Imperial College London *MSc in Epidemiology*
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Practical: Vector-borne microparasitic infections (Continued)
Model Answers

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Introduction

Vector-borne microparasitic infections, such as *Malaria*, can be characterised by high transmission intensity, and due to the linear dependency between the basic reproduction ratio and the vector to host ratio (V/H), by large values of (entomologically estimated) R_0. In this practical we will begin by gaining some understanding of the components which contribute to R_0 and how interventions such as vector control, bed-nets, and treatment (chemotherapy) influence transmission. Later in the practical you will construct a model of malaria transmission to look at the effects of latency in vectors, and seasonal variation of vector population numbers.

The primary aims of the practical will be to:

1. Familiarise yourselves with the components of the basic reproduction number for vector-borne microparasitic infections (reinforcing concepts already learned in the lecture).
2. Gain further familiarity with model construction (using Berkeley Madonna).
3. Investigate the effects of introducing the extrinsic incubation period.
4. Introduce seasonality (optional)

Part II. Building a model of malaria transmission

Now that you understand the basic reproduction number and its influence on transmission, we can solve the model numerically using Berkeley-Madonna. The full model for malaria transmission has four compartments, susceptible hosts (X_h), infected hosts (Y_h), susceptible vectors (X_v) and infected vectors (Y_v). For the purposes of this practical we shall ignore the per capita rate of human mortality in comparison with the per capita rate of recovery because the human life-span is measured in years in contrast to days for the duration of infection ($\mu_h << r$). To maintain population size constant, the per capita birth rate is equal to the mortality rate. We will ignore the complications of the vector life-cycle (holometabolous with eggs, larvae, pupae and imagoes) and assume that the rate of recruitment of susceptible (nulliparous) flies equals $\mu_v V$.

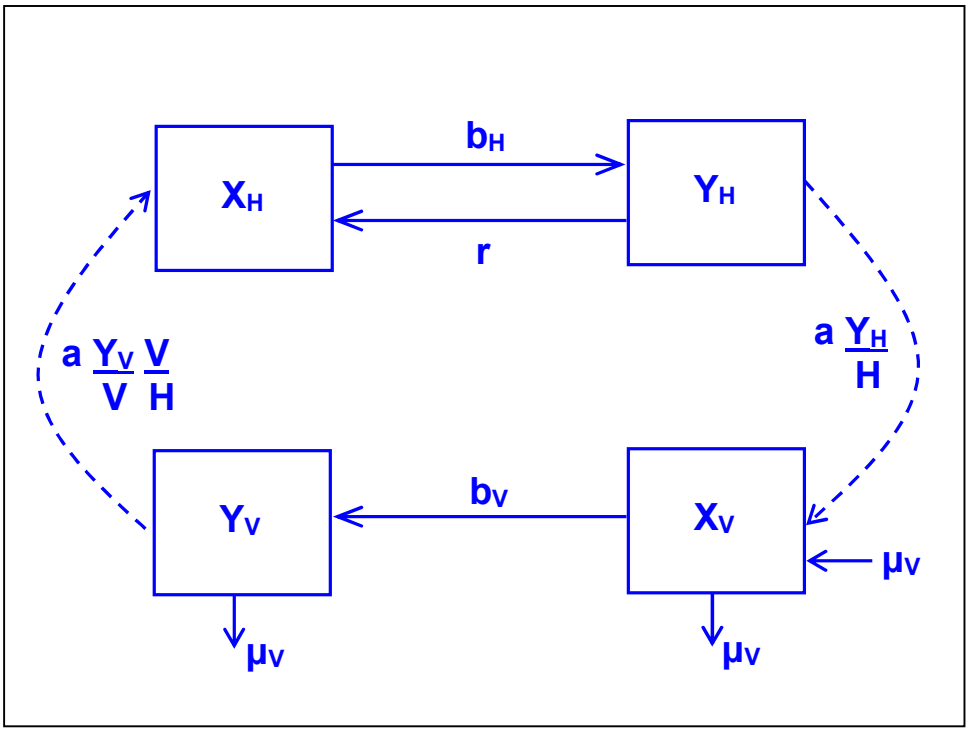
$$dX_h/dt = -(V/H)\, a\, b_h\, (Y_v/V)\, X_h + r\, Y_h \qquad \text{(eqn 1)}$$

