Protozoan predation in batch and continuous culture

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1 Introduction

The behaviour of predatory organisms has long been a topic of considerable fascination for artist and scientist alike. The image of strength and invulnerability that predators instil in the mind and their association with heroic masculine traits was exemplified in Beowulf, one of the earliest English epic poems, and continues today in the falcons and cougars that are so much a feature of men’s toiletry and car advertisements. The role of predation in natural communities and its effect on natural selection played a major part in Darwin’s development of his theories of evolution as is emphasized by Gause (1934) in
his basic text on the relationship between prey and predator populations. Since Darwin's time the underlying concepts of predation have been expanded to include political and economic considerations perhaps best seen in the works of Karl Marx who equated capitalists with predators and parasites, contending that societies based on such a system were unstable and would eventually die out.

With the exception of Gause's (1934) work, a decade ago it would have been difficult to find literature concerning the interactions of microorganisms except for purely qualitative descriptions, but now interest is gathering momentum and few microbiology meetings are held without some considerable reference to this topic. Bungay and Bungay (1968) reviewed several types of microbial interactions but we have limited ourselves to one - predation - and have restricted our terms of reference to protozoan predators only. In our view, of all interactions, predation is of prime importance since it is one of the major steps in the transfer of energy through a biotic community and is thus a significant component of community metabolism. Furthermore, to a large extent predation controls the numbers of prey present and partly determines the species composition of a community of microbes. Many protozoa are predatory and different species will feed on a great variety of microorganisms - from bacteria and algae to fungi and other protozoa (see Sandon, 1932, still the most complete account of the nonbiochemical aspects of the food of protozoa). In addition, the life-cycles of free-living protozoa are simple when compared with those of higher animals and so it is not surprising that protozoa are commonly used as experimental organisms.

Predation dynamics involve the way in which the populations of at least two species change with respect to time as well as to each other. In order to specify the nature of these changes, whether for purely descriptive purposes or more formally for proposing an hypothesis, it is necessary to formulate them in mathematical terms. This is not merely for convenience or efficiency but for the precision necessary if the description is to be fully understood or the hypothesis to be tested adequately. For these reasons much of the research that has been undertaken on prey-predator dynamics is of a theoretical nature and has involved the proposition and testing of mathematical models. The advantage of this approach is that the wide range of sophisticated methods available to the mathematician may be brought to bear on generating testable predictions from hypotheses. The manipulations
involved in developing a mathematical model are, perhaps, of greatest importance since they impose on the investigator insights into the system he is studying that would be difficult to gain from a purely qualitative approach to the same problem. The disadvantage of a mathematical approach is that the methods used are often unfamiliar to biologists. We sincerely hope that the section on theory will not immediately deter the biologist from reading it, since it is to him that we aim our remarks and for this reason have concentrated on the methodology underlying the basic mathematical models that have been proposed to explain prey–predator dynamics rather than attempting to detail the extent to which theoretical aspects of the subject have been developed.

In this paper we present some of our views about the way in which microorganisms have been used to study the relations between prey and predator; these studies have been undertaken to investigate both the behaviour of the microorganisms themselves and the prey–predator situation in general. We do not propose to review all the current literature in this field of study but rather to consider those methods of study we favour, prejudiced as this approach may appear to be.

2 Theory of microbial prey–predator interactions

In order to determine the way in which predatory organisms interact with their prey it is necessary to observe in some way the behaviour of the two populations. Observations alone, however, are unlikely to reveal the nature of the underlying mechanisms of interaction and so, within the framework of the scientific method, guesses or hypotheses are made that offer possible explanations for the observations. In order to either confirm or reject a hypothesis it must be stated in a form that allows testable predictions to be made. For dynamic systems this form appears to be of a mathematical nature and therefore much of the literature on prey–predator dynamics is concerned with mathematical hypotheses (or models) and the techniques involved in generating predictions from them.

We begin our discussion of microbial predation by approaching the subject with a simple hypothesis stated in generalized mathematical terms. We assume that the prey population \( H \) can grow in the absence of predator \( P \) but growth of the predator itself is dependent on the
presence of prey. Thus we are limiting our discussion to cases of obligate predation. We further assume that as the number of predators increases so the rate of decrease in the number of prey organisms increases and finally, that the death rates of prey and predator depend on their respective population densities. The equations for change of the two populations with respect to time can be written as

\[
\frac{dH}{dt} = g_1 H - d_1 H - f_1 P, \quad (1)
\]

\[
\frac{dP}{dt} = -d_2 P + f_2 P, \quad (2)
\]

where \( g, d \) and \( f \) are functions, which for the moment we will not specify, representing the way in which the populations grow and die and the effect of predation.

### 2.1 Kinetics of Predation

The functions \( f, d \) and \( g \) in equations (1) and (2) represent the kinetics of a prey–predator system. We now define these functions in the following manner:

\[\begin{align*}
g_1 &= \text{specific growth rate of prey} \equiv \frac{\dot{H}}{H} \text{ for } f_1 = d_1 = 0, \\
d_1 &= \text{specific death rate of prey} \equiv \frac{\dot{H}}{H} \text{ for } g_1 = f_1 = 0, \\
d_2 &= \text{specific death rate of predator} \equiv \frac{\dot{P}}{P} \text{ for } f_2 = 0, \\
f_1 &= \text{specific rate of predation} \equiv \frac{\dot{H}}{P} \text{ for } g_1 = d_1 = 0, \\
f_2 &= \text{specific growth rate of predator} \equiv \frac{\dot{P}}{P} \text{ for } d_2 = 0.
\end{align*}\]

We define the specific feeding rate, i.e. the effect of predation on the prey population, as \( f_1 H/P \).

The specific growth rate of microbial populations is often regarded as being, within limits, exponential. Application of this assumption to the equation for prey population change implies that \( g_1 \) is a constant which we shall call \( \alpha_1 \).

The assumption that microorganisms grow in an exponential fashion without limit is unreasonable, of course. The effect of population density on specific growth rate is incorporated in the Verhulst–Pearl logistic equation (Gause, 1934) which assumes a linear relationship

\[\frac{\dot{H}}{H} = \frac{g_1 H}{H} = \frac{g_1}{1 + \frac{g_1}{d_1}}, \quad (3)
\]

\[\frac{\dot{P}}{P} = \frac{f_2 P}{P} = \frac{f_2}{1 + \frac{f_2}{d_2}}, \quad (4)
\]

\[\alpha_1 = \frac{g_1}{1 + \frac{g_1}{d_1}} \text{ and } \alpha_2 = \frac{f_2}{1 + \frac{f_2}{d_2}}.
\]

\[\text{The dot over the symbol for a variable denotes its derivative with respect to time, e.g. } \dot{H} = \frac{dH}{dt}.
\]
between specific growth rate and population density. Thus

$$g_1 = a_1 - \gamma_1 H,$$

(3)

where $a_1$ and $\gamma_1$ are constants.

Introduction of a Verhulst term into the specific growth rate function takes account of the abiotic environment in a nonspecific manner. If it is assumed that a single nutrient limits the growth of the prey organism a more specific relationship between limiting substrate concentration ($S$) and specific growth rate, $\mu$, is given by the function suggested by Monod (1942):

$$g_1 = \mu = \frac{\mu_m S}{K+S},$$

(4)

where $\mu_m$ is the maximum specific growth rate and $K$, the saturation constant, is the concentration of limiting nutrient when the specific growth rate $= \mu_m/2$. It must be stated at the outset of this discussion that this relationship is an arbitrary one. Attempts have been made to analogize this expression with the Michaelis–Menten equation of enzyme kinetics. However, in the latter case a steady state is assumed to occur between the enzyme, substrate and enzyme–substrate complex and this assumption seems to be valid for enzyme systems. A nutrient–organism complex must also be assumed to be at steady state in order for the analogy to hold for the kinetics of microbial growth. It is clear that in a population of microorganisms that is either growing or dying this will not be true and that this complex will only be independent of time when the population of microorganisms itself is at steady state. Much of the experimental work that has been carried out on chemostat cultures has been with steady-state situations and it is not surprising that the results obtained are often in agreement with those predicted by the Monod relationship. However, as has been shown by Mateles et al. (1965), the way bacteria grow during transition periods is not predicted by this equation.

The specific death rate in many microbial populations growing under favourable conditions is small and often equated to zero. We will consider cases where this is applicable to the prey population but always assume that the predator dies in the absence of prey. The simplest assumption concerning the way the predator organisms die is that the process is exponential and so for the prey, $d_1 = 0$ and for the predator, $d_2 = \alpha_2$, a constant. We note that in continuous culture the rate of dilution of the system ($D$) may be regarded as a nonspecific death term.
The specific rate of predation and the specific growth rate of the predator are, of course, closely related functions as the process described by the former results in the latter. It is usually assumed that there is a proportionality constant between these two functions. On a biomass basis this is called a yield coefficient which may be defined by

\[ W = f_2/f_1 \]  

which expresses the predator biomass produced per unit of prey biomass. If \(f_1\) and \(f_2\) are assumed to be directly proportional to prey density then they become

\[ f_1 = \beta_1 H \quad \text{and} \quad f_2 = \beta_2 H, \]

where the constants \(\beta_1\) and \(\beta_2\) incorporate the growth yield.

Despite the shortcomings of the Monod relationship it does serve as a useful arbitrary description with which to analyse microbial growth and its application has been extended to describe the specific rate of growth of a microbial predator:

\[ \dot{f}_2 = \lambda = \frac{\lambda_m H}{L + H} \quad \text{and so} \quad \dot{f}_1 = \frac{\lambda_m H}{W(L+H)}, \]

where \(\lambda_m\) is the maximum specific growth rate of the predator and \(L\) is the saturation constant.

### 2.2 Prey–Predator Dynamics

Equations (1) and (2) represent the dynamic relationship between a prey and a predator population in which the kinetic functions are not specified. It is possible to predict in general terms the behaviour of the system in the form presented or by making further assumptions of a nonspecific nature. For example it might be proposed that the specific rate of predation is a function of the number of predators present, i.e. \(f_1 = f_1(P)\). In this way all the possible responses of the model may be predicted regardless of the specific nature of the functions involved. Analyses of this sort have been performed by Lotka (1923), Rescigno and Richardson (1967), Rescigno (1968), Rescigno and Jones (1972) and Waltman (1964). It is also possible to generalize equations (1) and (2) to multispecies systems and so investigate the dynamics of a total community. Such analyses have been carried out in statistical mechanics terms by Kerner (1957), Leigh (1968) and Samuelson (1971). Lefever and Nicolis (1971) and Nicolis and Prigogine (1971) employed
the theory of nonequilibrium thermodynamics while Goel et al. (1971) used a variety of mathematical techniques for these analyses. It is beyond the scope of this paper to detail these sophisticated mathematical treatments and we will confine our discussion to relatively simple analysis of deterministic mathematical models containing specified kinetic functions.

**TABLE 1**

Kinetic functions for dynamic models

<table>
<thead>
<tr>
<th>Model</th>
<th>Prey-specific growth rate ((g_i))</th>
<th>Prey-specific death rate ((d_i))</th>
<th>Predator-specific death rate ((d_p))</th>
<th>Specific rate of predation ((f_i))</th>
<th>Predator-specific growth rate ((f_p))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lotka-Volterra (batch)</td>
<td>(\alpha_1)</td>
<td>0</td>
<td>(\alpha_2)</td>
<td>(\beta_1 H)</td>
<td>(\beta_2 H)</td>
</tr>
<tr>
<td>Lotka-Volterra</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ Verhulst term (batch)</td>
<td>(\alpha_1 - \gamma H)</td>
<td>0</td>
<td>(\alpha_2)</td>
<td>(\beta_1 H)</td>
<td>(\beta_2 H)</td>
</tr>
<tr>
<td>Monod saturation (chemostat)</td>
<td>(\mu_m \frac{S}{K_s + S})</td>
<td>D</td>
<td>D</td>
<td>(\lambda_m H)</td>
<td>(\lambda_m H)</td>
</tr>
</tbody>
</table>

Application of the kinetic functions described in the last section to dynamic models is summarized in Table 1; they have been used to describe both batch and continuous systems and for convenience we describe in detail the way the simpler functions have been applied to batch cultures first and then extend the description to continuous systems.

