Chapter 2

Studies in Protozoan Population Ecology

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Reprinted from: Glase, J. C. and M. C. Zimmerman. 1992. Studies in protozoan ecology. Pages 19-66, *in* Tested studies for laboratory teaching, Volume 13 (C. A. Goldman, Editor). Proceedings of the 13th Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 191 pages.

Contents

Student Outline 20
Laboratory Objectives.....20
Population Growth Models.....21
Two-Species Interactions.....30
Protistan Species Available for Study 34
Investigations in Population Ecology 37
Techniques.....39
Schedule for Returning to the Laboratory 41
Literature Cited and Suggested Reading 42
Appendix A: Notes for the Instructor – Week 1 43
Appendix B: Notes for the Instructor – Week 2 60
Appendix C: Preparation of Cultures.....66

Student Outline
Laboratory Objectives

Conceptual

Describe the effect of the birth rate (natality) and the death rate (mortality) on population growth

2. Define the intrinsic rate of growth, *r*, and explain its relationship to exponential population growth.
3. Understand the major density dependent and independent factors limiting exponential growth.
4. Draw a logistic growth curve, and identify the exponential portion of the curve, the point on the curve where growth departs from exponential (inflection point), and the carrying capacity (*K*).
5. Describe the relative rate of population growth during the three portions of the logistic curve described in 4.
6. Explain how the expression $(K - N)/K$ converts the exponential growth model to the logistic model.
7. Understand the effects of interspecific competition on population growth and the possible outcomes that may occur when two species compete.
8. Understand the interactions of predator and prey and how each effects the population growth of the other.
9. Design a study to investigate some aspect of population growth.

Procedural

1. Learn some techniques for estimating the size of populations of protistans.

2. Learn some techniques for calculating population growth parameters, such as *r*, *K*, and the doubling time, from data collected from a growing population.
3. Plot data to obtain population growth curves and interpret the graphs.

Population Growth Models

☐ Complete the activities in this section before your laboratory. You will need a calculator that can calculate natural logarithms.

Population Growth Curves and Rates

The growth of many populations of organisms and the factors that control population growth are of interest to biologists. Population ecologists have developed several models that accurately describe population growth mathematically. In this laboratory we will initially consider two of these models and some of the basic steps used in the development of mathematical models. *Note:* The mathematics included are fairly simple; you should concentrate on the biological situation that is being modeled, not on the mathematics used. In the laboratory, you will study the growth of populations of single-celled organisms called protistans. You will design a study to investigate some aspect of protistan population ecology, such as competition or predation. This study will allow you to use some of the concepts developed in this introductory section.

We define a **population** as a group of individuals of the same species living in a well-defined area. In the following discussion, the system we will be modelling will be the growth of a population of flour beetles (*Tribolium* sp.) living in a container of flour. To census this population, you simply pour the flour through a sieve and count the beetles, returning them to the container when you are through. If you were to use this procedure to census your population every week for 7 weeks you might obtain the data shown in Table 2.1. To create a **growth curve** for this population, you simply plot the number of organisms on the *y*-axis versus time on the *x*-axis.

☐ Plot the data from Table 2.1 in Figure 2.1 and connect the data points to obtain the growth curve for your population of *Tribolium*.

Table 2.1. Number of individuals in a population of flour beetles (*Tribolium* sp.) living in a container of flour during a 7-week period.

Week	Individuals (*N*)	ln*N*	*dN/dt*
0	2		–
1	4		
2	9		
3	15		
4	36		
5	60		
6	131		
7	256		

The **growth rate** of a population is the change in the number of population members (ΔN) in a certain time period (Δt) or $\Delta N/\Delta t$. *Note:* Δ is a mathematical symbol meaning "change in." As an example, using the data collected on the first day (week 0) and at the end of week 1, the growth rate for our population would be

$$\frac{\Delta N}{\Delta t} = \frac{4 \text{ beetles} - 2 \text{ beetles}}{1 \text{ week} - 0 \text{ week}} = 2 \text{ beetles/week}$$

As a first step in modelling the population growth rate of such a system, the biologist tries to identify the factors that are most influential in causing changes in the system.

☐ What are some factors that might affect the growth of your population of flour beetles?

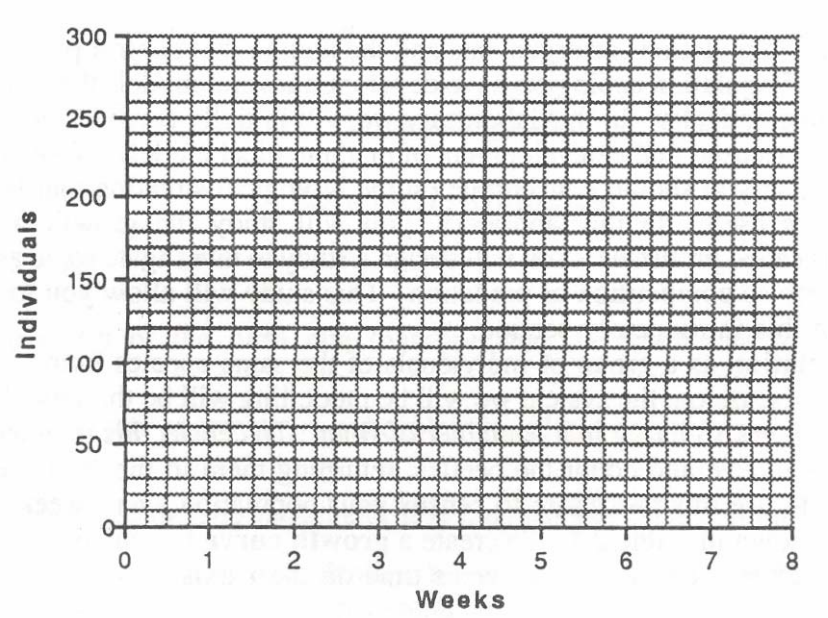

Figure 2.1. Growth curve of a population of flour beetles (*Tribolium* sp.) living in a container of flour.

Clearly, with respect to population growth, the rate of addition of individuals to the population minus the rate of loss of individuals from the population will directly determine the population's growth rate. **Birth** and **immigration** determine the rate of addition to the population. **Death** and **emigration** determine the rate of loss to the population. We incorporate these ideas mathematically setting the population growth rate equal to:

$$\frac{\Delta N}{\Delta t} = (B + I) - (D + E) \qquad (1)$$

where B and I are the number of new individuals added to the population due to birth (B) and immigration (I), and D and E are the number lost to death (D) and emigration (E) in a given period of time. Notice that if $(D + E) > (B + I)$, the population size will decrease with time and its growth will be negative.

To keep things simple, model-builders frequently assume that certain factors are constant while examining the effect of other variable factors. For example, if we assume that our beetle population is isolated, we can set *E* and *I* equal to zero and disregard these two factors. In addition, since the number of individuals born or dying in a population is directly dependent on the size of the population, we can express birth and death as **per capita rates**; that is, the number of births per population member, the **per capita birth rate (*b*)**, and the number of deaths per population member, the **per capita death rate (*d*)**. With these changes, equation 1 now becomes:

$$\frac{\Delta N}{\Delta t} = (bN - dN) \tag{2}$$

or, by regrouping terms, we get:

$$\frac{\Delta N}{\Delta t} = (b - d)N \tag{3}$$

That is, the population growth rate equals the per capita birth rate minus the per capita death rate times the population size.

To obtain the more conventional form of equation 3, two additional changes are required. First, the term dN/dt is substituted for $\Delta N/\Delta t$, to represent population growth as an instantaneous change. Second, because the difference between per capita birth rate and death rate is called the **intrinsic rate of increase** or *r* (called "little r"), we replace $(b - d)$ in equation 3 with *r* to get equation 4.

$$\frac{dN}{dt} = rN \tag{4}$$

This equation is the **model for exponential population growth**. Because *r* equals the difference between the per capita birth and death rates, it indicates *the number of new individuals added to the population per population member per unit time*. One estimates *r* when the population is growing rapidly so that its growth rate approximates the maximum population growth rate for the species in a specific environment. This model predicts that whenever *r* is positive (thus, $b > d$), the population's growth rate will continue to increase and the population will show **exponential growth**. The growth curve shown in Figure 2.1 is from a population that is growing exponentially.

Exponential Growth Model

If the rate of population growth of your flour beetles was constant, the growth curve in Figure 2.1 should be a straight line. Instead, Figure 2.1 shows that the population size accelerates through time; the curve becomes steeper and steeper. The acceleration in population size results because each new flour beetle can reproduce and leave offspring who can also reproduce and leave additional offspring. Consequently, the rate of population growth continues to increase and theoretically will eventually become infinite!

We can determine if a population is experiencing exponential growth by plotting our data in a slightly different way. In this case, we plot the *logarithm of population size* on the *y*-axis with time on the *x*-axis. If the population is growing exponentially and the data are plotted in this fashion, the resulting curve will be a straight line. Recall that a logarithm is an exponent. It is the power to which a base must be raised to obtain a specified number. Two commonly used bases are 10 in the Briggsian logarithm system and *e* in the natural or Naperian logarithm system (*e* has the approximate value of 2.72). In population ecology, natural logarithms are normally used.

☐ Use a calculator to determine the natural logarithms of the population sizes (ln*N*) of your flour beetle population and enter these data in column three of Table 2.1. Plot ln*N* versus time in Figure 2.2.

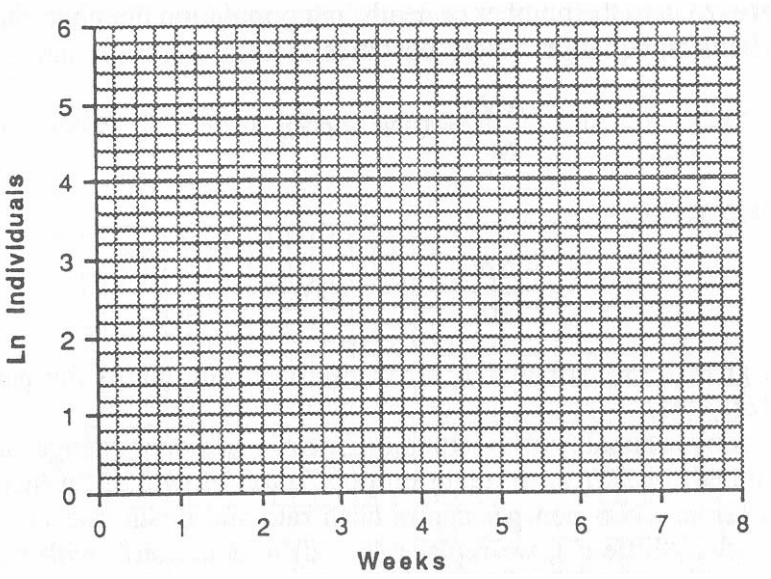

Figure 2.2. Growth curve of a population of flour beetles (*Tribolium* sp.) living in a container of flour plotted as the natural logarithm (ln) of population size.

To understand why exponential growth will be linear if plotted as the logarithm of population size versus time we must review what we know about logarithms. Recalling that a logarithm is an exponent, the linear growth shown in Figure 2.2 means that the logarithm of the population size increases uniformly with time. That is, the power to which e must be raised to equal the population size increases uniformly with time and the slope of the line ($\Delta y/ \Delta x$) in Figure 2.2 represents the magnitude of exponential growth. The slope of the line is our estimate of the species' intrinsic rate of increase, or *r*.

So, *r*, which equals the difference between the birth rate and the death rate, $r = b - d$, can be estimated as the slope of the curve during exponential growth or

$$r = \frac{\ln N_t - \ln N_0}{t} \tag{5}$$

So, for our flour beetle population, we could use data from week 0 and week 7 to estimate *r* as

$$r = \frac{5.54 - 0.69}{7} = 0.69 \text{ new beetles/beetle/week}$$

☐ Use equation 4 to determine the growth rates at each of the census times and enter these number in Table 2.1.

Different species have different intrinsic rates of increase. Obviously, a house fly will have a larger *r* value than an elephant. Both species are capable of exponential growth, but the rate of exponential growth will be greater for the fly than the elephant. Figure 2.3 shows arithmetic and logarithmic plots for three species, each growing at a different rate of exponential growth.

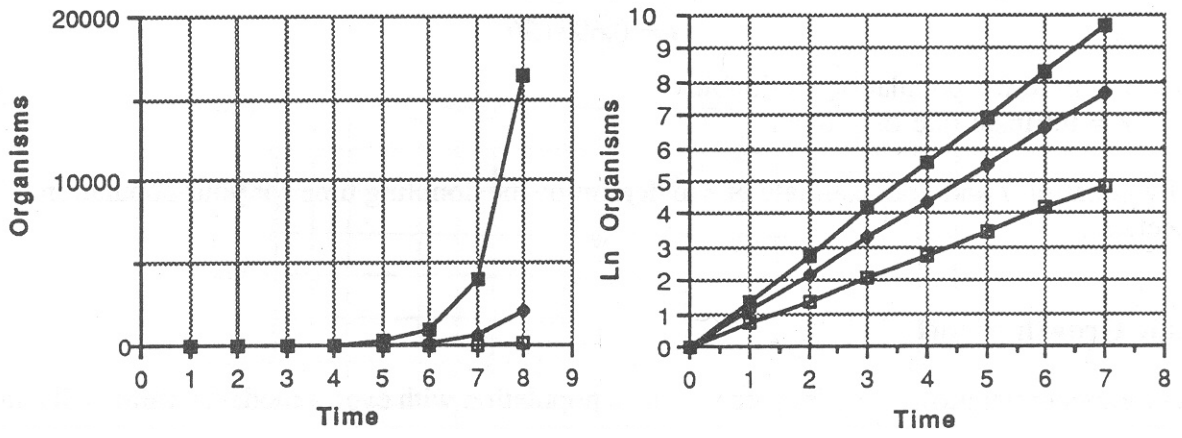

Figure 2.3. Arithmetic and logarithmic plots of the growth curves of three different species: Species 1 = ☐ ; Species 2 = ♦; Species 3 = ■.

☐ Use equation 5 to estimate *r* for each species based on data shown in Figure 2.3.

Species 1 =

Species 2 =

Species 3 =

If a population is increasing exponentially we can use *r* to determine the population size at a specific time (N_t) using the following equation:

$$N_t = N_0 e^{rt} \qquad (6)$$

where: N_0 = population size at time 0
N_t = population size at time *t*
e = base of the natural logarithms (= 2.72)
t = time measured in a set time interval

☐ Use equation 6 and your estimate of *r* to determine the expected number of flour beetles in your jar at the end of 1 year (52 weeks) if the population continues to grow exponentially.