$$dY_h/dt = (V/H)\, a\, b_h\, (Y_v/V)\, X_h - r\, Y_h \qquad \text{(eqn 2)}$$

$$dX_v/dt = \mu_v V - a\, b_v\, (Y_h/H)\, X_v - \mu_v X_v \qquad \text{(eqn 3)}$$

$$dY_v/dt = a\, b_v\, (Y_h/H)\, X_v - \mu_v Y_v \qquad \text{(eqn 4)}$$

Draw a flow diagram to represent the above equations



Begin by opening Berkeley Madonna. Choose **New** from the **File** menu. Madonna creates a new model (Untitled) and opens its equation window. You will need to start by choosing a method of numerical integration (choose Runge Kutta 4). You should also think about the time-scales we are interested in. All units have been expressed in terms of days. A typical time-step will be 1 day and a year 365 days. To gain sufficient accuracy with the model simulations set STOPTIME to run for 1 year (stop value of 365) with 365 output steps.

METHOD Euler (Change this to RK4)

STARTTIME = 0
STOPTIME=10 (Since we are working in days, change to 365)
DT = 0.02 (This is the default integration time-step)
DTOUT = 1 (the outputs will be in days)

Now give the initial values of your state variables. We will commence with 1 infective host and 99 susceptible hosts. For the mosquito population, we will start with 1000 susceptible mosquitoes and none infected.
E.g.

init (Xh) = 99

Proceed by writing your equations (1) to (4) as given above (for Madonna Syntax go to **Equation Help** in the **Help** menu).
E.g.

d/dt(Xh) = -(V/H)*a*bh*(Yv/V)*Xh + r*Yh

Since we must keep track of the total population size, add the variables H and V. Insert the equations **Xh+Yh** (for H) and **Xv+Yv** (for V). (eqns 5, 6)

You could add a variable for the basic reproduction number R_0, with equation

R0 = (V/H)* (a^2)*bv*bh/(muv*r) (eqn 7)

or the vectorial capacity, *C*,
C = (V/H)* (a^2)*bv*bh/(muv) (eqn 8)

and the Entomological Inoculation Rate (EIR)
EIR = (V/H)* a *(Yv/(Xv+Yv)) (eqn 9)

Now add the parameters from the following list as discussed above

a (in day^{-1})	bh	r (in day^{-1})	bv	muv (in day^{-1})
0.33 [1/(3d)]	0.2	0.02 [1/(50d)]	0.05	0.1 [1/(10d)]

The model is now ready to **Run**.
Graphs will be prepared with the first eight variables, which you can toggle to see represented. You can add pages and have each page representing a variable or a couple of variables. You can also choose which variables to plot: In the Window containing the graph, double click on the background and a Choose Variables Box dialogue will appear; add or remove variables and click on OK. The new variable tabs will appear in the bottom. Click on the tab. Click on **Run** to obtain the plot.

It is a good idea to check that the model is behaving in the way it should. Prepare a graph of the number of vectors, V, and of hosts, H against time and check that they remain constant and at the levels you set out at the beginning. Also inspect the graph for the basic reproduction number. Is it the same as you calculated in the first part of this practical?

To plot prevalences define two new variables hostprev (equation: **100*Yh/H**) and vectorprev (equation: **100*Yv/(Xv+Yv)**).

What do you predict will happen? Will the epidemic be severe? On a new graph plot the prevalences of host and vector infections. You can edit axes, series, graph names (Double click on the axes to edit them). What do you notice? Why is this the case?