2.2.1 Analysis of predation dynamics

a. **Batch culture** Batch culture of microorganisms is experimentally analogous to a closed ecosystem. Organisms are grown in containers and there is no input of matter or energy from the external environment except perhaps for gaseous exchange. The most widely employed theoretical framework for studying prey–predator dynamics is the Lotka–Volterra equations, independently derived by Lotka (1925) and Volterra (1926), which when applied to microorganisms assume the system to be closed and also independent of other abiotic factors. For two species these equations, in their simplest form, are

\[
\frac{dH}{dt} = \alpha_1 H - \beta_1 HP,
\]
\[
\frac{dP}{dt} = -\alpha_2 P + \beta_2 HP,
\]
from which we see that they are the simplest realization of equations (1) and (2) where \( g_1 = \alpha_1 \), \( d_1 = 0 \), \( d_2 = \alpha_2 \), \( f_1 = \beta_1 H \) and \( f_2 = \beta_2 H \) as indicated in Table 1.

The Lotka–Volterra equations are nonlinear and cannot be solved directly. It is possible to simulate solutions for them by numerical methods or on an analogue computer, as is shown in Fig. 1, but most insight into their behaviour has been gained by mathematical analysis.

In order to gain some understanding of the behaviour of the Lotka–Volterra equations it is instructive to consider how the prey population changes with respect to predator density, i.e. to eliminate the dependence of these variables on time. Dividing equation (8) by equation (9) yields

\[
\frac{dH}{dP} = \frac{(\alpha_1 - \beta_2 P)H}{(-\alpha_2 + \beta_2 H)P},
\]
which has the solution

\[
\beta_2 H - \alpha_2 \log H = \alpha_1 \log P - \beta_1 P + c.
\]
Here \( c \), the constant of integration, is dependent on the initial conditions, i.e. the sizes of \( H \) and \( P \) when \( t = 0 \). Equation (11) represents a family of closed curves as is shown in Fig. 2, each one predetermined by this constant. From this simple analysis we can expect quantitatively different responses from a Lotka–Volterra system depending upon the starting conditions.

Inspection of Fig. 2 reveals that the larger curves have distinctly flattened edges but the smaller the curves become, the more closely
Fig. 2. Solution of the Lotka–Volterra equations plotted in phase space.

they resemble ellipses. The centre of the curves is called the equilibrium point and the similarity of the curves to ellipses near the equilibrium point has been used by Pielou (1969) to obtain approximate solutions of the equations so that the behaviour of \( H \) and \( P \) with respect to time may be predicted. We describe here a slightly different approach used by Leigh (1968) (among others) which can be used as a basis for the study of other models of predation as well as the Lotka–Volterra equations. In essence we consider the behaviour of the differential equations near the equilibrium point where the values of \( H \) and \( P \) are small. Because of this characteristic the products of these variables are very small and their effect assumed to be negligible. Elimination of these second-order terms yields a set of differential equations which can be solved analytically.

The equilibrium point of a system is defined such that all the derivatives are equal to zero. Application of this definition to equations (8) and (9), i.e. \( dH/dt = dP/dt = 0 \), yields

\[
\tilde{H} = \frac{\alpha_2}{\beta_2} \quad \text{and} \quad \tilde{P} = \frac{\alpha_1}{\beta_1},
\]

where the tilde represents an equilibrium value. Both \( \tilde{H} \) and \( \tilde{P} \) will be finite, positive values but if we consider the variables defined by

\[
x_1 = H - \tilde{H} \quad \text{and} \quad x_2 = P - \tilde{P},
\]

then at the equilibrium point these new variables \( x_1 = \tilde{x}_1 \) and \( x_2 = \tilde{x}_2 \)
will both be zero. Substituting for $H$ and $P$ in equation (8) gives
\[
\frac{dx_1}{dt} = \alpha_1(x_1 + \bar{H}) - \beta_1(x_1 + \bar{H})(x_2 + \bar{P})
\]
(14)
which, when multiplied out, becomes
\[
\frac{dx_1}{dt} = \alpha_1 x_1 + \alpha_1 \bar{H} - \beta_1 x_1 x_2 - \beta_1 x_1 \bar{P} - \beta_1 x_2 \bar{H} - \beta_1 \bar{H} \bar{P}.
\]
As we are considering the behaviour of the system near the equilibrium point where $x_1$ and $x_2$ have small values, their product, $x_1 x_2$, will be very small and so we may neglect the term $\beta_1 x_1 x_2$. Furthermore, since from (12)
\[
\alpha_1 = \beta_1 \bar{P},
\]
then equation (14) reduces to
\[
\frac{dx_1}{dt} = -\beta_1 \bar{H} x_2.
\]
(15)
In a similar fashion substitution of the new variables into equation (9) followed by linearization yields
\[
\frac{dx_2}{dt} = \beta_2 \bar{P} x_1.
\]
(16)
We now differentiate equation (15) to obtain
\[
\frac{d^2x_1}{dt^2} = -\beta_1 \bar{H} \frac{dx_2}{dt}.
\]
After substitution of (16)
\[
\frac{d^2x_1}{dt^2} = -\beta_1 \beta_2 \bar{H} \bar{P} x_1,
\]
therefore from (12),
\[
\frac{d^2x_1}{dt^2} + \alpha_1 \alpha_2 x_1 = 0.
\]
(17)
This is the equation of a harmonic oscillator and has well-known properties. The solution of (17) is
\[
x_1 = A' \cos [(\alpha_1 \alpha_2)^{\frac{1}{2}} t + \phi],
\]
where $A'$ and $\phi$ are constants of integration. Converting back to the original variables we can obtain Pielou's (1969) equations:
\[
H = \frac{\alpha_2}{\beta_2} + \frac{\alpha_2}{\beta_2} A \cos [(\alpha_1 \alpha_2)^{\frac{1}{2}} t + \phi].
\]
(19)
Using similar methods the analogous expression for the predator can be shown to be
\[ P = \frac{\alpha_1}{\beta_1} + \frac{\alpha_2}{\beta_1} \left( \frac{\alpha_2}{\alpha_1} \right)^{1/2} A \sin \left[ (\alpha_1 \alpha_2)^{1/2} t + \phi \right]. \tag{20} \]

On the basis of these results we can state that, near the equilibrium point, the Lotka–Volterra equations predict that both prey and predator populations vary sinusoidally with equal periods, \( T = 2\pi/(\alpha_1 \alpha_2)^{1/2} \). In contrast, the amplitudes of the oscillations depend on the integration constant \( A \) and thus on the initial conditions; for the prey organism the amplitude is \( A \alpha_2/\beta_2 \) and for the predator, \((A \alpha_1/\beta_1)(\alpha_2/\alpha_1)^{1/2}\). The other constant of integration, \( \phi \), is the phase angle and depends on the value of the variables at zero time. If we let the prey population be at its maximum at this time then \( \phi = 0 \).

We also see that prey and predator are one quarter \( T \) out of phase with each other, with the prey increasing in density first, because prey density is described by a cosine function and predator density by a sine function.

The mean sizes of each population over one period or over many periods is equal to its equilibrium value:
\[
\frac{1}{T} \int_{t_0}^{t_0+T} H \, dt = \lim_{\theta \to \infty} \frac{1}{\theta} \int_0^{\theta} H \, dt = \frac{\alpha_2}{\beta_2}.
\]

\[
\frac{1}{T} \int_{t_0}^{t_0+T} P \, dt = \lim_{\theta \to \infty} \frac{1}{\theta} \int_0^{\theta} P \, dt = \frac{\alpha_1}{\beta_1}.
\]

In order to obtain a fuller understanding of the Lotka–Volterra equations and other models of predation it is necessary to understand how linear differential equations are solved. If we set \( \mathcal{D}^2 x_1 = \frac{d^2 x_1}{dt^2} \) and \( \mathcal{D} x_1 = \frac{dx_1}{dt} \) then equation (17) may be written as
\[
(\mathcal{D}^2 + c_1 \mathcal{D} + c_2) x_1 = 0,
\]
where \( c_2 = \alpha_1 \alpha_2 \) and \( c_1 = 0 \). We now substitute the differential operator \( \mathcal{D} \) by \( m \) and eliminate \( x_1 \) to obtain what is called the characteristic equation:
\[
m^2 + c_1 m + c_2 = 0.
\]

Now the general solution of equation (22) is
\[
x_1 = a_1 e^{m_1 t} + a_2 e^{m_2 t},
\]
where \( a_1 \) and \( a_2 \) are constants. The characteristic exponents, \( m_1 \) and \( m_2 \), are obtained by solving the characteristic equation for \( m \) using the quadratic formula:

\[
m = \frac{-c_1 \pm \sqrt{(c_1^2 - 4c_2)}}{2}. \tag{25}
\]

It is immediately apparent that the nature of \( m_1 \) and \( m_2 \) depend on the magnitude and sign of \( c_1 \) and whether the expression \( c_1^2 - 4c_2 \) is positive or negative. We will consider the possibilities in turn.

When \( c_1^2 > 4c_2 \) the characteristic exponents are either both positive, both negative or one is positive and the other negative. If both of the roots are positive the exponential terms in the characteristic equation become larger as time increases and the system in these cases is unstable. If, on the other hand, the characteristic exponents are both negative then \( x_1 \) will tend towards zero as time tends towards infinity. By definition, from equation (13), \( H = \dot{H} \) at \( x_1 = 0 \), so the system stabilizes at its equilibrium value. When both \( m_1 \) and \( m_2 \) have the same sign the equilibrium point generated is called a node. It is stable (Fig. 3) if both are negative and unstable if they are positive. If \( m_1 \) and \( m_2 \) are of opposite sign, a saddle point results. This is a very apt description of the behaviour of the solution trajectory in phase space as it travels like a marble rolling down from the pommel of a saddle towards the equilibrium point which is at the middle of the saddle. Unless the marble is on a perfectly placed trajectory it will not come to rest at this point, however, but will roll down one of the sides of the saddle. Thus a saddle point is unstable.

When \( c_1^2 < 4c_2 \) the term \( c_1^2 - 4c_2 \) in equation (25) becomes negative. This gives rise to what are called complex conjugate pairs of values for \( m \), i.e.

\[
m = n \pm pi, \tag{26}
\]

where \( i \) is defined as the square root of \(-1\) and \( n \) and \( p \) depend on the values of \( c_1 \) and \( c_2 \). Thus

\[
x_1 = a_1 \exp [(n + pi)t] + a_2 \exp [(n - pi)t].
\]

It can be shown (Euler's theorem) that this expression is equivalent to

\[
x_1 = [(a_1 + a_2) \cos pt + (a_1i - a_2i) \sin pt] \exp (nt).
\]

If \( a_1 \) and \( a_2 \) are unequal real numbers then \( x_1 \) has imaginary values,
which is an unrealistic result for the system we wish to represent. For real values of $x_1$ we can choose values for $a_1$ and $a_2$ such that

$$a_1i - a_2i = A'$$

and

$$a_1 + a_2 = B'.$$

Then

$$x_1 = (A' \sin pt + B' \cos pt) \exp (nt).$$

Equation (27) has both stable and unstable solutions depending on the value of $n$. If $n$, the real part of the conjugate root, is positive then $x_1$ increases sinusoidally towards infinity as $t$ becomes large and the system is unstable. Such an equilibrium is an unstable focal point. If $n < 0$ then the exponential term in equation (27) tends to zero as $t$ tends to infinity. In this case the oscillations of $x_1$ become smaller. As $x_1 = H - \dot{H}$ the prey population tends towards its equilibrium value. A similar result can be shown for the predator and the system is thus stable and the equilibrium a stable focal point (Fig. 3).
With \( n = 0 \) no damping occurs. This is the case with the transformed Lotka–Volterra variables in equation (17) where the coefficient \( c_1 \) is the characteristic equation (23) is zero so that equation (25) reduces to

\[
m = \pm \left( -\frac{4c_2}{2} \right)^{\frac{1}{2}} = \pm ( -c_2 )^{\frac{1}{2}} = \pm ( -\alpha_1 a_2 )^{\frac{1}{2}}.
\]

The solution can therefore be written as

\[
x_1 = A' \sin \left[ (\alpha_1 a_2)^{\frac{1}{2}} t \right] + B' \cos \left[ (\alpha_1 a_2)^{\frac{1}{2}} t \right],
\]

which is equivalent to equation (18) with \( A' = A/(\alpha_1)^{\frac{1}{2}} \) obtained by solving the equation for the initial conditions when it is found that the sine term vanishes.

