 e_0 s}{k_1 s + k_2 + k_3}$$

$$= \frac{-k_1 k_3 e_0 s}{k_1 s + k_2 + k_3}$$

as required.

(b) The membrane potential, V, of a cell obeys the following dynamics (in dimensionless form):

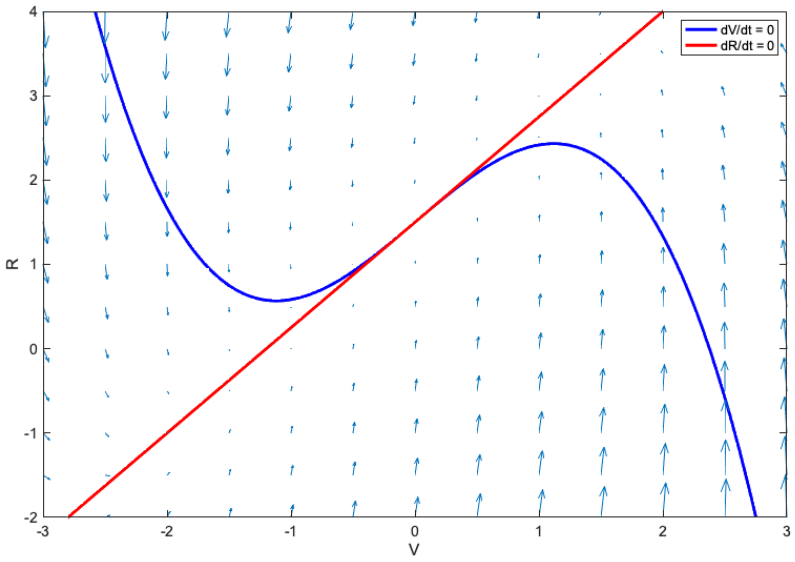
$$\dot{V} = 1.25V - \frac{V^3}{3} - R + 1.5 \quad (1)$$

$$\dot{R} = -R + 1.25V + 1.5 \quad (2)$$

where R is an adaptation variable.

(i) Sketch the phase plane of this system. [10%]

Phase plane (flow arrows optional):



(ii) Find the equilibria of this system and quantify their stability. [30%]

Solving:

$$0 = 1.25V - \frac{V^3}{3} - R + 1.5$$
$$0 = -R + 1.25V + 1.5$$

and substituting for R we obtain

$$1.25V - \frac{V^3}{3} - 1.25V = 0.$$

This has only one real solution at $V = 0$, which gives single equilibrium for the system at $(0, 1.5)$

The Jacobian at this equilibrium is

$$\begin{pmatrix} 1.25 - V^2 & -1 \\ 1.25 & -1 \end{pmatrix} = \begin{pmatrix} 1.25 & -1 \\ 1.25 & -1 \end{pmatrix}$$

and this has characteristic equation,

$$0 = (1.25 - \lambda)(-1 - \lambda) + 1.25 = \lambda(\lambda - 1/4)$$

so eigenvalues are $\lambda = 0, 0.25$. The fixed point is unstable.

(cont.

(iii) What can you deduce about the long term behaviour of this system? [10%]

The trajectories of the system are bounded: flow in all quadrants of the phase plane for large absolute values of V and R directs into a finite rectangle. This bounded region contains a single unstable fixed point. Therefore by the Poincare-Bendixon theorem, depending on initial conditions the system will either reside precisely at this fixed point or exhibit limit cycle oscillations of a fixed amplitude.

SECTION B

Answer **one** question.

3 Refer to the attached paper by Korobkova et al (2004) Nature.

(a) Summarise the aim, approach, findings and motivation of the paper in no more than 500 words. Provide an interpretation and comment on any limitations of the study. You may use diagrams if you wish. [65%]

A good answer is a clearly written, well structured answer that demonstrates an understanding of the material. A model example might cover the following points:

Aim: To measure the fluctuations of CW/CCW motor bias in E Coli in a way that allows identification of properties at the single cell level that might not be apparent in population-level measurements.

Approach: Immobilised E coli strains on a microscope slide and measured power spectrum of fluctuations of flagellar motion after attaching fluorescent beads. Used generically engineered strains to vary the copy number of key proteins in cells, allowing the origin of long-lived fluctuations to be identified.