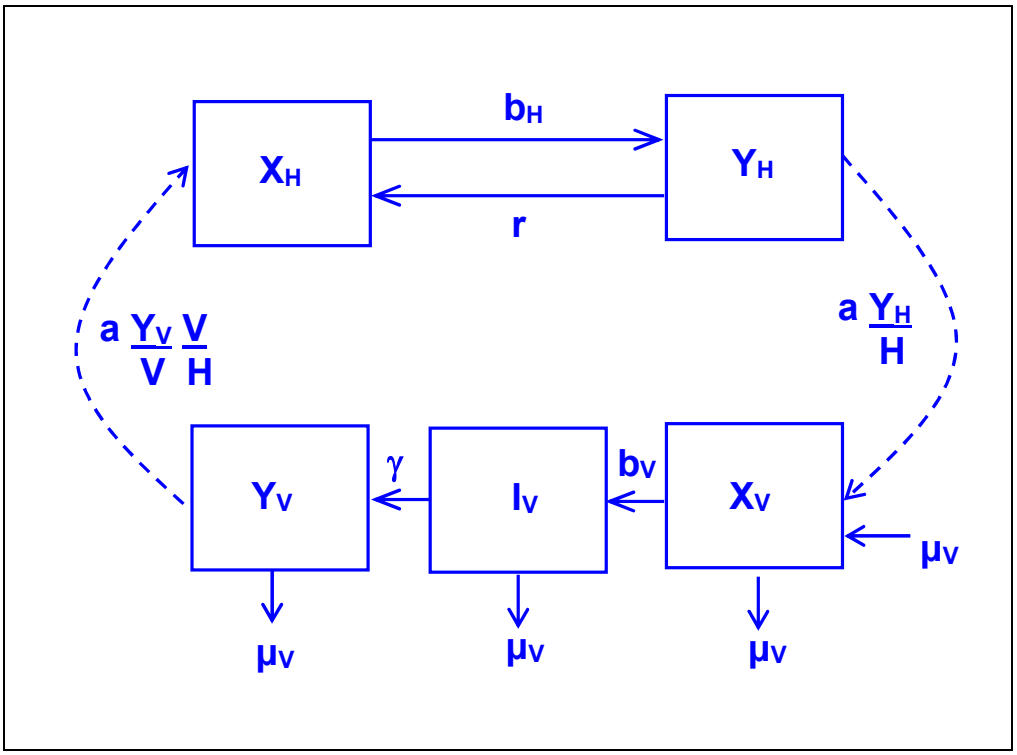
- Prevalence in hosts = 79% at equilibrium
- Prevalence in vector = 11.5% at equilibrium
- Same as calculated earlier (Host prevalence >> Vector prevalence)
- Since each introduced case generates on average ~6 new cases, yes, the epidemic would be severe

Save your model under a name (e.g. Vectors 1.mmd)

Observed endemic vector prevalences for a number of vector-borne infections are listed in Table 14.5 of Anderson & May (1991) and are in general much lower than the numbers predicted here (see Table 3 above). What has been left out of the model to give this discrepancy?

- Latency in the vector – the Extrinsic Incubation Period (EIP). As the EIP takes up a substantial amount of time in comparison with the vector's life expectancy, the expectation of infective life is lower; this contributes to explain the discrepancy. Other factors may include that not all blood-meals are taken on human hosts.

Can you think of how to modify the equations for the vector compartments to correct this? Write down a new model flow diagram, which includes a compartment, Iv, for vector incubation.



Suppose vectors incubate for a period of 9-12 days (see Table 27.1 of Kettle (1995) for latent periods of the four *Plasmodium* species which infect humans). In your **Equations Window** add a new parameter **gamma** and set its value to 0.0833 day^{-1}[1/(12 d)]. You will need to add a new variable to your model with Iv for "Incubating" or latent vectors and add its initial value, **init (Iv)**. Since we started the previous run with no infected mosquitoes, set the latent vectors to zero initially. Also you will have to change your equation for Yv to Iv (since infections now lead to an incubating phase rather than to infection). The loss terms for this equation will have to be modified to include loss of incubating mosquitoes that become infective (**-gamma*Iv**), and mortality of incubating mosquitoes (**-muv*Iv**). Now add an equation from the incubating stage Iv to the infective stage Yv. The new equation is **gamma*Iv** for the gain term, but don't forget that infective mosquitoes also die with net mortality **muv*Yv**. Lastly, to keep track of the number of vectors, change the definition of the variable V so that V = **Xv+Yv+Iv**, and the definition of vectorprev = **100*Yv/(Xv+Yv+Iv)**.

