POPULATION ECOLOGY:

EXPERIMENTS WITH PROTISTANS

ESA lab

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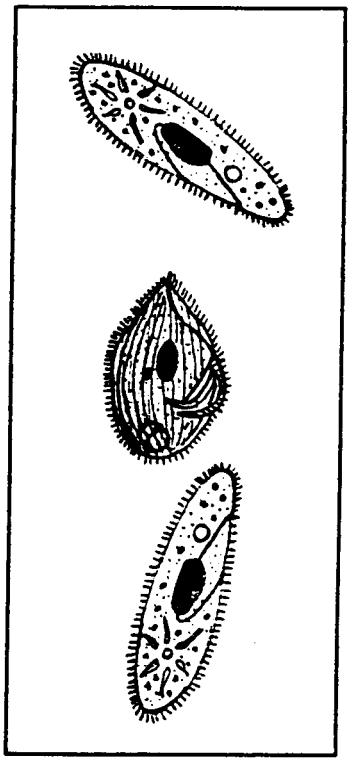
INTRODUCTION



During this two week investigative laboratory you will consider two-species interactions, such as competition and predation, that may affect the growth of populations of protistans (small, single-celled organisms). In student groups you will set up cultures of protistans in order to collect data on population growth. And you will design and conduct an investigation of competition, predator-prey interactions, or some other factor that may limit the growth of populations of these organisms. Your experiments will be completed during the second week and you will share your results and discuss them during class. Your instructor may also require a scientific paper and/or a written assignment on this research.

The growth of many populations of organisms and the factors that control their growth are of interest to biologists. To understand the various factors involved, population ecologists have developed several models that accurately describe population growth mathematically. As a part of this laboratory you will consider two of these models and some of the basic steps used in their development. Please note that the mathematics included are fairly simple; you should concentrate on the biological situation that is being modeled, not on the math used.

This study of population ecology is divided into two parts. In Part A you will 1. learn to identify five different protistans, 2. learn how to estimate the number of protistans in a culture, 3. learn how to dilute stock cultures to achieve specific concentrations of protistans, and 4. set up and carry out

a competion or predator-prey experiment of your design using one or more of the protistans available. Part B introduces you to the basic mathematical models describing exponential and logistic growth in populations of organisms. It will help you analyze, graph and interpret your data. Your instructor may use this part as a pre-laboratory out of class assignment, have you complete this part during an earlier laboratory period, or use this part as a follow-up exercise when your experiments are underway or after they have been completed.

LABORATORY OBJECTIVES

conceptual

1. Describe the effect of the birth rate (natality) and 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 affects the population growth of the other.
9. Design a study to investigate some aspect of population growth.

procedural

10. Learn some techniques for estimating the size of populations of protistians.
11. Learn some techniques for calculating population growth parameters, such as r, K, and the doubling time, from data collected from a growing population.
12. Plot data to obtain population growth curves and interpret graphs.

INVESTIGATIONS IN POPULATION ECOLOGY

PART A

MATERIALS

protistan cultures
stereoscopic binocular microscope
Pasteur pipettes and bulbs
volumetric pipettes and dispensers
depression slides or counting plates
sterile spring water
culture vials and plugs
concentrated liquid food

PROCEDURE FOR PROTISTAN OBSERVATIONS

PROTISTANS AVAILABLE FOR EXPERIMENTS

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 pipette and volumetric pipettes, all distinctly labeled. **Don't get the pipettes mixed up!** In the descriptions below, recall that 1.0 mm = 1000 μm.

initial observations

1. Use the stereoscopic, dissecting microscope to become familiar with all five species of protistans. Descriptions of each appear below.
2. Draw a sketch of each organism in the space provided.
3. Record the distinguishing features of each species in Table 1.

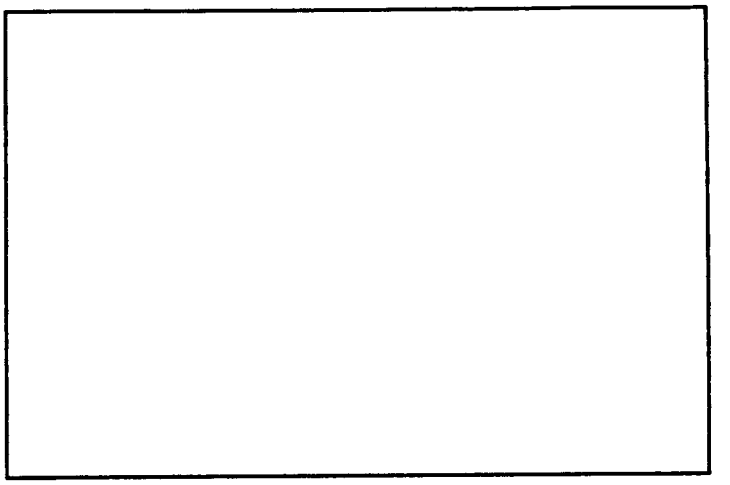
Paramecium caudatum

This species has a typical "paramecium" (slipper) shape and is large, about 250-300 μm in length.

your sketch of Paramecium caudatum

Paramecium bursaria

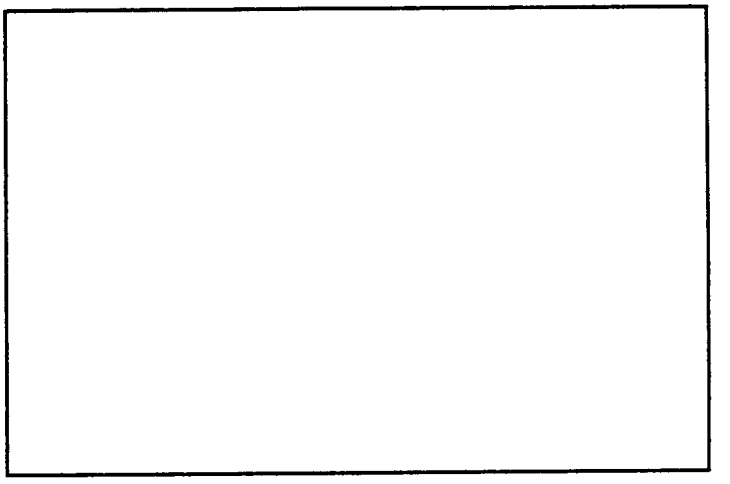
Several species of ciliates have symbiotic green algae living in their cytoplasm; *P. bursaria* is one of these. The markedly green appearance of *P. bursaria* is due to numerous algal cells in the genus *Zoochlorella* arranged much like chloroplasts around the periphery of *bursaria*'s cytoplasm. Examine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



your sketch of Paramecium bursaria

Spirostomum ambiguum

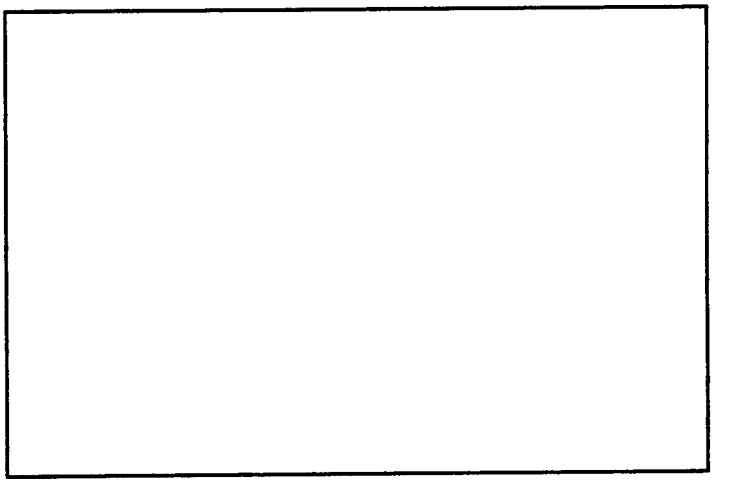
This is a very long (sometimes 2-3 mm!), club-shaped ciliate. Its size and shape are unlike any of the other species.



