The Growth of a Population of Yeast

"The elephant is reckoned the slowest breeder of all known animals, and I have taken some pains to estimate its probable minimum rate of natural increase; it will be safest to assume that it begins breeding when thirty years old, bringing forth six young in the interval, and surviving till one hundred years old; if this be so, after a period of from 740 to 750 years there would be nearly nineteen million elephants alive descended from the first pair."  Charles Darwin, Origin of Species, 1859.

Where are these elephants today? Obviously there must be some population checks and balances that limit the growth of elephants and all other species. Conditions limiting growth in numbers are generally called population dependent factors. These factors include food supply, disease, crowding, predation and resource depletion. Over time, a species grows until the environment is unable to support additional population members. At this point the population is said to have reached the carrying capacity of the environment.

In this laboratory investigation we will model Darwin’s elephant growth prediction. Luckily it won’t take 750 years to do this because it is possible to substitute yeast for elephants. Yeast cells divide rapidly so the time scale can be greatly compressed. You will begin the experiment with a few cells of one founder population growing in an ideal environment. Another population will be started and placed in a less then ideal environment. Then, over a period of five days, you will follow the change in the number of cells of each population using two different counting procedures.

Begin your study of population growth with a prediction. Turn to the analysis section of this laboratory. Draw a curve on the axis of the graph showing your prediction of how a population of yeast will change over time. Assume the initial growth conditions are ideal.
**Vernier Equipment**
LabPro Interface, Colorimeter, cuvette and cover

**Safety**
Wear safety goggles and lab apron. Tie back long hair and roll up long loose sleeves. Be especially careful when washing and drying slides and cover slips. Broken pieces are very sharp! Report any cuts immediately.

**Materials**
Safety goggles, microscope, microscope counting slide, cover slip, lens paper, sterile 5 ml. pipettes, (2) 250 ml Erlenmeyer flasks containing apple juice growth medium, sterile medicine droppers

**Procedure**
**Part 1 Day 0 - Cell counts by Absorbance.**
1. Label 2 Erlenmeyer flasks containing apple juice with your name and team number. Mark one flask A, and the other flask B. Remove the foil cap and using a sterile pipette transfer 5 ml of working yeast solution into flask A. Replace the foil cap. Remove the foil cap from flask B and using the same pipette transfer 5 ml of working yeast solution into flask B. Replace the foil cap on flask B. Save the pipette, keep it clean and sterile by placing it on a clean paper towel. Gently swirl each flask several times to mix their contents. Avoid splashing juice on the foil caps.

2. Check your computer lab station equipment for the following connections. The cable of the colorimeter is plugged into LabPro channel 1 (ch1). The LabPro power supply is plugged into the LabPro AC adaptor port and a 110 volt outlet. The USB cable is plugged into the LabPro and the computer USB port.

3. Examine the colorimeter. Open the cover by pushing the latch forward and lifting. Notice the
square opening in the center of the colorimeter. This is the sample well. Fill a cuvette with 3 ml of distilled water. Cover the cuvette with the plastic lid. Wipe the cuvette with a clean paper towel. Place the cuvette into the sample cell with one of the clear sides of the cuvette facing the arrow in the opening above the cell. Close the cover.

4. The green indicator light should be on and located below the 565 nm label. If the light is located in one of the other positions move it by pressing one of the two arrow pads.

5. Start the data collection software by double clicking on the LabPro icon found on the desk top. A data table and graph appear on the screen. The Y axis of the graph is labeled Absorbance and the X axis is labeled Time (sec). The Collect button at the top of the screen is highlighted.

6. Click the Collect button to activate the collection procedure. Data soon appears in the table, the graph and in a window above the graph. It is the window that we are most interested in. The window displays two readings, one transmittance and the other absorbency. The absorbance value should be close to 0.0. If the absorbance value is above 0.01 or below -0.01 the colorimeter should be calibrated. To calibrate the colorimeter, stop data collection, press the colorimeter’s cal pad and hold for 30 seconds. Click collect and the data should be at the 0.0 absorbance level.

7. Stop data collection, dump the distilled water and wash and dry the cuvette.

8. Measure the absorbance of the solution in each Erlenmeyer flask. Proceed as follows: swirl the flask gently to mix the yeast cells and the apple juice, continue swirling until all of the yeast found on the bottom of the flask is suspended in the solution. Expect some gas bubbles to be released. Do not shake the flask.