You may need to run the model for longer in order for it to reach endemic equilibrium (it will take longer to reach endemic prevalences of infection in humans and vectors).

Run the model. What are the new endemic prevalences (when including an additional compartment for incubating vectors)?

- In humans __56_____%
- In vectors __3.8_____%

Save your model with a different name (e.g. Vectors latency compartment.mmd).

Run a "sensitivity analysis" for various values of the incubation parameter **gamma**. From the **Parameters** menu choose **Parameter plot**. Choose **gamma** as the **Parameter**. In **no. of runs**

choose 10. Choose **Geometric** for **Series type**. Try from **Initial Value** 0.05 day^{-1} (20 day incubation), to **Final Value** 50 day^{-1} (incubation of 0.02 days or nearly no incubation). You should **Add** vectorprev and hostprev as Variables for the **Y axis** and tick **Final** for the output. The results are plotted against **gamma**. Double click on the **gamma** axis to change this (**x**) axis to Log scale. You should see that incubation period has a marked effect on vector prevalence. Why is this so?

- Longer incubation = lower prevalence in vectors. When there is nearly no incubation we recover the original infection prevalence in vectors of ~12%. If the EIP is very long (e.g. colder temperatures), the infection prevalence in mosquitoes will be very low. Actually, there will be an isotherm below which, no malaria transmission is possible (e.g., below 17 degrees Celsius in Russia).

Notice that the human host prevalence can be high but the vector prevalence can be very low. Section 14.4.1 of Anderson & May (1991) covers this subject in much greater detail.

You may also want to try a **Parameter plot** changing the values of the biting rate, ***a***, say from 0.01 (vectors are nearly zoophagic) to 0.33 (all blood-meals are taken on humans and vectors bite every 3 days, i.e. only once per gonotrophic cycle). If blood-meals were interrupted and vectors bite more than once per gonotrophic cycle, you may want to increase ***a*** up to 1 (vectors bite every day).

Different mathematical representations of latency in the vectors and their biological interpretations

When we incorporate latency by including an additional compartment, we are making the assumption that the rate of progression between the 'incubating' compartment and the 'infectious' compartment is constant. Therefore there will be an exponential distribution of latent periods; not all vector will become infective at once (but most of them will do almost immediately).

An alternative representation, which mirrors a different biological process, is to assume that the latent period is fixed, and that once the incubation period has elapsed all vectors become infectious. In this case, the differential equations are modified to include a delay (delay-type of differential equation), and if the survival of the vector is of the essence (which is, given the fact that latent periods are long in comparison to the vector's life expectancy), then the proportion of mosquitoes surviving the extrinsic incubation period is also included,

$$dY_v(t)/dt = a\, b_v\, [Y_h(t-\tau)/H]\, X_v(t-\tau)\, \exp(-\mu_v \tau) - \mu_v Y_v(t)$$

and the value of τ would be 1/gamma, or equal to n as in the lecture (the number of days necessary for an infected mosquito to become infective).

The Berkeley Madonna equations are:

```
d/dt(Xv)=muv*V-a*bv*(Yh/H)*Xv-muv*Xv
d/dt(Iv)=a*bv*(Yh/H)*Xv-a*bv*(delay(Yh/H,tau))*(delay(Xv,tau))*exp(-muv*tau)-muv*Iv
d/dt(Yv)=a*bv*(delay(Yh/H,tau))*(delay(Xv,tau))*exp(-muv*tau)-muv*Yv
```

and you must add parameter tau to your list,

tau=12 (this is equivalent to *n*)

Run the model. What are the new endemic prevalences (when including a fixed delay to vector infectiousness)?