The phase plane diagram which results from plotting \( x_1 \) against \( x_2 \) using the linearized Lotka–Volterra equations is similar to the smaller curves in Fig. 2 and is represented in Fig. 3, which summarizes the stable solutions we have discussed. We have seen that the curves for the Lotka–Volterra equations represent a stable system in that persistent, undamped oscillations occur. However, if the system is slightly perturbed the trajectory of any particular curve is changed and will not return to the original trajectory as the effect of the perturbation will be equivalent to changing the initial conditions. In this sense then the system is unstable. This sort of stability is called neutral stability and is defined by a characteristic equation which has complex conjugate roots with no real parts. The equilibrium point that it represents is called a vortex point (Fig. 3). It is of interest here to remark on a conclusion of Goel et al. (1971) which predicts that only an even number of species will be able to survive in a Lotka–Volterra system. We can now see how such a prediction arises. We have shown that for a two-species “Lotka–Volterra ecology” both species survive only if the characteristic equation produces complex conjugate roots. For a three-species system the quadratic root of equation (25) becomes a cubic root and three values of \( m \) are possible, instead of just two. As complex conjugate roots occur only in pairs it is only possible for two of these values to give rise to them. Thus only two of the three variables are capable of producing a stable result. In a stable situation, then, only two species can survive and the third will die out. Thus it can be seen readily that in any “Lotka–Volterra ecology” with an odd number of species the characteristic equation will not allow a stable solution.
Several modifications of the Lotka–Volterra equations have been proposed. We will discuss one which illustrates the effect of taking into account the abiotic environment by including a Verhulst term in the specific growth rate function of the prey. By doing this equations (1) and (2) become

\[
\frac{dH}{dt} = \alpha_1 H - \beta_1 HP - \gamma_1 H^2, \quad (28)
\]

\[
\frac{dP}{dt} = -\alpha_2 P + \beta_2 HP. \quad (29)
\]

The equilibrium values for these equations, i.e. solving for \(\frac{dH}{dt} = \frac{dP}{dt} = 0\), are

\[
\hat{H} = \frac{\alpha_2}{\beta_2},
\]

\[
\hat{P} = \frac{\alpha_1}{\beta_1} - \frac{\gamma_1 \alpha_2}{\beta_1 \beta_2}.
\]

Linearization gives rise to

\[
\frac{d^2x_1}{dt^2} + \gamma_1 \hat{H} \frac{dx_1}{dt} + \beta_1 \beta_2 \hat{H} \hat{P} x_1 = 0,
\]

where again, \(x_1 = H - \hat{H}\). The characteristic roots are

\[
m = \frac{-\gamma_1 \hat{H} \pm (\gamma_1^2 \hat{H}^2 - 4\beta_1 \beta_2 \hat{H} \hat{P})^{1/2}}{2}.
\]

For \(\gamma_1^2 \hat{H}^2 > 4\beta_1 \beta_2 \hat{H} \hat{P}\) the square root is a positive real value; if this value is smaller than \(\gamma_1 \hat{H}\) both \(m_1\) and \(m_2\) are negative and the result is of the form

\[
x_1 = a_1 \exp (-\delta_1 t) + a_2 \exp (-\delta_2 t),
\]

so as \(t \to \infty\), \(x \to 0\) and \(H \to \hat{H}\). Similarly as \(t \to \infty\), \(P \to \hat{P}\). Thus both prey and predator densities tend monotonically towards stable steady states because the roots of the characteristic equation are real and negative. In phase space this solution corresponds to the trajectory indicated in Fig. 3 and is a stable node.

When \(\gamma_1^2 \hat{H}^2 < 4\beta_1 \beta_2 \hat{H} \hat{P}\) the characteristic equation has complex conjugate roots and the equilibrium is a focal point. If the real parts of these roots are positive then the solution is unstable. For negative real parts the behaviour of the solution is oscillatory and of the form

\[
x_1 = A_c \exp (-ht) \cos (\omega_1 t + \phi),
\]
where \( h = \gamma_1 \bar{H}/2 \) and

\[
\omega_1 = \left[ \beta_1 \beta_2 \bar{H} - \left( \frac{\gamma_1 \bar{H}}{2} \right)^2 \right]^{1/2}.
\]

This equation is the same as that for a linear oscillator with friction. Strictly speaking the solution is not periodic but the time between successive maxima of \( x_t \), the conditional period, is constant and equal to \( 2\pi/\omega_1 \). The oscillations damp according to the value of \( h \) which is called the damping coefficient. The conditional amplitude of the oscillations, \( A_c \), and the phase angle, \( \phi \), are functions of the initial conditions of the system.

b. Continuous culture Population dynamics in a continuous culture are controlled by the rate of flow through the system. In chemostat culture, where the system is assumed to be well mixed and the rate of flow \( (F) \) into the culture vessel (of volume \( V \)) is the same as the rate of output, this flux is governed by the dilution rate, \( D = F/V \). For a single species of density \( X \) growth in a chemostat is described simply as

\[
\frac{dX}{dt} = \mu X - DX,
\]

where \( \mu \) is the specific growth rate of the organism. For a two-species prey–predator system the dynamics depend on the way in which prey and predator interact. If these interactions are of the type implicitly assumed by the Lotka–Volterra equations the defining equations for prey \( (H) \) and predator \( (P) \) are

\[
\frac{dH}{dt} = \mu H - DH - \beta_1 HP,
\]

\[
\frac{dP}{dt} = -\psi P - DP + \beta_2 HP.
\]

Here \( \psi \) is the specific rate of death of the predator. It can be seen readily that these equations are analogous to (8) and (9) with \( \alpha_1 = \mu - D \) and \( \alpha_2 = \psi + D \). Thus addition of a flow term to the Lotka–Volterra equations does not alter their form and the general solutions will be the same. The stable solution for these equations was found to be a vortex and this will not be changed by altering the experimental parameter, \( D \), within the limits of a stable result. What will change are the trajectories described in phase space and the values of \( \bar{H} \) and \( \bar{P} \) which define the equilibrium points. As the trajectories
are also dependent upon the initial conditions repeatable experimental results confirming these predictions would be difficult to obtain.

The effect of a Verhulst term added to a Lotka–Volterra open ecosystem is obtained by substituting for $\alpha_1$ and $\alpha_2$ in equations (28) and (29) in the same way. In this case the type of stable solution that is obtained depends on $\alpha_1$ and $\alpha_2$ and so will be determined, in part, by the value of $D$. In general an increase in $D$ will result in more rapid damping of the oscillations and eventually the disappearance of all oscillations. At low dilution rates, then, we might expect a stable focal point (Fig. 3) while at higher dilution rates a stable focus is generated.

Using Monod kinetics for prey and predator growth in a chemostat yields the following equations for populations change:

\[
\begin{align*}
\frac{dH}{dt} &= \frac{\mu_m SH}{K+S} - \frac{\lambda_m HP}{W(L+H)} - DH \\
\frac{dP}{dt} &= \frac{\lambda_m HP}{L+H} - DP.
\end{align*}
\]  

Here $W$ is the stoichiometric coefficient or yield of predator per unit of prey and is assumed to be constant. In contrast to the Lotka–Volterra equations with a Verhulst term, employing the Monod function allows us to specify the behaviour of the abiotic environment represented by the concentration of limiting nutrient:

\[
\frac{dS}{dt} = D(S_0 - S) - \frac{\mu_m SH}{Y(K+S)},
\]

where $S_0$ is the concentration of nutrient in the input medium and $Y$ is the yield constant for the prey organism.

These equations may be linearized near equilibrium and the trajectories of the solutions traced in three-dimensional (prey, predator and substrate) phase space. This analysis has been published in detail by Canale (1969, 1970) and will not be repeated here. The linearized equations admit to three equilibrium points defined by

\[
\begin{align*}
\hat{H} = \hat{P} = 0; \quad S &= S_0 \quad (a) \\
\hat{P} = 0; \quad \hat{S} + \hat{H}/Y &= S_0 \quad (b) \\
\frac{\hat{H}}{Y} + \frac{\hat{P}}{YW} + \hat{S} &= S_0 \quad (c)
\end{align*}
\]

The only equilibrium in which both prey and predator survive, and thus the only one of interest in the present context, is 36 (c). Equilibrium
16 (c) has three stable solutions depending on the dilution rate of the system. At comparatively high dilution rates such that neither species washes out, both prey and predator populations change monotonically towards steady-state values and a stable node (Fig. 3) is generated. At lower dilution rates a stable focal point (Fig. 3) is generated and the populations exhibit damped oscillations. At even lower dilution rates the system gives rise to sustained oscillations in all three variables. In phase space the trajectories are closed curves. Effectively what is happening is that the characteristic equation gives rise to an unstable focal point but the solution trajectory is bounded so that the variables cannot exceed values imposed by the parameters of the system. This type of solution is called a limit cycle (Fig. 3) and is different from the vortex point of the Lotka–Volterra equations in that the cycles are independent of the initial conditions and after small perturbations the solution trajectory will return to its original path. A system which gives rise to limit cycles may thus be regarded as strictly stable (i.e. according to the definition of Liapounof (Andronov et al., 1966)). Limit cycles cannot be found directly by analysing the linearized equations as they are a result of second-order terms.

Several modifications of the Monod (1942) term have been proposed (Contois, 1959; Kono and Asai, 1969; Topiwala, 1971; Yano and Koga, 1973) and recently Jost et al. (1973b) have proposed what they call a multiple saturation model to represent the specific rate of growth of a predatory microorganism in chemostat culture. This expression takes the form

$$\lambda = \frac{\lambda_m PH^n}{\prod_{i=1}^{n} (L_i + H)}.$$

The growth of the predator is assumed to take place in $n$ stages, each stage with an effective saturation constant of $L_i$. For $n = 1$ the expression reduces to the Monod term. The authors derive this expression by assuming that "pseudo-steady states" of the hypothetical prey–predator complexes occur for each of the $n$ stages of predator growth in the same way that has been suggested for the proposed nutrient–biomass complex by analogy to enzyme kinetics. As has been pointed out, however, it is unlikely that such complexes do remain independent of time in changing systems and this is particularly inappropriate for describing prey–predator dynamics due to the oscillatory responses observed.
The point of studying mathematical models is to gain some insight into the possible mechanisms underlying a given system. The prey–predator models we have discussed so far show that qualitatively different types of response may exist and provide a great deal of theoretical information about the possible behaviour of microbial populations. What these models have not done, and what is needed, is to relate the population dynamics of the system to the way in which the individual prey and predator organisms behave although at least an attempt has been made with the saturation models. There are two ways in which this problem may be attacked; either individual prey and predator organisms may be studied to determine their mode of interaction or the population behaviour may be analysed and the behaviour of the individuals inferred from the results. It is the latter method for which chemostat culture is most useful. To date there appears to be no satisfactory theory which does relate cellular behaviour to the behaviour of a population even for single-species systems. Ramkrishna et al. (1967) have proposed what they call structured models which take explicit account of the physiological state of the microorganisms, and Fredrickson et al. (1970) put forward convincing arguments in support of such an approach. In effect they say that the physiological state and therefore the rate of reaction of a cell changes as a function of its age and that representing a population of cells by a single time-dependent variable is insufficient; the population is composed of cells in different states and each state should be represented on a population basis. A specific example of a structured model is that proposed by Williams (1967) for a single species growing in either batch or continuous culture. Merely by regarding a cell as being divided into a structural-genetic and a synthetic portion several fundamental observations of growth dynamics are predicted, including the distinction between change in biomass and number densities. The chief argument against employing models of this type for multispecies systems is that too much complexity is involved in the final mathematical formulations. Although it is quite possible to produce sets of models for such systems based on the approach of Williams (1967), the resulting equations contain a great many coefficients whose values are unknown and variables which would be difficult, if not impossible, to measure. In general, increasing the number of parameters in a mathematical expression will increase the variety of responses that can be expected. Models of this sort then would be very difficult to test and most probably at this stage in such
investigations represent little more than exercises in curve fitting. On the other hand it might be possible to apply analyses of the form proposed by Prigogine and his co-workers (Prigogine and Nicolis, 1971) which specifically relate to the behaviour of nonlinear systems operating far away from equilibrium. Another possible line of approach lies in the new field of mathematics originated by René Thom which appears to provide a mathematical way of studying the occurrence of sudden changes in systems. This "catastrophe theory" has been applied to morphogenetic events (Thom, 1970) and its extension into other biological phenomena is to be expected.

