# Assessing Population Growth

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## Part 1: The Growth of a Yeast Population

For better or worse, the historical starting point for much of our current knowledge of population growth stems from an elegant group of laboratory experiments performed early in this century by Carlson and later analyzed by Pearl. In these experiments the growth of the populations of a variety of microorganisms and protozoans were followed through time. Yeasts, for example, undergo rapid cell division in culture medium, and the growth of the population follows a **logistic growth** curve. There is an initial phase of slow population growth, a phase when the population grows very rapidly, and eventually a period when the population reaches a maximum size, the **Carrying Capacity** or $K$. Even in the highly artificial environment of a culture flask, the population does not remain at the maximum size but declines because of resource limitations pathogens, etc.

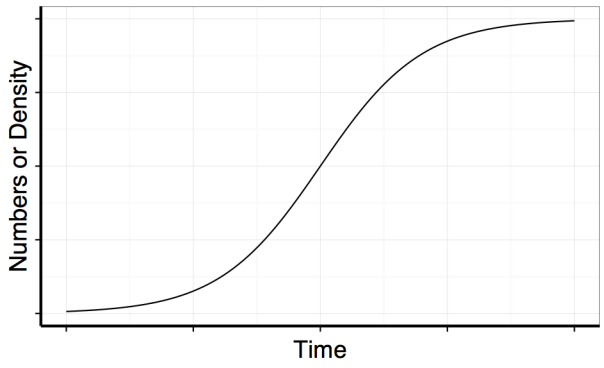


Figure 1: A generic figure of logistic growth

In the first of two experiments, we will repeat the classic Carlson-Pearl experiment using Baker's yeast. You have probably noticed the ability of microorganisms to cloud initially clear liquid if the container is left open at room temperature for several days. Although the individual cells cannot be seen with the unaided eye, the microorganisms become so numerous that the populations clouds the liquid. The extent of this cloudiness is directly proportional to the number of microorganisms and thereby provides a convenient means to measure their numbers using a spectrophotometer.

A spectrophotometer accurately measures changes in a beam of light passing through a liquid. The values may be measured either as transmittance (i.e., the amount of light that passes through a medium when the initial amount of light is known) or as absorbance (the amount of light absorbed by the medium). We will use absorbance in this lab, which varies from 0.0 (totally transparent) to 2.0 (opaque) on these instruments. As microorganisms become more numerous in liquid culture they will block the light passed through the liquid. The amount of light they block is directly proportional to their density, assuming the liquid is routinely stirred or shaken.

## Procedure

A yeast culture can take several hours to grow to measurable densities, but by 48 h it is probably reaching its maximum density (or even starting to decline). Consequently, the TAs will have set up series of cultures over the previous days with ages from 1 h to 2 days. The date and time when these cultures began growing is indicated on the flask. Your job, then, is simply to measure the densities (absorbance) of the several ages of cultures.

