Name_____

Pre-Lab: Effects of Nutrition and Temperature on Population Growth of Bacteria

1. Draw two simple, labeled graphs, one for exponential population growth and one for logistic population growth over time. No population can sustain exponential growth forever. What changes an exponentially growing population into a logistically growing population?

2. Find the common log (base 10) for the numbers 12, 43 and 8548 to 2 decimal places.

3. A spectrophotometer is an instrument that measures the amount of light absorbed by a solution (as well as the amount of light transmitted through a solution). Explain how the spectrophotometer will allow you to determine the bacterial population in your test tubes.

Effects of Nutrition and Temperature on the Population Growth of Bacteria

Objectives

- Examine the effects of environmental variables on the growth of *E.coli* cultures
- Measure bacterial growth by taking optical density readings on a spectrophotometer and showing the results graphically

Introduction

A **population** is a group of individuals of the same species living together in a certain area. The size and structure of any population changes over time due to a variety of factors, including changing rates of birth, death, and migration. Ecologists study these changes in order to learn more about how populations of different species interact with each other and with their environment, as well as how to manage the growth of populations of special interest to humans, such as crop species or disease organisms.

When a population has access to **unlimited** resources (an infinite amount of food, water, living space, etc.), it can experience unrestricted growth. Under these conditions, each individual has access to all the necessary resources and can produce offspring at a consistently high rate. As a result, the **per capita growth rate** (the average contribution an individual makes to the growth of the population) remains constant. Population size can increase quite rapidly under these conditions. For example, suppose a population of bacteria starts off with 10 individuals in the first generation. If each individual divides to produce two offspring, the second generation will contain 20 individuals (an increase of 10 individuals). If each of these 20 divides, generation three will contain 40 individuals (an increase of 20); generation four will have 80 (an increase of 40); generation five will have 160 (an increase of 80), and so forth. The population gets larger each generation, and each generation adds a larger number of individuals than the previous one. This is called **exponential** or **unrestricted growth** (see Figure 1).

Any population of any species can undergo exponential growth when resources are unlimited. However, in most realistic situations, populations cannot continue to grow forever. **Limited** resources prevent the population from expanding beyond a certain size. For example, there may only be enough food to feed 300 individuals, or only enough space for 40 individuals to live. This limit on the population size is called the **carrying capacity**.

When a population is much smaller than the carrying capacity, there are plenty of resources available for each individual and exponential growth can occur. As the population gets closer and closer to the carrying capacity, resources become scarce, and the population cannot grow as fast. Individuals have less access to resources and cannot produce as many new offspring. As a result, the per capita growth rate decreases. When the population reaches the carrying capacity, growth stops and the population remains at a constant size. This pattern is called **logistic** or **restricted growth** (see Figure 2). Different populations will have different carrying capacities depending on the amounts of resources such as food, water, and space that are available for the population to use.

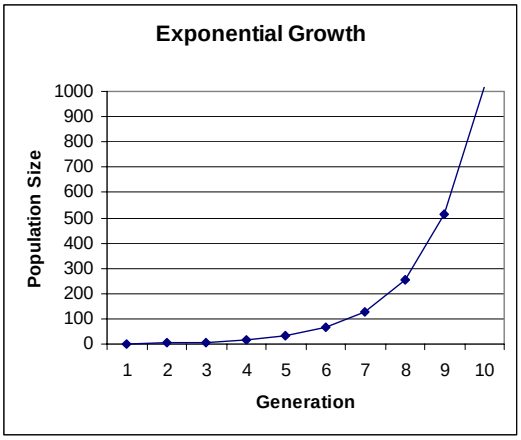


Figure 1: exponential (unrestricted) population growth.

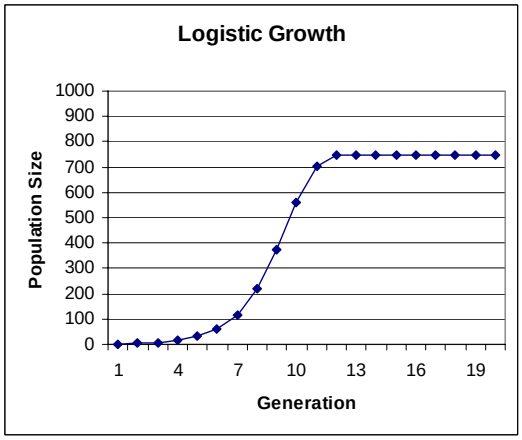


Figure 2: logistic (restricted) population growth. Carrying capacity = 750.