2.2.2 Simulation techniques

In order to test any particular dynamic model it is necessary to compare experimental data to the behaviour of the time-dependent variables of the theory. As many biological models are nonlinear and cannot be solved directly by analytical methods it is therefore necessary to generate reliable estimates of these values by some other means. Two methods are commonly employed for accomplishing this: the system of differential equations may be represented by an electrical circuit and solved on an analogue computer or the solutions may be approximated, to any degree of accuracy required, by numerical integration. These methods have the advantage over the linearization technique described in the last section in that the effects of nonlinear terms are taken into account and transient responses to perturbations can be determined with precision.

Details of analogue computer methodology applied to ecological systems are given by Patten (1971); the solution of the Lotka–Volterra equations illustrated in Fig. 1 was obtained by this technique and Bungay (1968) and Williams (1967), among others, have used this method for investigating microbial population behaviour. The main advantage of the analogue computer is that it brings the operator into the closest possible contact with the equations under analysis and results can be obtained instantaneously whereas most digital systems do not afford this facility. For this reason small analogue computers are valuable teaching aids but as the magnitude of the parameters in any particular set of equations is restricted by the voltage output of the machine, in many cases the equations must be scaled in order to be analysed by this method. Scaling can be a very complicated
process and it is mainly for this reason that most workers now
tend to use numerical techniques in conjunction with a digital
computer.

Numerical approximation of the solution of differential equations
is accomplished by repeatedly calculating the integrals after very
short time intervals, the size of which is called the step size. The smaller
the step size, the more accurate are the results. Because step sizes are
comparatively small and because for each step considerable arithmetic
is involved the only practical way of solving equations in this way is
by using a digital computer.

Numerical integration techniques have been considerably simplified
by the introduction of purpose-built simulation languages which
provide all the facilities of an analogue computer without the restrict-
ions imposed by scaling. Such languages have several advantages for
biologists with limited mathematical background: firstly, they are
simple to learn so that the ecologist can concentrate on the problem at
hand rather than details of programming; secondly, the transient
behaviour of a model can be examined without recourse to detailed
mathematical analysis. Furthermore, for a system whose parameters
are known the output from such simulation can be used as a basis for
designing experiments and also for fitting the results to those predicted
by the model being tested.

Several simulation languages exist but the one most widely used in
studies of microbial population dynamics is CSMP (Continuous System
Modelling Program) designed for use on IBM 360 series computers.
Instructions for using CSMP are described in the S/360 Users' Manual
IBM 20-0367-2 and also by Patten (1971) who emphasizes its applica-
tion to ecological models. For convenience we reproduce in Table 2 a
CSMP program which represents the interaction between bacterial
prey and a protozoan predator under conditions of continuous culture,
assuming growth of both organisms to obey Monod kinetics, i.e. the
solutions to equations (32), (33) and (34) are simulated. The program
may be divided into three parts:

a. Data statements  These assign numerical values to constants and
parameters as shown in Table 2. As it is not possible to keypunch
subscripts or lower case letters the symbols employed are necessarily
different from those used in equations (32), (33) and (34) and the
following equalities hold: \( SO = S_0, D = D, MUMB = \mu_m, KS = K, \)
A prey–predator computer program written in the simulation language CSMP/360. (After Curds, 1971a.)

<table>
<thead>
<tr>
<th><strong>TABLE 2</strong></th>
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<tbody>
<tr>
<td><strong>CONTINUOUS SYSTEM MODELLING PROGRAM</strong></td>
</tr>
<tr>
<td><strong>PROBLEM INPUT STATEMENTS</strong></td>
</tr>
<tr>
<td>PARAM</td>
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<tr>
<td>PARAM</td>
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<td>INCON</td>
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DYNAM

\[
\begin{align*}
MUB &= \frac{MUMB \times S}{KS + S} \\
MUC &= \frac{MUMC \times B}{KB + B} \\
SDOT &= D \times SR - D \times S - MUB \times B/Y \\
BDOT &= MUB \times B - D \times B - MUC \times C/YC \\
CDOT &= MUC \times C - D \times C \\
S &= \text{INTGRL} (SA, SDOT) \\
B &= \text{INTGRL} (BA, BDOT) \\
C &= \text{INTGRL} (CA, CDOT) \\
\end{align*}
\]

TIMER | DELT = 0·01, FINTIM = 240·0, PRDEL = 1·0, OUTDEL = 1·0 |
PRINT | S, B, C, MUB, MUC |
PRTPLT | S, B, C |
TITLE | DYNAMIC MODEL PREDATOR–PREY |
LABEL | DYNAMIC MODEL PREDATOR–PREY |
END |
ENDJOB |

\[YB = Y, \quad MUMC = \lambda_m, \quad KB = L, \quad YC = W.\] In order to obtain numerical results from differential equations the initial conditions must be specified; these are the concentrations of prey, predator, and limiting nutrient at zero time. They are symbolized by BA, CA and SA respectively.

b. Structural statements These describe the functional relationship (DYNAM) between the variables of the model and so define the system to be simulated. The bacterial population is symbolized by B, the predator by C and the nutrient concentration by S. Statements are written in standard FORTRAN. The first two statements calculate the specific growth rates of the prey and the predator and the next three statements define new variables SDOT, BDOT and CDOT. The last three statements contain the CSMP function INTGRL which integrates these variables using initial conditions defined by data statements.
c. *Control statements*  These specify the options relating to the translation and execution of the program. They include the length of time over which integration is required (FINTIM), the step size, which in this program is fixed (DELT) but under CSMP need not be, and allow the numerical values of the variables to be printed (PRINT) and/or plotted (PRTPLT) at specified intervals (PRDEL and OUTDEL, respectively). Other options are available for which the Users' Manual should be consulted.

The values of the parameters assigned in Table 2 are those determined experimentally by Curds and Cockburn (1971) for the bacterium *Klebsiella aerogenes* and the ciliate *Tetrahymena pyriformis*. Typical results of the simulation are given in Fig. 4. All five variables (bacteria,
substrate and ciliate concentrations and the specific growth rates of the two organisms) oscillate in a regular fashion without damping. The stability of the system can be tested by using the analytically calculated equilibrium values as the initial conditions. Even when high precision is used the system begins to oscillate immediately (Curds, 1971a), the amplitude increasing and the wavelength decreasing until stable limit-cycle oscillations are established. Thus even minor perturbation from the equilibrium position generated within the computer results in movement away from the equilibrium point towards regular periodic motion.

Fig. 5. Effect of dilution rate upon theoretical steady-state values of limiting nutrient and bacteria (a) in the absence of a predator and (b) when a predatory ciliated protozoan is present. (Adapted from Curds, 1971a.)
The relationship between dilution rate and the population variables for a single-species chemostat culture is shown in Fig. 5(a). In comparison the values obtained when a predator is present, assuming steady states are achieved, are shown in Fig. 5(b). It can be seen that when a predator is present the steady-state concentration of substrate decreases as the dilution rate is increased whereas the prey and predator populations increase. This is contrary to the situation when a single organism is present. At dilution rates in excess of the critical rate for the ciliate the culture becomes a single species continuous culture and the curves are identical.

The types of equilibria derived analytically may be demonstrated by simulation; for the kinetic constants used in Table 2 dilution rates from 0.02 to 0.33 h⁻¹ produce stable limit-cycle oscillations; at \( D = 0.34 \) and 0.55 h⁻¹ damped oscillations occur indicating a stable focal-point solution; at \( D = 0.36 \) h⁻¹ a stable node results as the concentrations of bacteria, ciliate and substrate tend to steady-state values monotonically. At dilution rates above 0.37 h⁻¹ the ciliate population washes out and (see equation (45)) the bacteria asymptote to steady-state levels (stable node).

Under limit-cycle conditions the frequency of the oscillations depends upon the dilution rate in the way illustrated in Fig. 6. At low dilution rates the frequency is low and increases to a maximum after which it decreases to zero, i.e. steady state is obtained.

The magnitude of the maximum specific growth rate of the predator has a significant effect upon oscillation frequency. High values produce oscillations with low frequencies and as the maximum specific growth rate decreases so the frequency increases. The value of the saturation constant of the ciliate affects both the oscillation frequency and the extreme limits of the oscillating population. Frequency increases with the saturation constant. At high values of the saturation constant the maximum population densities obtained are reduced while the minimum population sizes are not so small. Small saturation constants produce the reverse effect.

The effect of varying other parameters can be estimated by varying them one at a time and simulating the results. Increasing the maximum specific growth rate of the prey gives rise to an increase in the frequency of the oscillations; the saturation constant of the prey has little effect on the periodicity of the system but it does affect the maximum concentration of ciliates that can be obtained. The value of \( K \) also determines
the rate of change in substrate concentration; at low values the decline in substrate becomes very rapid and simulation often allocates negative values to substrate concentrations in such cases. This, of course, is theoretically and physically impossible and the explanation of the result lies in the mechanics of the numerical technique employed in the simulation language. Several numerical integration methods are available but all of them depend on the step size over which each approximation is performed. For functions which change rapidly such as the case indicated here, the step size must be very small otherwise the result estimated will be inaccurate and the magnitude of the innacuracy may increase as integration proceeds. For the particular example of substrate concentration at low $K$ values the rapid decrease in $S$ results in the calculation of a negative value for this variable as $S \to 0$ which the integration routine cannot rectify. In this case the error is obvious but inaccurate results, both qualitative and quantitative, may arise which are not so easily noticed. It is therefore wise to include some sort of error analysis when simulation techniques are employed.
Probably the simplest check, but not the most rigorous nor the most efficient, is to run some programs twice using different step sizes to determine if the results differ significantly.

The simulation technique of analysing population dynamics is valuable for studying multispecies systems and for testing specific kinetic functions. Bungay and Paynter (1971) used computer simulation methods to investigate the dynamic behaviour of multicomponent models and included first-order time delays between substrate changes and the response of the growth rate of the microorganisms. These authors argue that when the limiting nutrient concentration is suddenly increased the cells cannot immediately establish the specific growth rate predicted by the Monod function and there is a lag period during which the cellular machinery necessary for the adaptation is produced. On the other hand they state that the growth rate can decline rapidly with no apparent lag if nutrient concentration is decreased. Young, Bruley and Bungay (1970), working with the yeast, \textit{Saccharomyces cerevisiae}, tested several mathematical ways of representing a delay mechanism and found that a first-order lag fitted their data reasonably well. For these reasons Bungay and Paynter (1971) used unmodified Monod functions when substrate concentrations were declining and the same functions with first-order time lags when substrate concentrations increased. They report that such a time delay caused the substrate concentration to oscillate with much greater amplitude and in particular caused the simulated nutrient concentration to drop to low values. It should be noted, however, that a smaller saturation constant could also give the same result.

The work of Bungay and Paynter (1971) on complex microbial systems also included the effect of a predator feeding on two competing prey organisms. Curds (1974) also tested a number of complex microbial food chains using slightly different kinetic functions. Bungay and Paynter (1971) infer, and Curds (1974) states that the magnitude of the kinetic constants have a more significant effect on the dynamics of the populations than the detailed form of the kinetic functions.

As computer simulations of microbial interactions are so much simpler to perform than the appropriate laboratory experiments no doubt this theoretical approach will be increasingly employed by microbial ecologists, perhaps at the expense of experimental investigation. The danger, already becoming apparent, is that a host of models
will be simulated and appear in the literature. As there are an infinite number of theoretical models such a process is without limit unless it is clearly recognized that solving a set of differential equations will not add to our understanding of ecological processes unless the equations themselves are based on ecological observations. If the introduction of simulation languages does not increase the number of experiments performed on ecological systems, or, even worse, switches the attention of ecologists from observational or experimental approaches to purely theoretical ones, they will have hindered rather than enhanced the progress of ecological research. We believe that theoretical and experimental approaches should be integrated; construction of a mathematical model imposes upon the investigator the responsibility of rigorously defining his assumptions and allows explicit predictions to be made. Experiment is needed to determine how close these predictions, and thus the assumptions on which they are based, mirror reality. At present our models are incomplete and usually only agree in a qualitative manner with results from laboratory cultures. They are even less accurate in describing natural communities of microorganisms. The role of computer simulation as a means of testing dynamic hypotheses and comparing experimental results with theoretical predictions, is rapidly becoming an indispensable technique and in the future will also become of increasing importance in forecasting the behaviour of ecosystems both in the laboratory and in nature.

