Protozoan Population Ecology and Interactions

Laboratory Learning Outcomes

Conceptual

1. Describe the effect of birth rate and death rate on population growth.
2. Apply the concepts in BMEs 24.1, 24.2 and 27.1 to study growth of protozoan populations.
3. Design an experiment to test for a factor (interspecific interaction or environmental) that may limit population growth.

Procedural

1. Learn some techniques for estimating the size of populations of protozoans.
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 Curves and Rates

You and your group will study the growth of a focal population and how it might be affected by an interaction with another species. You will relate the growth of your population to the mathematical situations discussed in Bio-Math Explorations 24.1 and 24.2 on exponential and logistic growth rates. In those BMEs you also explored growth rates and doubling times, parameters that we can measure in our test populations and compare between control and treatment situations. You will design a study to investigate some aspect of population ecology of single-celled organisms called protozoans, such as competition or predation.

Two-Species Interactions

You will consider a relatively complex situation in ecology 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 competition. We will then consider the interesting situation where one species is a predator and the other its prey.

Competition

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.

To develop these ideas consider the pioneering work done by G. 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 protozoans), first separately and then in two-species populations, carefully noting the effects of each species on the growth of the other. Figure 1 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.

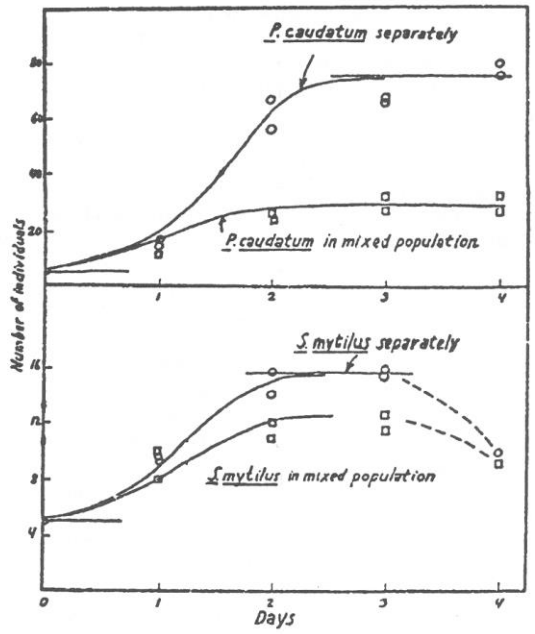

Figure 1. The growth in numbers of individuals of *Paramecium caudatum* and *Stylonychia mytilus* cultured separately and together (from Gause, 1934).

Based on Figure 1, estimate the carrying capacities for each species grown alone (K_{alone}) and in mixed population. To assess the effect of competition on each species, calculate the ratio of the carrying capacity in mixed population to the carrying capacity when grown alone for each species.

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. *Stylonychia* eventually replaced *Paramecium* in Gause's cultures. No two species that compete for the same essential, limited resource can long exist together in the same place and time.

In another experiment where other species of bacteria were available as food, 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, as was suggested in Section 27.2. 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 similar resource requirements occur together in the same time and place, there is a selection pressure for the less competitive species to adapt.

Predation

In this two-species interaction, you 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. Predator populations will increase in direct proportion to the number of prey available minus the death rate of the predators, as you learned in BME 27.1. 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.

The model in BME 27.1 suggests that a cyclical relationship between the number of prey and the number of predators in a system. As predators increase, prey abundance must decrease. However, as the number of prey decreases, this 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.

Gause (1934) tested the model for predator-prey relations using the protozoans *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 extinction (Figure 3A). Gause observed oscillations only when he introduced (as immigrants) one *Paramecium* and one *Didinium* into the experimental set-up every third day (Figure 3B).

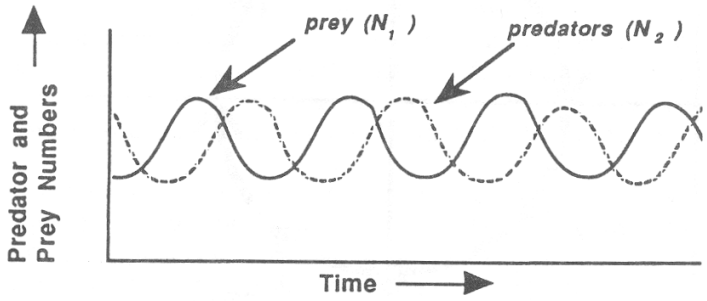

Figure 2. Fluctuations in the number of prey (*N*1) and predators (*N*2) through time. (Modified from Wilson and Bossert, 1971.)