A population's **doubling time** is the time required for the size of the population to become twice as large. For a population showing exponential growth this can occur very quickly. Recall equation

$$N_t = N_0 e^{rt} \quad or \quad \frac{N_t}{N_0} = e^{rt}$$

If we are interested in estimating the doubling time for a population growing exponentially, then

$$\frac{N_t}{N_0} = 2 \quad and \quad 2 = e^{rt} \quad or$$

$$\ln(2.0) = rt \quad so$$
$$t = 0.69315/r \tag{7}$$

where: t = time for population to double
r = intrinsic rate of growth

☐ Use equation 7 and your estimate of r to determine the doubling time for your population of flour beetles.

Logistic Growth Model

The exponential growth model predicts that a population with even a modest r value will continue to increase and in a short time will contain more population members than atoms in the universe. Although young populations may undergo exponential growth for certain limited time periods, growth very quickly becomes progressively less and eventually comes to fluctuate around zero. Figure 2.4 shows an idealized population growth curve for organisms such as your beetles. Notice that, although the growth during the first 8 weeks was exponential, on about week 8, and thereafter, the growth rate began to slow. By week 15 the population size stabilized at a fixed value. This stable population size, where $dN/dt = 0$, is called the **carrying capacity** symbolized by K.

☐ Estimate K from the data presented in Figure 2.4.

K represents an equilibrium population size that the population will reach despite its initial growth rate. To improve our model so that it better reflects what we know about the growth of real populations, we must make the model more complex and account for the effect of the carrying capacity on population growth.

The fundamental problem with our model is the assumption that the birth (b) and death (d) rates are constant through time and independent of N. Obviously, in real populations, as growth occurs and limited resources, such as food and space, become more scarce, thus influencing the survival and reproduction of population members, there will be a tendency for b to decrease and d to increase. That is, the birth and death rates are said to be **density dependent**. We must modify the exponential growth model to reflect the dependence of b and d on N.

Without evidence to the contrary, biologists usually assume that a linear relationship exists between two variables. In this case, the independent variable is population size (N) and the dependent variables of concern are b and d. As you know, the general formula for a straight line is y

= $a + mx$ where y is the dependent variable and x the independent variable. The slope of the line is m and a the y-intercept (where $x = 0$). We can show the dependence of the birth rate (b) on N in the following manner:

$$b = b_0 - k_b N \tag{7}$$

Notice that the population's current birth rate (b) is dependent on the maximal birth rate when the population is very small (b_0) *minus* the product of the current population size (N) times a constant (k_b) that quantifies the dependence of the birth rate on N (k_b is the slope of the decrease for the birth rate). Equation 8 shows that as population size increases, the birth rate will directly decrease. Similarly we can show the dependence of the death rate on population size as follows:

$$d = d_0 - k_d N \tag{8}$$

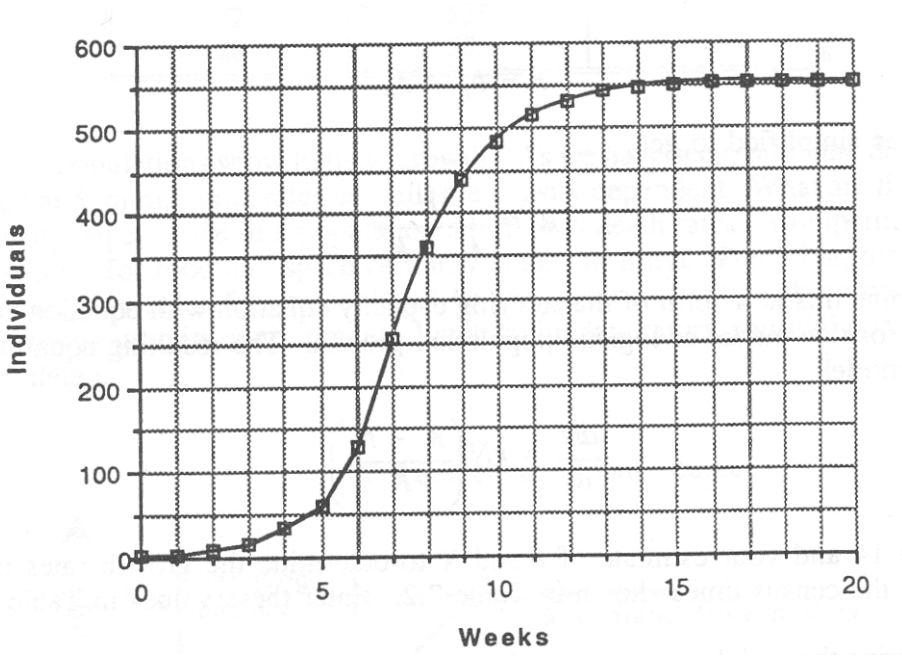

Figure 2.4. Idealized population growth curve for flour beetles (*Tribolium* sp.) living in a container of flour.

The current death rate (d) will depend on the minimal death rate when the population is very small (d_0) plus the product of the current population size (N) times a constant (k_d) that quantifies the dependence of the death rate on N (k_d is the slope of the increase for the death rate). Equation 9 shows that as population size increases, the death rate will also increase.

We now wish to combine both equations and relate them to population growth.

$$\frac{dN}{dt} = \left[(b_0 - k_b N) - (d_0 + k_d N) \right] N \tag{9}$$

This model, called the **logistic growth equation**, accurately describes the pattern of growth and regulation characteristic of many populations. Notice that as N increases, the first expression within brackets ($b_0 - k_b N$) will decrease, while the second expression ($d_0 + k_b N$) will increase. This equation predicts that zero population growth will occur when the birth rate equals the death rate; in other words, when:

$$b_0 - k_b N = d_0 + k_d N \tag{10}$$

With several algebraic manipulations we can convert the equation showing the conditions necessary for zero population growth into the following equation:

$$N = \frac{b_0 - d_0}{k_b + k_d} \tag{11}$$

At zero population growth the population size is stable through time. This value of N is called the carrying capacity of the environment and is usually given the symbol K. K represents an equilibrium value of population size that any population will ultimately reach regardless of its initial growth rate. Since

$$r = b_0 - d_0$$

equation 12 can be simplified to get

$$K = \frac{r}{k_b + k_d} \tag{13}$$

We can combine this new form of the carrying capacity equation with equation 10 to obtain the differential form for this model of logistic population growth. The resulting equation is called the **logistic growth model**:

$$\frac{dN}{dt} = r N \left(\frac{K - N}{K} \right) \tag{14}$$

☐ Use equation 14 and your estimate of r and K to determine the growth rates of a *Tribolium* population at the census times shown in Table 2.2. Enter these values in Table 2.2.

Notice that during the initial stages of growth the two models make the same predictions. Compare the values dN/dt from Table 2.1 and Table 2.2. During this phase, N is very small so that the expression $(K - N)/K$ is close to 1 and dN/dt approximates rN. As N becomes larger and larger, the expression $(K - N)/K$ becomes more and more influential in the model and dN/dt is decreased appropriately. When the population size equals the carrying capacity ($N = K$), the population has reached **zero population growth** ($dN/dt = 0$).

The **logistic growth model**, accurately describes the pattern of growth and regulation characteristic of many populations. Figure 2.5 graphically contrasts the model for exponential growth (curve A) with the model for logistic growth (curve B). The difference between the two curves represents the difference in the predicted numbers of population members for the two models. This difference between the potential population size and the realized population size is due to so-called **environmental resistance**, that is, the resistance that the environment offers to the continued exponential growth of a population. The point on the growth curve where the population departs from exponential growth is called the **inflection point**. The inflection point is sometimes called the point of **maximum sustained yield**, because this is the population size when the growth rate is maximal. Resource managers try to maintain a population at its inflection point in order to maximize the number of organisms that can be "cropped" from the population.

☐ Draw in your estimate of the inflection point for the growth curve in Figure 2.4.

Table 2.2. Growth rate of a population of flour beetles (*Tribolium* sp.) living in a container of flour during a 20-week period.

Week	Individuals (*N*)	*dN/dt*
1	4	
4	36	
8	360	
12	535	
20	555	

Why do many populations show logistic growth? As we have seen, birth and death rates (in some cases emigration and immigration rates as well) are density dependent. What are the *ultimate* factors causing the density dependence of a population's birth and death rates? Competition with members of one's own species for food and space certainly comes to mind. Predation, disease, competition with other species, plus other factors could all be important in limiting the growth of a particular population interacting with its environment. Later in this laboratory you will consider two of these factors in more detail.

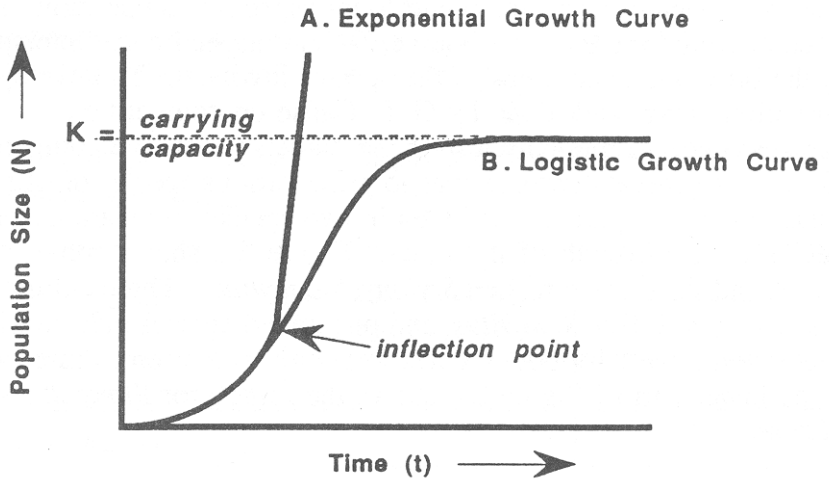

Figure 2.5. The exponential and logistic growth curves. *K* estimates the carrying capacity for a population showing logistic growth.

The carrying capacity seen in closed systems, such as your flour beetle population, will only be maintained for a short period of time. Because the food resources within the system are finite and constantly decreasing, the beetle population size will start to drop after reaching its peak and eventually all the beetles will die when the food supply is depleted. This contrasts markedly with carrying capacities seen in the natural ecosystems involving communities of organisms. The carrying capacity that an environment has for a given predator species is dependent upon the supply of prey, for example, several species of herbivores. The carrying capacities that the environment

has for the herbivore species depends, in turn, on the biomass of plant species that serve as the herbivores' food supply. Finally, the carrying capacity values for the plant species are determined, in part, by the amount of solar energy available. Thus, carrying capacities in the real world are maintained by considerably more complex and dynamic processes than those in the closed systems that you have studied in this laboratory.

Two-Species Interactions

☐ Complete the activities in this section before your laboratory.

Based on the preceding section dealing with the growth of a single-species population, we are now ready to consider the more complex situation found in a population where two different species exist together. We will first examine the interactions occurring between two different species competing for some of the same resources, and how their population growth rates are mutually influenced by the competition. We will then consider the interesting situation where one species is a predator and the other its prey.

Competition

In the broadest sense, competition exists whenever two organisms require the same limited resource. In **intraspecific** competition both competing organisms are of the same species; in **interspecific** competition they are of different species. The logistic growth of a population results from the density dependence of the birth and death rates due, in part to intraspecific competition. In cases where two or more species are competing for the same resource, both intraspecific and interspecific competition will be influencing the birth and death rates of the species involved. To develop these ideas let us consider some of the pioneering work done by G. F. Gause on competition.

Gause was interested in experimentally testing the models for simple competition developed by Vito Volterra in 1926. His general approach was to grow various species of organisms (he worked with yeast and protistans), first separately and then in two-species populations, carefully noting the effects of each species on the growth of the other. Figure 2.6 shows some of Gause's data for *Paramecium caudatum* and the closely related *Stylonychia mytilus*. Three cultures were started: one with five *P. caudatum*, one with five *S. mytilus*, and one mixed-species culture with five individuals of both species. Population growth for both species was compared when cultured separately and with the other species. The lower part of Figure 2.6 shows the results for *S. mytilus*, the upper part, the results for *P. caudatum*.

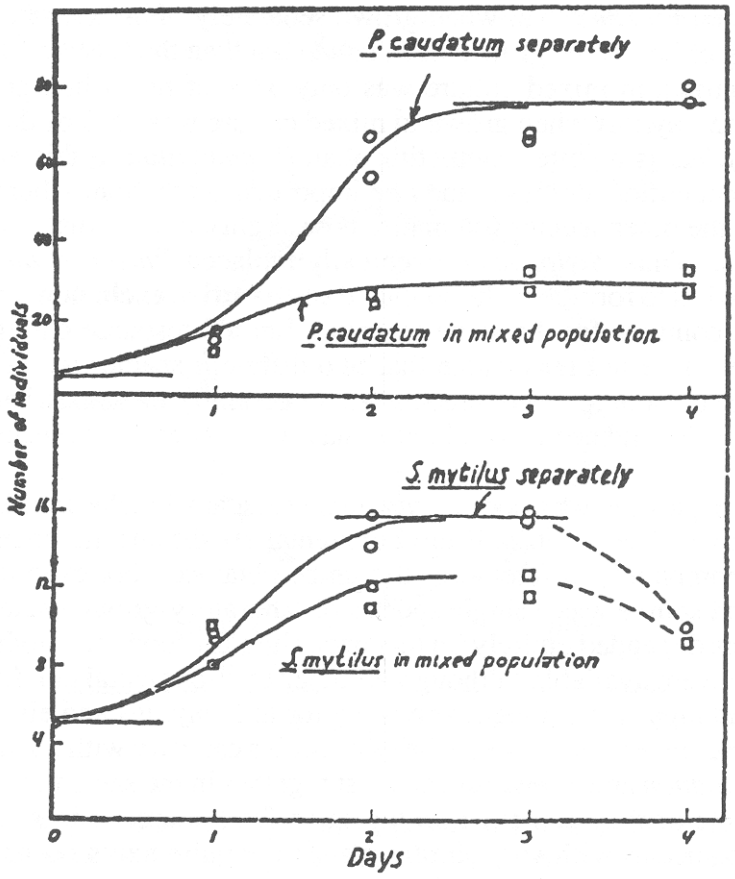

Figure 2.6. The growth in numbers of individuals of *Paramecium caudatum* and *Stylonychia mytilus* cultured separately and together (from Gause, 1934).

☐ Based on data in Figure 2.6, estimate the carrying capacities for each species grown alone (K_{alone}) and in mixed population (K_{mixed}).

Species	Carrying capacity	
	Alone (K_{alone})	Mixed (K_{mixed})
Paramecium caudatum		
Stylonychia mytilus		

☐ To access the effect of interspecific competition on each species, calculate the ratio of the carrying capacity in mixed population to the carrying capacity when grown alone for each species.