Findings: It had been assumed that switches in flagellar motion in single cells were memoryless, e.g. exhibited exponential distribution of switching times. However, the study identified a trend in the power spectrum of switching events at low frequencies. This suggests that there are long-lived variations in the CW/CCW bias of the motor. The authors used inducible mutants to vary the copy numbers of key proteins in the chemotaxis pathway, identifying the methytransferase CheR as a major contributor to long-lived fluctuations.

Motivation: Individuality in motor switching fluctuations is directly linked to a cell's tendency to explore an environment or dwell in a locality. Since this phenotype is difficult for individual cells to modify adaptively, any evidence for how explore/exploit behaviour might vary at the individual level is relevant to its fitness in changing environments.

Interpretation: the long dwell times indicate that individual cells can shift their explore/exploit behaviour over time. This indicates that there is both population-level variability and within-individual variability in this key property over time

Limitations: as with all genetic alterations there could be unanticipated side-effects of manipulating protein expression level. The study also does not directly assess how this phenotypic variability affects chemotactic behaviour.

(cont.

(b) What is meant by "non-genetic individuality" in the context of this study, how is hypothesized to arise in the signalling network being studied, and what relevance might it have for bacterial chemotaxis? [35%]

- Non-genetic individuality means when there are detectable differences in phenotype within a clonal population
- in this context it means that there is diversity in the steady state CW/CCW switching behaviour of the flagellar motor
- this is due to variation in the copy number of signalling proteins in each cell (e.g. CheR)
- the relevance to chemotaxis is that this determines the steady state tumble probability and thus the average steady state run length; this will lead to variation in behavioural phenotype with some cells exploring large areas with long runs and others tumbling more frequently and exploring smaller areas

4 Refer to the attached paper by Veraart et al (2012) Nature.

(a) Summarise the aim, central hypothesis, approach and findings of the paper in no more than 500 words. Provide an interpretation and comment on any limitations of the study. You may use diagrams if you wish. [65%]

A good answer is a clearly written, well structured answer that demonstrates an understanding of the material. A model example might cover the following points:

Aim: to test a theory-based approach for inferring the proximity of a critical transition (abrupt, qualitative change in dynamics or steady state behaviour) in a model living system that is relevant to a wider ecosystem.

Approach: authors use a bioreactor with cyanobacterial cultures whose growth rate depends on light being maintained in a healthy range. Under normal conditions incoming light is shielded by the biomass, resulting in a stable population density. Increasing light intensity above a health range causes cell death, which reduces shielding and eventually causes collapse of the population. To measure the proximity to collapse, they gradually increase light intensity and perturb the culture with dilution, measure optical density (proportional to cell density) and estimate recovery rate. They also estimate variance and autocorrelation of the optical density timeseries. The experiment is repeated in two cultures.

Findings: as predicted by previous theory, recovery rates grow as the populations approach collapse, so too does autocorrelation time. However there is no detectable trend for variance.

Interpretation: even in a complex, uncertain nonlinear system it may be possible to detect signs of an abrupt transition by looking at changes in the recovery rate of state variables; this is because many transitions necessitate slowing down of the flow field near a bifurcation (e.g. "ghost" near a saddle node bifurcation).

Limitations: only two replicates; population density has clear decreasing trend for both cultures in Figure 1, would help to hold light and observe a steady-state; this is a closed, laboratory controlled system and a questionable model for a complex ecosystem, so some of the claims about wider utility remain open; the lack of a trend in variance is not convincingly explained.

(b) Comment on the methods put forward for inferring the proximity of an ecological system to a critical transition. Are these methods practical and can you foresee issues with their reliability? [35%]

Key points include:

- two methods are proposed: (1) passively monitoring variance/autocorrelation time from timeseries and (2) estimating relaxation time (slowing) through direct perturbation
- both methods have problem that proximity to transition is hard to infer
- both methods may be insensitive to certain types of abrupt transitions, where parameter range for slowing is narrow (together with previous point contributes to false negatives)
- slowing is not a sufficient condition for a critical transition (can give a false positive)
- need to perturb the system for (2) which is not always possible and could even destabilise it
- usual measurement issues: robust estimates of variance and exponential decay are sensitive to noise/data hungry

END OF PAPER