In this lab we will be using populations of bacteria to investigate population growth. Bacteria are ideal for this activity because of their rapid reproduction; new generations are produced in a matter of minutes. This will allow us to follow the growth of our populations over many generations in a short period of time. We will be using the species *Escherichia coli*, which is a common inhabitant of mammalian intestines (including our own).

We will investigate the effects of two factors on the growth of bacterial populations: growth medium (which supplies nutrition) and temperature. Different types of bacteria require different sets of nutrients in order to grow. Many different types of growth media are used for growing bacteria, including the nutrient broth and yeast extract that we will be using in this lab. The nutrient broth is composed of beef broth and peptone. Peptone is an enzyme derived digest of protein and provides nitrogen, amino acids, and vitamins in culture media. It is distinguished by low cystine and tryptophan content. Yeast extract is the other media that we will use to grow our *E. coli* culture. It contains yeast extract, tryptone and salt. Yeast extract is the water-soluble portion of autolyzed yeast and it is often used to supply B-complex vitamins in bacteriological media as well as flavor to snack foods and pet foods. Tryptone is a digest of the milk protein casein; it provides nitrogen, amino acids and vitamins in media. Your experiments should help you determine which medium is better suited for *E. coli*.

You will also determine whether temperature has an effect on the growth rate of *E. coli*. This species normally grows at a mammalian body temperature of approximately 37° C. You will culture populations at this temperature and at a cooler temperature of 21° C.

Each group of students will culture four populations of bacteria under the following conditions:

Flask a - nutrient broth, 21° C
Flask b - nutrient broth, 37° C
Flask c - yeast extract, 21° C
Flask d - yeast extract, 37° C

You will monitor the growth of these populations using a **spectrophotometer**. A spectrophotometer is an instrument that measures the amount of light that is absorbed by a solution. Absorbance (or optical density, O.D.) is proportional to the number of particles absorbing light. As bacteria multiply, they increasingly cloud the solution they are growing in, because each tiny bacterium scatters and/or absorbs light to some extent. You will measure the O.D. of the solution and use this reading as a proxy for the size of the bacterial population at twenty minute intervals throughout the lab period.

You will graph the O.D. of each bacterial population vs. time and use the results to determine whether the population is undergoing restricted or unrestricted growth. On regular graph paper, you will also graph the **logarithm** of the O.D. x 100 vs. time to monitor the per capita growth rate for the population. Most calculators have a LOG key that will compute the common log of any number larger than 0. The relationship between a number and its logarithm is exponential ($\log_{10} x = y$ if $x = 10^y$). Because of this relationship between numbers and their logs, changing from an arithmetic plot (y vs. x) to a semi-log plot (log y vs. x) changes an exponential curve to a straight line. For a graph of population growth, the slope of the semi-log plot indicates the instantaneous per capita growth rate. A straight line (constant slope) indicates a constant per capita growth rate.

Procedure:

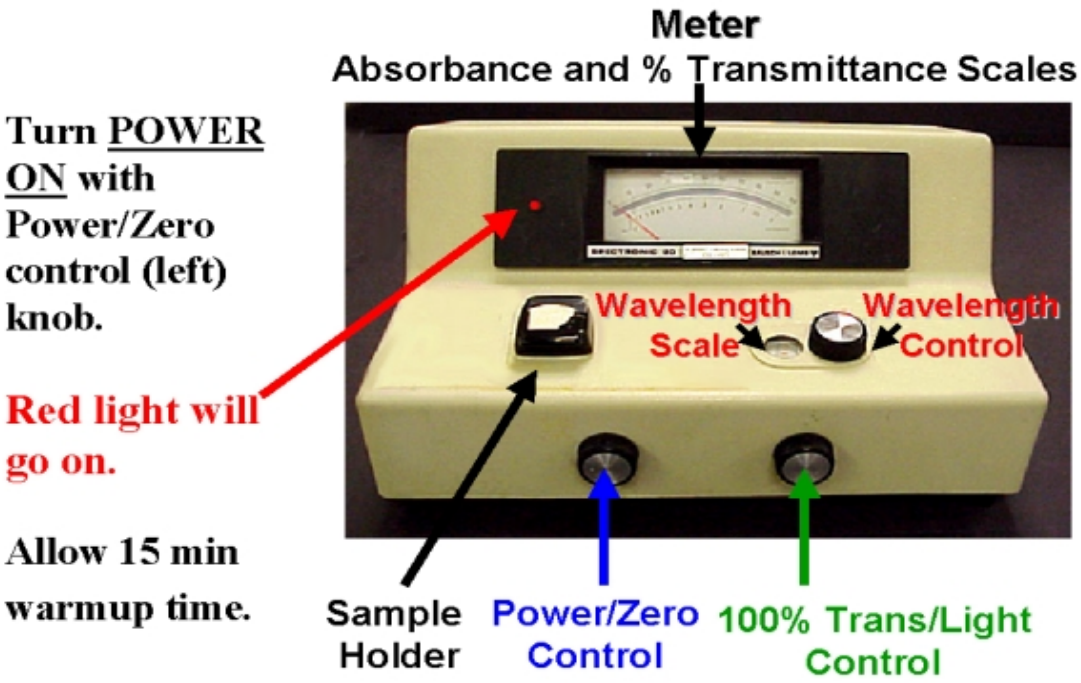
Note: *E.coli* culture cannot be poured directly down the sink; please use the *E.coli* waste jars on your table for any *E.coli* culture that you no longer need. Once a tube or flask is emptied of *E.coli*, it may be rinsed and that water may go down the sink.

Setup:

1. Turn on the spectrophotometer and let it warm up.
2. Label 4 - 125 ml flasks "a", "b", "c", "d", and your group name with tape towards the top of the flask.
3. Label 4 spectrophotometer test tubes "a", "b", "c", "d", and your group name with tape at the top of the tubes.
4. Label 1 spectrophotometer test tube "BN" for blank nutrient broth and one test tube "BY" for blank yeast extract with tape at the top of the tubes.
5. Put on gloves. If you are allergic to latex, use the vinyl gloves and let the TA know. Safety glasses are available to protect your eyes from splashes.
6. Measure 5 ml of the nutrient broth solution using the 10 ml graduated cylinder and pour it into the tube marked "BN" (nutrient broth blank). **You need to keep both blanks for the entire experiment.** Rinse the graduated cylinder with DI (deionized water) in the sink.
7. Measure 50 ml nutrient broth solution using the 25 ml or the 50 ml graduated cylinder and pour it into flask "a". Repeat with flask "b".
8. Measure 5 ml of the yeast extract solution using the 10 ml graduated cylinder and pour it into the tube marked "BY" (yeast extract blank).
9. Measure 50 ml of yeast extract solution and pour it into flask "c". Repeat with flask "d".
10. Measure 25 ml of the *E.coli* solution and pour it into flask "a". Repeat with flasks "b", "c", and "d".

11. Record what you have put into each flask. Start your timers and let them run throughout the experiment without stopping. Record the time each time you take a sample reading.

Overview Spectronic 20 Controls



Setting up the spectrophotometer to obtain optical density (O.D.) readings:

- Read through the directions for using the spectrophotometer below and use one of the blank test tubes ("BN" or "BY") to blank the machine. Tell your TA when you are ready to read the optical density (O.D.) of your samples. The TA will make sure you understand the operation of the Spectronic 20 instrument; do not hesitate to ask for more instruction if you are not sure you are using the machine properly.
- Fingerprints on the test tubes can alter the optical density readings so wipe down your test tubes with a Kim wipe each time you put them in the spectrophotometer. You must use the blank of the solution that you want to sample to set the machine to the proper levels. For example, if you want to read flask "a", you must first "blank" the spectrophotometer with the test tube "BN" because flask "a" contains nutrient broth.
- The wavelength has already been set. Without any tube in the sample holder and using the left-hand dial on the front (or top) of the machine, set the needle to zero. Place your proper blank test tube into the tube holder. Close the cover. Turn the right-hand knob until the needle reads zero optical density (which is also 100% transmittance). Remove the blank tube. The spectrophotometer is now ready to read your sample's O.D. Be sure to record the absorbance (O.D.) of each sample, not the transmittance, which can also be read from the machine.

Take your samples:
Note: Questions from this lab will be on the lab practical possibly including the use of pipets and the spectrophotometer. Every student must know how to take

samples, blank the spectrophotometer and find the O.D. so take turns using all of the equipment.