	Paramecium caudatum	*Stylonychia mytilus*
K_{mixed}/K_{alone} =		

In general, you can see that both species experience slower growth and attain a lower carrying capacity (K) when cultured together than when grown separately. Furthermore, *S. mytilus* seems to have a greater depressing effect on the growth of *P. caudatum* than the reverse. The carrying

capacity for *P. caudatum* when grown in mixed culture was only 33% of that when grown alone, whereas, the carrying capacity for *S. mytilus* when grown in mixed culture was 75% of that when grown alone. This suggests that *S. mytilus* is a better competitor than *P. caudatum* in this situation.

Overall, Gause's competition studies made one important prediction: whenever one species has a competitive edge over the other species (no matter how slight), in time that species will completely replace the other species. Thus, *Stylonychia* eventually replaced *Paramecium* in Gause's cultures. Gause's results led biologists to formulate the so-called **competitive exclusion principle**, which states that no two species that compete for the same essential, limited resource can long exist together in the same place and time. It is not reasonable that two different species would be exactly equal in their usage efficiency of a resource. One species would certainly be somewhat more efficient than the other, and the more efficient species would eliminate the less efficient species wherever the two occurred together.

You may now ask the question why does *P. caudatum* exist at all in the world, given that *S. mytilus* so clearly out competes it? The point is, if environmental conditions are changed or if a different food source is offered, completely different results can be obtained. For example, in the experiment described above, the food source was a single species of laboratory-grown bacteria, *Bacillus subtilis*. Both species of protistans depended entirely on this bacterium for food. In another experiment where other species of bacteria were available (although all other environmental conditions were identical), Gause found that *P. caudatum* was competitively superior to *S. mytilus* and eliminated it. Even with the same food source the competition between the two species can vary with changes in environmental conditions. Thus, *P. caudatum* and *S. mytilus* can exist together in the same time and place if alternate food sources are available or they can exist on the same food source if in slightly different places or times. Whenever two species with very similar resource requirements occur together in the same time and place, there is a selection pressure for the less competitive species to diversify. That is, those individuals of the less competitive species that have genetically-determined adaptations allowing them to reduce competition with the more competitive species (by either utilizing alternate food sources or changing their time or place utilization of a common resource) will survive. The competitive exclusion principle, in this sense, helps explain the great diversity of resource utilization patterns exhibited by organisms in the real world.

Predation

In this second two-species interaction, we will consider the effect of a predator population on the growth of a prey population and vice versa. Intuitively, we would predict that the rate of increase of the prey population will equal its natural tendency to increase minus the number of prey consumed by predators. Predator populations will, on the other hand, increase in direct proportion to the number of prey available minus the death rate of the predators. In this fashion the density of prey influences the growth of the predator population, and the density of predators, in turn, influences the growth of the prey population. Alfred J. Lotka (1925) and Vito Volterra (1926) independently developed mathematical models expressing these relationships, known today as the Lotka-Volterra model.

The Lotka-Volterra model suggests that a cyclical relationship will develop between the number of prey and the number of predators in a system. As the number of predators increases, the number of prey must decrease. However, as the number of prey decreases, this ultimately causes the number of predators to decrease. This now allows the prey to recover and the entire cycle is begun again. These changes are diagrammed in Figure 2.7.

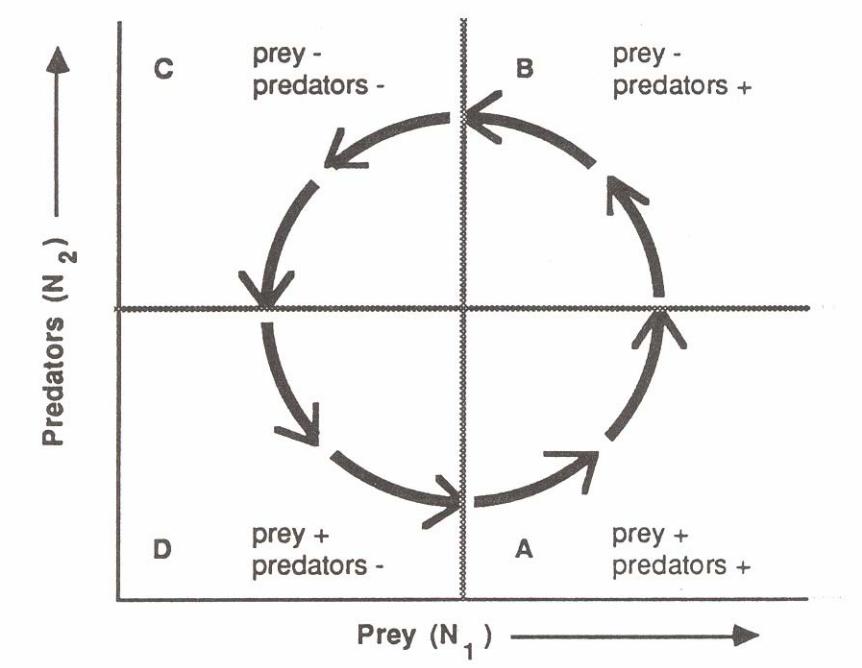

Figure 2.7. Predator-prey interactions as predicted by the Lotka-Volterra model. Arrows depict the relationship between prey numbers (N_1) and predator numbers (N_2); + = increasing, - = decreasing. (Modified from Wilson and Bossert, 1971.)

In Figure 2.7, N_1 (prey numbers) and N_2 (predator numbers) are plotted and the arrows represent the curve describing the relationship between these two variables. For discussion the curve is divided into four equal segments (A, B, C, D). In segment A the numbers of both predators and prey are increasing. In segment B the number of predators has reached a level where they increasingly reduce the number or prey. In segment C the decrease in prey now causes a decrease in the growth rate of predators due to a lack of food. Finally, in segment D, prey start to recover, due to a constantly decreasing number of predators. Through time these sorts of fluctuations in predators and prey show the type of pattern diagrammed in Figure 2.8.

☐ On Figure 2.8 circle representative areas of the two curves corresponding to the four segments (A, B, C, D) shown in Figure 2.7.

Gause (1934) was the first to make an empirical test of the Lotka-Volterra model for predator-prey relations. He reared the protistans *Paramecium caudatum* (prey) and *Didinium nasutum* (predator) together. In these initial studies, *Didinium* always exterminated *Paramecium* and then died of starvation; that is, instead of the predicted oscillations, Gause observed divergent oscillations and extinction (Figure 2.9A). This result occurred under all the circumstances Gause used for this system: making the culture vessel very large, introducing only a few *Didinium*, and so on. The suggestion was that the *Paramecium-Didinium* system did not show the periodic oscillations predicted by the Lotka-Volterra model. Gause then introduced a complication into the system: he used a medium with sediment. *Paramecium* in the sediment were safe from *Didinium*, which never entered it, in this type of system *Didinium* again eliminated the *Paramecium*, but only from the clear fluid medium; *Didinium* then starved to death, and the *Paramecium* hiding in the sediment (which acted as a refuge) emerged to increase in numbers (Figure 2.9B). The experiment

ended with many prey, no predators, and no oscillations. After further experimentation, Gause was able to observe oscillations only when he introduced (as immigrants) one *Paramecium* and one *Didinium* into the experimental set-up every third day (Figure 2.9C). Apparently, considerable environmental complexity is essential to the establishment of a balanced predator-prey system.

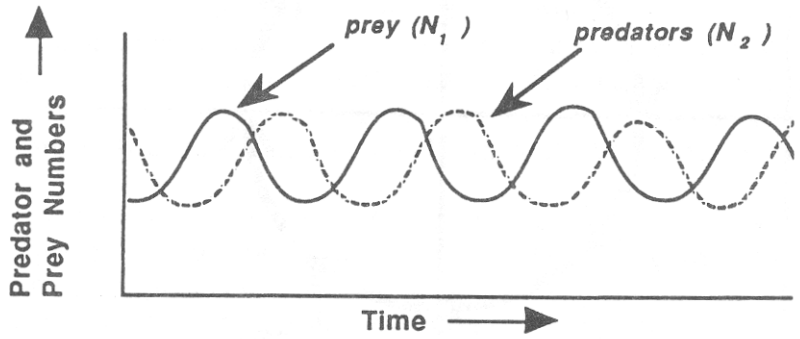

Figure 2.8. Fluctuations in the number of prey (N_1) and predators (N_2) through time as a result of the type of predator-prey interaction depicted in Figure 2.7. (Modified from Wilson and Bossert, 1971.)

Protistan Species Available for Study

You will have access to five species of ciliated protistans: *Paramecium caudatum*, *Paramecium bursaria*, *Spirostomum ambiguum*, *Blepharisma lateritium*, and *Didinium nasutum*. With the exception of the predator *Didinium*, these species feed on organic particles and bacteria, which they filter from the water in which they live. Each species will be available in a separate stoppered flask as a single-species culture. Each culture will have its own Pasteur pipet and volumetric pipets, all distinctly labelled. *Don't get these pipets mixed up.* In the descriptions below, recall that 1.0 mm = 1000 μm.

☐ Use the stereoscopic, dissecting microscope to become familiar with all five protistan species.

Paramecium caudatum: This species has a typical "paramecium" shape and is large, about 250–300 μm in length.

Paramecium bursaria: Several species of ciliates have symbiotic green algae living in their cytoplasm; *P. bursaria* is one of these. The markedly green appearance of *P. bursaria* is due to numerous algal cells of the genus *Zoochlorella* arranged much like chloroplasts around the periphery of *P. bursaria*'s cytoplasm. Also, notice its more rotund shape in comparison with *P. caudatum*. *P. bursaria* is 100–150 μm in length. Examine *P. bursaria* at high magnification and you can readily see the individual algal cells.

Spirostomum ambiguum: This is a very long (sometimes 2–3 mm!), club-shaped ciliate. Its size and shape are unlike any of the other species.

Blepharisma lateritium: This pink ciliate is pear-shaped and about 175 μm in length.

Didinium nasutum: This predaceous protistan is barrel-shaped and has two girdles of cilia. Although its size is variable, depending on how recently it has eaten, its markedly different shape and locomotory behavior readily distinguish it from any of the other species. *Didinium* swims in a wide-spiralling fashion upward through the culture. If it should make solid contact with a prey species (Figure 2.10), *Didinium* discharges special toxin-containing trichocysts that quickly immobilize the prey and physically attach it to the predator. The prey is now pulled into the expanded and highly modified oral groove and cytostome of the predator. After killing and engulfing a prey, *Didinium* returns to the bottom, where it gently rotates for several hours until the meal is digested. It has been estimated that the predator requires from two to three prey before dividing mitotically.

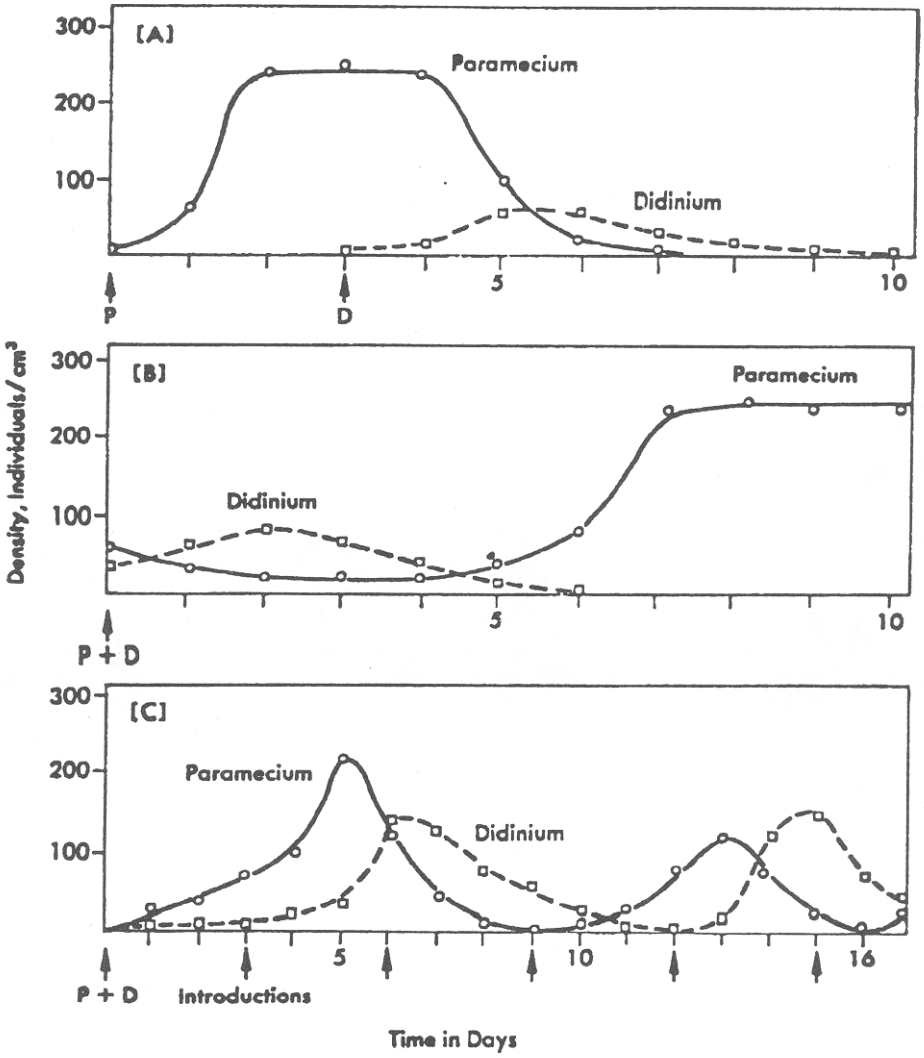

Figure 2.9. Predator-prey interactions between *Paramecium* and *Didinium* under different conditions (see text for discussion). (Modified from Gause, 1934.)

☐ If *Didinium* cysts are available, examine these as well as the active organisms. As you make your observations, record the distinguishing features of these five species in Table 2.3.

Table 2.3. Distinguishing features of five species of ciliated protistans.