your sketch of Spirostomum ambiguum

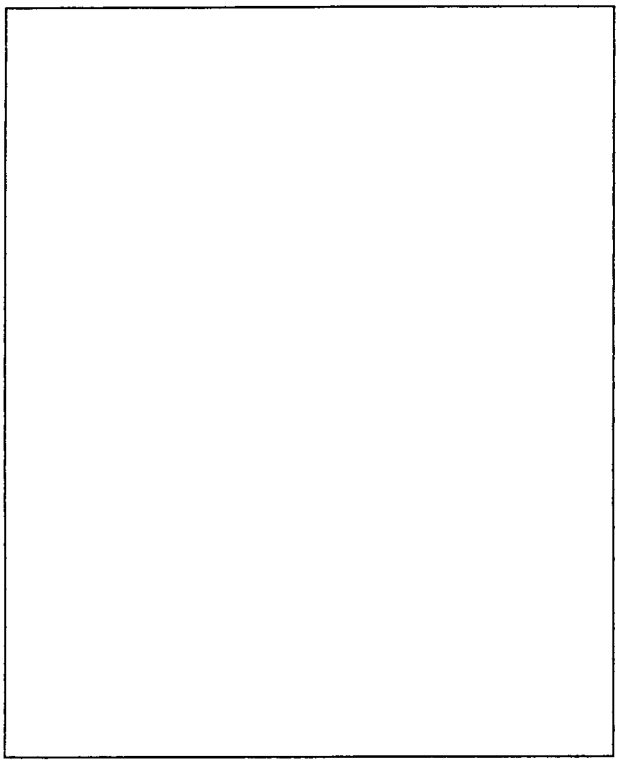
Blepharisma lateritium

This pink ciliate is pear-shaped and about 175 µm long.



your sketch of Blepharisma lateritium

Didinium



your sketch of Didinium

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 distinguishes it from any of the other species. *Didinium* swims in a wide-spiraling fashion upward through the culture. If it should make solid contact with a prey species, *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, 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. If *Didinium* cysts are available, examine these as well as the active organisms. If you find small, imobile, organisms that look somewhat like *Didinium* in your culture, these are probably cysts.

TABLE 1. Distinguishing features of five species of ciliated protistans.

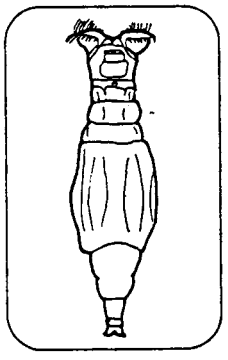
	shape	size	color	behavior
Paramecium caudatum				
Paramecium bursaria				
Spirostomum ambiguum				
Blepharisma lateritium				
Didinium nasutum				

OTHER SPECIES

protistans

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 protistans. As long as the community of these organisms is essentially the same in all the cultures you will set up, you can ignore them.

rotifers



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 protistan. Rotifers are usually attached to the bottom by their foot, but they also can 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. *Philodina*, a common rotifer in protistan cultures is shown at the left. This rotifer is about 750 μm long.

EXPERIMENTAL PROCEDURES

COUNTING INDIVIDUAL ORGANISMS

thorough mixing is critical

depression slides or plate

replicate samples

1. **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 pipettes so you don't contaminate cultures with other species.

2. 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 distribute the organisms randomly. While **vigorously** stirring the culture with a Pasteur pipette, squeeze and release the bulb to fill the pipette.

3. Hold the pipette at a 45^0 angle and carefully release two or three drops back into the culture. The next drop should be put into a depression on a slide or counting plate. This operation must be done quickly so that organisms don't begin to settle within the pipette.

4. Release the rest of the sample back into the culture vial.

5. Repeat step 1, 2, and 3 until five different sample drops have been removed from the culture and placed in individual depressions.

6. Focus on the first slide or first depression in the counting plate, with the black stage disk in position, and carefully count all the organisms.

7. Repeat this for all five samples and calculate an average from these numbers.

8. 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 (or two series of depression slides) 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 the in appropriate depression of the second plate.

dilute sample if necessary

9. There are 20 drops in a 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 15 organisms in one drop of the diluted sample the number per ml in the original culture would be:

   (number in diluted sample) x (dilution factor) x 20 drops/ml
   or
   15 organisms/drop x 4 x 20 drops/ml = 1200 organisms/ml

   The dilution factor simply equals the total number of drops in the diluted sample.

calculate number/ml

ESTIMATING STOCK CULTURE DENSITY

After you have designed your experiment and determined the experimental protocol you will need to make population estimates of the appropriate stock cultures. Each species' stock culture will have an associated labeled Pasteur pipette 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.

DESIGNING YOUR EXPERIMENT

competition studies

In studying competition, the approach used by Gause (1934) 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 interspecific 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.

suggestions

Design your experiment to include two sets of five replicate 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 replicate 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.

Example calculations and chart to set up experimental cultures.

Density of stock culture	Total desired # of ind.	Vol. of stock culture needed	End volume desired	Vol. of distilled water needed	Final density
Example: 32 ind./ml	800	$\frac{800}{32} = 25$ ml	40 ml	40-25 = 15 ml	$\frac{800}{40} = \frac{20 \text{ ind.}}{\text{ml}}$
Your Cultures: sp. A alone	800		40 ml		$\frac{20 \text{ ind.}}{\text{ml}}$
sp. B alone	800		40 ml		$\frac{20 \text{ ind.}}{\text{ml}}$
Mixed: sp. A mixed	800		20 ml		$\frac{40 \text{ ind.}}{\text{ml}}$ $\frac{20 \text{ ind.}}{\text{ml}}$ after combined
Sp. B mixed	800		20 ml		$\frac{40 \text{ ind.}}{\text{ml}}$

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 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 pipette, and when you are sure there are exactly three in the pipette, add them to the *Paramecium* culture, rinsing out the pipette several times with prey culture. If you are examining the effect of the predator on prey population growth (and the effect of prey on predator population growth), you should have a set of cultures with prey only and another set with predators only.

suggestions

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?

cysts

As previously mentioned, 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 media, culture media in which *Paramecium* were living (but now have been removed by filtration), and culture media with *Paramecium* in causing *Didinium* to emerge from encysted form. Each culture should receive 10-20 cysts.

other questions

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?

SETTING UP YOUR CULTURES

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

determine density

1. Determine the density of the appropriate stock cultures (see previous section).

determine necessary dilutions

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 its total volume, then you need 800 individuals (20 individuals/ml x 40 ml) of species A. This can be obtained by adding 2.50 ml of stock culture (320 individuals/ml x 2.50 ml = 800 individuals) to 37.5 ml of autoclaved spring water. However, to achieve the most uniformity in the 5 replicate cultures, you should place 12.5 ml (5 x 2.5 ml) of stock culture into a large flask and add 187.5 ml (5 x 37.5 ml) of autoclaved spring water. You can then dispense 40 ml from this vessel to each of 5 culture vials, making sure to keep it well stirred while dispensing.