3 Practice

There are two basic methods of experimentally investigating prey-predator relationships; the first is to use the batch-culture method in which the populations of organisms are isolated from the external environment, and the second is to use the continuous-culture technique in which explicit account is taken of input and output of energy and matter. In the latter method stable conditions such as steady states or sustained oscillations may occur which are of considerable advantage, as experiments may be run over extended periods of time.
3.1 Batch Culture

Until the introduction of the continuous-culture technique by Monod (1950) all work on the growth kinetics, physiology and biochemistry of microorganisms was based on batch-culture methods. Continuous cultures have not replaced batch methods since the latter have several advantages over the former. The batch-culture method is a comparatively quick and easy way of obtaining reasonable estimates of kinetic data and yield coefficients without major apparatus; indeed batch studies usually precede continuous-culture work. It is possible to work with low population densities and to make the environment spatially heterogeneous, both of which are inapplicable in continuous culture. However, there are also several major disadvantages inherent in batch cultures which can be overcome by the use of continuous-culture techniques. At the onset of batch cultures, the nutrient is usually in excess and there are few organisms, but as the latter grow the nutrient diminishes; at the same time, the physicochemical properties of the environment change and there will be an accumulation of potentially harmful metabolic products. In spite of the disadvantages, batch culture will continue to play a vital role in prey–predator research and the following account will serve to illustrate its use and adaptability.

3.1.1 Qualitative experimental work

a. Food preferences of protozoa Several authors have reported upon the use of batch cultures in their experiments concerned with the food preferences of protozoa. Many of these workers (Burbanck, 1942; Curds and Vandyke, 1966; Groscop and Brent, 1964, and others) have used bacteria as the prey and these have been presented, either as suspensions or as streaks on agar plates, to protozoa as their sole food source. This type of work has clearly demonstrated that not all bacteria, in isolation, are suitable for the prolonged survival of all protozoa. For example, Curds and Vandyke (1966) presented five species of ciliate separately with an excess supply of nineteen strains of different bacteria as the sole food source and they were able to divide the prey into three major categories—toxic, unfavourable and favourable—according to their effect upon the predators.

Apparently certain bacteria, particularly pigmented varieties, are toxic to various protozoa (Chatton and Chatton, 1927; Kidder and
Stuart, 1939; Singh, 1942, 1945, 1946; Brent, 1948; Groscop, 1963; Groscop and Brent, 1964; Curds and Vandyke, 1966) and the evidence available suggests that the bacterial pigment is frequently the toxic agent. It should be remembered, however, that these data have been obtained from laboratory studies and the significance of these observations when applied to the natural environment remains to be defined. According to Curds and Vandyke (1966) some bacteria – unfavourable ones – although nontoxic will not support the growth of protozoa indefinitely, whilst others – favourable ones – will do so. In addition, a bacterial strain may be favourable to one protozoon but unfavourable to another. Even within a group of favourable bacteria there is a degree of favourability for a given protozoan species since it has been demonstrated (Curds and Vandyke, 1966) that an excess of different favourable bacteria will support different maximum growth rates of ciliated protozoa. Perhaps this is not surprising as it is analogous to the situation in osmotrophic microorganisms where the growth rate is dependent upon the identity of the nutrient supplied. Burbanck and Gilpin (1946) even suggested that the measurement of the growth rate of the ciliate *Colpidium colpoda* could be used as a method for the identification of some medically important intestinal bacteria.

Although most work of this nature has considered bacteria as the prey organisms some information is available on the feeding activities of amoebae on soil fungi. Heal (1963) presented four species of amoebae with 35 species of fungi; all of the 19 species of yeast were eaten to varying extents and it was suggested that yeasts are a possible food source for soil amoebae. Although the sporangiospores of the 16 fungal species presented were ingested, only those of *Paecilomyces elegans* and *Polyscytalum fecundissimum* were actually digested and supported the growth of the amoebae.

b. Food selection Data from batch experimental work imply that protozoa are able to select the organisms to be ingested (Singh, 1942). For example, Schaeffer (1910) recorded that the ciliate *Stentor coeruleus* ingested 12 of the 15 *Phacus* sp. cells supplied while all 13 of the sulphur particles introduced were rejected. Furthermore, *S. coeruleus* apparently was able to discriminate between two species of *Phacus*, predominantly accepting *P. trignet* and rejecting *P. longicaudus*. However, no data were given on whether either of the two algal species were actually digested and it is well known that ciliates will ingest apparently useless
particles such as carmine. Many carnivorous protozoa will feed upon certain prey protozoa to the exclusion of others; for example the ciliate *Didinium nasutum* feeds exclusively upon the ciliate *Paramecium caudatum* and there are many other well-documented examples (Sandon, 1932). More evidence on food selection has been furnished by Lee *et al.* (1966) who used tracer-labelled organisms as a method of studying prey–predator relationships among the foraminifera. They presented more than 50 $^{32}$P or $^{14}$C-labelled axenic species of protists to ten species of foraminifera and found that although all these organisms occur in the natural habitat, the foraminifera selected only certain organisms for ingestion. They found that the yeasts, cyanophytes, dinoflagellates, chrysophytes and most of the bacteria tested were not eaten whereas certain species of diatom, chlorophytes and bacteria were eaten in large quantities. Later, Müller and Lee (1969) found that the identification of potential food organisms by these methods did not necessarily indicate whether or not they would support the growth of the foraminifera. These two authors reported their failure to establish bacteria-free cultures of the four foraminifera species supplied with one or two species of algae and observed that in addition bacteria were required for the prolonged survival of the protozoa. This type of work indicates that ingestion of prey is of little significance unless it is accompanied by data on the growth-supporting potentials of the prey.

Almost all the experimental work on the selective abilities of protozoa has been aimed at demonstrating their abilities rather than the quantitative aspects of the problem and there is clearly an urgent need for work of this nature.

### 3.1.2 Quantitative experimental work

#### a. Zoological methods

Salt (1967) used a purely zoological approach when he carried out a series of batch experiments on the predatory ciliate *Woodruffia metabolica*. He found that this ciliate would feed upon three species of the genus *Paramecium* but not upon bacteria, algae and some other ciliates tested. Salt's (1967) novel method was to introduce washed animals into 0·1-ml drops of inorganic salts medium under paraffin oil. The oil prevented evaporation yet allowed gaseous exchange and the introduction and removal of organisms during an experiment. A ciné camera was set to photograph the complete drop at 50-minute intervals so that animals could be counted from the photographic
records at convenient times. The usual form of experiment was to introduce 2–3 \( W. \) \( \text{metabolica} \) along with 200 \( P. \) \( \text{aurelia} \) (prey) into the drop and the predator was supplied with additional prey as necessary. Few bacteria were present and so it was assumed that the \( P. \) \( \text{aurelia} \) did not multiply during the experimental period.

Using these techniques, Salt (1967) estimated that \( W. \) \( \text{metabolica} \) spends some 15 per cent of its time hunting and that only 2 per cent of its encounters with prey result in a successful catch, that is \( W. \) \( \text{metabolica} \) ingests every fiftieth \( P. \) \( \text{aurelia} \) it meets. Although no correlation could be found between feeding rate and prey concentration, it is likely from Salt's (1967) calculations that prey concentration was not limiting. Furthermore, he suggested that there were only two feeding rates for \( W. \) \( \text{metabolica} \): maximum, when the prey is above a critical concentration, and zero (\( W. \) \( \text{metabolica} \) encysted), when below the threshold. Evidence was presented by Salt (1967) to suggest that feeding rate, hunting time and the number of prey consumed per new \( W. \) \( \text{metabolica} \) produced (equivalent to reciprocal of yield coefficient) decreased with increasing predator density while the growth rate remained constant. Unfortunately no statistical evidence was given and it is not always obvious from the graphs that Salt (1967) is strictly correct in coming to these conclusions. In a later paper concerning the predation of \( A. \) \( \text{proteus} \) upon \( P. \) \( \text{aurelia} \), Salt (1968) admits that there was little statistically significant evidence to substantiate his claims that increases in predator density affects feeding rate etc. as he had reported for \( W. \) \( \text{metabolica} \). It is apparent from one of Salt's (1967) graphs that the scatter of feeding-rate data increases with predator density and this suggests that perhaps the rate of feeding might be dependent upon the number of prey available per predator as has been reported for other organisms (Curds and Cockburn, 1968).

b. Microbiological methods Microbiologists have long used batch cultures to measure growth rates, saturation constants and yield coefficients, and these techniques are of great value to workers investigating prey–predator relationships. The work of Gause (1934) on growth and predation of microorganisms remains a classical example of the integration of theory and experiment. Gause used a variety of microbes in his experiments and compared his data to the predictions of the Verhulst and Lotka–Volterra equations. For details the reader is referred to Gause's excellent book.
Proper and Garver (1966) were probably the first to employ modern microbiological methods to study protozoa. They presented a few predators, the ciliate *Colpoda steinii*, with various known concentrations of prey, 8–940 mg l\(^{-1}\) *Escherichia coli*, and counted the ciliate populations at appropriate intervals of time. The peak populations of predator obtained during the batch growth cycle were then plotted against the initial concentrations of prey (see Fig. 7). Provided all prey have been consumed, then the slope of such a line will equal the yield coefficient and a linear relationship indicates that the yield is constant. Using these methods Proper and Garver (1966) found that a linear relationship was obtained and the yield of *C. steinii* feeding on *E. coli* was calculated to be 0.78. Figure 7 shows a similar plot for *Tetrahymena pyriformis* feeding upon *Klebsiella aerogenes* taken from the work of Curds and Cockburn (1968). Table 3 gives examples of yields and kinetic constants that have been obtained using the batch-culture method. It will be seen from Table 3 that the yield estimates obtained lie in two groups – the two high ones of Proper and Garver (1966) and Canale *et al.* (1973) and the other three in the region of 0.4–0.5. It should be remembered here that the magnitude of the yield depends upon the accuracy of the estimation of the dry weights of the organisms.
The estimation of the dry weight of the predator is perhaps technically
the most difficult measurement to carry out unless it can be cultivated
axenically. When it is not cultivated axenically it is very difficult to
wash sufficient numbers of protozoa from bacteria and debris in order
to obtain reliable weight estimates.

Curds and Cockburn (1968) calculated that *Tetrahymena pyriformis*
had an effective yield of 0·09 (based on total carbon in nondefined
medium to ciliate carbon) in axenic culture but 0·37 when based on a
balance of the carbon actually utilized. The yield was somewhat
higher (0·5) when the ciliate was fed upon *Klebsiella aerogenes*. From
Table 3 it can be seen that the yield coefficients range from 0·37 to 0·78

<table>
<thead>
<tr>
<th>Predator</th>
<th>Prey</th>
<th>Yield ((W))</th>
<th>(\lambda_m) (k^{-1})</th>
<th>(L) (mg l^{-1})</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acanthamoeba sp.</td>
<td><em>Saccharomyces</em></td>
<td>0·37</td>
<td>0·07</td>
<td>—</td>
<td>Heal (1967a)</td>
</tr>
<tr>
<td></td>
<td><em>cerevisiae</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colpoda steinii</td>
<td><em>Escherichia coli</em></td>
<td>0·78</td>
<td>0·23</td>
<td>6·0</td>
<td>Proper and Garver (1966)</td>
</tr>
<tr>
<td>Entodinium caudatum</td>
<td><em>Escherichia coli</em></td>
<td>0·50</td>
<td>—</td>
<td>—</td>
<td>Coleman (1964)</td>
</tr>
<tr>
<td><em>Tetrahymena</em></td>
<td><em>Klebsiella</em></td>
<td>0·50</td>
<td>0·22</td>
<td>11·6</td>
<td>Curds and Cockburn (1968)</td>
</tr>
<tr>
<td><em>pyriformis</em></td>
<td><em>aerogenes</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Tetrahymena</em></td>
<td><em>Aerobacter</em></td>
<td>0·73</td>
<td>0·10</td>
<td>6·1</td>
<td>Canale <em>et al.</em> (1973)</td>
</tr>
<tr>
<td><em>pyriformis</em></td>
<td><em>aerogenes</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uronema sp.</td>
<td><em>Serratia</em></td>
<td>—</td>
<td>0·15</td>
<td>0·49 (as C)</td>
<td>Hamilton and Preslan (1969)</td>
</tr>
<tr>
<td></td>
<td><em>marinorubra</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

and even if the two high yields were ignored the range is much higher
than is generally recorded for metazoan invertebrates; and Heal
(1967b) suggested that the differences between microorganisms and
metazoan invertebrates are that the microbes grow at relatively high
rates and do not possess a stage in their life-cycle equivalent to the adult
metazoan. Microorganisms generally grow to a certain size, divide and
proceed to grow again, whereas the macro-invertebrates generally pass
through a series of larval stages before becoming adults which only
continue to grow in the sense that tissues such as reproductive cells
etc. are produced and replaced. Engelmann (1961) showed that slow-
growing or nonreproducing organisms tended to convert less food to
protoplasm than did fast-growing animals. MacFadyen (1963) stated that macro-invertebrates use about 90 per cent of their assimilated energy in respiration over their complete life-cycle.