9. Remove the foil cap from the flask and hold it to prevent contamination. Another team member should transfer 3 ml of the yeast apple juice mixture into the cuvette using the sterile pipette. Replace the foil cap. Hold the pipette to keep it sterile.

10. Wipe the sides of the cuvette with a clean paper towel. Place the cuvette into the colorimeter sample well and click on Collect.

11. Allow a few seconds for the absorbance recording to stabilize. Record the absorbance value in your team data table and in the class data table.

12. Remove the cuvette from the colorimeter. Dump the contents of the cuvette. Wash and dry the colorimeter before testing the second yeast culture.

13. Place flasks A in the room temperature incubator. Place flask B in the refrigerator.

**Figure 2. Counting Chamber**

[Diagram of a counting chamber with a reservoir filled with yeast solution]
Part 2 Day 0 - Cell counts by observation.
1. Obtain a microscope, counting slide, cover slip, and lens paper.

2. Clean the lenses of your microscope and polish the counting slide and cover slip.

3. Place the counting slide on the stage of your microscope. Place the cover slip on the slide so that it is supported by the two cover slips glued to the slide. (See Figure 2)

4. Bring a small sample of the working yeast solution to your lab station. Swirl the working solution before removing the sample.

5. Swirl your working yeast sample. Place a small drop of the working yeast solution at the edge of the cover slip. The solution should run back under the cover slip and fill the well of the counting chamber.

6. Using low power, focus on the cells in the well of the slide. Adjust the light intensity for better clarity. A few cells should appear in the field of view.

7. Rotate the revolving nosepiece of your microscope to bring the high power lens into position. Focus the high power lens using the fine adjustment knob of your microscope. Adjust the light intensity again to improve the clarity of your view. Now the cells should appear larger but less numerous.

8. Count all of the cells you see in the high power field. Some cells may have buds extending out from their wall. Count buds as separate cells. You may also find small clumps of cells. Count the cells in the clump individually as well. Record the number of cells in Table 2. Move the slide to a new position. Refocus the lens and count the number of cells in this new field of view. Record this number in Table 2. Continue this process until you have counted ten fields or two hundred cells.

9. Clean up your working area. Wash and dry the counting slide and cover slip.

10. Complete Analysis Day 0.

Part 1 Days 1,2,3, and 4 - Cell Counts by Absorbance
1. Measure the absorbance of yeast cultures A and B by following the procedure used on day 0. It is very important to suspend the cells in the culture by swirling the flask gently several times. The bottom of the flask should be clear after swirling. Do not shake the flask. Record the data in Table 1 and in the master class data table. Save the yeast solution from the cuvette in a clean test tube. Keep each cuvette solution separate and identified.

Part 2 Days 2 and 4 - Cell Counts by Observation
1. Count the cells in flasks A and B using the same procedure followed on day one. This time however, you will count the cells in the flasks instead of those in the working solution. Use a dropping pipette to transfer a small drop of yeast solution from the test tube containing culture A to the slide. This is the liquid you saved from the cuvette. Use a new dropping pipette for each transfer to avoid cross contamination. Count ten fields or stop after counting 200 cells. On day 4, the number of cells in the high power field may be too large to count. If this happens make a dilution of the culture you are counting. Combine 1.0 ml of culture with 9.0 ml of water. Mix the two solutions together and then count the number of cells in the diluted solution. Multiply the number of cells counted by ten to obtain an estimate of the cell count in the original culture.
2. Record the data in Table 1 and in the master class data table.


Analysis Day 0.

1. What environmental condition is being varied in this investigation?

![Figure 3. Volume Occupied by Cells Counted]

2. Assume the area of the high power field of your microscope is 0.139 mm$^2$ and the depth of the counting well is 0.25 mm. (See figure 3.) Solving for the volume of the cylinder you observed through the microscope we get 0.035 mm$^3$. Your teacher may alter these values slightly if your microscope is not a standard model.

3. What is the average number of cells you counted in one field of view? _______

4. What is the volume of this field of view? ______________

5. How many cells would you estimate there would be in 1 mm$^3$? Solve for X using the following formula: Ave. cell count / 0.035 = X / 1.0

6. How many cells would you estimate there would be in one ml. of the working solution? 1000 mm$^3$ = 1 ml.

7. How many cells did you transfer from the working solution into Flasks A and B?
8. How many cells are in each ml. of cultures A and B at time zero? Remember the volume of the culture flask is 100 ml.