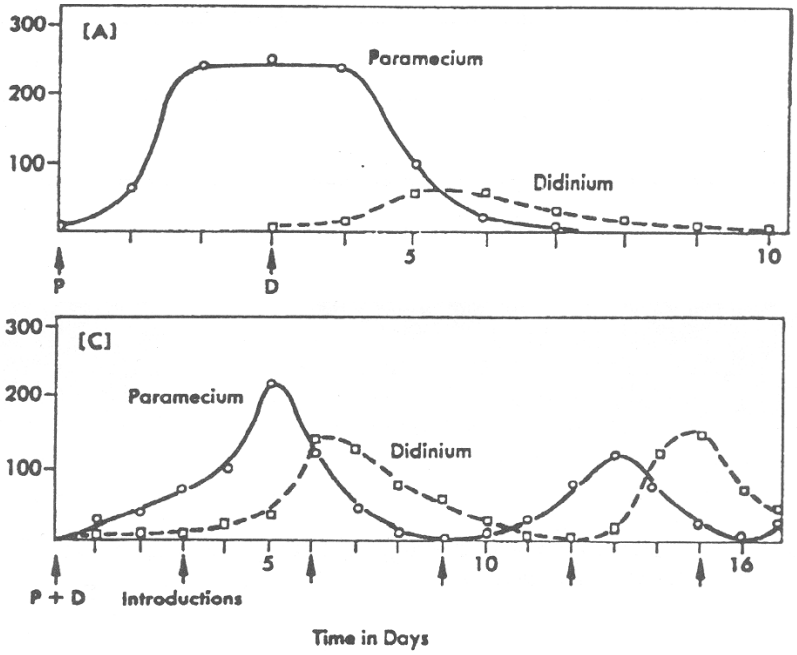

Figure 3. Predator-prey interactions between *Paramecium* and *Didinium* under different conditions (see text). (Modified from Gause, 1934.)

Protozoans Available for Study: Observing the Species

You will have access to five species of ciliated protozoans (Table 1). With the exception of the predator *Didinium*, these species feed on organic particles and bacteria, which they filter from the water. Each species will be available in a separate stoppered flask as a single-species culture. Each culture will have its own pipets, all distinctly labelled. ***Don't get these pipets mixed up***. Observe each species and use your observations to fill in Table 1.

- *Paramecium caudatum*: has a typical "paramecium" shape and is ~250–300 µm in length.
- *Paramecium bursaria*: has a symbiotic green algae living in its cytoplasm. Has a more rotund shape in comparison with *P. caudatum*. Around 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 (up to 2–3 mm!), club-shaped ciliate. Its size and shape are unlike any of the other species.
- *Blepharisma lateritium*: a pink ciliate, pear-shaped and about 175 µm in length.
- *Didinium nasutum*: a predaceous protozoan, barrel-shaped with two girdles of cilia. Has markedly different shape and locomotory behavior 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, *Didinium* discharges toxin-containing trichocysts that immobilize the prey and physically attach it to the predator. The predator requires two to three prey before dividing mitotically.

Other Species in Your Cultures

You probably noticed many other inhabitants in the cultures. Most of these are considerably smaller than the key species and include other protozoans and rotifers (tiny animals). These species are a

natural part of the community in which the species described above live. In your experiments you will not be collecting data on these smaller organisms. Since the community of these organisms is essentially the same in all the cultures you will set up, we will ignore them.

Table 1. Distinguishing features of five species of ciliated protozoans.

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

Potential Investigations in Population Ecology

Competition Studies

You can design a competition study using any species except *Didinium*. We will discuss design of competition studies. The approach used by Gause was to grow both species separately and together. If two species do not compete there should be no difference in their growth rates, whether cultured separately or together. 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 treatment vials be used. We will discuss the dilution procedures. Being able to distinguish and accurately count the two species is critical.

Calculate averages at each sample time for all three culture types and plot your growth curves. 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

Because of the voracious appetite of *Didinium*, you must start with a large prey density and few predators. You should use *undiluted* prey stock culture in your replicates. Only three *Didinium* should be added to those cultures that will receive the predator. Dilute the stock predator culture so you get only three within the drop on a depression plate. As with competition studies, we will discuss experimental design and how to calculate estimates of *r* and *K* for both prey and predator species.

Miscellaneous Studies

- What environmental factors might affect protozoan 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

Microscopes and counting plates 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. Protozoans tend to cluster on the bottom of the vessel, so gently but thoroughly agitate the culture to be sampled to obtain representative samples. Carefully swirl the culture vial and while vigorously stirring the culture with a 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 relevant organisms.
6. Repeat 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, 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) 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 per drop and convert to #/ml. Use a dilution factor if you had to dilute your sample prior to counting. For example, if you 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 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 tape labels to your pipets so you don't contaminate cultures with other species.

Estimating the Population Density of the Stock Culture

All studies will 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 Initial Cultures

If sufficient equipment and biological material are available, five replicate cultures should be set up for each treatment 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 above).
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 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 protozoan 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 will be useful for later data analysis.
5. Each group member must be able to identify the species involved 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 natural log of N over time, as in Figure 2. To calculate *r*, use $r = [\ln(N_t) - \ln(N_0)/t]$ and data from the *linear* portion of the curve, where population growth is exponential.

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 on your graph of natural log of N over time. *Note:* If a curve does not level off or goes down after reaching a peak, use the highest population level attained as your estimate of *K*.

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 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: Prior to sampling, the content of each vial must be mixed *very* thoroughly. Throughout the week, sediment containing many protozoans will accumulate on the bottom of each vial. The culture must be mixed sufficiently before sampling.
4. Please be certain to clean up after yourself and cover the microscope.

Literature Cited

Gause GF (1934) The Struggle for Existence. Williams and Wilkins, Baltimore, 163 pp.

Lotka AJ (1925) Elements of Physical Biology. Williams and Wilkins, Baltimore, Maryland, 460 pp. (Reprinted in 1956 as Elements of Mathematical Biology, Dover Publications, New York, 460 pp.)

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Wilson, EO, WH Bossert (1971) A Primer of Population Biology. Sinauer, Stamford, Connecticut, 192 pp.