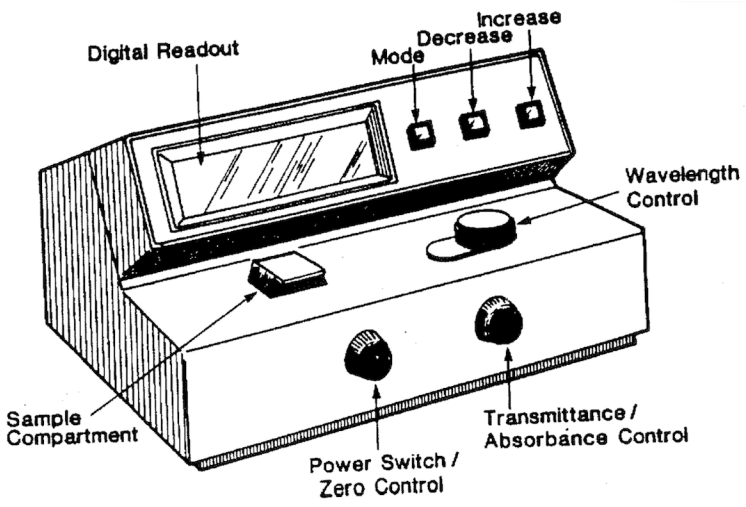


Figure 2: The spectrophotometer

1. **Calibrate the Spectrophotometer:**

    a. Turn on the Power Switch and allow the instrument to warm up for at least 15 min. (This will probably have been done for you.)
    b. Set the Wavelength Control at 540 nm, a wavelength at which microorganisms absorb light.
    c. Set the instrument in the **Transmittance mode** by pressing the Mode control until "Transmittance" is seen on the lighted display.
    d. With the sample compartment closed (and without a sample in the chamber) zero the instrument using the Power Switch/Zero control.

**IMPORTANT**: In the next steps care must be taken that nothing is spilled into the cuvette chamber in the spectrophotometer.

e. Change the Mode to Absorbance.

2. **Calibrate with a reference blank.** In our case the blank will be a cuvette of the culture media that has not been inoculated with yeast.

   a. Wipe a cuvette clean with Kimwipes and then fill it about 3/4 full. Be careful not to spill any of the medium on the outer walls of the cuvette. Also be careful to handle the cuvette with Kimwipes only. (Finger prints will block the light and cause error.)
   b. Insert the reference blank in the sample chamber. Make sure the index line on the cuvette is aligned with the mark on the cuvette holder. The cuvettes should never be twisted once they are placed in the spectrophotometer. (Twisting causes scratches which in turn causes permanent error with the cuvette).
   c. Adjust the display to 0% absorbance using the Transmittance/Absorbance Control.
   d. Remove the blank cuvette. Cuvettes should be cleaned only with repeated rinsing and then dried with Kimwipes. (Brushes should never be used.) Cuvettes should be stored in their container when not in use and should not be left lying on the counter tops. The same cuvette should be used for all readings by each group of students in the lab section.

3. **Measure the absorbance of each yeast culture.** Be sure to swirl the culture flask or tube to avoid settling, then fill the cuvette 3/4 full with a sample from a previously inoculated flask. Place the sample in the chamber and read the Absorbance.

4. Repeat with all of the cultures, recording the absorbance for each.

## Part 2: The Growth of a Lemna Population

If given unlimited resources, a population of *any* species can rapidly grow at a rate limited only by its intrinsic ability to reproduce (age of first reproduction, numbers of offspring per reproductive event, frequency of reproductive events). In the second experiment we will begin following population growth in *Lemna minor* (Duckweed), a common aquatic plant. Although *Lemna* can flower and produce seeds, it usually reproduces by vegetative propagation or budding. With adequate light, temeprature, and nutrients budding proceeds rapidly. *Lemna* and other aquatic vascular plants (e.g., *Wolffia*, *Azolla*, and giant duckweed, *Spirodela polyrhiza*) can occur in great numbers in lakes or ponds. Indeed, it is often considered a nuisance because it carpets ponds, but it is actually an important food for fish and birds and shelter for small aquatic species. It can also grow in impressively nutrient rich waters (read: wastewater). Duckweed (usually *S. polyrhiza*) is being developed as means of treating effluent as well as a bioenergy fuel stock.

In this context, it is important to determine how to sustainably harvest the greatest abundance or biomass from a growing population. This is a common issue in wildlife management,

fisheries, forestry, and increasingly in situations where microbes are used in industrial applications. It seems reasonable to assume that harvesting too heavily will cause the population to decline, potentially to extinction, but to little harvesting leaves valuable biomass unused and the population growing too slowly. Where exactly is the right level?