Species	Shape	Size	Color	Behavior
Paramecium caudatum				
Paramecium bursaria				
Spirostomum ambiguum				
Blepharisma lateritium				
Didinium nasutum				

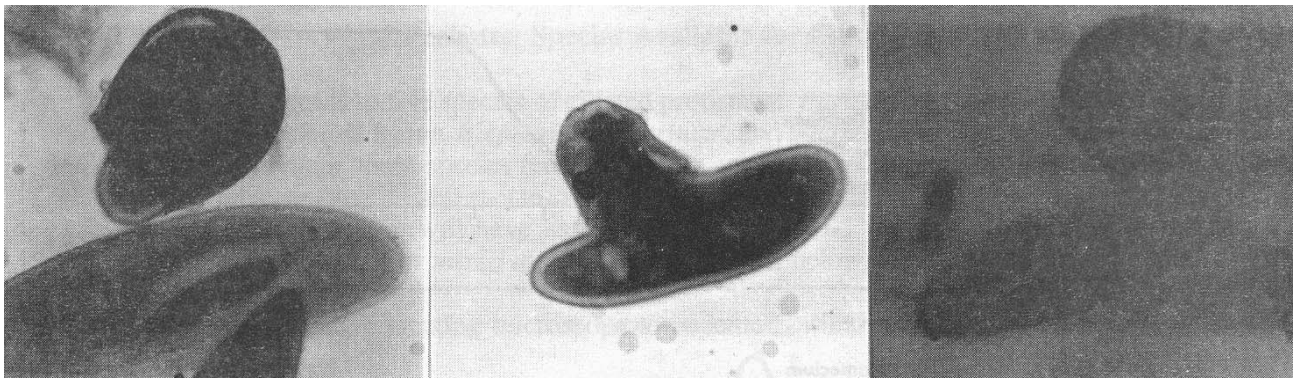

Figure 2.10. Stages in prey capture and consumption by the predaceous protistan *Didinium nasutum*. Prey shown here is *Paramecium caudatum*.

Other Species in Your Cultures

You have probably noticed many other inhabitants of the cultures that you have been observing. Most of these are considerably smaller than the key species you will be studying. Some are small ciliates or flagellates. These species are a natural part of the community in which the species described above live. In the studies you will do in this lab, you will not be collecting data on these smaller protists. Since the community of these organisms is essentially the same in all the cultures you will set up, you can ignore them.

Rotifers are another incidental species you are likely to encounter in your study of protistan ecology. Rotifers are multicellular (in fact, about 1000 cells), but they are only slightly larger than a good-sized protist. Figure 2.11 shows a diagram of a typical rotifer. Rotifers are usually attached to the bottom by their foot, but they can also be seen swimming about or crawling along the bottom like an "inch worm." The unique ciliated feeding organ on the expanded head end will help you differentiate rotifers from the protists. As with the smaller protists, you should ignore rotifers when you collect data.

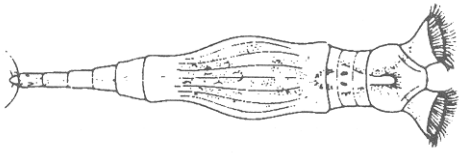

Figure 2.11. *Philodina* sp., a common rotifer in protistan cultures; actual size about 750 μm.

Investigations in Population Ecology

Competition Studies

We have two species of *Paramecium*, *Spirostomum ambiguum*, and *Blepharisma lateritium* available for competition studies. In studying competition, the approach used by Gause was to grow both species separately, starting each culture with equal numbers of organisms. He then compared the single-species cultures with a third set of cultures which were initiated by adding equal numbers of *both* species. Thus, although the total number of organisms in this third set of cultures was double the number in the other culture sets, the number of organisms of each species was the same as the corresponding single-species cultures. Comparing population growth for each species when cultured separately with population growth in the two-species cultures measures the effect of each species on the growth of the other. This is true because both *intra-* and *inter*specific competition occur in the two-species cultures whereas only intraspecific competition occurs in the single-species cultures. *Under these conditions, if two species do not compete at all, there should be no difference in their growth rates, whether cultured separately or together.*

Establish two sets of five 40-ml cultures with each species grown separately. The initial density should be 20 individuals per ml (800 individuals/culture). A third set of five cultures should contain 1600 individuals per culture, 800 of each species. In this study it is critical that good initial estimates of the stock cultures be made and that the correct dilution procedure for setting up the

three sets of vials be used. Obviously, being able to distinguish between and accurately count the species involved is also critical.

- ☐ Calculate averages at each sample time for all three culture types and plot your growth curves as shown in Figure 2.6. To help you interpret your data, you should calculate *r* and *K* for each population, and compare these parameters for each species when grown alone and in mixed culture. Also, you will want to look at the ratio of carrying capacities for each species when grown alone and in mixed culture.

Predator-Prey Studies

A number of different kinds of studies involving the predator *Didinium* and its *Paramecium* prey can be attempted. In general, because of the voracious appetite of *Didinium*, you must start with a large prey density and few predators. We suggest five replicate cultures per condition, each containing 40.0 ml of *undiluted* prey stock culture. Only three *Didinium* should be added to those cultures that will receive the predator. To add *Didinium* to a prey culture, it is best to dilute the stock predator culture so you get only three within the drop on a depression plate. Carefully remove three individuals with a Pasteur pipet, and when you are sure that there are exactly three in the pipet, add them to the *Paramecium* culture, rinsing out the pipet several times with prey culture. If you are examining the effect of the predator on prey population growth (and effect of prey on predator population growth), you should have a set of cultures with prey only and another set with predators only.

Studies could be designed to determine if *Didinium* shows a preference for a prey species, if offered a choice. Others might try to examine the effect of physical factors (such as a larger culture volume or a fiberglass screen sediment) on the predator-prey system. How does *Didinium* detect and capture prey and what could you do to the environment to interfere with these processes and test the validity of your ideas?

Many ciliates form inactive resting stages called cysts when conditions become unfavorable. For example, *Didinium* encysts when it runs out of prey and can remain in this stage for many months. Later, if prey become available to *Didinium*, it will emerge as an active predator. How does the encysted *Didinium* detect the presence of prey? You might test the effectiveness of plain culture media, culture media in which *Paramecium* were living (but have now been removed), and culture media with *Paramecium* in causing *Didinium* to emerge from encysted form. Each culture should receive 10–20 cysts.

- ☐ In your predator-prey studies, calculate averages at each sample time for all your culture types and plot your growth curves for both predator and prey. Concentrate on graphical interpretations of the data. Note when (and if) the prey become extinct, and what effect this has on *Didinium* population growth. Also calculate estimates of *r* and *K* for both prey and predator species.

Miscellaneous Studies

- What environmental factors might affect protistan growth and how can you study them?
- Does *Paramecium bursaria* obtain any benefit from the photosynthetic activities of its algal endosymbiont? Does the endosymbiont make *P. bursaria* a better competitor with other species?

Techniques

Counting Population Numbers

A stereoscopic binocular microscope and a counting plate with multiple depressions will be used for determining the numbers of organisms present in one-drop samples taken from your cultures. Pasteur pipets will be used to remove samples. Carefully use the following procedure in all data collection:

1. Because protistans tend to cluster on the bottom of the vessel, the culture to be sampled must be agitated *thoroughly* in order to obtain representative samples. Carefully swirl the culture vial to randomly distribute the organisms. While vigorously stirring the culture with a Pasteur pipet, squeeze and release the bulb to fill it.
2. Hold the pipet at a 45° angle and carefully release two or three drops back into the culture. The next drop should be put into a depression on the counting plate. This operation must be done quickly so that organisms don't begin to settle within the pipet.
3. Release the rest of the sample back into the culture vial.
4. Repeat steps 1, 2, and 3 until sufficient samples have been removed from the culture.
5. Focus on the first depression in the counting plate, with the black stage disk in position, and carefully count all the organisms.
6. Repeat this for all samples on the plate. Use at least five drops to estimate the population density of a culture and calculate an average from these numbers.
7. If more than 15 individuals per drop are present in your cultures, you should dilute the sample to make counting easier. Use two counting plates to do this.
    a) First add the one-drop samples to the depressions on the first plate.
    b) Add enough drops of spring water to dilute the samples.
    c) Now, while stirring the contents in the depressions of the first plate, remove one drop and put it in the appropriate depression of the second plate.
8. There are 20 drops in 1 ml, so each drop equals *0.05 ml*. Calculate the average number of organisms in 1.0 ml for the culture sampled. Use a dilution factor if you had to dilute your sample prior to counting. For example, if you had diluted one drop of culture with three drops of spring water and then counted 16 organisms in one drop of the diluted sample the number per ml in the original culture would be:

    (number in diluted sample) × (dilution factor) × 20 drops/ml
    or
    16 organisms/drop × 4 × 20 drops/ml = 1280 organisms/ml

    The dilution factor simply equals the total number of drops in the diluted sample.
9. *Caution:* Use only intact Pasteur pipets for sampling. If the end is broken it will release a larger drop and should be discarded. Carefully attach masking tape labels to your pipets so you don't contaminate cultures with other species.

Estimating the Population Density of the Stock Culture

Most studies that you might undertake will initially require an accurate estimate of the population density of the stock cultures appropriate to your study. Each species' stock culture will have associated labelled Pasteur pipet that you should use in making this estimate, employing the procedure outlined in the previous section. Because this initial estimate is critically important, base it on at least *10* single-drop samples.

Establishing Your Initial Cultures

If sufficient equipment and biological material are available, five replicate cultures should be set up for each treatment group in your study. The density of organisms in stock cultures may be quite high and, in most studies concerned with population growth rates, will need to be diluted. Follow these guidelines in setting up cultures for studying the rate of population growth under the conditions with which your study is concerned.

1. First, determine the density of the appropriate stock cultures (see previous section).
2. Calculate the volumes of stock culture and spring water needed to obtain 40-ml cultures with 20 organisms per ml. For example, if the estimated density of Species A stock culture is 320 individuals per ml and you want your replicate cultures to have 20 individuals per ml and equal 40 ml in total volume, then you need 800 individuals (20 individuals/ml × 40 ml) of Species A. This can be obtained by adding 2.50 ml of stock culture (320 individuals/ml × 2.50 ml = 800 individuals) to 37.5 ml of autoclaved spring water. However, to achieve the most uniformity in the five replicate cultures, you should place 12.5 ml (5 × 2.5 ml) of stock culture into a large flask and add 187.5 ml (5 × 37.5 ml) of autoclaved spring water. You can then dispense 40.0 ml from this vessel to each of five culture vials.
3. Add 1.0 ml of concentrated liquid food supplement to each vial. *Note:* The supplement consists of one protistan pellet (dehydrated young grass stems, obtained from Carolina Biological Supply Co.) per 100 ml of spring water.
4. Put a foam plug in each plastic culture vial and label the vial. In addition to including the names of group members, room number, and lab time on each label, you should number each replicate culture individually. As you collect data on population density changes throughout the week, keep the data for each culture separate by associating them with the replicate number. These paired data values from each culture may be useful for later data analysis.
5. Each group member must be able to identify the species involved in the study and be proficient and uniform in the use of sampling procedures if unnecessary variation in data collection is to be avoided.
6. Store your vials in a designated area in the laboratory room.

Estimating *r*, *K*, and Doubling Time

Intrinsic Rate of Increase (r)

To estimate *r*, plot the growth data as in *N* versus time, as in Figure 2.2. To calculate *r*, use equation 5 and data from the *linear* portion of the curve, where population growth is exponential. Estimated where it exists before factors start to limit it.

Carrying Capacity (K)

Although more sophisticated methods exist, the simplest way to determine the population's carrying capacity (*K*) is to visually estimate where the growth curve becomes horizontal. This is most easily done if the natural log of *N* is plotted versus time as in Figure 2.2. *Note:* If a growth curve does not level off or goes down after reaching a peak value, use the highest population level attained as your estimate of *K*.

Doubling Time

Use equation 7 and your estimate of *r* to determine the doubling time for your populations.

Schedule for Returning to the Laboratory During the Population Ecology Study

1. Your group should plan to return on three or four occasions throughout the week to monitor population growth in your cultures.
   a) For predator-prey studies, return about 24, 48, 72, and 96 hours after the study was initiated.
   b) For all other studies, return about 48, 72, and 96 hours after the study was initiated.
2. Final counts will be done the following week on the seventh day (169 hours).
3. Important Cautions:
   a) Prior to sampling, the content of each vial must be mixed *very* thoroughly. Throughout the week, sediment containing many protistans will accumulate on the bottom of each vial. The culture must be mixed sufficiently to distribute this sediment uniformly before sampling.
   b) Use only *intact* Pasteur pipets for sampling.
4. It will take your group about *45 minutes* to make counts of the cultures in your study.
5. Please be certain to clean up after you are through and put away the stereoscopic binocular microscope and lamp you have used.

Literature Cited and Suggested Readings

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APPENDIX A
Notes for the Instructor – Week 1

Suggestions for Introduction to Population Ecology

Included here are a series of questions and replies that you may find useful in covering important concepts presented in the introductory section of this laboratory. This should be a good occasion to use questioning since all students will have had an opportunity to think about this material in the process of completing the activities there.

Relevance of Population Ecology

Why study population ecology? Important for understanding problems in conservation, endangered species, resource management, human demographics.

Population Growth Curves and Rates

1. Ask the following questions as you consider the transparency showing Figure 2.1.

   What does the shape of the growth curve for the flour beetle population (Figure 2.1) indicate about growth of this population? Population growth is not constant through time; population growth increases through time.

   What would the shape of a population growth curve be like if the population size increased at a constant rate through time? A straight line.

   How should the rate of growth ($\Delta N/\Delta t$) vary in a population with a straight line growth curve? Population growth rate would be constant through time and equal to the slope of the line.

   Why does population size accelerate through time in the flour beetle population (as shown in the graph)? Population growth depends, in part, on the number of new individuals added to the population. Addition of new individuals depends on the number of present population members. As population size increases, the rate of addition of new individuals increases.

   Why do we call this type of population growth curve (as shown in the graph) an exponential curve? Because the number of individuals in the population increases in the same way that a base raised to an exponent increases: 2^1=2, 2^2=4, 2^3=8, 2^4=16, 2^5=32, 2^6=64, etc.

   How should the rate of growth ($\Delta N/\Delta t$) vary in a population growing exponentially? Population growth rate would increase through time.

2. Ask the following questions as you consider the transparency showing Figure 2.2.

   Why should the population data in Table 2.1 produce a straight line when plotted as the natural log of N versus time? Remember that a logarithm is an exponent to which a base must be raised to produce a specified number (as in $10^2 = 100$, $\log 100 = 2$). If a population is increasing at an exponential rate, then the logarithm of population size (the exponent to which N must be raised) should increase uniformly (be linear) with respect to time.

   In the equation describing the rate of exponential population growth, $dN/dt = rN$, what is r? r is the intrinsic rate of growth; it is the difference between the per capita birth and death rates.

   How can we estimate r from population growth data? Plot the growth curve as the natural logarithm of population size against time. r is the slope of the line. Specifically, $r = (\ln N_t - \ln N_0)/t$.

What are the units for r? Since *r* is the difference between the per capita birth and death rates, *r* is expressed as the number of new individuals per current population member per unit time.