- In humans _____36_____%
- In vectors _____1.7_____%

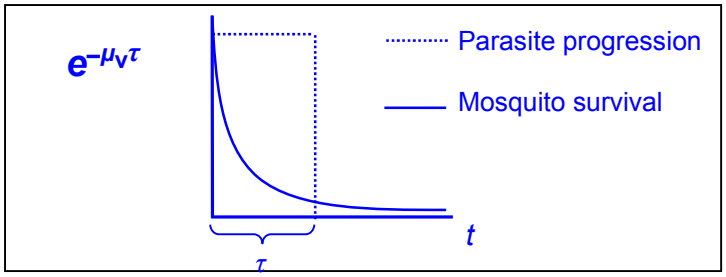
Save your model with a different name (e.g. Vectors latency delay.mmd).

Write the expression for the proportion of vectors becoming infectious when there is a constant rate of progression, gamma (γ), between the latent and the infectious compartment, and the mosquitoes die at a constant rate μ_v. Calculate the value of such expression.

$$\frac{\gamma}{\gamma + \mu_v}$$ **This is equal to 0.45**

45% of mosquitoes progress to infectiousness.

Write the expression for the proportion of mosquitoes becoming infective when vectors become infectious after a fixed period τ and die at a constant rate μ_v. Calculate the value of such expression. Compare the values obtained by the two approaches and discuss the reasons for any difference.



This is equal to 0.30; 30% of mosquitoes progress to infectiousness.

The reason for the difference is that when we assume a constant rate of progression, gamma, most mosquitoes progress at once according to the exponential distribution of latent times. When instead, we assume a fixed delay, mosquitoes are not progressing until tau days are completed, but they are still dying at a constant mortality rate, with an exponential distribution of survival times.

Your endemic infection prevalences should be somewhat smaller with the delay equation when compared with the model including an additional 'Incubating Mosquitoes' compartment.

In practice, progression to infectiousness is likely to be a mixture of these two processes, with a delay for oocyst maturation, but with some oocysts bursting before others, so that there will be a distribution of times sporozoites reach the salivary glands and the mosquitoes become infectious. Adding more than one incubating compartment, leads to a gamma distribution for the rate of progression (see Smith *et al.* 2004, and Dawes *et al*. 2009).

See Holmes, Bartley & Garnett (1998) for a discussion of these two alternative representations in the context of dengue transmission.

The basic reproduction ratio with latency

We end up this part of the practical by remembering the expression of R_0 with latency in the vectors given in the lecture notes:

$$R_0 = (V/H)\, a^2\, b_h\, b_v\, D_h\, L_v\, p^n \text{ , or}$$

$$R_0 = (V/H)\, a^2\, b_h\, b_v\, D_h\, L_v \exp(-\mu_V\, n)$$

where p is the probability of mosquito daily survival; n (=tau) = 1/gamma; μ_V = -ln (p); D_h is the duration of infectiousness in humans = $1/r$, and L_v is the life expectancy of vectors = $1/\mu_V$. In our case p = 0.90 (= $e^{-0.1}$), implying that 90% of vectors survive from one day to the next.

NB. In the lecture notes, you will notice that we have called L_v as the 'longevity factor' of Garrett-Jones (1964), i.e., the expectation of infective life. In this case, L_v is defined as the product of the probability of having survived n days and the remaining life expectancy, which in the exponential, ageless model is the reciprocal of the vector mortality rate.

Modify the expression for R_0 in your model accordingly.

Now you can see that R_0 is linearly related to vector density; it will change mildly non-linearly with changes in a (which is squared because the vector needs to bite at least twice to pick up and transmit the infection), but it will respond strongly non-linearly with changes in p (which is to the power of n, the average extrinsic incubation period). Compare reducing by 50% the value of p, with the changes in R_0 exerted by those explored in Table 5 above. (Remember the relationship that exists between p, μ_V and L_v.)