3. If time permits, determine the density of the replicate cultures you have just set up.

add food supplement

4. Add 1.0 ml of concentrated liquid food supplement to each vial.

plug and label vials

5. Put a foam plug in each plastic culture vial and label the vial. Include the names of group members on each label and 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.

6. 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.

7. Store your vials in a designated area in the laboratory room.

COLLECTING DATA

1. Your group should plan to return on three or four occasions throughout the week to monitor population growth in your cultures.

   schedule

   For predator-prey studies, return about 24, 48, 72, and 96 hours after the study was initiated

   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 (168 hours).

3. Important Precautions

   precautions

   a. Prior to sampling, the content of each vial must be very thoroughly mixed. Throughout the week, sediment containing many protistians will accumulate on the vials' bottoms. The culture must be mixed sufficiently to distribute this sediment uniformly before sampling.
   b. Use only intact Pasteur pipettes for sampling.

4. It will take your group about 45 minutes to make counts on the cultures in your study.

Complete part B of this lab to gain the necessary background for your data analysis.

5. Calculate averages at each sampling time for all culture types. Plot your data as arithmetic and log growth curves (see Part B). To help you interpret your data you should also 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. For the predator-prey studies, concentrate on graphical interpretations of the data. Note if and when the prey become extinct, and what effect this has on *Didinium* population growth.

   average and plot data

6. To estimate r, use the lnN versus time growth curve. To calculate r, use equation 5 (Part B) and data from the linear portion of the log curve, where population growth is exponential. Estimated in this fashion, r is a measure of the maximum growth rate for the population in the particular environment where it exists before factors start to limit it.

   calculate r

7. 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 (see Figure 6, Part

   estimate doubling time

B) when the natural log of N is plotted versus time. 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.

estimate doubling time

8. Use equation 7 (Part B) and your estimate of r to determine the doubling time for your populations.

INTERPRETATION AND DISCUSSION OF YOUR DATA

YOUR EXPERIMENT

EXPERIMENTAL CONDITIONS:

STUDENTS IN GROUP:

HYPOTHESIS:

PROTOCOL:

SCHEDULE FOR DATA COLLECTION: assign each member of the group specific times to return to the lab.

Data Chart (modify if necessary)

Experimental Conditions _____

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

Vial	drop #	24 hrs A	24 hrs B	24 hrs C	48 hrs A	48 hrs B	48 hrs C	72 hrs A	72 hrs B	72 hrs C	96 hrs A	96 hrs B	96 hrs C	168 hrs A	168 hrs B	168 hrs C
1	1															
	2															
	3															
	4															
	5															
	mean															
2	1															
	2															
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	4															
	5															
	mean															
overall means																

Counts made by _____ (your name)

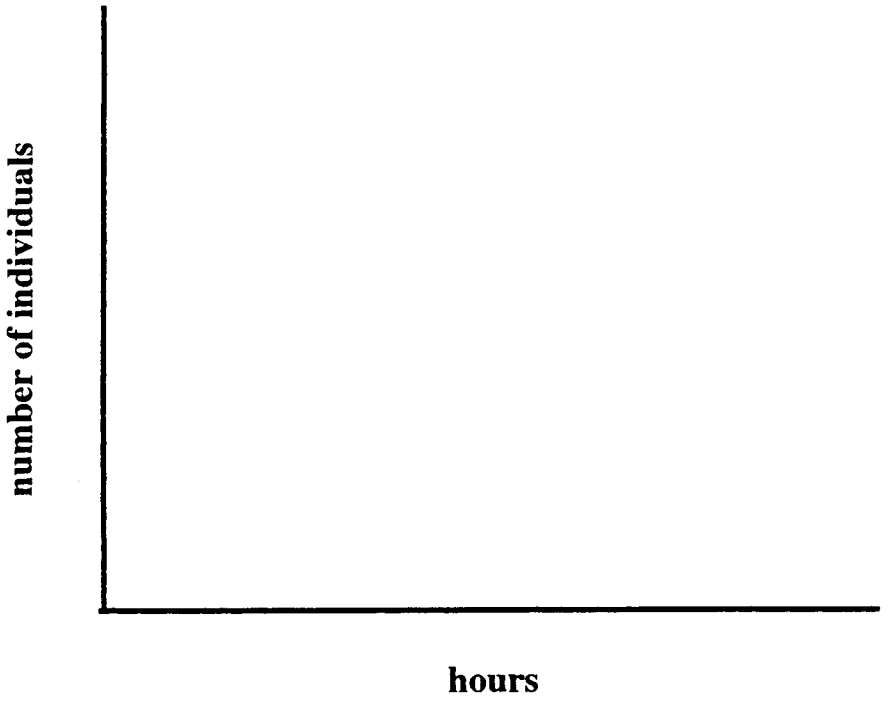


FIGURE 1.
arithmetic plot
of your data

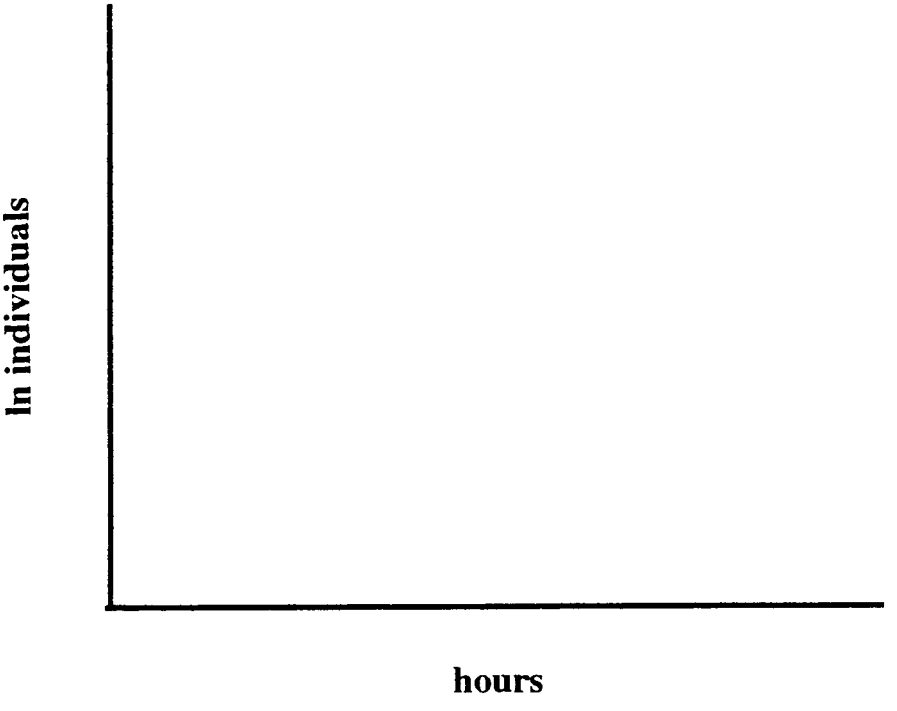


FIGURE 2.
log plot
of your data

PART B

BASIC POPULATION ECOLOGY

To complete the work in this part of the lab, you will need a calculator that can show natural logs.

population growth

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 seven weeks you might obtain the data shown in Table 2. 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.

TABLE 2. Number of flour beetles (*Tribolium*) each week in a flour culture .