3. Ask the following questions as you consider the transparency showing Figure 2.3.

   Can different populations grow at different rates of exponential growth? Yes. Depending on their species-typical reproductive potential, different species can grow at different rates of exponential growth. Elephant and house fly populations can both show exponential growth; the rate of exponential growth for the house fly population will be greater.

   Looking at the growth curves shown in Figure 2.3, which species would have the greatest r value? The smallest *r* value? Species 3 has the largest *r*; Species 1, the smallest *r*. Species that grow at greater rates of exponential growth have larger *r* values than species that grow at lesser rates of exponential growth.

   How can we estimate the size of a population growing exponentially at some time (t) in the future knowing the species' r value and the starting population size (N_0)? Use the equation $N_t = N_0 e^{rt}$. Review the example calculating four beetle population size after 52 weeks of exponential growth.

4. Ask the following questions as you consider the transparency showing Figure 2.4.

   Why do populations not show exponential growth for very long? Populations run out of resources, which increases intraspecific competition, resulting in enhanced death rates and reduced birth rates. Birth and death rates are density dependent.

   For what type of resources do species members compete? Food, oxygen, CO_2 and light (for photosynthetic autotrophs), water, mates, space free of parasites, wastes, and predators, etc.

   Describe the shape of a population growth curve that is more typical for populations in the long term. It is "S" shaped, with rapid initial growth tapering off to zero growth at a fixed population size called the carrying capacity.

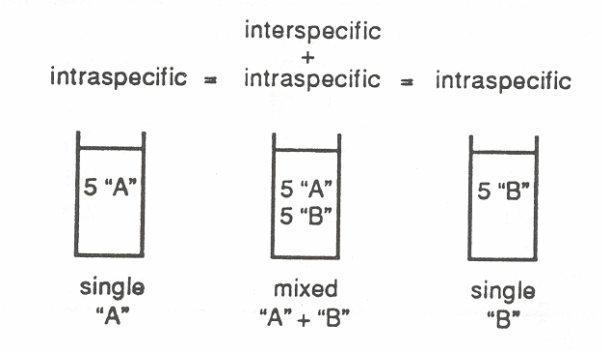
   How should the rate of population growth vary for different portions of the logistic growth curve? The growth rate will continue to increase up to the inflection point and then it will decrease until it equals zero at the carrying capacity. Consider the data in Table 2.2.

   Why is estimating the inflection point important for resource managers, for example, fisheries biologists? Because a population can be harvested at a maximum rate at that level since its growth rate is greatest at the inflection point. Thus, the inflection point is sometimes called the *point of maximum sustained yield*.

   Why does the expression (K - N)/K in the logistic growth model make that model better than the exponential growth model at predicting growth in most populations? $(K - N)/K$ scales the exponential growth model to reflect the effect of population size on birth and death rates. Consider the difference between *dN/dt* values for the two models at different population sizes (Tables 2.1 and 2.2).

5. Ask the following questions as you consider the transparency showing Figure 2.6.

   In Gause's studies of competition, why did he have the same number of individuals of each species in the mixed species cultures that he did in each of the single species cultures? Gause wanted to study the effect of interspecific competition. To separate the effects of intraspecific and interspecific competition, he established cultures so that initially the amount of intraspecific competition in single species cultures would be the same. Therefore, any eventual difference between species' growth in single and mixed culture would be due to interspecific competition. See the following figure:



Simply by examining Figure 2.6, can you predict which species is most affected and which is least affected by interspecific competition? Yes. The species with the greatest relative reduction in growth in mixed culture is most affected by the presence of the other species. Discuss how to quantify this by calculating the ratio of carrying capacities (K_{mixed}/K_{alone}).

6. Ask the following questions as you consider the transparency showing Figures 2.7 and 2.8.

   Why did Lotka and Volterra predict out-of-phase predator and prey population cycles, with the prey leading the predator cycle, instead of logistic growth for both (with a fixed carrying capacity)? Because each population influences the growth of the other population; the density of prey has a positive influence on the growth of the predator population and density of predators has a negative influence on the growth of the prey.

   How would you create Figure 2.7 from data represented in Figure 2.8? Plot prey numbers on the *x*-axis versus predator numbers on the *y*-axis through the various cycles. The resulting curve should be closed (an oval).

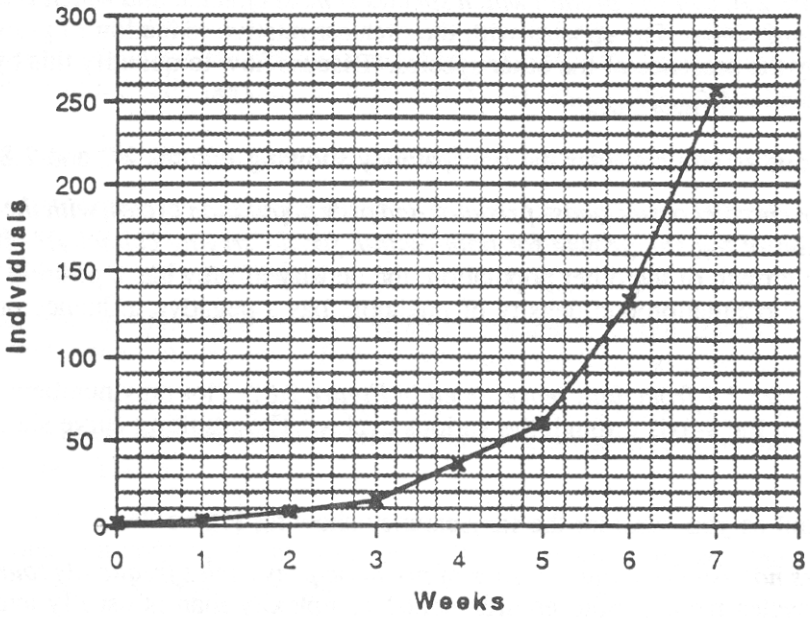
7. Ask the following question as you consider the transparency showing Figure 2.9.

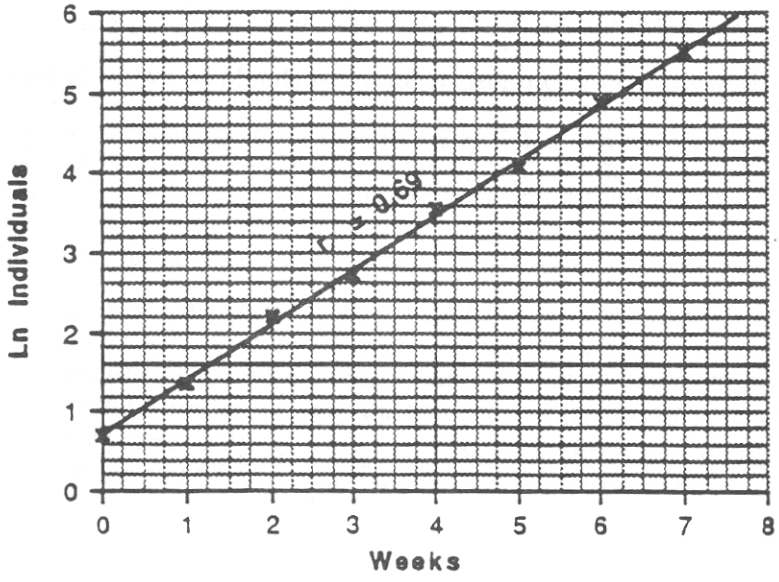
   Why do laboratory studies not usually show the types of predator-prey cycles frequently found in nature? Balanced predator-prey cycles require more environmental complexity than is usually available in the laboratory, including refugia for prey, immigration of prey, multiple prey species, etc.

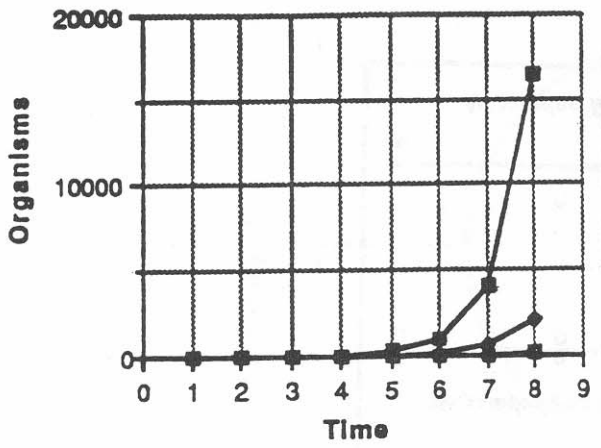
Transparencies

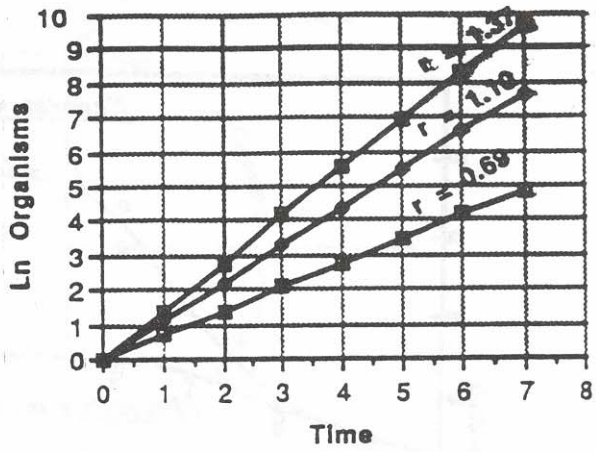
The following four pages show the overhead transparencies that should be available for introducing this laboratory exercise.

Week	Individuals(N)	LnN	dN/dt
0	2	0.69	–
1	4	1.39	2.76
2	9	2.20	6.21
3	15	2.71	10.35
4	36	3.53	24.84
5	60	4.09	41.40
6	131	4.87	90.39
7	256	5.54	176.64