In this experiment you will experiment with two levels of harvesting to determine which maximizes the overall yield of *Lemna*. We will replicate the experiment with two levels of nutrients so you can see how the results change with resource levels. Your TAs will grow and count control populations for you; your job is to grow and harvest your own populations and then compare them to each other and to the controls. You will need to decide as a lab section what your two levels of harvesting will be.

| Materials | | |
|---|---|---|
| *Lemna minor* colonies | large culture tubes | Saran wrap |
| Hoagland's culture medium | test tube rack | forceps |

## Procedure

1. The Teaching Assistant will explain the basic morphology of *Lemna*, particularly the basic unit of its organization, the frond. Buds will emerge from these fronds, and clusters of individuals often form.

2. Add 2 mL of Hoagland's solution to each of two tubes and 15 mL to two other tubes. Then add distilled water to each tube to a total volume of 30 mL. These will be your two nutrient levels. At each level of nutrients you will apply one level of harvesting to each tube, for a total of four treatments (low nutrient-low harvesting, low nutrient-high harvesting, high nutrient-low harvesting, and high nutrient-high harvesting).

3. Using forceps, place several separated fronds in a culture tube with the culture medium for *Lemna*. The lab section will set up several cultures. Label the tube with the date, the treatment, and the initial number of fronds.

4. Cover the tube with Saran Wrap. Punch several small holes in the Saran Wrap with the forceps. Then place the culture tube in the tube rack under the bank of fluorescent lights in the lab.

5. Count and record the number of fronds in each week over the next 4 weeks. After recording the number of fronds, harvest the appropriate number from each tube and record the number harvested.

## Population Growth Analyses

In our analyses we will (eventually) fit two models of population growth to your data on the yeast populations and the control *Lemna* populations. Remember that unbounded populations with overlapping generations grow exponentially, but when resources are limited the population will grow logistically. The exponential growth equation is:

$$N_t = N_0 e^{rt} \tag{1}$$

and the logistic growth equation is:

$$N_t = \frac{K N_0 e^{rt}}{K + N_0(e^{rt} - 1)} = \frac{K}{1 + \left(\frac{K}{N_0} - 1\right) e^{-rt}}, \tag{2}$$

where $N_t$ is the population size at time $t$, $N_0$ is then the initial population size, $r$ is the intrinsic growth rate (per capita growth rate when resources are unlimited), and $K$ is the carrying capacity.

Our initial goal, however, is for you to develop a bit of intuition for these models and parameters relative to your own hard-won data. So first we will make some informative plots, and then we will fit these two models to our data. Lastly, we will return to the question of harvesting rates in our *Lemna* populations.

**HINT** Complete the steps and analyses for one type of organism (e.g., yeast), then copy all of your calculations and graphs into a new workbook. You can then just change the data to that of the new population (e.g., *Lemna*) without having to re-enter everything.

1. For the yeast and then the control *Lemna* populations that the TAs provide, **plot the number of organisms ($N_t$) against census time ($t$)** in hours or days. In the case of the yeast, you will be using absorbance in place of actual numbers. Describe in your own words what happened to the populations.
   What are the similarities and differences between them?
   Where on the graph is each population growing the fastest (i.e., the slope the steepest)?
   Which model of population growth seems to best describe your results?

   It should be pretty obvious which model of growth fits these data best, but let's explore what that means a bit.

2. **Plot the population growth against population density**. How can we estimate the population growth rate? Again, it is the slope of your previous graphs. The slope is simply the amount that a population grew in a time interval (e.g., from hour 1 to hour 4) divided by that time interval (here, 3 hours), or more generally, $slope = (N_{t+1} - N_t)/(time_{t+1} - time_t)$.
   Create a column for the population growth rate over every interval. Note you will not have a growth rate for the last time point because that was when we stopped counting. We want to plot this against the population size, $N_t$, but should we use the population size at the start or end of the interval ($N_t$ or $N_{t+1}$)? The best strategy is to use the *average* population size during the interval, $(N_t + N_{t+1})/2$. So create a new column

called "average N" and calculate the average population size for each time-step. Now plot the population growth rate against the average $N$ during each interval.

Where is the population growth rate greatest? Where is it the least?