Week	Individuals (N)
0	2
1	4
2	9
3	15
4	36
5	60
6	131
7	256

growth rate

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 zero) and at the end of week two, the growth rate for population would be

$$\Delta N/\Delta t = (9 \text{ beetles} - 2 \text{ beetles})/(2 \text{ week} - 0 \text{ week}) = 3.5 \text{ beetles/week}.$$

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?

Plot the data from Table 2 in Figure 3 (below) and connect the data points to obtain the growth curve for this population of Tribolium.

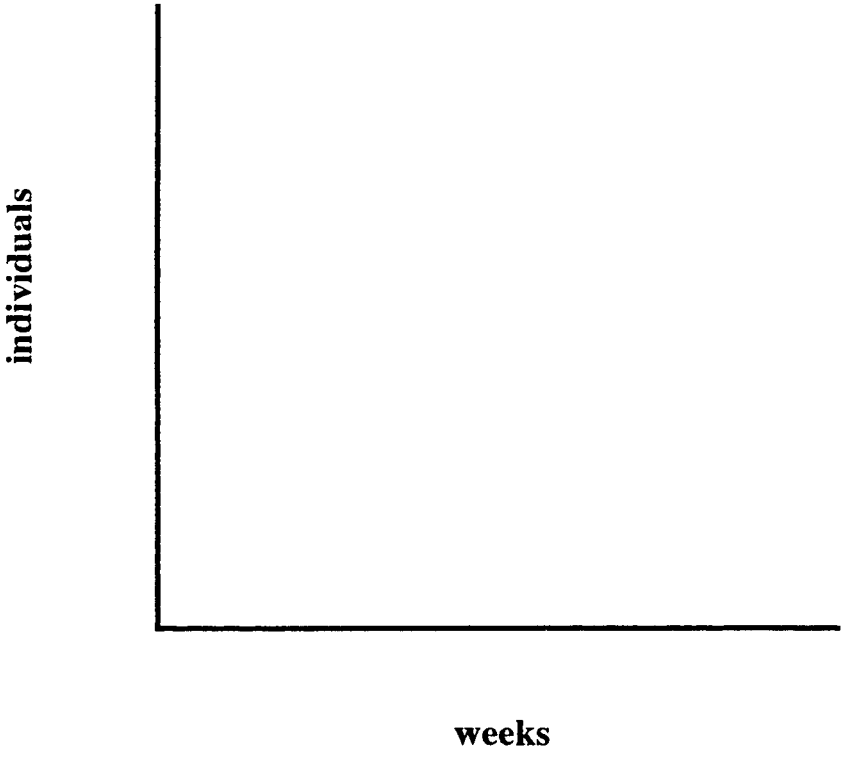


FIGURE 3. ***Your plot*: number of beetles/week - on arithmetic population growth curve**

REGULATING FACTORS

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:

$$\Delta N/\Delta t = (B + I) - (D + E)$$ *(equation 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) $\geq$ (B+I), the population size will decrease with time and its growth will be negative.

constant vs variable factors

To keep things simple, model-builders frequently assume that certain factors are constant while examining the effect of the 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:

(equation 2)

$$\Delta N/\Delta t = (bN - dN)$$

or by regrouping we get

(equation 3)

$$\Delta N/\Delta t = (b - d)N$$

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

intrinsic rate of increase

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)

$$\Delta N/\Delta t = rN$$

EXPONENTIAL GROWTH MODEL

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 possible for the species in that specific environment.

exponential growth

The 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 1 is from a population that is growing exponentially.

If the rate of population growth of the flour beetles were constant, the growth curve in Figure3 should be a straight line. Instead, Figure 3 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 which also can 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 relationship 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 logs are normally used.

logarithm of population size

natural logs

Use a calculator to determine the natural logs of the population sizes (ln N) of your flour beetle population. Enter these data in column 3 of Table 3 . Plot ln N versus time in Figure 4.

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

TABLE 3. Number and natural logs for a *Tribolium* population living in a container of flour over a seven week period.

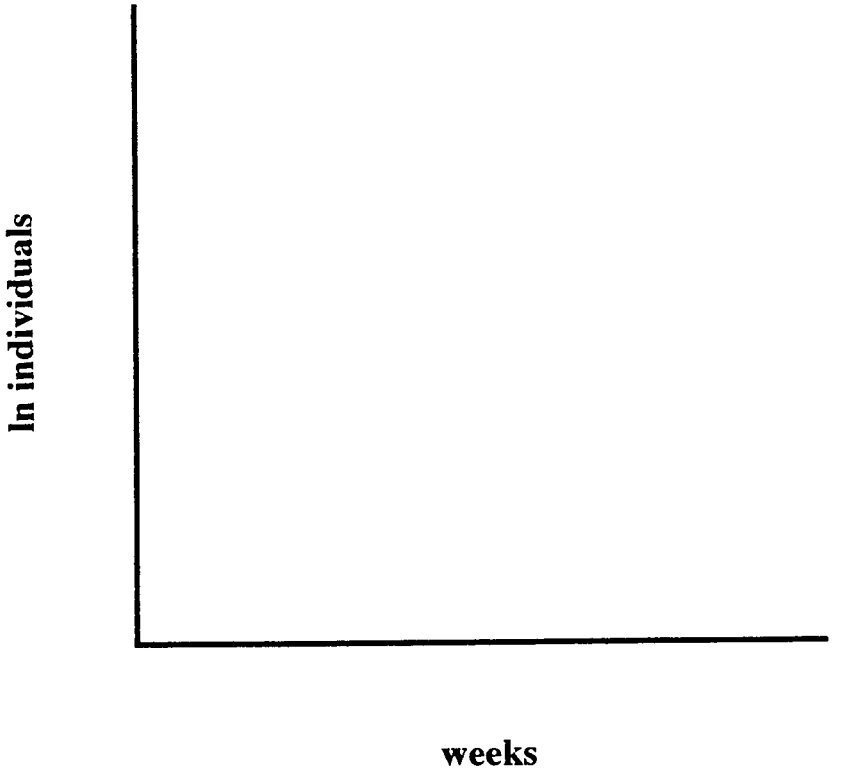


FIGURE 4. *Your plot* of the natural logs for the *Tribolium* population over time.

GROWTH RATE

intrinsic rate of increase (r)

To understand why exponential growth will be linear if plotted as the logarithm of population size verses time we must review what we know about logarithms. Recalling that a logarithm is an exponent, the linear growth shown in Figure 4 means that the logarithm of the population size increases uniformly with time, and the slope of the line ($\Delta Y/\Delta X$) in Figure 4 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 the exponential growth or

(equation 5)

$$r = (\ln N_t - \ln N_0)/t$$

So, for the flour beetles population, we could use data from week zero and week seven to estimate r as

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

Determine the growth rates (r) for the beetle population at each of the census times and enter these in Table 4.

TABLE 4. Number and growth rates for *Tribolium* population over seven week period.

Week	Individuals (N)	$\Delta N/\Delta t=(rN)$
0	2	
1	4	
2	9	
3	15	
4	36	
5	60	
6	131	
7	256	

Different species have different intrinsic rates of increase. Not surprisingly, house flies have a larger r value than elephants. Both species are capable of exponential growth, but the rate of exponential growth will be greater for the fly than the elephant. Figure 5 shows arithmetic and log plots for three different species, each growing at a different rate of exponential growth.