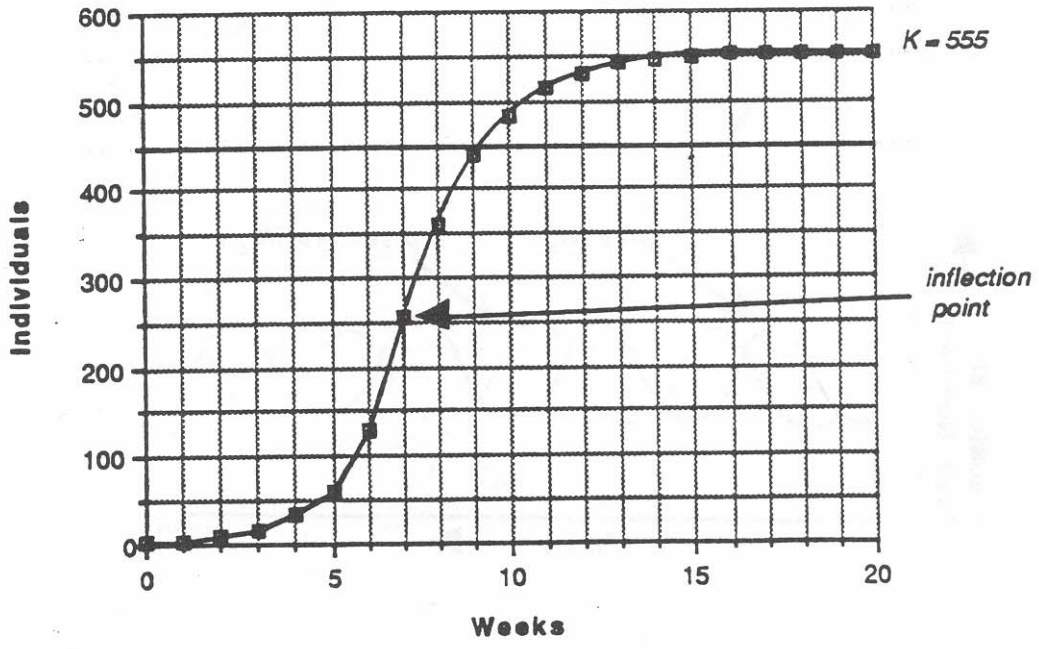


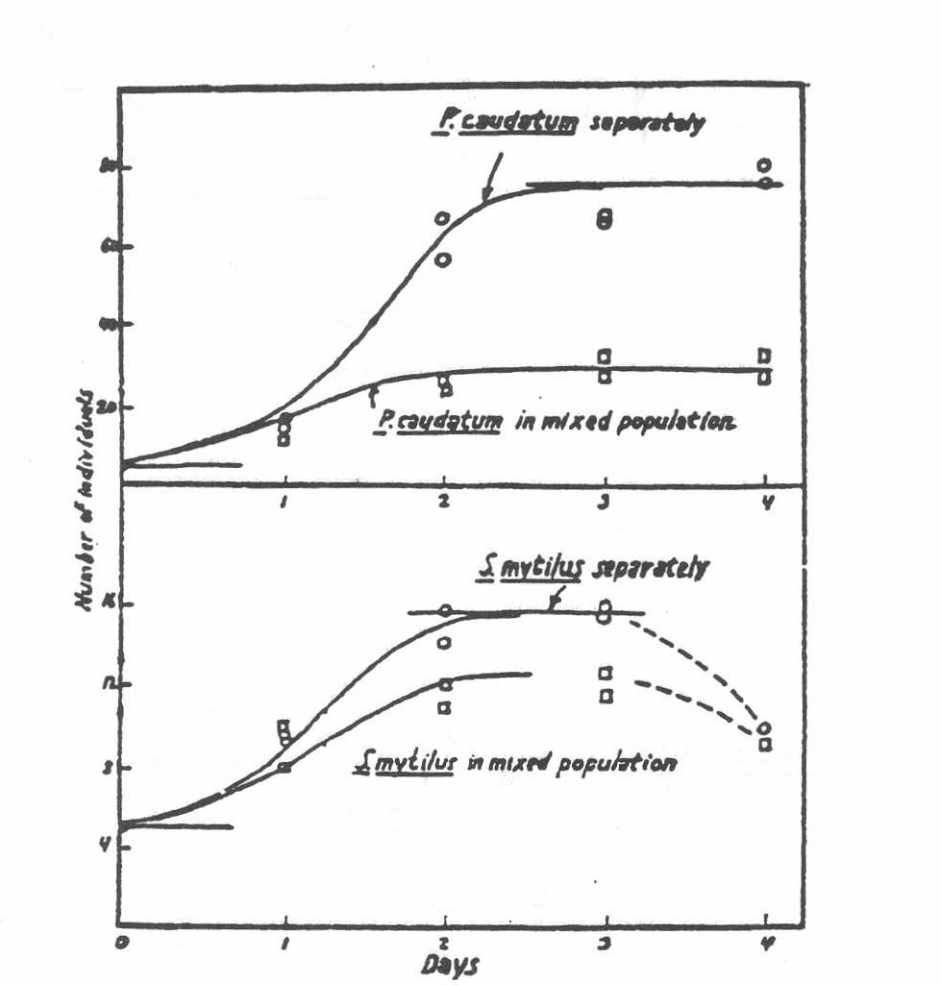


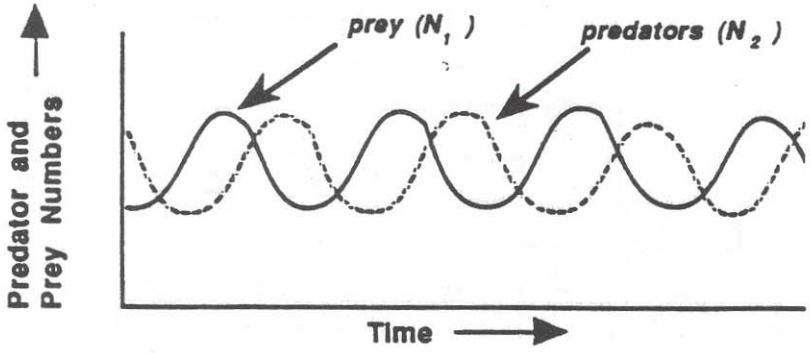


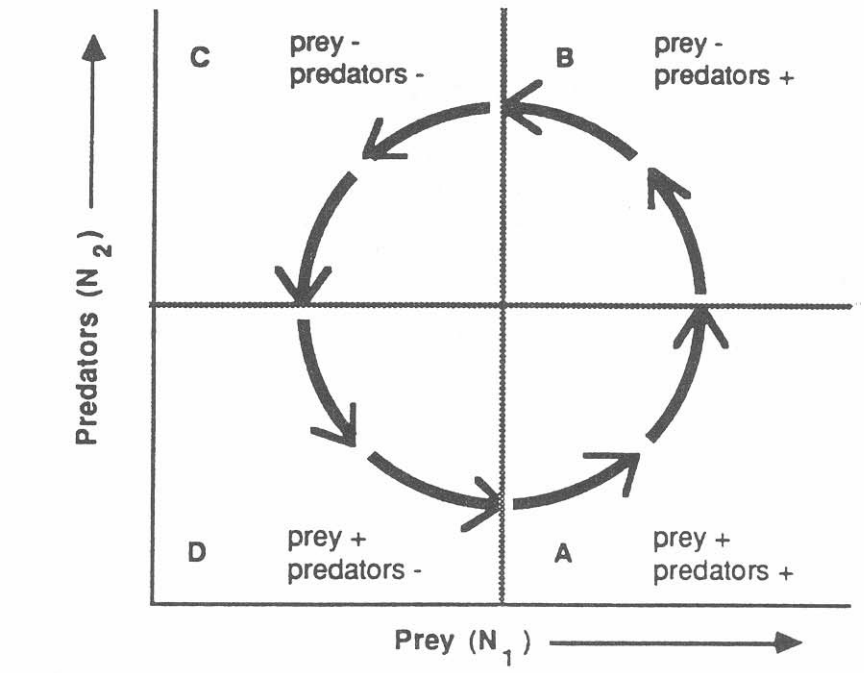


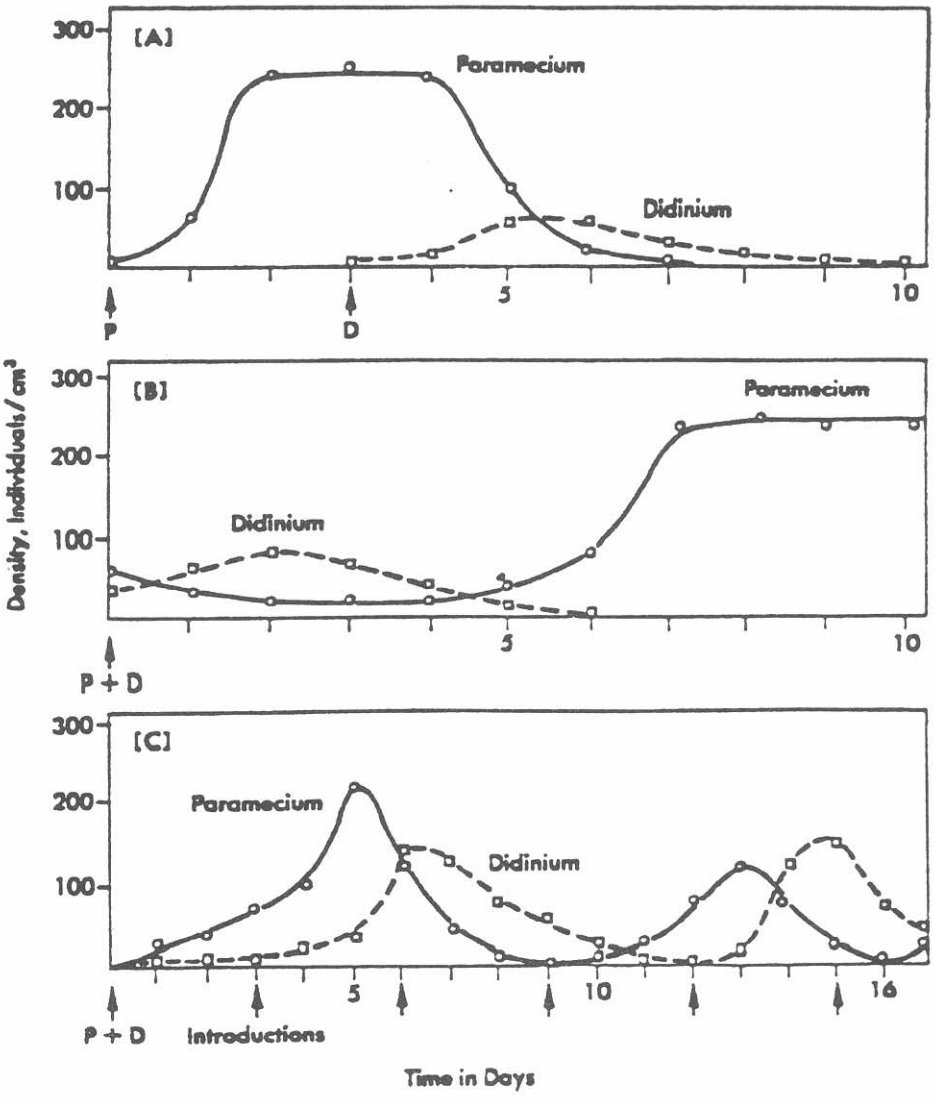
Week	Individuals (N)	dN/dt
1	4	2.74
4	36	23.21
8	360	87.81
12	535	10.07
20	555	0.0




P. caudatum separately
P. caudatum in mixed population
S. mytilus separately
S. mytilus in mixed population
Number of individuals
Days


prey (N_1)
predators (N_2)
Predator and Prey Numbers
Time


C
prey -
predators -
B
prey -
predators +
D
prey +
predators -
A
prey +
predators +
Predators (N_2)
Prey (N_1)


[A]
Paramecium
Didinium
300
200
100
P
D
5
10
[B]
Paramecium
Didinium
P + D
[C]
Paramecium
Didinium
Introductions
16
Density, individuals/cm³
Time in Days

Laboratory Activities and Timing

The class will examine the five protistan species available for these studies and determine the population densities of all stock cultures except the predator *Didinium* culture. These density estimates will be needed when students set up their own cultures for the study they design. You should now interact with groups to help them design a study that is feasible within your equipment and supplies constraints. Part of the design process will be determining how to set up the replicate cultures groups will need to accomplish the objectives of their study. The remainder of the lab period will be used to establish the replicate cultures and coordinate group data collection throughout the week.

Activity	
Introduction to population ecology	45 minutes
Census stock cultures	40 minutes
Design studies	20 minutes
Set up studies	45 minutes

Introduction to Population Ecology

You should start by providing an overview of the objectives and activities of this investigative sequence. The pre-lab activities section of the Student Outline is designed to help students learn the major concepts needed to design meaningful population ecology studies and understand their results. These concepts include population growth, growth curves, growth rates, exponential growth, logistic growth, *r*, *K*, and doubling time as measures of population growth, and the types of population interactions expected between competing species and predator and prey. I suggest you go over the pre-lab activities with the class as an introduction to this laboratory. You will have transparencies of most of the tables and figures from this chapter to use in your introduction. The following material shows the results of these activities and answers all in-text questions.

1. Plot the data from Table 2.1 in Figure 2.1 and connect the data points to obtain the growth curve for your population of *Tribolium*.

Table 2.1. Number of individuals in a population of flour beetles (*Tribolium* sp.) living in a container of flour during a 7-week period.

Week	Individuals (*N*)	ln*N*	*dN/Dt*
0	2	0.69	–
1	4	1.39	2.76
2	9	2.20	6.21
3	15	2.71	10.35
4	36	3.53	24.84
5	60	4.09	41.40
6	131	4.87	90.39
7	256	5.54	176.64

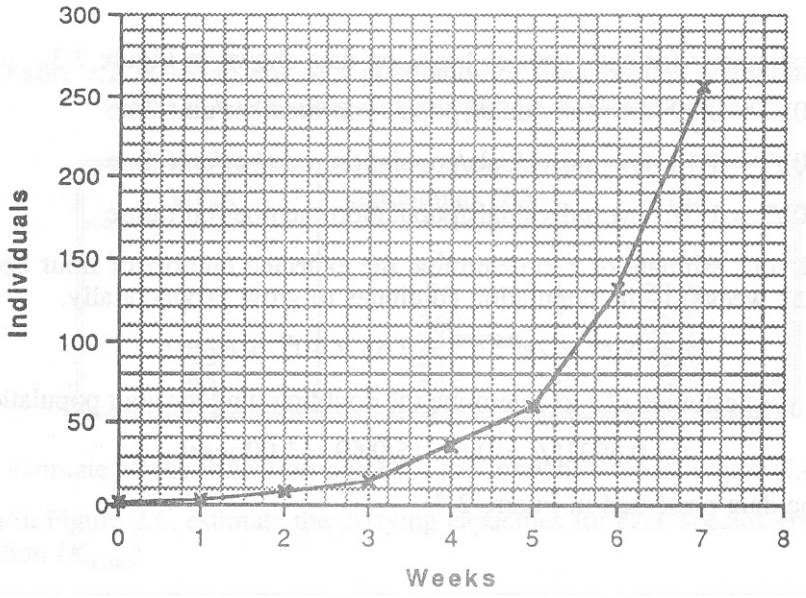


Figure 2.1. Growth curve of a population of flour beetles (*Tribolium* sp.) living in a container of flour.

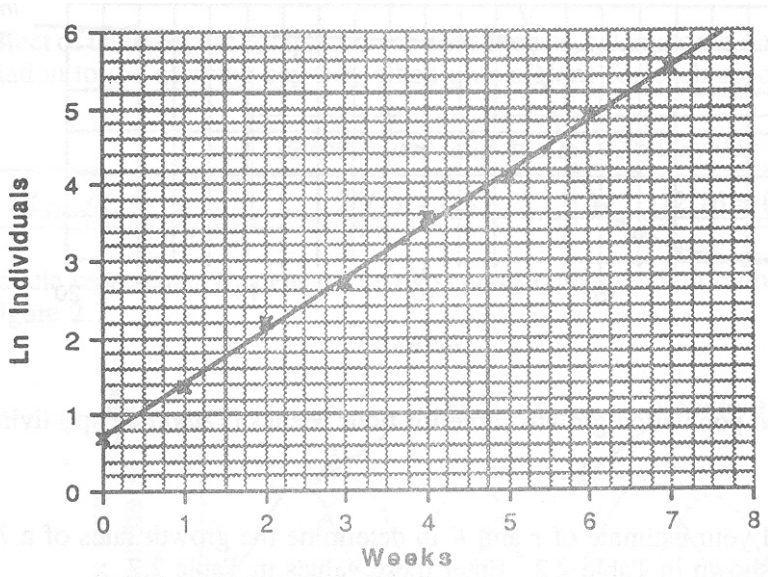


Figure 2.2. Growth curve of a population of flour beetles (*Tribolium* sp.) living in a container of flour plotted as the natural logarithm (ln) of population size.

2. What are some factors that might affect the growth of your population of flour beetles? Rates of birth, death, immigration, and emigration.

3. Use equation 5 to estimate *r* for each species based on data shown in Figure 2.3.

   Species 1 = (4.8 - 0)/7 = 0.69 new individuals/population member/unit time

   Species 2 = (7.7 - 0)/7 = 1.10 new individuals/population member/unit time

   Species 3 = (9.6 - 0)/7 = 1.37 new individuals/population member/unit time

4. Use equation 6 and your estimate of *r* to determine the expected number of flour beetles in your jar at the end of 1 year (52 weeks) if the population continues to grow exponentially.

   $N_t = N_0 e^{rt} = 2e^{(0.69)(52)} = 7.65 \times 10^{15}$ *beetles!!*

5. Use equation 7 and your estimate of *r* to determine the doubling time for your population of flour beetles.

   $t = 0.69315/r = 0.69315/0.69 = 1.00$ *week*

6. Estimate *K* from the data presented in Figure 2.4.

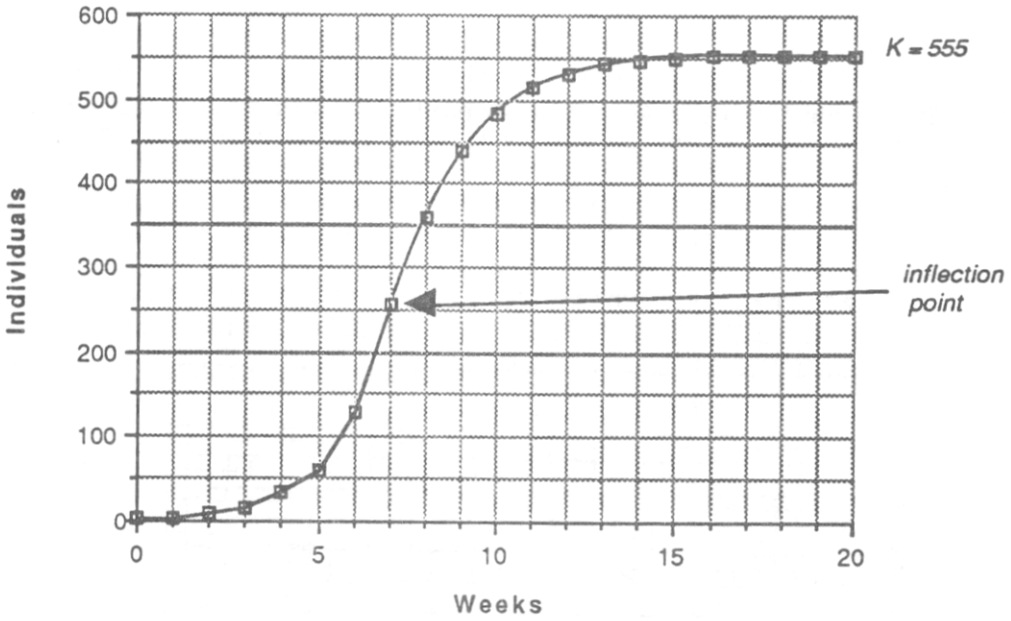


Figure 2.4. Idealized population growth curve for flour beetles (*Tribolium* sp.) living in a container of flour.