3. Now, **plot the per capita growth rate against population density**. This is simply the population growth rate, which you just calculated as the slope, divided by the average abundance (or absorbance) during the time interval.

   How does *per capita* growth differ from *population* growth in these two graphs? Where is the per capita growth rate greatest? Where is it the smallest? Why?

   It is worth noting that real-world data can be very messy and thus deviate from theoretical expectations by quite a lot. Don't get too worried about, say, negative growth rates or values that seem really large. Had we taken more measurements at more regular intervals you would probably see a clearer, smoother trajectory. In our case, you may have to squint a little see the pattern.

4. Now let us try to **fit the *exponential* population model to our data**. (Fitting the logistic model will be very similar, but the exponential model is simpler.) Let's take this in steps:

   a. Create a new column next to your data called "Exponential fit."

   b. Then, somewhere below all of the cells with your data, create a cell with $N_0$ in it. To make it easier to refer to this cell, you can name it "N0". Look for the box with the "name" of the cell in it, for instance `F2`, click in it, and type "R0". Now you can refer to cell `N0` in your formulas and the spreadsheet will know what you mean. You want to put the label "N0" in the cell adjacent to it for your own benefit.
      For the yeast data we do not have an actual count of the initial density (or absorbance), but we can use a value of 0.0001, which is the absorbance that is just below readable. For the *Lemna* population, we know how many individuals we started with, so just enter that value.

   c. Next, create a cell for the value of the intrinsic growth rate, $r$. Label it "r_exp", as above, since this is the value of $r$ for the exponential model. Pick a value of $r$ to try out. You will get to change this later.

   d. Now, in the first entry of the "Exponential fit" column, you will need to type in the model $N_t = N_0 e^{rt}$ in terms that the spreadsheet understand. For example, it would be `=N0*EXP(r_exp*A2)` in a spreadsheet where the first time value is in cell `A2`.

   e. Copy this formula for the rest of the column, applying it to all of the time points. Then plot the predicted values of the exponential model *on the same plot as your data*. (Double-click on the graph, then go to `Format/Data Ranges` to get a window that will let you add new data series.)

How well does the line fit your actual data? Can you adjust the value of `r_exp` to get it closer? To part of the data?

f. Next, we will formalize our metric of how well our model predictions fit our actual data. A common metric is called the sums of squares or sum of squared differences, often abbreviated, SS. We simply calculate the difference between the observed value and the predicted value (often called the "expected" value) at every time point, square these differences, and then add them up. In mathematical terms, this is $SS = \Sigma(obs - exp)^2$.

So create a column called "(obs-exp)^2" and enter the formula to have it calculate these squared differences at every time step. Then below all of these cells use the `sum()` formula to add up all of these squared differences. This is your sums of squares value.

Now try to tweaking the value of `r_exp` to *minimize* the sums of squares. (Why minimize rather than maximize the SS?) If you're adventurous you can also try tweaking `N0` as well as `r_exp` to get a better fit.
What is the lowest SS you can achieve? What is the value of `r_exp` associated with this best fit? (Note, you may want to check with your neighbors to see how they did.) Write these values down.

Best-fit value of $r$: _____ Best-fit value of SS: _____

5. You will likely not be surprised that there is are more direct methods for fitting the exponential model to your data. Create a new column called "ln(Nt)" and in it take the natural logarithm of population size (or absorbance), $\ln N_t$, using the function, `log()`. Now plot these values against time and fit a linear trend line to the data. Be sure it prints the equation. Remember that:

$$N_t = N_0 e^{rt}, \tag{3}$$

so,

$$\ln(N_t) = \ln\left(N_0 e^{rt}\right)\ \ln(N_t) = \ln(N_0) + rt. \tag{4}$$

This looks like the slope of a line ($y = mx + b$, where we replace $m$ with $r$, $x$ with $t$, and $\ln(N_0)$ for $b$). Thus the slope of the line fit the natural log of $N_t$ against time should be a pretty good estimate of the value of $r$ that best fits your data (even though it is likely to miss the actual dynamics of your populations... resources were limited, right?). Similarly, the intercept is is the natural log of the starting population size, so $N_0 \approx exp(intercept)$.