Table 5. Sensitivity of the basic reproduction number to changes in parameter values (Part II)

50% reduction	Parameter value	R_0
In vector numbers (V)	i.e. 500	0.82 Linear
In biting rate per vector on humans (*a*)	1/6	0.38 Non-linear
In duration of infectiousness (1/ν)	25	0.82 Linear
In the probability of daily survival (*p*)	0.45	3.75×10^{-4} Very non-linear

What is the role of adulticidal insecticides (as opposed to larvicides) in malaria control programmes? (Think of this in terms of IRS (indoor residual spraying), ITNs (insecticide-treated nets), ULV (ultra-low volume, usually knock-down insecticides), and novel pesticides such as entomopathogenic fungal infections.)

To kill mosquitoes during this latency period, therefore preventing them from transmitting. IRS kills mosquitoes when, after a blood-meal, they rest on the walls indoors. They may have picked up infection but will not survive the EIP. ITNs are treated with synthetic pyrethroids that kill by means of knock-down effects as mosquitoes rest on the surface of the nets to try and feed. They may also have an excito-repellent effect that diverts mosquitoes away. Not only ITNs kill mosquitoes but also they reduce the vector-human contact and possibly the biting rate on humans (mosquitoes may feed on nonhuman blood-hosts). ULV insecticides are aimed at flying mosquitoes (e.g. dengue vectors). Fungal biopesticides can kill adult mosquitoes, but if they do it more slowly (allowing blood-fed mosquitoes to develop a batch of eggs and oviposit), their selective pressure on components of reproductive fitness will be lower.

Bibliography

Chapter 14: Indirectly transmitted microparasites. In: Anderson, R.M. & May, R.M. (1991) *Infectious Diseases of Humans. Dynamics and Control.* Oxford: Oxford Science Publications.

Chapter 5: The population dynamics of malaria (J.L. Aron & R.M. May). In: Anderson RM, ed. (1982). *Population dynamics of Infectious Diseases. Theory and Applications*. London: Chapman and Hall.

Bailey, N.T.J. (1982). *The Biomathematics of Malaria*. London: Charles Griffin & Co.

Dawes, E.J., Zhuang, S., Sinden, R.E. & Basáñez, M.-G. (2009). The temporal dynamics of *Plasmodium* density through the sporogonic cycle within *Anopheles* mosquitoes. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 103(12): 1197-1198.

Dye, C. (1986). Vectorial capacity: must we measure all its components? *Parasitology Today* 2(8): 203-209.

Dye, C. (1990). Epidemiological significance of vector-parasite interactions. *Parasitology* 101: 409-415.

Dye, C. (1992). The analysis of parasite transmission by bloodsucking insects. *Annual Review of Entomology* 37: 1-19.
Garrett-Jones, C. (1964). Prognosis for the interruption of malaria transmission through assessment of the mosquito's vectorial capacity. *Nature* 204: 1173-1175.

Gilles, H.M. (1993). *Bruce-Chwatt's Essential Malariology*. Third Edition. London: Arnold.

Holmes, E.C., Bartley, L.M. & Garnett, G.P. (1998). The emergence of dengue: past, present, and future. Chapter 10, In: *Emerging Infections*. London: Academic Press, pp. 301-325.

Kettle, D.S. (1995). *Medical and Veterinary Entomology*. 2nd edition. CABI Publishing.

Koella, J.C. & Antia, R. (2003). Epidemiological models for the spread of anti-malarial resistance. *Malaria Journal* 2(3): 1-11.

Macdonald, G. (1956). Theory of the eradication of malaria. *Bulletin of the World Health Organization* 15: 369-387.

Magesa, S.M. et al. (1991). Trial of pyrethroid impregnated bednets in an area of Tanzania holoendemic for malaria. Part 2. Effects on the malaria vector population. *Acta Tropica* 49: 97-108.

Pull, J.H. & Grab, B. (1974). A simple epidemiological model for evaluating the malaria inoculation rate and the risk of infection in infants. *Bulletin of the World Health Organization* 51: 507-516.

Rogers, D.J. (1988). The dynamics of vector-transmitted diseases in human communities. *Philosophical Transactions of the Royal Society of London* (series B) 321: 513-539.