7. Use equation 14 and your estimate of *r* and *K* to determine the growth rates of a *Tribolium* population at the census times shown in Table 2.2. Enter these values in Table 2.2.

Table 2.2. Growth rate of a population of flour beetles (*Tribolium* sp.) living in a container of flour during a 20-week period.

Week	Individuals (*N*)	*dN/dt*
1	4	2.74
4	36	23.21
8	360	87.81
12	535	10.07
20	555	0

8. Draw in your estimate of the inflection point for the growth curve in Figure 2.4.

9. Based on data in Figure 2.6, estimate the carrying capacities for each species grown alone (K_{alone}) and in mixed population (K_{mixed}).

Species	Carrying capacity	
	Alone (K_{alone})	Mixed (K_{mixed})
Paramecium caudatum	75	25
Stylonychia mytilus	16	12

10. To access the affect of interspecific competition on each species, calculate the ratio of the carrying capacity in mixed population to the carrying capacity when grown alone for each species.

	Paramecium caudatum	*Stylonychia mytilus*
K_{mixed}/K_{alone} =	25/75 = 0.33	12/16 = 0.75

11. On Figure 2.8 circle representative areas of the two curves corresponding to the four segments (A, B, C, D) shown in Figure 2.7.

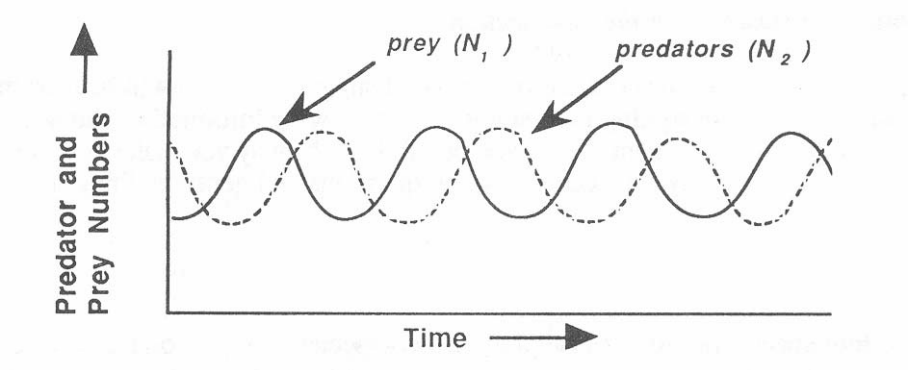


Figure 2.8. Fluctuations in the number of prey (N_1) and predators (N_2) through time as a result of the type of predator-prey interaction depicted in Figure 2.7. (Modified from Wilson and Bossert, 1971.)

Laboratory Logistics

When you receive your stock cultures (*Paramecium caudatum*, *Paramecium bursaria*, *Spirostomum ambiguum*, *Blepharisma lateritium*, and *Didinium nasutum*), put each with its associated Pasteur pipet into a separate, clearly-labelled enamel pan and place in separate parts of the room. Hopefully, this will reduce the chances of contamination with another species. When students are removing aliquots of the stocks to set up their studies (each group will have its own collection of pipets) be sure they have labelled the pipets and admonish them to be careful in making the transfers. *The contents of each flask must be thoroughly mixed before sample removal.*

We have sufficient culture and materials so that each lab should have four investigative groups. Having four people involved in a study will be advantageous because the group will be returning several times during the first week. Return trips to monitor population growth can be made by half of the group and be accomplished in about 1 hour.

Studies fall into three categories: (1) predator-prey, (2) competition, and (3) others. Because the predator-prey studies must use undiluted *Paramecium* culture, you may need to restrict the numbers of this type of study, depending on how dense your cultures are. In all studies where population growth is being studied (non-predator/prey studies) it will be necessary to reduce the density of the stock cultures to 20 individuals per species per ml. All culture vials should contain 40 ml of culture + 1.0 ml of concentrated food medium.

Each group will have a 15-hole culture rack for holding their culture vials during the study. Each lab will have two light tables on one of the side benches to provide uniform illumination for the cultures. If the study design requires, wrap vials with aluminum foil to eliminate light. The light tables will be marked off into lab section areas and all cultures racks from a lab should be placed on the light table in the appropriate area. This will make it easier for groups to find their cultures when they return later in the week.

Estimating the Population Density of the Stock Cultures

A good estimate of the density of the stock cultures is needed when each group sets up the replicate vials for their study. To accomplish this, have each student count one one-drop sample for each of the four non-*Didinium* stock containers, using the procedures indicated in the Student Outline. *Thorough mixing of cultures before and during sample withdrawal is essential.* Also, dilution may be necessary before counting if population densities are especially high in some stock stocks. While counting, students should learn how to identify the four species and record their observations in Table 2.3. A census of the *Didinium* culture is not needed, but students should look at a sample of this culture in a counting plate depression to learn its morphology and behavior. Record class data on the board and calculate the mean densities as number individuals per ml.

Investigations *(See summary in the next section)*

Because the Student Outline contains only general suggestions, you will have to interact extensively with each group while they are designing their study. The following information should serve as the basis for the recommendations you make to them. However, be subtle in the way you make these suggestions. Don't simply tell them how to set up a study; proceed by asking questions and gently guiding them in the most productive direction.

Competition Studies

We have four species of ciliates available for two-species competition studies. In all cases students must initially determine the stock culture densities. Two sets of five 40-ml cultures should be established with each species grown separately (20 individuals/ml) and a third set of five should contain 20 individuals of one species + 20 individuals of the other species. Comparing the growth of each of the two species when grown separately with the condition when cultured together will measure the amount of competition between the

two species. This can be best summarized by calculating the ratio of carrying capacities (K_{mixed}/K_{alone}) as discussed in the Student Outline and plotting the growth curves as in Figure 2.6.

Species A: 5 vials (20 individuals/ml)
Species B: 5 vials (20 individuals/ml)
Species A + B: 5 vials (40 individuals/ml; one-half A, one-half B)

In this study it is critical that good estimates of the stock cultures be made and that the correct procedure for setting up the three sets of vials be used. Obviously, being able to distinguish between and accurately count the species involved is also crucial for all group members.

Predator-Prey Studies

Because of the voracious appetite of *Didinium* it is necessary to start with a large prey density and few predators. The following results were obtained by adding three individuals of *D. nasutum* to 40 ml (thus 0.75/ml) of *P. caudatum* culture (mean value of three replicates):

Species	Density (number/ml)						
	Day						
	0	1	2	3	4	5	6
P. caudatum	372	427	213	28	0	0	0
D. nasutum	0.075	0	53	622	813	1263	1016

We suggest that 12 cultures of *P. caudatum* be set up, each 40 ml in volume. Half of these should receive three *Didinium*, the other half will serve as controls, allowing continued growth in the absence of the predator. *Note: P. caudatum* is the preferred prey for *Didinium* and it is best to use this species in a simple predator-prey study. A maximum of 1100 ml of *P. caudatum* is available in each lab for all studies that might require it; therefore keep track of the volume requested by groups and modify accordingly. Adding the three *Didinium* is a critical step in the study. It is best to dilute the stock predator culture until you get just three per drop (i.e., three per depression well). Then remove the entire well contents with a pipet. Check to make sure the *Didinium* are in the pipet by holding it up to the light, or checking under the dissecting scope. When students are sure that there are exactly three in the pipet, add them to the prey culture, rinsing out the pipet several times with prey culture.

Other predator-prey studies might be concerned with factors that interfere with predation by *Didinium*. Gause found that the presence of refugia help to delay elimination of the prey by predators. Fiberglass screening can be used to create refugia for the prey.

Prey preference can also be studied. The following design is recommended:

Culture set 1: *Didinium* and Species A (6 replicate cultures)
Culture set 2: *Didinium* and Species B (6 replicate cultures)
Culture set 3: *Didinium* and Species A and B (6 replicate cultures)
Culture set 4: Species A and B without *Didinium* (6 replicate cultures)

Again, all cultures should equal 40.0 ml. Adjust by dilution with spring water the most dense species' culture to equal the least dense species' culture. Each culture in set 1 and 2 should contain 40 ml of the adjusted stock cultures (equal densities). Each culture in set 3 and 4 should be composed of 20 ml of Species A and 20 ml of Species B so that the total number of organisms equals culture sets 1 and 2. This study should be done by two groups of students.

Miscellaneous Studies

An interesting problem that you might suggest to a group that is undecided on a project (or perhaps interested in an uninteresting or infeasible study) would be to determine how much benefit *P. bursaria* receives from its symbiotic green algae. Results obtained in the past show that *P. bursaria* clearly grows best in the light. This question could be studied by setting up cultures of 40-ml volume with 20 individuals/ml and maintaining one set in the light and the other set in the dark (covered by aluminum foil). A more comprehensive design for two groups is suggested in the summary section.

Below is a summary of an investigation of the symbiotic relationship carried out by Parker (1926):

1. In *Paramecium bursaria*, the symbiotic algae (*Chlorella vulgaris*) utilize the CO_2 produced by the animal in the presence of light.
2. The presence of the symbiotic algae is not essential for survival of *P. bursaria* afforded an adequate external food supply.
3. *P. bursaria* deprived of an external supply of food die when the culture is exposed to a continuous stream of CO_2-free air over a period of time.
4. Similar protists without symbiotic algae survive a similar exposure to CO_2-free air.
5. In the absence of light, the algae derive some sustenance from the host.
6. The symbiotic algae may, under certain conditions, be digested by the host.

Summary of Suggested Studies

The following is a summary of possible investigative studies and suggestions for management of student groups. Each student group should have four members, with a maximum of four groups per lab. Keep the following limitations in mind:

Stock cultures available per lab:
1100 ml *P. caudatum*
500 ml all other species

Culture vials: 60 per lab (15 per group)

Because of these limitations, each lab section can accommodate at most two groups doing predator-prey studies. We suggest ways these two groups could work together and share data to get more significant results. Other suggested combinations are also included. Be aware that predator-prey studies use large amounts of prey stock cultures, which may not leave enough for other studies unless you plan *carefully*.

Studies	Replicate cultures	Number of student groups
Competition: Two species (A + B) (stock cultures are diluted to give 20 individuals/ml)		
Set 1: A only Set 2: B only Set 3: A + B	5 5 5 Total = 15	1

Studies		Replicate cultures	Number of student groups
Predatory-prey: Basic study (480 ml prey stock culture)			
Set 1: Prey only Set 2: Prey + predator		6 6 Total = 12	1
Predatory-prey: Screening (960 ml prey stock culture)			
Without screening	Set 1: Prey only Set 2: Prey + predator	6 6	2
With screening	Set 3: Prey only Set 4: Prey + predator	6 6 Total = 24	
Predatory-prey: Prey preference (960 ml each prey stock culture)			
Set 1: Prey A + predator Set 2: Prey B + predator Set 3: Prey A + prey B + predator Set 4: Prey A + prey B only		6 6 6 6 Total = 24	2
Endosymbiosis studies (stock cultures diluted)			
Set 1: *P. bursaria* in light Set 2: *P. bursaria* in dark		5 5	2
Set 3: Some other species in light Set 4: Some other species in dark		5 5 Total = 20	
P. bursaria: Competition study with other species in light and in dark (stock cultures diluted)			
In light	Set 1: *P. bursaria* alone Set 2: Other species alone Set 3: Two species	5 5 5 Total = 15	2
In dark	Set 1: *P. bursaria* alone Set 2: Other species alone Set 3: Two species	5 5 5 Total = 15	

Establishing Initial Cultures

In all cases other than the predator-prey studies, the stock cultures should be diluted to 20 individuals/ml for a total of 40.0 ml per replicate culture. Help students determine how many ml of the stock they will need in order to get this required density. Each group will have several 1.0-ml and 10.0-ml volumetric pipets for establishing these cultures. After the students calculate the ml of stock cultures needed to make up each replicate vial, multiply that volume by the number of required replicate cultures and have them make up one large culture of a given density. They can then divide that culture up into the replicate vials. This gives more uniformity in initial population sizes than if each vial was made up separately. Thus a group doing competition (for example) might have the following cultures:

Individual species	5 vials with 40 ml of Species A @ 20 individuals/ml 5 vials with 40 ml of Species B @ 20 individuals/ml	
Mixed	5 vials with 40 ml:	20 ml of Species A @ 40 individuals/ml 20 ml of Species B @ 40 individuals/ml

They would have to make four large cultures:

Individual species	Species A: 5 vials × 40 ml/vial = 200 ml of 20 individuals/ml Species B: 5 vials × 40 ml/vial = 200 ml of 20 individuals/ml
Mixed	Species A: 5 vials × 20 ml/vial = 100 ml of 40 individuals/ml Species B: 5 vials × 20 ml/vial = 100 ml of 40 individuals/ml

After replicate cultures are set up, 1.0 ml of liquid food supplement should be added to each vial. Apply a piece of masking tape to each vial, give it a number, and add group names, lab day and time, and room number. Place all group vials into a single 15-hole culture rack and locate on the appropriate section of the light table.

Data Collection

Help students set up a schedule for returning to the lab during the week. Predator-prey study groups should come back about 24, 48, 72, and 96 hours after the cultures are set up. All other groups can return about 48, 72, and 96 hours after the cultures are set up. Make sure all group members know for which data collection times they are responsible. Also, suggest that all group members exchange names and phone numbers so they can communicate during the week if the schedule needs to be adjusted for whatever reason.

Obviously each group member must be able to distinguish the protozoan species included in the study. The group should go over the counting procedure in the lab during the first week so members use exactly the same protocol in gathering data. Help each group develop a common data collection table that the whole group can use throughout the week. A generic example is given on the next page. Five one-drop samples should be counted for each replicate culture and the data should be associated with the vial number. This may allow a paired statistical analysis in answering certain questions. They can record raw data as number per drop and later convert to number per ml or per 40 ml culture.