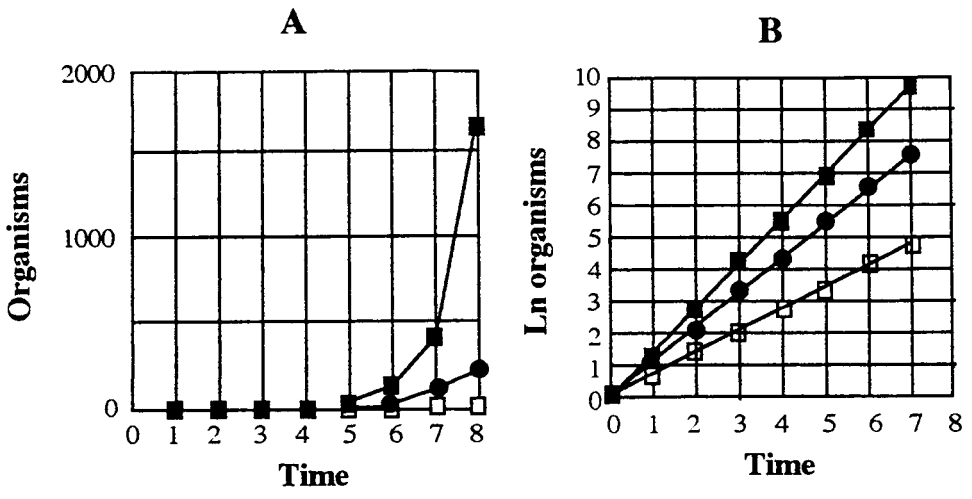


FIGURE 5.
Arithmetic (A) and logarithmic (B) plots of the growth curves of three different species: species 1 =■; species 2 =●; species 3 =□.

Estimate r for each species based on data shown in Figure 5.

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:

$$N_t = N_o e^{rt} \qquad \textit{(equation 6)}$$

where

N_o = population size at time zero
N_t = population size at time t
e = base of the natural logarithm (=2.72)

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

doubling time

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 6:

$$N_t = N_0 e^{rt} \text{ or } N_t/N_0 = e^{rt}$$

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

(equation 7)

$$N_t/N_0 = 2 \text{ and } 2 = e^{rt} \text{ or}$$
$$\ln(2.0) = rt \text{ so}$$
$$t = 0.69315 / r$$

where

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

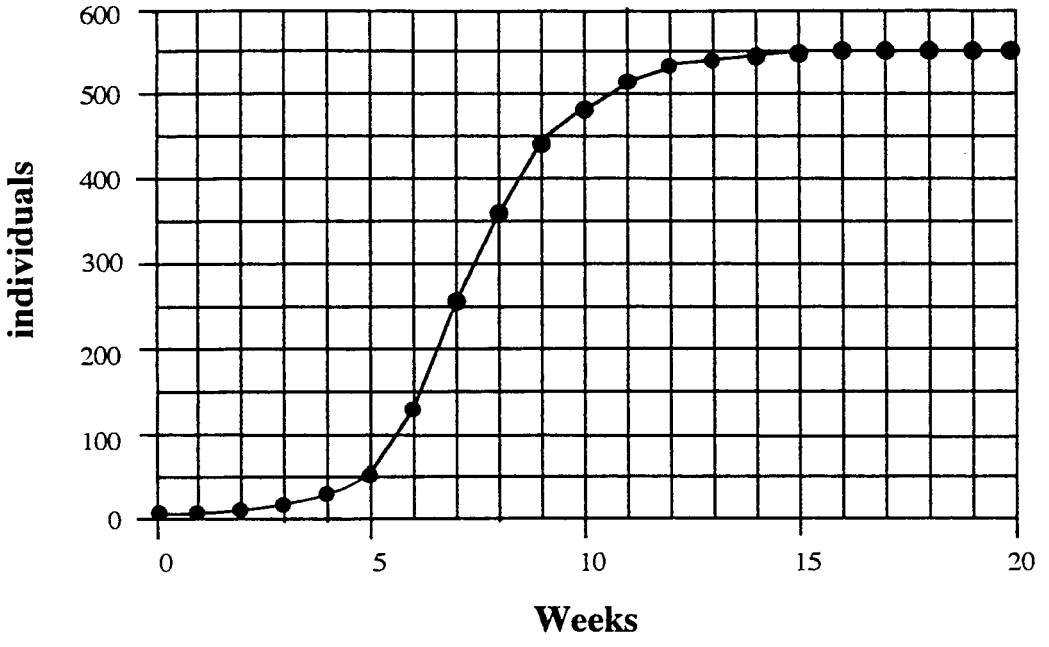
Use equation 7 and your estimate of r to determine the doubling time for the population of flour beetles.

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 can eventually fluctuate around zero. Figure 6 shows an idealized population growth curve for organisms such as the flour 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**.

carrying capacity

FIGURE 6. Idealized population growth curve for flour beetles (Tribolium sp.) living in a container of flour.



Estimate K from the data presented in Figure 6.

K represents an **equilibrium population size** that the population will reach independently of its initial growth rate. To improve our mathematical model of population growth so that it better reflects what we know about real populations, we must account for the carrying capacity.

equilibrium population size

The fundamental assumption that the birth (b) and death (d) rates are constant through time and independent of N is not met in most real populations. As growth occurs and limited resources, such as food and space, become more scarce, 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.

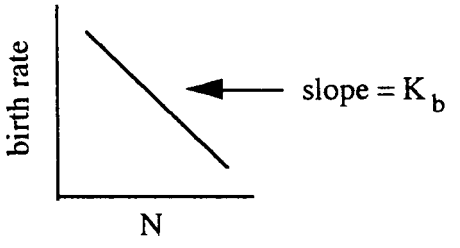
density dependency

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:

(equation 8)

$$b = b_0 - k_b N$$

or, graphically

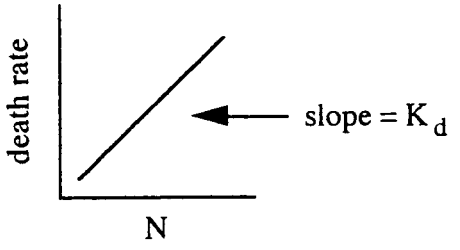


Notice that the population's current birth rate (b) is dependent on the maximal birth rate when the population is very small (b_0) minus the product of the current population size (N) times a constant (k_b) that quantifies the dependence of the birth rate on N (k_b is the slope of the decrease for the 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:

(equation 9)

$$d = d_0 + k_d N$$

or, graphically



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. Substituting the density dependent birth and death rates in equations 8 and 9 for r = b-d in equations 3 and 4, we get:

(equation 10)

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

logistic growth equation

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 ($b_0 - k_bN$) will decrease, while the second expression ($d_0 + k_dN$) will increase. This equation predicts that zero population growth will occur when the birth rate equals the death rate; in other words, when:

$$b_0 - k_bN = d_0 + k_dN \qquad \textit{(equation 11)}$$

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

$$N = \frac{(b_0 - d_0)}{k_b + k_d} = K \qquad \textit{(equation 12)}$$

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

$$r = b_0 - d_0 \qquad \textit{(equation 13)}$$

Equation 12 can be simplified to get:

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

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.

logistic growth model

The resulting equation is called the **logistic growth model**

$$\frac{dN}{dt} = rN\left[\frac{K - N}{K}\right] \qquad \textit{(equation 14)}$$

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 5 and enter these values in the table.

TABLE 5. Growth rate of *Tribolium* population over a twenty week period.

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

ZERO GROWTH

Notice that during the initial stages of growth the two models make the same predictions. Compare the values dN/dt from Table 3 and Table 4. 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 **zero population growth** (dN/dt = 0).