Try plugging these values into the equation for the exponential model. Do they minimize the sums of squares any better than what you had guessed? Do you think the exponential model is a reasonable description of the population dynamics you observed?

6. Finally, let us **fit the logistic model of population growth to your data**. Proceed as you did for the exponential model:

a. creating a column for the logistic fit using the parameter values in `N0_logist`, `r_logist`, and `K`;

b. plotting this line on the same graph as your data;

c. calculating the sums of squares of the logistic fit to your data; and

d. adjusting parameter values to minimize the sums of squares.

Best-fit value of $r$: _____ $K$: _____ SS: _____

Does the logistic model fit your data better by the exponential model? How can you tell?

7. **Optional.** The approximation of $r$ from the regression line in step 5 does not work for the logistic model. There is another way to estimate $r$ and $K$, however. Remember that the logistic model describing the *change in population growth* with population abundance or density is:

$$\frac{dN}{dt} = rN\left(1 - \frac{N}{K}\right). \tag{5}$$

The *per capita* growth rate is thus,

$$\frac{1}{N}\frac{dN}{dt} = r\left(1 - \frac{N}{K}\right) = r - \frac{r}{K}N. \tag{6}$$

This looks like the equation for a line again. We simply need to plot the per capita growth rate against the population size. You will notice that we already calculated both the per capita growth rate and the average population size, so this should be straightforward. Here $r$ is the intercept of the best fit-line and the slope estimates $-r/K$, so $K = -r/slope$. (Alternatively, you can estimates $K$ as the point where the line crosses the x-axis, i.e., where per capita growth = 0. Why does this work?)
Try entering these estimates of $r$ and $K$ into your logistic predictions to see if it fits your data better. You may have to tweak the estimate of $N_0$ a bit to get the line to fit well.

8. **Compare harvesting strategies.** You should now have a good idea of how your *Lemna* population grows, and in particular where the growth rate is highest. We would expect that a harvesting strategy that kept the population near it's maximum population growth rate would produce the greatest yield. So before you actually measure the yields, write down your prediction for which strategy will have yielded the most *Lemna*.

Prediction: _____

Now calculate the average number of *Lemna* that each group harvested over the entire experiment from each treatment (i.e., harvesting strategy). Which strategy won? Does this match your predictions? If not, why?

If you are interested, try calculating how many *Lemna* you would have *expected* to get under each harvesting strategy. You already have the equations in your spreadsheet that calculate the population size through time for the logistic model. All you need to do is calculate the number of *Lemna* harvested at each time point, reduce the population by that many, and let it grow until the next time.

## Lab Assignment

You will present three figures from your *Lemna minor* data with descriptive captions.

**Figure 1:** Show the population dynamics of the control *Lemna* populations through time, along with the predictions from the best-fit population model. Make sure your caption describes both the data and the model, including the parameter values. (4 points)

**Figure 2:** The second figure will illustrate how population growth changed with population density. The caption should include your predictions for the level of harvesting that would maximize overall yield. (3 points)

**Figure 3:** The last figure will show the actual yields in the two harvesting treatments. It should represent the average and standard error from the class data. The caption should include your conclusion(s) based on these data (and can refer to figure 2 if you need). (3 points)

Points will be assigned according to this rubric:

| | Fig 1 | Fig 2 | Fig 3 | Total | Possible |
|---|---|---|---|---|---|
| Correct axis labels (with units) | ____/1 | ____/1 | ____/1 | | /3 points |
| Clear, concise, and adequate presentation of data | ____/2 | ____/1 | ____/1 | | /4 points |
| Clear, descriptive caption | ____/1 | ____/1 | ____/1 | | /3 points |