12. Swirl flask "a" well to mix the contents. Remove 5 ml of solution using a sterile graduated pipette and green pi-pump and place the solution into test tube "a". Put the pipette back into the wrapper it came in to keep it clean. You will use this pipette each time for flask "a". Mark an "a" on the wrapper or on the pipette. Record the O.D. of flask "a" to two decimal places; record the time. Pour the 5 ml sample that you just obtained a reading from back into the flask it came from and replace the stopper. Take 5 ml of solution from flask "b" with a clean pipette in the same manner and record its O.D. and time. Reblank the spectrophotometer with the "BY" and then find the O.D. of flasks "c" and "d".

13. Place flasks "a" and "c" in the room temperature shaking water bath and place flasks "b" and "d" in the 37°C shaking water bath. Be sure your flasks have your group label on them so you will know which flasks are yours. There is no need to rinse the test tubes between readings.

Continue the procedure:

14. At approximately 20 minute intervals, but record the exact time using the timer, turn off the shaker, remove your flask, swirl it and take a 5 ml sample with the proper graduated pipette. Place it into the proper test tube, find the optical density, return the solution to its flask and return the flask to its water bath. Remember to reblank the machine each time the solution you are sampling changes. Continue taking readings throughout the class.

Analysis:

15. While your cultures are incubating, each student should fill in the Bacterial Population Data Table.
16. Graph the O.D. (dependent variable on the y-axis) vs. time (independent variable on the x-axis) on regular graph paper. The maximum optical density you will record is ~1.00 and the maximum time will be about 150 minutes. Use these numbers to set up the scale of your graph. Start to plot your results as you receive them. Graph each solution separately so that there will be four lines showing optical density over time for each of the four populations of *E.coli* that you followed. Each line should be distinguished by different colors or different symbol which should be shown in the legend.
17. On regular graph paper, graph the log (O.D. x 100) vs. time. Again show 4 lines, one for each population. See appendix B for help in plotting graphs.

Your report on this lab should focus on two issues: 1) the nature of population growth in your *E.coli* cultures and 2) a comparison of population growth of *E. coli* in the 4 different

environmental conditions. See Appendix A for help with writing a lab report. Be sure to address the following questions (a-e)

a. Does **population size** of *E. coli* change as time passes in your cultures? How do you know?

b. Does the **rate** of *E. coli* population growth change over time in your cultures? How do you know?

c. Are the "test-tube" environments limited, so that *E. coli* populations show restricted growth, or unlimited, so that *E.coli* shows unrestricted growth? Give an answer for each bacterial population.

d. How are the shapes of these growth curves likely to change if the experiment is continued for a substantially longer period of time? Why?

e. Does inspection of your graphs reveal differences in growth of *E. coli* populations under different environmental circumstances? Do these differences make sense in terms of what you know of the natural habitat and niche of *E. coli*?

Clean Up

Remove all tape from flasks and test tubes. Please place all *E.coli* cultures into the *E.coli* waste jars on your tables. Place used flasks, test tubes and beakers in the containers next to the sink. Blank solutions may go down the sink. Place the pipettes in the pipette waste jars. Place your gloves in the trash. Wash your hands.

Honor Pledge

Please sign and date the following honor pledge to give your word that the lab report you submit is completely your own work. Attach the signed honor pledge to the front of your lab report.

HONOR PLEDGE

- **I pledge that this work is entirely my own.**
- **I understand that plagiarism is copying words or original thoughts without proper acknowledgement of the source.**
- **I understand that this includes copying straight from the lab manual, a partner or other student, other lab reports, the internet, texts, etc.**
- **I understand that plagiarism will result in a zero for the report, and that other consequences may follow.**

Print your name: _____

Sign: _____

Date: _____

Bacterial Population Data Table						
flask	broth	temp °C	sample time (min)	O.D.	O.D x 100	log (O.D. x 100)
a	NB					
a	NB					
a	NB					
a	NB					
a	NB					
a	NB					
a	NB					
b	NB					
b	NB					
b	NB					
b	NB					
b	NB					
b	NB					
b	NB					
c	YE					
c	YE					
c	YE					
c	YE					
c	YE					
c	YE					
c	YE					
d	YE					
d	YE					
d	YE					
d	YE					
d	YE					
d	YE					
d	YE					