The **logistic growth model**, accurately describes the pattern of growth and regulation characteristic of many populations. Figure 7 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.

ENVIRONMENTAL RESISTANCE

inflection point

Draw in your estimate of the inflection point for the growth curve in Figure 6.

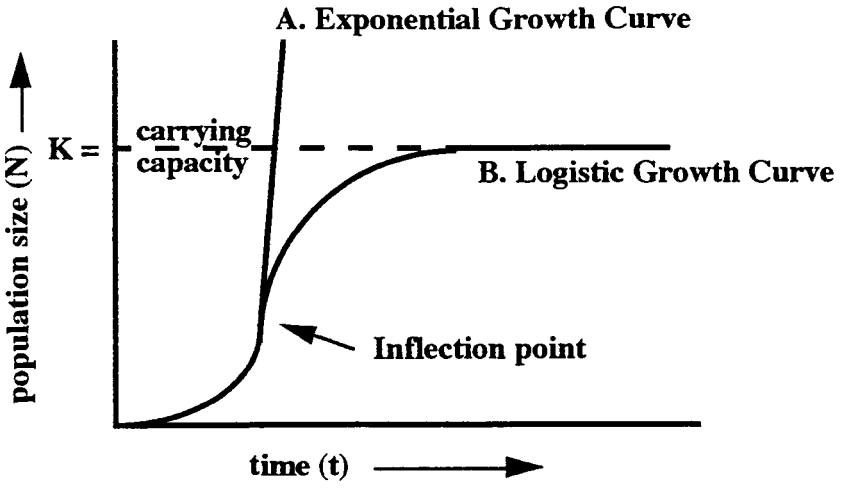
Why do many populations show logistic growth? As we have seen, birth and death rates (in some cases emigration and immigration rates as well) are density dependent. What are the ultimate factors causing the density dependence of a population's birth and death rates? Competition with members of one's 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. In completing this lab you will consider one or more of these factors in more detail.

REGULATING FACTORS

carrying capacities in natural systems

The carrying capacity seen in closed systems such as the 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 natural ecosystems involving communities of organisms. The carrying capacity of an environment for a given predator species is dependent upon the supply of prey which, for example might be several species of herbivores. The carrying capacities 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 investigate in this laboratory. It is not surprising, then, that real populations rarely demonstrate simple patterns described by mathematical models. For instance, non-density dependent influences can be very important.

FIGURE 7.
Exponential and logistic growth curves. K estimates the carrying capacity for a population showing logistic growth.



TWO SPECIES INTERACTIONS

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 then will consider the interesting situation where one species is a predator and the other its prey.

COMPETITION: inter- and intra-specific

In the broadest sense, competition can occur 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 8 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.*

Gause's experiments

mytilus, and one mixed-species culture with five individuals of both species. Population growth for both species was compared when cultured separately and in the mixed culture. The upper part of Figure 68shows the results for *P. caudatum* and the lower part shows the results for *S. mytilus*.

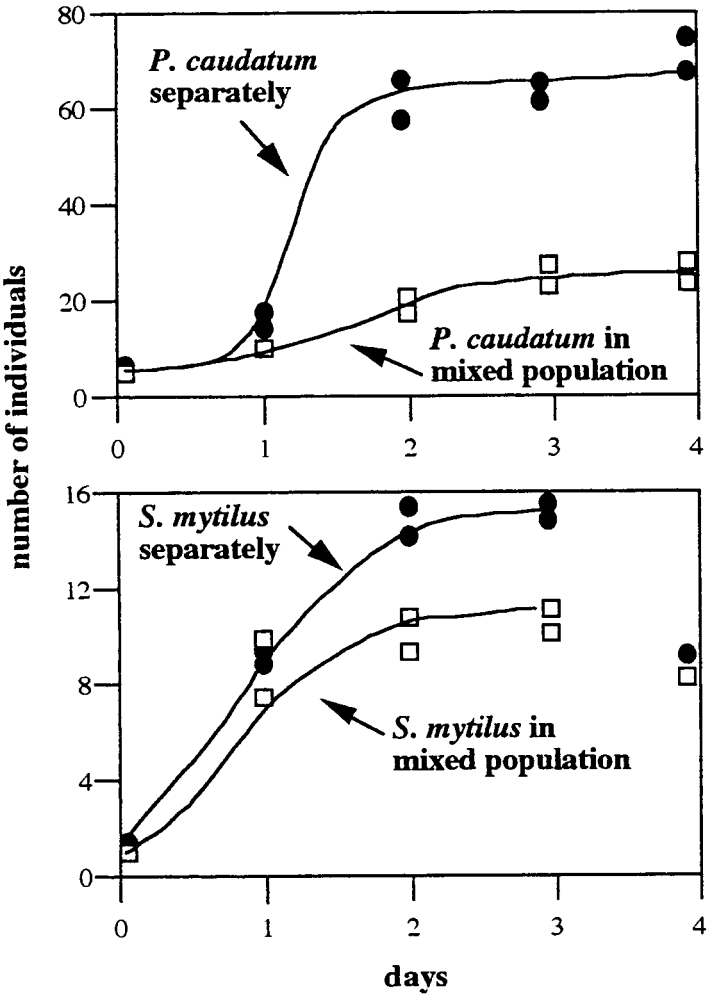


Figure 8.
Growth in numbers of individuals of *Paramecium caudatum* and *Stylonychia mytilus* cultured separately and together (redrawn from Gause, 1934).

Based on the data in Figure 8, estimate the carrying capacities for each species when grown alone (K_{alone}) and in mixed population (K_{mixed}). Enter your results in the chart on the following page.

estimated carrying capacity

	carrying capacity	
	alone (K_{alone})	mixed (K_{mixed})
Paramecium caudatum		
Stylonychia mytilus		

To assess 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. Enter the results in the chart below.

the effect of competition

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

growth in mixed culture

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, *S. mytilus* eventually replaced *P. caudatum* 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 of a resource. One species would certainly use more of the resource and eliminate the other species whenever the two occurred together.

competitive exclusion principle

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 place if alternate food sources are available or they can exist using the same food source 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.

change in food supply

change in environment

diversity in resource utilization patterns

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 the 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 influencesthe 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.

Lotka-Voltera 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.

TESTING THE MODEL

extinction of Paramecium

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 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* then starved to death, and the Paramecium hiding in the sediment (which acted as a refuge) emerged to increase in numbers (Figure 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* to the experimental set-up every third day (Figure 9C). Apparently, considerable environmental complexity is essential to the establishment of a balanced predator-prey system.

extinction of Didinium

oscillating populations

REFERENCES

Berger, J. 1980. Feeding behavior of *Didinium nasutum* on *Paramecium bursaria* with normal and apochlorotic zoochlorellae. Journal of Microbiology 118:397-404.

Gause, G.F. 1934. The struggle for existence. Williams and Wilkins, Baltimore MD.

Karakashian, S.J., M.W.Karakashian and M.A. Rodzinka. Electron microscopic observations of symbiosis of *Paramecium bursaria* and its intracellular algae. Journal of Protozoology 15: 113-128.

Lotka, A. J. 1925. Elements of physical biology. Williams and Wilkins, Baltimore MD. As reprinted in Elements of Mathematical Biology, Dover Publications, Inc., NY, 1956.

Luckingbill, L.S. 1973. The effects of space and enrichment on a predator-prey system. Ecology 55:1142-1147.