An example of a paper incorporating latency in the vector as we have here:
Ghani, A. et al. (2009). Loss of population levels of immunity to malaria as a result of exposure-reducing interventions: consequences for interpretation of disease trends. *PLoS One* 4(2): e4383.

An example of a paper incorporating realistic incubation periods within the mosquito is (see in particular the Supplementary Information protocol):

Smith, D.L., Dushoff, J., McKenzie, F.E. (2004). The risk of a mosquito-borne infection in a heterogeneous environment. *PLoS Biology* 2(11): e368.

For those of you who may speak Spanish, the following may be a useful reference:
Basáñez M-G & Rodríguez D (2004). Dinámica de transmisión y modelos matemáticos en enfermedades transmitidas por vectores (Transmission dynamics and mathematical models of vector-borne diseases). *Entomotropica* 19: 113-134.

Comparisons of IRS and ITNs can be found in:
Curtis, C.F. & Mnzava, A.E.P. (2000). Comparison of house spraying and insecticide treated nets for malaria control. *Bulletin of the World Health Organization* 78: 1389-1400.

And rationale and models for novel approaches in:
Read, A.F. et al. (2009). How to make evolution-proof insecticides for malaria control. *PLoS Biology* 7(4): e1000058.

Hancock, P.A. (2009). Combining fungal biopesticides and insecticide-treated bednets to enhance malaria control. *PLoS Computational Biology* 5(10): e1000525.

The following section of the practical is entirely *optional*, and you may wish to complete it at your leisure.

At this point you may want to save your model with a different name as suggested above, and introduce the modifications that follow. This way, you will have a model without seasonality (the previous exercise) in one file, and a model with seasonality (next exercise) in a different file, and be able to compare both.

The effect of variable mosquito density

Macdonald (1956) suggested that areas of "stable" high transmission malaria should be less sensitive to fluctuations in mosquito density than areas of low transmission. The basic model you have constructed can be used to test this very easily. Suppose that the vector population fluctuates seasonally with rainfall (affecting availability of breeding sites and therefore recruitment into the vector population) but has a constant background component. In the **Window** menu, reselect **Equations**, and change the equation for the variable V to read

1000*(1+0.45*Sin(2*PI*TIME/365))

where PI is ~3.1416. This is a sinusoidal function with period 365 days and goes from a minimum background of V about 500 vectors to a maximum of approximately 1500 during the course of a year with a mean of about 1000. Now rerun the model for 10 years (STOPTIME = 3650). You should see the seasonal fluctuations clearly. For more clarity as to the scale of the X Axis, double click on it and change it from Auto to Minimum 0, Maximum 3650, Div 10. This should allow you to see clearly the number of years in the X Axis.

What happens to R_0?

- R_0 oscillates between approximately 1.8 and 4.6.

What do you think this means in terms of a critical vector density?

- We are varying the vector density (V) seasonally, leaving unchanged the vector competence (the probability that a successful infection establishes within the vector, per bite, b_h), the vector mortality rate (μ_V) and the biting rate per vector on humans (a). Given these parameters, the critical vector density (threshold biting rate or *TBR*, above which R_0 = 1) would be the same, but the value of R_0 would be (linearly) proportional to vector density as this varies seasonally,

  $R_0 = (V/H)\, a^2\, b_h\, b_v\, D_h\, L_v \exp(-\mu_V\, n)$
  $TBR = (r\, \mu_V)/(a\, b_h\, b_v \exp(-\mu_V\, n))$
  TBR = 2.01 bites per day = 734.5 bites per year