Data Sheet

Condition: _____

Species:A = _____, B = _____, C = _____

Vial	Drop	48 hours			72 hours			96 hours			168 hours		
		A	B	C	A	B	C	A	B	C	A	B	C
1	1												
	2												
	3												
	4												
	5												
	Mean												
2	1												
	2												
	3												
	4												
	5												
	Mean												
3	1												
	2												
	3												
	4												
	5												
	Mean												
4	1												
	2												
	3												
	4												
	5												
	Mean												
5	1												
	2												
	3												
	4												
	5												
	Mean												
Overall mean													
Standard deviation													

APPENDIX B
Notes for the Instructor – Week 2

Data Analysis

General

1. First, plot growth curves for each vial. Calculate means and standard deviations for each vial at the different sampling times. Plot growth curves as ln of *N* versus time. How similar are the growth curves for replicate cultures subjected to the same treatment? Why might there be differences?
2. Estimate the carrying capacity and *r* for each culture. If growth curves do not level off, use the greatest population size achieved as an estimate of the carrying capacity. Use the steepest part of the curve to estimate *r*. Doubling time can also be used to compare the magnitude of exponential growth in different populations.
3. Combine data from different vials under the same conditions, unless there is reason not to or to omit certain vials. Determine means for replicates and calculate the standard deviation of these means. Plot a growth curve for cultures grown under the same conditions.

Competition Studies

Use the rank sum test to statistically compare K_{alone} and K_{mixed} for each species as estimated from the growth curves for each replicate vial. Numbers to compare would be K_{alone} for Species A (and Species B) versus K_{mixed} for Species A (and Species B). Similarly, use the rank sum test to statistically compare *r* alone and *r* mixed for each species as estimated from the growth curves for each replicate vial.

Calculate carrying capacity ratios (K_{mixed}/K_{alone}) for pairs of vials. A problem here is how to associate values from the five different single-species and five different mixed-species vials. One approach might be to arbitrarily match single-species and mixed-species vials based on your estimate of their carrying capacity. So the two single-species and mixed-species vials with the largest estimated carrying capacities would be matched, the two with the next largest matched, and so on. You could then calculate carrying capacity ratios from these matched vials and use the rank sum test to compare the carrying capacity ratios for the two species to see if they differ significantly. You could also use this approach to do plots in the format of Figure 2.6 for each pair of vials.

Predator-Prey Studies

For the basic predator-prey study, students can plot prey alone, prey with predator, and predator alone (if included) on the same graph. Estimates of *r* for the predator and *K* for prey-only cultures should be attempted. The rank sum test can be used to compare prey numbers with and without predators at each sampling time to determine when significant differences occur. This can also be done for predators alone or with prey at each sample time. For studies with another treatment (i.e., presence or absence of refugia), you will probably want plots of prey alone, prey with predators, predators alone for each condition on separate graphs. The rank sum test can be used to compare prey numbers and predator numbers at each sample time for the two conditions. Also, one could use the rank sum test to compare *r* values for predators under the two conditions.

For prey preference studies, two graphs might show changes in numbers for each prey by itself with the predator and with the other prey and the predator. A third graph could display predator numbers with each species alone and with both available. A fourth graph could show growth curves for the two species without predators. Rank sum tests can be used to test various sets of data having to do with the affect of the predator on disappearance of each prey species and the affect of each prey species on the predator's growth. For example, a rank sum test could be done at each sample time to determine when the number of Prey A and Prey B differ significantly in vials where the predator had access to both or to compare numbers for each prey

species when alone with the predator or with the other prey species and the predator. Also, one could use the rank sum test to compare *r* values for predators with access to each prey species alone or with access to both species.

Endosymbiosis Studies

Growth curves can be plotted for *P. bursaria* in the light and dark on the same graph. If two groups are doing a competition study between *P. bursaria* and some other species in the light and in the dark, they could do a Gausian plot for each species in the light and another in the dark. Rank sum tests can be used to compare the carrying capacities, K_{alone} and K_{mixed}, and carrying capacity ratios in the light and in the dark for each species. See suggestions under competition studies above for a way to match vials to calculate these ratios.

About the Rank Sum Test

This test should meet most of the statistical needs of your students in the population ecology study. It is designed (see White, 1952) to compare two groups of data (unpaired) and determine if the numbers in the two groups differ significantly. Data are first converted to ranks (separately) and then the rank sums are compared. Sample sizes can be unequal and the minimum sample size is four.

Advanced Analysis of Competition: Calculations of the Competition Coefficient

In order to quantify the effect of one species on the growth of the other, Gause developed what he called **competition coefficients** (α and ß). Expressed in simplest terms, the competition coefficients represent the number of individuals of the first species that are ecologically equivalent to one individual of the second species. These are obtained by calculating the potential for growth showed by one species when grown alone and comparing it with the realized growth when grown with the second species. Before calculating α and ß let us relate them to the equation for logistic growth.

The logistic growth shows how growth can be expected to proceed for one species (N_1);

$$\frac{dN_1}{dt} = r_1 N_1 \left(\frac{K_1 - N_1}{K_1} \right) \tag{15}$$

where the subscript 1 indicates that the specified parameters apply to Species 1. Now, if a second competing species is involved, its presence will reduce the environment's carrying capacity for the first species. This reduction will be equal to the number of the second species present (N_2) time the number of individuals of Species 1 to which Species 2 is ecologically equivalent, or αN_2. Therefore, the logistic equation for Species 1 can be modified in the following manner to show the effect of the second species:

$$\frac{dN_1}{dt} = r_1 N_1 \left(\frac{(K_1 - \alpha N_2) - N_1}{K_1} \right) \tag{16}$$

Similarly, the effect of Species 1 on the growth of a population of Species 2 would be:

$$\frac{dN_2}{dt} = r_2 N_2 \left(\frac{(K_2 - \beta N_1) - N_2}{K_2} \right) \tag{17}$$

Technically, the competition coefficients are calculated by rearranging equation 2 to solve for α:

$$\alpha = \frac{K_1 - \left(\frac{\frac{dN_1}{dt} \times K_1}{r_1 N_1} \right) - N_1}{N_2} \tag{18}$$

and equation 3 to solve for ß:

$$\beta = \frac{K_2 - \left(\frac{\frac{dN_2}{dt} \times K_2}{r_2 N_2} \right) - N_2}{N_1} \tag{19}$$

Values to be submitted into these equations are obtained from graphs similar to those depicted in Figure 2.6 where:

K_1 and K_2 = carrying capacity of the first and second species when grown *separately*;

r_1 and r_2 = intrinsic rates of growth for the first and second species when grown *separately*, as determined using equation 5; and

N_1 and N_2 = numbers of the first and second species in a *mixed* population at a particular time period; and

dN_1/dt and dN_2/dt = instantaneous rate of growth of the first and second species during time period *t when growing together*, as determined using the logistic growth equation 14, $dN/dt = rN[(K - N)/K]$. The *K* values here are the carrying capacities for each species when grown *together*.

Since competition coefficients calculated from data at different points along the mixed-species population growth curve may vary somewhat, it is best to average values calculated for several points along the curve when your populations are increasing in size. Thus, Gause calculated at α = 5.5 (effect of *Stylonychia* on *Paramecium*) and ß = 0.12 (effect of *Stylonychia* on *Paramecium*). That is, each individual *Stylonychia* utilizes resources that could support 5.5 *Paramecium* and each *Paramecium* utilizes resources that could support 0.12 *Stylonychia.* As we see from these data, *Stylonychia* clearly outcompetes *Paramecium* under the particular conditions of culture.

In general, there are four possible outcomes of interspecific competition which can be predicated from the relationships of the competition coefficients and the carrying capacities for the two species. Assuming that α is the effect of Species 2 on Species 1 (that is, the number of individuals of Species 1 that are absent because of the presence of one individual of Species 2), that ß is the effect of Species 1 on Species 2 (number of individuals of Species 2 that are absent because of the presence of one individual of Species 1), and that K_1 and K_2 refer to the carrying capacities of Species 1 and 2 when grown alone, the four outcomes of interspecific competition are:

$\alpha > \frac{K_1}{K_2} \quad \beta > \frac{K_2}{K_1}$	Depending on the relative sizes of the two starting populations, one species or the other will survive.
$\alpha < \frac{K_1}{K_2} \quad \beta < \frac{K_2}{K_1}$	Both species survive in some sort of equilibrium coexistence.

$\alpha < \frac{K_1}{K_2} \quad \beta > \frac{K_2}{K_1}$ Species 2 will be eliminated.

$\alpha > \frac{K_1}{K_2} \quad \beta < \frac{K_2}{K_1}$ Species 1 will be eliminated

Using this sort of analysis and the values for α, ß, K_1, and K_2 presented above, what outcome would you predict for *Stylonychia* and *Paramecium*? See pages 156–164 in *A Primer of Population Biology* for a graphical summary of these outcomes.

Calculation of Competition Coefficients (α and ß): An Example

Species	Organisms/ml (averages, $N = 5$)			
	Time (hours)			
	0	48	72	168
Species 1:				
Alone	20	160	240	334
Mixed	20	172	188	205
Species 2:				
Alone	20	78	307	684
Mixed	20	88	228	272

Calculation Procedure

1. K_1 and K_2: Estimated from the single-species curves. If the curves do not level off perceptibly, estimate K_1 and K_2 as best you can. If growth declines after reaching a maximum value, this value should be considered K.

$K_1 \approx 350$ organisms
$K_2 \approx 700$ organisms

2. r_1 and r_2: Estimated from exponential part of growth in single-species cultures. These are estimated based on growth during the first 48 hours for Species 1 grown along and the first 72 hours for Species 2 grown alone.

$$r_1 = \frac{\ln N_t - \ln N_0}{t} = \frac{\ln(160) - \ln(20)}{48} = \frac{5.08 - 3.00}{48} = 0.04 \text{ organisms/population/member/hour}$$

$$r_2 = \frac{\ln N_t - \ln N_0}{t} = \frac{\ln(307) - \ln(20)}{72} = \frac{5.73 - 3.00}{72} = 0.04 \text{ organisms/population/member/hour}$$

r_1 and r_2 represent the intrinsic rates of growth during the exponential phase for each species cultured separately.

3. dN_1/dt and dN_2/dt: Estimated using logistic growth equation and data from mixed-species cultures. *Note:* The K values here are estimated as in 1 above, but from the data on growth in *mixed-species culture*.

48 hours

$$\frac{dN_2}{dt} = r\,N\left(\frac{K - N}{K}\right) = (0.04)(88)\left(\frac{280 - 88}{280}\right) = 2.41 \text{ organisms/ml/hour}$$

72 hours

$$\frac{dN_1}{dt} = r\,N\left(\frac{K - N}{K}\right) = (0.04)(172)\left(\frac{210 - 172}{210}\right) = 1.25 \text{ organisms/ml/hour}$$

$$\frac{dN_1}{dt} = (0.04)(188)\left(\frac{210 - 188}{210}\right) = 0.79 \text{ organisms/ml/hour}$$

168 hours

$$\frac{dN_2}{dt} = (0.04)(228)\left(\frac{280 - 228}{280}\right) = 1.69 \text{ organisms/ml/hour}$$

$$\frac{dN_1}{dt} = (0.04)(205)\left(\frac{210 - 205}{210}\right) = 0.20 \text{ organisms/ml/hour}$$

$$\frac{dN_2}{dt} = (0.04)(272)\left(\frac{280 - 272}{280}\right) = 1.31 \text{ organisms/ml/hour}$$

These values are the actual growth rates for the two species grown together.

4. Calculate α and ß at various times during study:

$$\alpha\ 48\ hours = \frac{K_1 - \left(\frac{\frac{dN_1}{dt} x\ K_1}{r_1 N_1}\right) - N_1}{N_2} = \frac{350 - \left(\frac{(1.25)(350)}{(0.04)(172)}\right) - 172}{88} = 1.3$$

$$\alpha\ 72\ hours = \frac{350 - \left(\frac{(0.79)(350)}{(0.04)(188)}\right) - 188}{228} = 0.55$$

$$\alpha\ 168\ hours = \frac{350 - \left(\frac{(0.20)(350)}{(0.04)(205)}\right) - 205}{272} = 0.50$$

$$\alpha = \frac{1.3 + 0.55 + 0.50}{3} = 0.78$$

$$\beta\ 48\ hours = \frac{K_2 - \left(\frac{\frac{dN_2}{dt} x\ K_2}{r_2 N_2}\right) - N_2}{N_1} = \frac{700 - \left(\frac{(2.41)(700)}{(0.04)(88)}\right) - 88}{172} = 0.77$$

$$\beta\ 72\ hours = \frac{700 - \left(\frac{(1.69)(700)}{(0.04)(228)}\right) - 228}{188} = 1.82$$

$$\beta\ 168\ hours = \frac{700 - \left(\frac{(0.31)(700)}{(0.04)(272)}\right) - 272}{205} = 1.99$$

$$\beta = \frac{0.77 + 1.82 + 1.99}{3} = 1.53$$

5. **Conclusion:** These results predict that Species 1 will be eliminated by Species 2, since:

$$\alpha\ (= 0.78) > \frac{K_1}{K_2}\ \left(= \frac{350}{700} = 0.50\right) \qquad \beta\ (= 1.53) < \frac{K_2}{K_1}\ \left(= \frac{700}{350} = 2.00\right)$$

APPENDIX C
Preparation of Cultures

Protozoan stock cultures can be obtained from Carolina Biological Supply Co. (2700 York Rd., Burlington, NC 27215). Base cultures should be started no later than 2 weeks prior to the laboratory. Keep in mind that if base cultures do not grow to large enough densities, you can purchase more stock and add to the base culture during the week of the laboratory. Base cultures can be reared in 1-gallon glass jars at room temperature under low light intensity. Over the years, we have tried using local spring water to rear the base cultures; this usually resulted in mixed success. You can try a local source of spring water and autoclave it before use, or purchase pasteurized spring water and *Paramecium* medium from Carolina Biological Supply Co. (which comes with rearing instructions). Your goal is to be sure and have base cultures of organisms with greater than 50 organisms/ml (> 100 preferred) and enough volume so that each lab can make dilutions down to 20 organisms/ml in culture vials (plastic fruitfly vials with foam plugs from Carolina Biological Supply Co. work well). A 1000-ml stock/base culture of *Paramecium caudatum* (with > 50 organisms/ml) along with 500-ml cultures (with > 50 organisms/ml) of the other protozoans should be plenty for a 30-student laboratory section. If predator-prey studies are done, you do not have to rear *Didinium* early (since only two organisms per vial are used), just have a culture delivered a few days before use. Typically, 15 fruitfly vials should be anticipated as replicates for each student group. Replicate vials can be held together by rubber bands or holes (matching the diameter of the culture vial) can be drilled into sections of 2" × 4" wooden planks and used to support the vials. Students should also be made aware of such variables as light intensity and temperature.