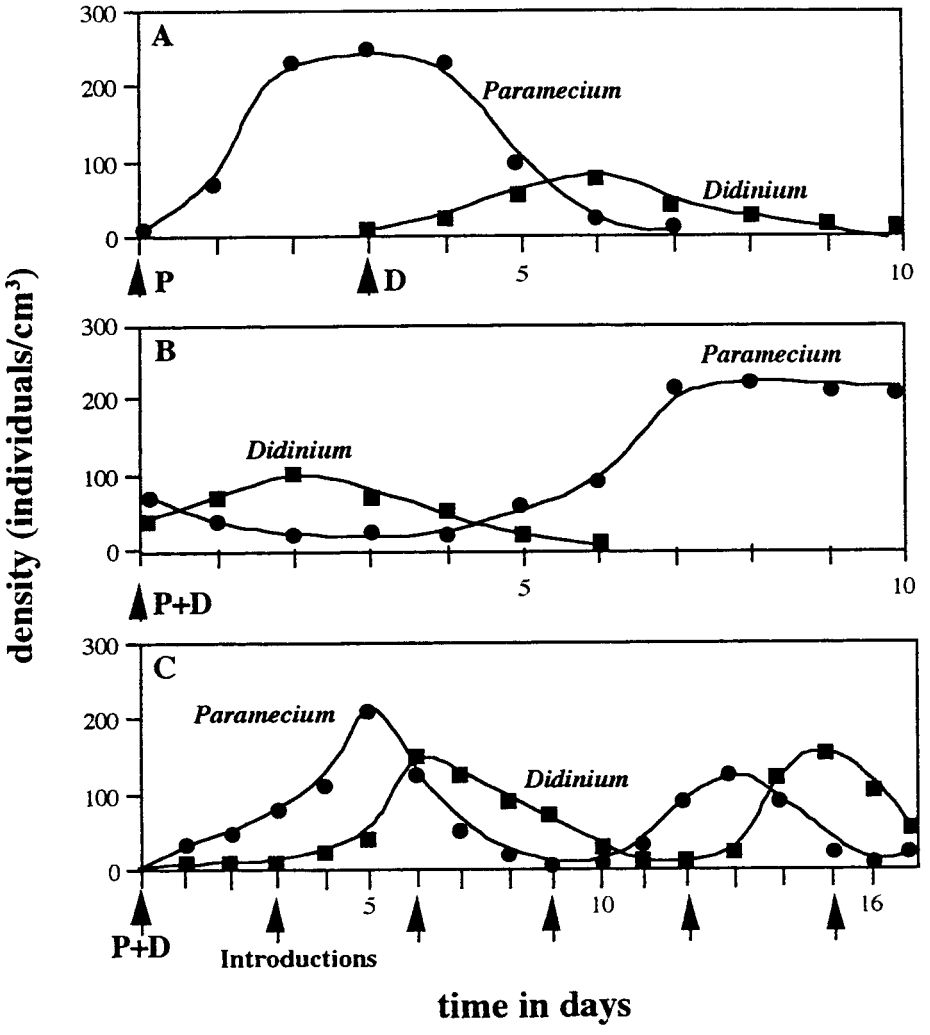


FIGURE 8.
Gause's results for predator-prey studies:
A. extinction of *Paramecium*
B. extinction of *Didinium*
C. oscillating populations

REFERENCES (Continued)

Parker, R.C. 1926. Symbiosis in *Paramecium bursaria*. Journal of Experimental Zoology 46:1-12.

Ricklefs, R.E. 1990. Ecology, third edition, W.H. Freeman and Company, NY, 896 pp.

Volterra, V. 1926. Variations and fluctuations of the number of individuals of animal species living together. In: R.N. Chapman (ed.), Animal Ecology, McGraw-Hill, New York, 409-448.

Whittaker, R.H. 1975. Communities and Ecosystems, 2nd ed. MacMillan Publishing Company, NY.

White, C. 1952. The use of ranks in a test of significance for comparing two treatments. Biometrics 8: 33-41.

Wilson, E.O. and W.H. Bossert. 1971. A primer of Population Biology. Sinauer Associates, Stamford CT.

NOTES TO THE INSTRUCTOR

LABORATORY LOGISTICS

source for stocks

culture treatment

Stock cultures of *Paramecium caudatum, Paramecium bursaria, Spirostomum ambiguum, Blepharisma lateritium,* and *Didinium nasutum* can be obtained from Carolina Biological or other supply companies. You will need to reserve at least several weeks to grow the large volumes of culture required for this lab. Place about 200 ml of dense culture from the biological supply company in a wide-mouth glass container with a capacity of several liters (gallon institutional food containers work well). Add an equal volume of glass-distilled water (spring water also works, but is less reliable) and keep at room temperature or cooler, under normal to low room lights. The containers should be left open, but covered with a piece of cheese cloth. Set up several duplicate cultures, in case one crashes. Add several grains of boiled wheat to each culture and allow it to grow for 4-5 days. Check the density of cultures regularly and when they have about doubled, add an equal volume of water and continue with this procedure until you have the required volume needed and a density of at least 100 individuals per ml (200/ml is preferred). Add more wheat as needed; however, too much wheat can lead to a bacterial bloom and the protistan population may crash. Adding several mls of protozoan pellet infusion (1 protozoan pellett: 100 ml sterile culture water) will speed growth somewhat, but may also lead to a population crash. Some have found that a combination of several wheat grains and 1-5 ml protozoan pellet infusion works best. Experiment with culturing conditions (water, light, temperature, food well in advance of this lab so you can discover what works best for you. You do not need to rear the Didinium cultures since only are needed per replicate culture. Simply order them to arrive when the lab is to begin. If they arrive too early you will need to feed them dense *Paramecium* cultures to keep them alive.

culture distribution

When you are ready to distribute cultures to the student laboratories, filter each stock culture through several layers of cheese cloth to remove the organic debris that accumulates on the bottom and makes counting difficult during these studies. Before lab, place each culture container and several pipettes into a clearly labeled pan. During lab, arrange the cultures, their pans and associated pipettes in different parts of the lab room, to reduce the likelihood of cross contamination. When students are removing aliquots of the stocks to set up their studies, be sure each group labels and uses its own pipettes. The contents of each flask **must** be thoroughly mixed before sample removal.

culture vials

Plastic fruit fly vials with foam plugs like the type sold by Carolina Biological Supply (#17-3076) work well for student use. Groups of vials

can be held together with large rubber bands or placed into a wooden rack with holes drilled to accomodate them. The racks can be kept on light tables to ensure uniform lighting during the experimental period. If the study requires, the vials can be wrapped with foil to eliminate light. Students will need to clearly label all vials and the vial rack.

lab groups

We recommend that students work in groups of four on their experiments. 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 45 minutes to an hour.

types of experiments

Studies fall into three categories: 1. predator-prey, 2. competition, and 3. others (!?). The predator-prey studies must use undiluted *Paramecium* culture. Either restrict the number of this type of study or obtain more stock of this species. 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 ml. All experimental cultures should be 40 ml + 1 ml of concentrated food medium (1 protistan pellet + 100 ml sterile, spring water).

ESTIMATING POPULATION DENSITY

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 drop samples for each of the four non-*Didinium* stock containers, using the procedures indicated in the student's section. Again, thorough mixing of the cultures before and during sample withdrawal is essential! Counting *Didinium* is not necessary because these are counted as they are added to culture vials, but the students should look at it on a culture plate to learn its morphology. Dilution may be necessary before counting if population densities are especially high. While counting, the students will learn how to identify the species. Have the class record their data on the board and calculate the mean densities as number of individuals/ml.