In the same way that the value of the yield coefficient of a bacterium varies with the identity of the limiting nutrient (Hadjipetrou et al., 1964) so does the yield coefficient of a protozoon depend upon the identity of its prey. Recently, Dive (1973) showed that the yield coefficient of *Colpidium campylum* was usually greatest when the ciliate was fed upon Gram-negative organisms, and perhaps this is ecologically understandable since freshwater bacteria tend to be Gram-negative rods. The yield of the ciliate was greatest when fed upon *Enterobacter cloacae* but approximately halved when fed upon similar numbers of *Serratia marcescens*. When both bacteria were supplied as a mixture of equal numbers, the value of the yield coefficient was found to be approximately an average of the two former coefficients.

The relationship between the growth rate of the predator and concentration of its prey can be investigated at the same time as yield coefficient. The growth rate of the predator, *Colpoda steinii*, feeding on *Escherichia coli* is given in Fig. 8(a) which is taken from the work of Proper and Garver (1966). A Lineweaver-Burk plot may be obtained by plotting the reciprocal of the growth rate against the reciprocal of the prey concentration as in Fig. 8(b). In that form of plot, the negative intercept on the y-axis is the saturation constant, $L$, and the slope equals the maximum growth rate. This form of plot tends to be

![Fig. 8. Batch culture growth studies on *Colpoda steinii* feeding on *Escherichia coli*. (a) Effect of prey concentration upon growth of predator. (b) A reciprocal (Lineweaver-Burk) plot of data given in (a). (After Proper and Garver, 1966.)](image-url)
excessively biased as most of the data become crowded towards the x-axis. A better method (Dowd and Riggs, 1965) is to plot predator growth rate against prey concentration, where the slope is equal to $1/(\text{maximum specific growth rate})$ and it intercepts the abscissa at $L$. Application of minimization routines such as the Simplex method are now easy to use in computer programs and allow parameter estimation from nonlinear functions. Such methods are to be preferred as they are more accurate.

Whereas most authors (Proper and Garver, 1966; Curds and Cockburn, 1968; Hamilton and Preslan, 1969; Canale et al., 1973) agree that a reciprocal plot of the specific growth rate of a predator against the concentration of its prey (Fig. 8(b)) gives an acceptable linear relationship from batch data, there is a certain amount of evidence which suggests that the specific feeding rate of an organism is not only related to the concentration of prey present but also to the concentration of predators themselves. Curds and Cockburn (1968) presented known concentrations of Tetrahymena pyriformis with bacteria for a feeding period of five hours. At the end of this time the numbers of bacteria and ciliates were estimated once more. It was found from studies such as these that when the average specific rates of predation were plotted against the mean concentration of bacteria present during the feeding period, a wide scatter of points was obtained (Fig. 9(a)). However, when the specific rate of predation (weight of bacteria consumed per thousand ciliates per hour) was plotted against the ratio of bacteria available per ciliate (Fig. 9(b)) the scatter was considerably decreased. This suggested that the concentration of predator and prey play important roles in controlling the feeding rate of the ciliate. It should also be noted (Fig. 9(b)) that the curve tends to overshoot before settling to its saturation value; this is a frequently observed phenomenon (Canale et al., 1973; Bazin et al., 1974) for which no explanation has yet been offered.

Contois (1959) found that when Aerobacter aerogenes was grown in a chemostat, the specific growth rate of the bacterium was dependent not only on the concentration of the limiting nutrient but also on the population density of the growing bacteria; he modified Monod's (1942) equation as follows:

$$\mu = \frac{\mu_m S}{C_0 S + S},$$  \hspace{1cm} (37)

where $C$ is "a growth parameter which is constant under defined conditions" and $X$ is the bacterial population density.
Fig. 9. Effect of bacterial concentration upon the specific predation rate of *Tetrahyena pyriformis* in batch culture. (a) Plot obtained when numbers of predator cells are ignored. (b) Plot of same data when predator cell numbers are taken into account. (After Curds and Cockburn, 1968.)

When equation (37) is expressed in terms of ciliate feeding then

\[ f_1 = \frac{\lambda_m H}{W(CP_n + H)}, \]

where \( f_1 \) and \( \lambda_m \) are the specific and maximum specific rates of predation, \( H \) is the concentration of bacteria available as food and \( P_n \)
is the number of ciliates per unit volume. If we let
\[
\frac{\lambda_m}{W} = f_m
\]
then rearrangement of (38) yields
\[
\frac{H}{P_n f_1} = \frac{C}{f_m} + \frac{1}{f_m} \frac{H}{P_n}. \tag{39}
\]
Therefore from equation (39), when \(H/P_n f_1\) is plotted against \(H/P_n\) a straight line will be obtained if the equation fits the data (38). Furthermore, the slope of the line will equal \(1/f_m\) and will intercept the abscissa at a value equal to the constant \(C/f_m\). Figure 10 is such a plot and illustrates that there was indeed a linear relationship. Equation (38) describes a three-dimensional surface and this is shown isometrically in Fig. 11.

Fig. 10. Linear form of feeding data illustrated in Fig. 9(a).
(After Curds and Cockburn, 1968.)
3.2 CONTINUOUS CULTURE

Although a considerable body of literature exists on the theoretical aspects of predation relatively few experiments have been reported on chemostat culture of microbial prey–predator systems; this is surprising since this method represents the most efficient way of experimentally testing the various hypotheses which have been proposed. The basis of a theoretical consideration of predation dynamics lies in making simplifying assumptions (Williams, 1971); therefore an experimental system used to test a theory must reflect these simplifications as closely as possible. Chemostat culture of microbial prey and predator organisms is the method that is most likely to reflect the assumptions implicit in the models we have discussed although the species must be chosen with care. For example, the limiting nutrient should be metabolized only by the prey organism so that the predator density is entirely dependent on that of the prey; the predator must be capable of feeding in the
absence of a solid support and neither organism should adhere to the walls of the culture vessel so that the culture remains well mixed. In order to simplify analysis of the results it is obviously advantageous if the metabolic products do not inhibit the growth of either organism.

The variables, $H$ and $P$, in the mathematical models we have discussed refer to prey and predator population densities respectively. Theoretical considerations imply that these represent biomass concentrations (Gause, 1934; Tsuchiya et al., 1972a). In order to test the models rigorously, then, it is necessary to obtain biomass estimates of both prey and predator populations. It is usually simple to distinguish prey from predator by microscopic examination and to estimate their numbers using a counting chamber. However, such methods are usually insufficient due to the large amount of data that need to be collected in order to test a model. The possible oscillatory response makes a great deal of data necessary if the maxima, minima, slopes and frequencies of the experimental variables are to be estimated adequately. It is for these reasons that the only practical method of enumerating prey and predator populations is by the aid of electronic techniques such as the Coulter Counter. To distinguish the populations by this method it is essential that the prey organism is significantly different in volume from the predator.

The measurement of biomass concentrations of both prey and predator organisms in mixed culture is more difficult. Williams (1971) has shown for the green alga, *Chlorella pyrenoidosa*, that there is a linear relationship between dry weight and the product of the mean cell volume and cell number. As dry weight is a commonly accepted measure of biomass this product, expressed as a concentration which we will call the volume density, can be used in analysing mixed cultures provided that this property extends to other organisms and assuming that the mean cell volumes of the organisms involved can be determined. This is usually possible with the comparatively large prey organisms and this method has been used by Canale et al. (1973) and by Curds and Cockburn (1971) for *Tetrahymena* and by Rapa and Bazin (1973) for slime mould amoebae. Estimating the mean cell volume of prey organisms, especially if they are bacteria, is more difficult due to their smaller size. Both Canale et al. (1973) and Curds and Cockburn (1971) estimated the density of their prey organisms by centrifuging the mixed culture and measuring the optical density of the remaining
bacterial suspension. The possible objection to this technique is that the mean size of bacteria is a function of their specific growth rate (Schaechter et al., 1958) and bacterial size changes during the prey–predator cycle have been shown by Rapa and Bazin (1973). Thus any fixed centrifugation regime will sediment a variable amount of bacteria resulting in inaccuracies in the method. Rapa (personal communication) has shown that for low predator densities in a Dictyostelium–Escherichia system the effect of the amoebae on the turbidity of the bacterial suspension is small and that reliable estimates of prey biomass can be made from optical density measurements of the mixed suspension during these periods of culture. Further, as has already been pointed out by Bazin et al. (1974), the mean cell volume of the amoebae varies sinusoidally with the number density but out of phase with it. It is thus possible in this system to obtain estimates of the predator biomass density from cell number data and, for some periods in the culture of these organisms, the biomass concentration of the prey by turbidometric methods.

Prey–predator relationships can be studied in two fundamentally different types of continuous culture systems, the choice being determined by the data required. A two-stage system, where the growth of the two organisms is physically separated, is most suitable for determining kinetic data while prey–predator population dynamics are best studied in a single-stage system in which both organisms are grown together. The single-stage system is more complex and for this reason will be considered after two-stage systems.

3.2.1 Two-stage continuous culture

a. Methods A two-stage continuous culture is one way of arranging that the prey organism does not grow in the presence of the predator; all growth of the prey takes place separate from the predator in the first reactor. An outline of the apparatus used by Curds and Cockburn (1971) is shown in Fig. 12. The first stage is a simple chemostat which is used merely as a means of converting the soluble limiting nutrient into a steady-state population of prey organisms. The dilution rate of the first stage is kept as low as possible in order to keep the nutrient concentration low which precludes further growth of the prey in the second reactor. A drop in the concentration of bacteria is sometimes observed in the second reactor even when predators are not present
and this is probably due to cytolysis. It is preferable to operate the first reactor at a constant dilution rate during a set of experiments on the growth of the predator as the prey will then be kept in as physiologically constant a state as possible. If the first stage is fitted with a constant-volume device and the prey suspension is independently pumped to the second stage, it is possible to operate the second reactor over a wide range of dilution rates without changing that of the first. The two-stage system shown in Fig. 12 has a first-stage reactor working volume larger than that of the second stage which can therefore be operated at dilution rates both higher and lower than that of the first reactor. A simple cascade system, where all the contents of the first reactor overflow into the second, does not give this adaptability and the dilution rate of the second stage becomes largely dependent upon that of the first stage.

Fig. 12. Two-stage continuous-culture apparatus as used by Curds and Cockburn (1971). A₁, sterile air input for stirring and aeration at rate of 1 volume per minute; A₂, sterile air input to prevent contamination of feed line; C, cotton wool plug; F, air filter; M, magnetic stirrer; O, upward flow constant-level device overflowing to waste; P, peristaltic pump; T, tap; SD, sampling device to prevent nonsterile air being sucked back into system; SR, sterile growth medium input.
Hamilton and Preslan (1970) used a rather different approach to the problem of presenting a continuous supply of a nondividing population of prey to the predators. They grew the bacterium *Serratia marinorubra* separately in batch cultures which were then harvested, resuspended and finally pumped to a reactor containing the predatory marine ciliate *Uronema* sp. The actual method chosen is purely a matter of personal preference and convenience; however, we prefer a two-stage system since this has proved to be simpler and less susceptible to contamination.

b. *Establishment of a steady state* Whereas the Monod (1942) theory predicts that the population of prey and predator will quickly attain a steady-state situation without oscillations in a two-stage system, some workers have experienced practical problems in achieving this. Hamilton and Preslan (1970), for example, found that steady-state populations of *Uronema* sp. feeding upon *Serratia marinorubra* were quickly reached in their chemostat and could be maintained for several weeks. The populations did not exhibit any oscillatory behaviour but they did find that the predators were washed out at low dilution rates (relative dilution rate, $D/\mu_m < 0.5$) while they survived and agreed well with Monod theory at higher dilution rates. They suggested that the protozoa were washed out because something other than food concentration was limiting. These observations will be dealt with later but it should be remembered here that when $D/\mu_m = 0.5$ the steady-state food concentration equals that of the saturation constant.