9. Enter the starting yeast cell counts for day 0 in Table 1 and in the master class data table.

**Day 2 and 4**

1. What is the average number of culture A cells counted in one field of view?

2. What is the volume of this field of view?

3. How many cells would there be in one mm$^3$ of culture A? See step 4 above.

4. How many cells would there be in one ml of culture A? See step 5 above.

5. Enter the day 2 and 4 yeast cell counts for culture A in Table 1 and in the master class data table.

6. What is the average number of culture B cells counted in one field of view?

7. What is the volume of this field of view?

8. How many culture B cells would there be in one mm$^3$?

9. How many culture B cells would there be in one ml?

10. Enter the day 2 and 4 yeast cell counts for culture B in Table 1 and in the master class data table.

**Final Analysis**

1. Prepare a graph showing change of the flask A yeast population (cells / ml.) with absorbance. Plot the data on 4 cycle semi log graph paper. Let the X axis represent absorbance and the Y axis represent the yeast population. Set the absorbance range from 0 to 100. Set the population range from $10^4$ (100,000) to $10^8$ (1,000,000,000). Make a mark at the intersection of the first pair of data points. Label this point day 0. Similarly label the other points of intersection day 2 and 4. Join the data points with a "best fit" line. This line can now be used to estimate cell counts on days 1 and 3. Repeat this procedure for flask B using a dotted line. Write a title for the graph.

2. Calculate the average of the team readings from the master class data table. These averages may already be available to you if the data was entered into a master class spreadsheet.

3. Plot a new graph on a second sheet of semi log graph paper. Plot the population size (cells / ml.) on the Y axis and the age of the culture (days) on the X axis. Plot team data and class average data for cultures A and B. Connect the sets of data points with "best fit" lines. Use some coding method to identify the four curves drawn on the graph. Write a title for the graph.

4. Examine the class average curve for culture A. Concentrate on a region of the curve where it is linear and rising rapidly. Use this region of the graph to estimate the time it takes for the yeast population to double. ______________

5. Estimate the doubling time for culture B using the procedure described above. __________
6. How do you account for the difference in the doubling time between culture A and B?

7. Scientists have to be careful to avoid drawing conclusions based on erroneous data. How reliable is your data? Compare your estimates of yeast population growth to that of the other teams in your class. Is there any variation from team to team? Can you find any reasons for the differences found? How would you change the experiment to make the data more reliable?

8. Examine the class average curve for culture A again. Is the rate of growth of the population constant? Does the rate of growth of the population slow as the culture ages? Identify any population dependent factors that might cause a change in the rate of growth of a population of yeast cells.

9. Discuss the advantages and disadvantages of the two methods you used for estimating yeast population growth.

Going Further
1. Continue your study of how temperature affects the growth of yeast by incubating the cultures in warm water baths. Use aquarium heaters as a source of constant temperature.

2. Wine is a product of yeast fermentation. The literature suggests that fermentation ends when the alcoholic concentration of the wine reaches 11 - 12%. Study the growth of yeast in various concentrations of denatured ethanol and culture medium. Does alcohol limit both fermentation and cell division?

3. Modify the experiment to study the growth of nonpathogenic cultures of bacteria. Study the growth of these populations in the presence of antibiotics.
Table 1. Team Absorbance and Cell Counts

<table>
<thead>
<tr>
<th>Day</th>
<th>Culture Flask A</th>
<th>Culture Flask B</th>
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<tbody>
<tr>
<td>Day 0 Absorbance</td>
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<tr>
<td>Day 0 Cell Count</td>
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<tr>
<td>Day 1 Absorbance</td>
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<td>Day 2 Absorbance</td>
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<tr>
<td>Day 2 Cell Count</td>
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<td>Day 3 Absorbance</td>
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<tr>
<td>Day 4 Absorbance</td>
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<tr>
<td>Day 4 Cell Count</td>
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Table 2. High Power Field Cell Counts

<table>
<thead>
<tr>
<th>Field Number</th>
<th>Yeast Cells Counted (A)</th>
<th>Yeast Cells Counted (B)</th>
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<tbody>
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<td>1</td>
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<tr>
<td>Average</td>
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