STUDENT EXPERIMENTS

The lab itself contains only general suggestions and the instructor will need 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, 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

There are 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/ml of one species + 20 individuals/ml 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. This can be best summarized by calculating the ratio of carrying capacities as previously discussed, and by plotting the curves.

In this study it is critical that: 1. good estimates of the stock cultures are made, 2. the correct procedure for setting up the three sets of vials is used, and 3. the students are able to distinguish between and accurately count the species involved.

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 3 individual *Didinium nasutum* to 40 ml of *Paramecium caudatum* cultures, using 3 replicates.

FIGURE 10. Interaction between *Didinium* and *Paramecium* in mixed culture

day	density (#/ml) *Paramecium*	*Didinium*
0	372	3/40 ml
1	427	0
2	213	53
3	28	622
4	0	813
5	0	1263
6	0	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. Because of its size, *Spirostomum* is not suitable prey! Keep track of the amount of *P. caudatum* available and modify if necessary to accommodate the students' requests. Adding only three *Didinium* is a critical step. It is best to dilute the stock of this predator until you get just 3/drop (i.e. 3 per depression well). Then remove the entire well contents with a pipette.

Check to make sure the *Didinium* are in the pipette by holding it up to the light, or checking under the dissecting scope. When students are sure that there are exactly three in the pipette, they should add them to the prey culture, rinsing out the pipette several times with prey culture.

refugia

Other predator-prey studies might be concerned with factors that interfere with predation by *Didinium*. Gause (1934) 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

Prey preference can also be studied. Perhaps the following design is best:

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, no *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 <u>two</u> groups of students.

endosymbiosis

An interesting study is to determine how much *P. bursaria* benefits 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 below.

Parker's study of Paramecium bursaria

The following is a summary of the results of an investigation by Parker (1926) of the symbiotic relationship between *P. bursaria* and its associated algae (*Chlorella vulgaris*).

1. In *P. bursaria*, the symbiotic algae 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 with 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.

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 ml per replicate culture. Help students determine how many ml of the stock they will need in order to get this required density. Give each group several 1.0 and 10.0 ml volumetric pipettes for establishing their 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 want to have the following cultures:

experimental vials

for individual species culture	- 5 vials with 40 ml of sp A @ 20 indiv/ml - 5 vials with 40 ml of sp B @ 20 indiv/ml
for mixed culture	- 5 vials with 40 ml:20 ml sp A @ 40 indiv/ml and 20 ml sp B @ 40 indiv/ml

make larger cultures for setting up experimental vials

They would first make 4 large cultures:

for individual species culture	- Species A: 5 vials x 40 ml/vial = 200 ml of 20 indiv/ml - Species B: 5 vials x 40 ml/vial = 200 ml of 20 indiv/ml
for mixed	- Species A: 5 vials x 20 ml/vial = 100 ml of 40 indiv/ml cultures - Species B: 5 vials x 20 ml/vial = 100 ml of 40 indiv/ml

mix, then split the large cultures

They would mix each large culture, then split it into the desired five replicates.

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. We also suggest ways that 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.

competition experiments

Competition Studies (two species -- A and B)
Stock cultures are diluted to give 20 individuals/ml

	replicate cultures	
set one -- A only	5	*one*
set two -- B only	5	*student*
set three -- A + B	5	*group*
	15	

predator-prey experiments

basic study

Predator-Prey (basic study) - 480 ml prey stock culture

	replicate cultures	
set one -- prey only	6	*one*
set two -- prey + predator	6	*student*
	12	*group*

with screen

Predator-Prey (screening) - 960 ml prey stock culture
Stock cultures are diluted to give 20 individuals/ml

		replicate cultures	
w/o screen	set one -- prey only	6	
	set two -- prey + predator	6	*two student*
with screen	set three -- prey only	6	*groups*
	set four -- prey + predator	6	
		24	

prey preference

Predator-Prey (prey preference) - 960 ml each prey stock culture

	replicate cultures	
set one -- prey A + predator	6	
set two -- prey B + predator	6	*two*
set three -- prey A + prey B	6	*student*
set four -- prey A + prey B only	6	*groups*
	24	
set one -- *P. bursaria* in light	5	*one*
set two -- *P. bursaria* in dark	5	*student*
	10	*group*

better design
prey preference

P. bursaria (better study design) - stock cultures diluted

	replicate cultures	
set one -- *P. bursaria* in light	5	
set two -- *P. bursaria* in dark	5	two student groups
set three -- some other species in light	5	
set four -- some other species in dark	5	
	10	

competition
inlight and dark

P. bursaria -- competition study with other species in light and in dark - stock cultures diluted

		replicate cultures	
in light	set one -- P. bursaria alone	5	
	set two -- other species alone	5	
	set three -- two species	5	
		15	*two student groups*
in dark	set one -- P. bursaria alone	5	
	set two -- other species alone	5	
	set three -- two species	5	
		15	

DATA COLLECTION DURING THE WEEK

Help your students set up a schedule for returning to 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.

species identification

recording data

Each group member must be able to distinguish the protozoan species included in their study. The group should go over the counting procedure during lab so members use exactly the same protocol in gathering data. Help each group modify the data collection table on p. 11 if needed by their study. Five one drop samples should be counted for each replicate culture and the data should be associated with the vial number. They can record raw data as number per drop and later convert to number per ml or per 40 ml culture.

determining means

DATA ANALYSIS

general

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.

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. 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_{alone}/K_{mixed}) 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. Students 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 8 for each pair of vials.

basic 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 also can be done for predators alone or with prey at each sample time.

prey with refugia

For studies with another treatment (i.e. presence or absence of refugia), the students can plot prey alone, prey with predators, and 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.

prey preference

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 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.

the rank sum test

This test should meet most of the statistical needs of your students in the population ecology study. It is designed 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 4. (See White, 1952.)

further analysis

For more sophisticated data analysis, refer to Gause's competition coefficients and incorporate them into the studies.

EDITOR'S NOTE

Since the classic studies of Lotka and Volterra in the 1920's and that of Gause in the 1930's many different models of predator-prey interractions have been tested. Ricklefs (1990) gives an interesting summary of some of these studies. (See References, p. 69).

EXPERIMENTS TO TEACH ECOLOGY FEEDBACK FORM

POPULATION ECOLOGY: Experiments with Protistans

Please complete this form after you have used this experiment and mail it to the address given on the reverse side of this page.

1. Was the introduction clear and informative? What changes would you suggest?

2. Was the list of materials complete? Would you suggest any additions or modifications?

3. Was Part A easy to follow? What changes would you suggest?

4. Is Part B clearly written? Are there modifications you would suggest? Did you use Part B as introductory material or as a supplement to Part A?

5. Were the illustrations and data charts adequate? What others would you include?

6. Were the instructor's notes complete? If you noted any omissions, what were they?

7. What level of students used the experiment? Was it suitable for this level? If not, what changes would you suggest?

8. Did the experiments work? Please explain any problems you had when using these experiments?

10. On a scale of 1-10, with 10 as outstanding and 1 as terrible, how would you rate this experiment? Would you recommend this experiment to others?

11. Was it helpful for this laboratory exercise to have been written for the student rather than as an instructor's guide?

Please mail the completed form to: Dr. Jane M. Beiswenger
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Laramie, WY 82071