In the case of the ciliate *Tetrahymena pyriformis* feeding on *Klebsiella aerogenes*, Curds and Cockburn (1971) found that several weeks elapsed before steady states of the two organisms could be established. Soon after the ciliates had been inoculated they were found to be few in number but large in size; over a period of a week or two the ciliates progressively became smaller and more numerous. At the same time the concentration of bacteria in the second reactor decreased with time. After this comparatively long transient period, steady-state populations of bacteria and protozoa (numbers and mean cell volumes) were obtained without sign of oscillation. The steady-state mean cell volume of the ciliate population was found to be low at low growth rates and high at high growth rates within the limits of $5 \times 10^5 - 50 \times 10^5 \mu m^3$. This is in agreement with the behaviour of bacteria that are known to be largest at high growth rates and with some early batch culture data of Harding (1937) who was the first to demonstrate that the
size of a holozoic ciliate could be controlled by the concentration of bacteria available as a food source. The lowest relative growth rate used throughout this continuous culture work was 0.1 but there were never any signs of washout of the ciliate.

Jost et al. (1973a) studied the growth of *Tetrahymena pyriformis* in chemostats receiving flows of nondividing *Acetobacter vinelandii* and *Escherichia coli*. In both cases they obtained steady-state populations of ciliate and bacterium without oscillations within the period of about a week. In the same paper Jost et al. (1973a) reported upon the dynamic behaviour of all three organisms growing together in the same reactor and this will be mentioned later in the appropriate section. In view of the evidence on food selection and preference it is a pity that these authors did not carry out the intermediate experiments of feeding *T. pyriformis* on mixed populations of nongrowing bacteria instead of immediately investigating a far more complex system.

c. Results Curds and Cockburn (1971) found that the data obtained from a two-stage chemostat of *Tetrahymena pyriformis* and *Klebsiella aerogenes* did not quite fit the Monod (1942) function. This they attributed to the changes in ciliate size which affected both kinetic constants, $\lambda_m$ and $L$. Caperon (1967) proposed the following equation to describe the specific growth rate of a population of microorganisms,

$$\frac{1}{X} \frac{dX}{dt} = \frac{SK_3c_0/qP_n}{(K_3/K_1) + S},$$  (40)

where $c_0/P_n$ is the number of adsorption sites per individual, $q$ is the mass of food required to form a new individual, $S$ is the concentration of food available and $K_1$ and $K_3$ are the rate coefficients for the adsorption of food at the site and the freeing of the adsorption site with dimensions of h$^{-1}$ and ng h$^{-1}$ respectively.

If it is assumed that the adsorption site of a holozoic ciliate is the cytostome or mouth, and since there is only one mouth per individual ciliate, then here $c_0/P_n = 1$. Taking the yield coefficient with respect to cell mass as a constant,

$$q = M/W,$$  (41)

where $W$ is the yield coefficient and $M$ is the mean mass of the cell. In the case of the ciliate, $H$, the concentration of prey, replaces $S$. Incorporating these relationships into equation (40) the specific growth
rate of a ciliate predator becomes

\[
\lambda = \frac{(K_3W/M)H}{(K_3/K_1) + H},
\]

(42)

In the case of Tetrahymena pyriformis, it is likely that the size of the ingestion site is proportional to cell mass. That is, the larger the cell the larger is its mouth, so that the rate coefficient for adsorption of food at the ingestion site is greater. If this assumption is valid and \(K_1\) is linearly dependent on \(M\) then \(K_1 = K_4M\) where \(K_4\) is a constant, and the specific growth rate would be better described by replacing the term \(K_3/K_1\) in equation (40) by \(K_3/K_4M\).

Under steady-state conditions the specific growth rate of a population in a chemostat, \(\lambda\), is equal to the dilution rate \(D\) of the reactor. Substitution into equation (42) gives

\[
D = \frac{(K_3W/M)\dot{H}}{(K_3/K_4M) + \dot{H}}.
\]

(43)

In equation (43) the quantity \(K_3W/M\) has units of \(h^{-1}\) and is equivalent to the maximum specific growth rate, \(\lambda_m\), which in this case is dependent upon the mean cell mass. Simplifying equation (43),

\[
D = \frac{K_4K_3W\dot{H}}{K_3 + K_4M\dot{H}},
\]

(44)

whence

\[
\frac{\dot{H}}{D} = \frac{1}{K_4W} + \frac{1}{K_3W}\dot{H}.
\]

If equation (43) is applicable, a plot of \(MW\dot{H}\) (or \(v\dot{H}\) when \(v\), the mean cell volume, is directly proportional to its mass) against \(\dot{H}/D\) would be linear, the slope of the line would equal \(1/K_3W\) and it would intercept the abscissa at \(1/K_4W\). Figure 13 is such a plot using the data of Curds and Cockburn (1971); the correlation coefficient for the plot was 0.92 whereas a conventional Lineweaver–Burk plot of \(1/D\) against \(1/\dot{H}\), appropriate if body size has no effect upon \(\lambda_m\) and \(L\), showed a much poorer correlation with a coefficient of 0.72.

Equation (43) describes a three-dimensional surface and this is illustrated in Fig. 14. It is important to note that the following statements about Fig. 14 are merely for descriptive purposes and are not intended to denote the order of events in a growing culture. In Fig. 14
Regression equation: $y = 37.712 + 0.125x$

$1/K_4W = 37.71$, $K_4W = 0.026$

$1/K_3W = 0.125$, $K_3W = 7.97$

Fig. 13. Linear form of growth rate data using equation (44).
(After Curds and Cockburn, 1971.)

Fig. 14. Three-dimensional model of effect of bacterial concentration and ciliate mean cell volume on the specific growth rate of *Tetrahymena pyriformis* in continuous culture. (After Curds and Cockburn, 1971.)
at any chosen mean cell volume, the specific growth rate of *Tetrahymena pyriformis* increases with an increase in the concentration of bacteria (food) until a plateau (the maximum specific growth rate) is reached. It is important to note that the height of the plateau is dependent upon the mean ciliate cell volume. At any chosen bacterial concentration the specific growth rate is lower at higher mean cell volumes. When a continuous culture is first inoculated with ciliates, food in the form of bacteria is usually in excess, the ciliates respond by feeding rapidly and so become relatively large in size. As the concentration of bacteria drops due to predation the ciliate cells become smaller but more efficient (higher $\lambda_m$) and these adjustments continue until a steady-state situation is attained.

The population effect observed in batch cultures by Curds and Cockburn (1968) was not found in continuous cultures. This was explained (Curds and Cockburn, 1971) in terms of the Monod (1942) theory based on Michaelis–Menten enzyme kinetics to be due to the fact that the ciliate population (equivalent to enzyme concentration in Michaelis–Menten kinetics) in batch cultures could have been too great for the concentration of bacteria (equivalent to substrate concentration) whereas a basic assumption made to derive the Michaelis–Menten equation is that the enzyme concentration must be small compared with the substrate concentration. Continuous cultures of the chemostat type are self-regulating and the populations are in dynamic equilibrium; it is not possible to reach a steady-state condition at which the ciliate population is too great for the bacterial population.

Hamilton and Preslan (1970) found that the cell volume of *Uronema* sp. also varied considerably but they stated that these changes did not reflect changes in specific growth rate but apparently varied in response to the concentration of bacteria in the feed reservoir. According to chemostat theory the concentration of bacteria (nutrient for ciliates) in the reservoir should only influence the concentration of protozoa in the reactor and so Hamilton and Preslan (1970) related protozoan size to numbers. They concluded that the equations of Herbert et al. (1956) are suitable for the description of the populations of *Uronema* sp. in a chemostat over a wide range of inflowing concentrations of bacteria and that its growth was regulated by prey concentration in the manner suggested by Caperon (1967) but with two exceptions: at low protozoan populations some factor other than food becomes limiting and yield based on cell carbon may not be constant. In fact Hamilton and Preslan
(1970) did not demonstrate a population factor. They observed that the protozoa were washed out when the dilution rate and substrate concentration in the inflowing medium were low simultaneously; they appear to be unaware of the relation between the critical dilution rate for an organism and the concentration of substrate in the inflowing medium. The relationship was shown by Herbert et al. (1956) to follow the equation

\[ D_c = \frac{\mu_m S_0}{K + S_0}, \]  

(45)

where \( D_c \) is the dilution rate at which the organism will be washed out of the system. Two of the three occasions when Uronema sp. was washed out could be predicted by using equation (45).

The yield coefficient of bacteria is known to vary with growth rate (Herbert et al., 1956) and is not constant as is usually assumed. Although Hamilton and Preslan (1970) found that the yield coefficient of Uronema sp. varied from 0.16-0.05 (based on bacterial carbon to ciliate carbon) and seemed to be dependent upon the concentration of bacteria in the reservoir, they could find no change in yield when based upon the numbers of organisms; we have already stated that numbers are not usually a good estimate of population. Curds and Cockburn (1971) could find no significant evidence to suggest that the yield of Tetrahymena pyriformis varied with its growth rate.

The yield coefficient of a predatory organism in two-stage continuous culture can be derived from the equation given by Herbert et al. (1956),

\[ \tilde{P} = W(H_0 - \bar{H}), \]  

(46)

where \( \tilde{P} \) is the concentration of predator, \( W \) is the yield coefficient and \( H_0 \) and \( \bar{H} \) are the concentrations of prey entering and leaving the second reactor respectively. It follows from equation (46) that a plot of \( \tilde{P} \) against \( (H_0 - \bar{H}) \) will give a line whose slope is equal to \( W \). This is illustrated in Fig. 15 and the yield has been calculated to be 0.54. The two groups of points in Fig. 15 were obtained by using two concentrations of bacteria in the first-stage reactor.

The vast majority of work concerning protozoan predation in two-stage continuous culture has dealt with bacteria as the food source. However, Taub and McKenzie (1973) and Taub (1973) used a two-stage chemostat system for studying primary production by the green alga, Chlamydomonas reinhardtii, in the first flask and its consumption by Tetrahymena vorax in the second flask. The relationship between light
Regression equation: \( y = 0.5 + 0.54x \)
Slope = yield = 0.54

Fig. 15. Relation between the population of *Tetrahymena pyriformis* in the second stage of a chemostat system and the quantity of bacteria consumed. (After Curds and Cockburn, 1971.)

intensity, limiting nutrient, dilution rate and population density was studied in association with a mathematical model based on modified Monod kinetics. The authors demonstrated the range of experimental conditions over which predation can reduce algal concentration.

3.2.2 Single-stage continuous culture

a. Slime mould–bacteria systems  The first experimental investigations of prey–predator dynamics in chemostat culture were carried out by Dr H. M. Tsuchiya and his co-workers at the University of Minnesota in 1963 and this group is still very active in the study of microbial interactions. Initially a system consisting of amoebae of the cellular slime mould *Dictyostelium discoideum* feeding on *Escherichia coli* with glucose as the sole carbon source was used (Tsuchiya et al., 1972a). One of the most obvious properties of this and other similar biological
systems is that despite strict control of the external environment the populations often exhibit highly nonlinear responses that do not correspond to the simple cyclical changes predicted by deterministic mathematical models. This behaviour is due partly to measuring cell numbers rather than biomass concentrations for, as has been shown by Williams (1971), the former parameter is inherently more variable than the latter. Further, two-species microbial prey–predator systems are particularly sensitive to minor environmental perturbations; in our experimental studies we have noticed that even small changes in the rate of aeration or stirring are sufficient to cause major changes in prey and predator concentrations. As we will discuss later, it is also likely that the biological properties of the cells themselves lead to behaviour that is not directly associated with predatory activity.