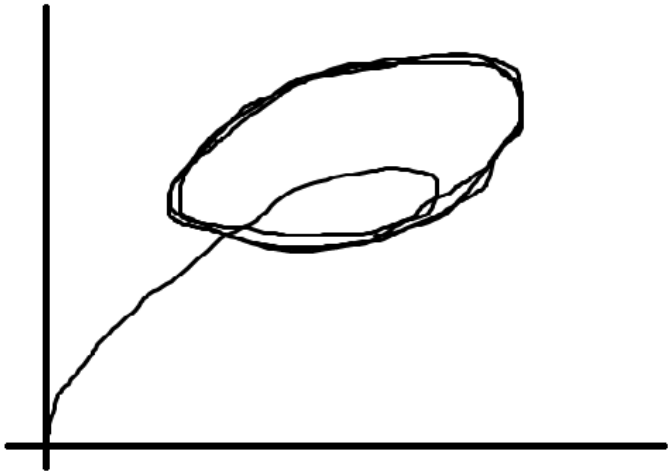
Draw a **Phase Plane Plot** of hostprev against vectorprev. In the **Help** menu, go to **How do I**

Make a Phase Plane Plot

• Run the model
• Double click anywhere in the x-y plane of the graph (or select *Choose Variables* from the *Graph* menu)
• Remove all variables from the Y axis with the exception of hostprev.
• Replace TIME on the x axis with vectorprev by selecting vectorprev in the drop down *X Axis* menu.
• Click *OK*. You may have to double-click in the X Axis and return to Auto.

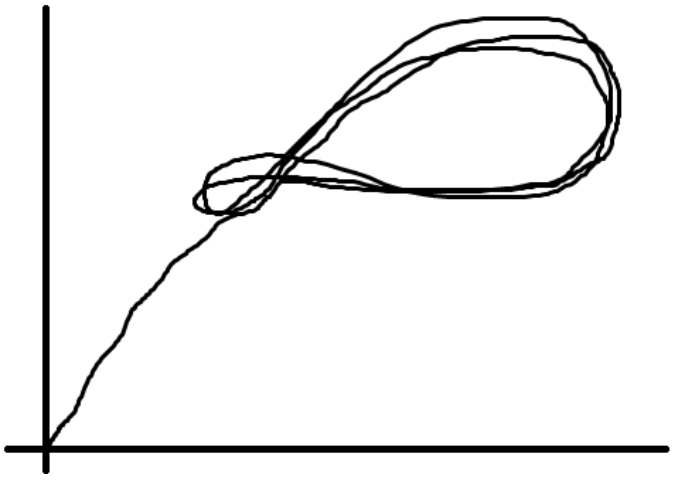
Can you see the cycles now?

- Yes, after an initial period of increase, the value of hostprev against vectorprev rapidly settles into regular cycles, as evidenced by the phase plane plot produced, which looked like this:



To see the influence of minimum and maximum vector numbers, and in order to get a mean prevalence which is closer to your previous values, the mean no. of vectors would have to be 1000 (as above). Go back to the Equations window and change V to read **1000*(1+0.9*Sin(2*PI*TIME/365)).** This is a sinusoidal function with period 365 days and goes from a minimum background of V about 100 vectors to a maximum of approximately 1900 during the course of a year with a mean of about 1000. Re-run the model, prepare graphs of V vs. time, of R_0 vs. time, and of the prevalence of infection in humans and mosquitoes vs. time. Take a look at the average values and also at how the peaks and troughs of the different variables relate to each other. You may wish to change the Y Axis to Log (Tick Log and Auto).

- The resulting phase plane plot looks like the following :



Explain the following:

- R_0 mirrors Vector density
  - R_0 depends linearly on the value of V and therefore, as V increases or decreases, R_0 will follow these cycles.

- Peak infection prevalence in vectors takes place at lowest vector density values
  - This would be because when the denominator value of V is at its lowest, the infection prevalence in vectors (the ratio of infected vectors to total vector population) would be at its highest

- How infection prevalence in hosts and vectors track each other?
  - Infection prevalence in vectors and hosts tracked each other quite closely; there was a slight lag between the rise in abundance of infected vectors and the rise in abundance of infected hosts, this lag was in the region of 10 days.

You should now have a feeling for the dynamic properties of vector-borne infections.

Reference:

Macdonald, G. (1956). Epidemiological basis of malaria control. *Bulletin of the World Health Organization* 15: 613-626.