It is evident from the data of Tsuchiya et al. (1972a) that the response of the amoeba and bacterium agrees in general with that expected; both populations oscillate, with the prey increasing and declining in advance of the predator. After extended culture periods the oscillations damped out and as the dilution rate was increased the wavelength of the oscillations decreased. In general terms therefore the response they observed was one that would be predicted by an equilibrium of the stable focal point type. The authors quoted the work of Gause (1934) to support the analysis of their results in terms of the Monod saturation function, rather than the Lotka–Volterra equations, by assuming that the response of their system was independent of the initial conditions but offered no specific evidence that this was in fact the case. They were unable to obtain a good quantitative fit to their data but suggested that the development of a resistant bacterial strain and time lags in the kinetics of the system might explain these discrepancies between theory and practice.

One of the features of the results of Tsuchiya et al. (1972a) was that the wavelengths of the prey, predator and nutrient oscillations varied. We have also observed this characteristic in some of our experiments with this system. This phenomenon may be one of the most difficult to explain unless stochastic events are included in the theoretical consideration. After the oscillations had damped out, the comparatively high bacterial concentration and low predator density remained constant and considerable numbers of amoebae accumulated on the walls of the culture vessel just above the air–liquid interface. Examination of the culture medium revealed clumps of three to ten amoebae
and the presence of spores. We shall attempt to explain these observations in the light of some of our results later.

A *Dictyostelium discoideum*–*Escherichia coli* system is being further investigated by one of us (M. J. B.) and as we have already indicated, much of the work of Tsuchiya et al. (1972a) has been confirmed. However, our chief aim is to represent the dynamic behaviour of the system in terms of biomass rather than in numbers of organisms. Our reasons for this choice have already been explained and the disadvantage of measuring cell numbers have been well illustrated by the behaviour of the mixed population during the early stages of chemostat culture (Rapa and Bazin, 1973). At the beginning of such experiments *E. coli* was grown as a batch culture in a chemostat vessel. When a high bacterial density was obtained, spores of the slime mould were inoculated. After germination the nutrient flow was started. The initial response of the prey population was oscillatory with a wavelength of about 3-5 hours but there were no complementary oscillations in the bacterial numbers. Since the wavelength of the oscillations corresponds to the doubling time of the predator population, the behaviour of the culture could be explained if the amoebae were dividing synchronously.

The behaviour of *Dictyostelium discoideum* and *Escherichia coli* after this initial period is illustrated in Fig. 16(a), (b). These data have yet to be fully analysed but they will serve as the basis for a qualitative description of what we consider to be the major biological events in the association of these organisms. For descriptive purposes the response of the culture has been divided into three periods. The first phase (Fig. 16(a), (b)) is one in which damped oscillations occur, although the damping coefficient does not seem to be constant since the amplitudes of the first two oscillations are approximately equal. In agreement with the results of Tsuchiya et al. (1972a) and some of our other observations on the same system, but in contrast to the predictions of the Lotka–Volterra theory and Monod saturation kinetics, the wavelength of the oscillations do not seem to be constant. The shapes of the curves generated by the data are in qualitative agreement with the Monod theory as predicted by numerical integration (see Fig. 4 and Curds, 1971a; Tsuchiya et al., 1972a). A feature of the predator curve, which is not predicted by any of the models, is the appearance of secondary peaks which correspond to the maxima in the glucose concentrations. We do not believe this to be an anomalous result as
we have now observed this response on numerous occasions. Similar results have been obtained in the spread of influenza virus in an isolated human population where it was explained by postulating that two strains of the disease had been introduced. Although it is possible that two strains of *D. discoideum* were present in the experimental system we feel that a more likely explanation would have to take into account the relationship between predator and glucose concentration. As the secondary peaks occur at high glucose levels it is possible that the
sugar affects the amoebae despite the report of Wright and Bloom (1961) that the vegetative cells are impermeable to glucose.

After the oscillations have damped out, the experimental variables may be regarded as having reached steady-state values. The culture then enters the second phase (Fig. 16(c)) where the amoebae increase in number at the expense of the bacteria and new steady-state levels are obtained. We believe that this might be a biological response unrelated to the prey–predator dynamics of the system; during the normal cycle of cellular slime moulds the amoebae aggregate to form differentiated structures called pseudoplasmodia. Formation of pseudoplasmodia occurs on a solid substrate in response to starvation and results in the production of spore-bearing structures. In chemostat culture the physical environment precludes pseudoplasmodia formation but it is possible that the amoebae attempt to aggregate. As a prelude to aggregation in the chemostat we suggest that the amoebae increase their rate of predation (Fig. 16(c)) in order to store sufficient reserve nutrient for the nonvegetative phase of growth. If it is assumed that after this period the amoebae then attempt to aggregate but cannot do so because of the enforced homogeneity of the system, behaviour of the sort shown in Fig. 16(c) might be expected. The amoebae in this stage are presumably partly committed to forming pseudoplasmodia and are no longer capable of growing vegetatively as efficiently as before. Thus the concentration of bacteria increases and the number of amoebae declines. The interpretation of the final stages in the chemostat in terms of the natural history of the slime mould rather than as a result of prey–predator dynamics is supported by the results of Tsuchiya et al. (1972a) who found that the amoebae tended to clump and adhere to the solid surface of the culture vessel in the final stages of continuous culture. They also found spores in the culture medium which suggests that some of the organisms had been at least partly successful in completing the spore-forming stage of their life-cycle.

The results of a similar experiment, corresponding to the first stage shown in Fig. 16(a), (b), have been plotted in phase space by Bazin et al. (1974). According to the analysis of Canale (1969), based on Monod kinetics, the trajectory of such a plot should tend towards a plane in prey–predator-nutrient phase space. The data of Bazin et al. (1974) does show such a tendency, as is shown by constructing a three-dimensional model of the results which is shown isometrically in Fig. 17. An even better fit to a plane is obtained if the logarithm of bacterial
The movement of the trajectory towards the centre of the plot indicates damped oscillations and an equilibrium of the stable focal point type. (Original data of V. Rapa.)

numbers is used on the prey axis rather than untransformed bacterial concentration. These authors suggested that this might be due to plotting number rather than biomass densities.

b. *Ciliate-bacteria dynamics* The data of Canale et al. (1973) who used chemostat cultures of *Tetrahymena pyriformis* feeding on *Aerobacter aerogenes* with sucrose as the limiting nutrient are more difficult to interpret. Prey and predator populations oscillated in a rather erratic fashion but many of the results do not show a complementary periodicity in the concentration of carbohydrate which in many cases appeared to remain constant. The authors were not able to explain their results in terms of the simple Monod equation, but suggested an extension of the same model using the same type of kinetics but including equations for both refractile and usable carbohydrate. They proposed that only part of the carbohydrate that they measured was in a form suitable for bacterial growth and the rest was the result of metabolic product formation. This new model did not fit the data during transient periods of growth and they suggested that higher-order effects, such as time delays, and the self-regulatory and adaptive ability of the predator,
which are not incorporated into the model, are important factors that cannot be disregarded.

Adaptation is also regarded as an important phenomenon by van den Ende (1973) as a result of his observations of chemostat cultures of *Tetrahymena pyriformis* and *Klebsiella aerogenes*. After extended periods of culture it was found that the bacteria tended to adhere to the sides of the culture vessel. Van den Ende (1973) suggested that this was a defensive mechanism resulting from the selective pressure of predation and that mutant bacterial strains arose which were more prone to sticking to the sides of the vessel than the parental strain. As a result of this property the bacteria become less susceptible to predation as the forces binding the bacteria to the walls are greater than those created by the ciliary feeding currents of the protozoa. However, in the absence of predators the majority of bacteria tend to stick to solid surfaces in continuous culture and this is one of the commonest difficulties encountered in this method of microbial propagation. In mixed cultures we have never found that a predator population was sufficiently efficient to devour an entire bacterial population; in fact it is the predator that is most likely to be washed out in continuous culture. This is due to the comparatively high potential growth rates of the bacteria and also to their smaller size so that on a weight for weight basis it is less likely that a bacterium will disappear from the system. This argument does not exclude the possibility of bacterial mutation resulting in wall growth as a defensive mechanism but considerably more evidence is needed than is offered by van den Ende (1973) in support of such a hypothesis.

Jost *et al.* (1973a) and Tsuchiya *et al.* (1972b) investigated the predation of *Tetrahymena pyriformis* in a chemostat with both *Azotobacter vinelandii* and *Escherichia coli* as two and three species cultures. The results obtained with the two species systems showed damped oscillations even at low dilution rates where according to the Monod equations sustained limit cycle oscillations are predicted. In addition, the residual nutrient (glucose) concentrations were higher than predicted by this model. The authors explain their results in terms of the double saturation model which has already been mentioned (see section 2). In spite of the better fit obtained with this model, it predicts the behaviour of biomass concentrations while the authors measured only numbers and, as we have already pointed out, these parameters are not linearly related.
When the two prey species were cultured together with the single predator all three organisms survived and essentially reached steady states. This is strong evidence against the simple Lotka–Volterra equations as a model for predation in a chemostat as it will be remembered that such equations predict that any three species communities will be unstable.

4 Applied aspects

Whereas kinetic and dynamic data gathered from experimental and mathematical models were initially used as an aid to explain and predict the behaviour of microbial populations in the natural habitat, in recent years there has been an increasing emphasis for work of this nature to be applied to aerobic biological waste-treatment processes. One of these, the activated-sludge process, is a type of continuous-culture system and is used for the treatment of sewage and industrial wastes. After aeration in a reactor, the microbial populations in the form of a sludge are settled in a sedimentation tank. Most of the solids are returned back to the reactor but some are wasted at a rate sufficient to keep the solids concentration in the reactor constant. Thus the system is a continuous culture with feedback of microbial solids.

It is well known that activated sludge contains large populations of protozoa (Curds and Cockburn, 1970) and it is common to find numbers of ciliated protozoa in the order of tens of thousands per millilitre in the mixed liquor. Curds et al. (1968) showed that ciliated protozoa were responsible for effluent clarification and suggested that this could be attributed to their predatory activities on the populations of suspended bacteria. Data collected from batch and continuous cultures enabled Curds (1973a) to state that if protozoa in activated sludge feed at rates similar to those in pure culture then the ciliates in that process could easily be responsible for the removal of suspended bacteria by predation alone.

Continuous culture studies also made it possible for Curds (1971b) to model and simulate microbial populations in an activated-sludge system. The models considered the fate of two groups of bacteria: those which flocculate and settle on sedimentation and those suspended in the sewage which never flocculate. It was assumed that ciliates would feed only on those bacteria that remained in suspension and not upon flocculated bacteria since it was argued that protozoa do
not have the appropriate oral apparatus to deal with large masses of bacteria. The population dynamics of these organisms were simulated on a computer and steady-state concentrations were obtained without oscillations. It would appear that the populations did not oscillate because a stable concentration of prey organisms was contained in the sewage flow. The prey did not grow appreciably in the reactor because of competition with the relatively large population of sludge bacteria for a common substrate. At steady state, the concentration of soluble substrate in the effluent was determined by the growth rate (fixed by the sludge-wastage rate) of the sludge bacteria. The concentration of dispersed bacteria in the effluent was similarly determined by the growth rate of the predatory ciliates. It was found that the growth rate of a free-swimming ciliate would be greater than that of an attached ciliate and at steady state would equal the sewage-dilution rate and sludge-wastage rate respectively. Thus the model predicted that the habit of the predators would have a considerable effect on effluent quality. A plant containing only free-swimming ciliates would produce a fairly turbid effluent whereas a plant containing attached ciliates would produce a highly clarified effluent. Activated-sludge plants that contain no protozoa were predicted to deliver very turbid effluents. These three predictions are in general agreement with what has been observed in practice on full-scale plants.

The model failed to explain why protozoan populations often change radically over short periods of time (Brown, 1965). Curds (1973b) found that diurnal variations in sewage flow and bacterial content would induce diurnal rhythms in ciliate populations. Further, the introduction of certain carnivorous protozoa could also produce predator–prey limit-cycle or damped oscillations in a microbial system that otherwise would attain a steady state. Similar models also enabled Curds (1971b, 1973b) to explain the succession of different protozoan types during the establishment of an activated sludge on a basis of growth kinetics and settling properties.

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