Name _____

Date _____ Per. _____

Population Growth in *Lemna minor*

Background

A population is a group of individuals of the same species living in the same area at the same time. In stable environments, the number of individuals in a population remains relatively constant. In rapidly changing environments, however, many individuals may die within a short period, leaving only a few survivors to perpetuate the species. In either case, the survival of the population depends on its ability to increase in number.

Models of population growth help scientists understand how a population's size changes over time. These models have applications in microbiology, wildlife management, pest management, agricultural productivity, and toxicology.

Lemna minor, or duckweed, is a member of the family Lemnaceae. This tiny organism is ideal for population growth experiments because it reproduces quickly, requires minimal space to grow, and requires no maintenance. This freshwater plant can be identified by its buoyant, leaf-like structures, called fronds (see *Fig*. 1a). Duckweed plants have one root, and grow by vegetative budding (see *Fig*. 1b). *Lemna* is found in still waters from temperate to tropical zones (though it prefers temperatures around 25˚C). Duckweed is the smallest flowering plant.

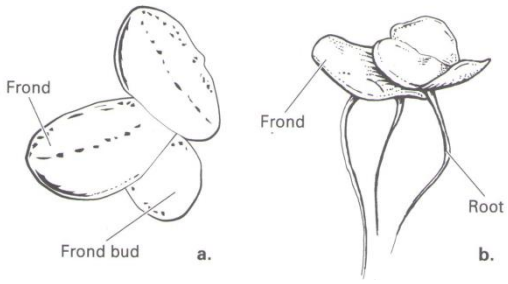

Fig. 1 (a) top view; (b) side view

On average, fronds live four to five weeks. Due to its rapid growth rate, duckweed is utilized in a variety of governmental and commercial practices. For example, the US EPA requires companies that make pesticides to determine how the chemicals affect aquatic plant biology. Many companies use duckweed as the model plant, measuring a pesticide's toxicity by observing its effect on the growth rate of duckweed.

Duckweed is also used to remove nitrogen and phosphorous from wastewater. Nitrogen and phosphorous are plant nutrients that in high concentrations (as in wastewater) promote rapid plant growth. When wastewater is released into the environment untreated, new plant growth can quickly clog waterways and cause eutrophication. To remove nutrients from wastewater, *Lemna* plants are added to treatment tanks to act as natural filters. As the plants grow, they take up nitrogen and phosphorous from the wastewater. When the duckweed plants die, they are harvested, composted, and used as mulch. The treated wastewater continues to the next stage of water purification.

Besides applications in toxicity testing and water treatment, duckweed performs well as a food resource. *Lemna* has high nutrient content and is a viable livestock alternative. Duckweed gives an advantage in poultry feed by naturally offering pigments that are otherwise provided through expensive supplements. Additionally, *Lemna* can be used by wildlife as shelter and food. Duckweed is beneficial in pond communities because it reduces the rate of evaporation and restricts algal blooms.

During this lab, you and your classmates will test *Lemna minor*'s population growth rate and carrying capacity in a variety of growing mediums or treatments. The treatments being studied are conditions that may be present in natural waterways. Your class will test the following conditions: salinity, phosphate level, nitrate level, amount of shade, and pH level. One group will also monitor a control test tube held under normal growing conditions, as shown in Table 1.

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Your group will test one environmental condition at three different treatment levels. For example, salinity is tested at relative low, medium, and high levels. All parameters are tested using three replicates, and frond growth is monitored over 21 to 28 days. After making observations and gathering and analyzing data, you will identify the optimal growing conditions for *Lemna minor*.

Materials: petri dish, spatula, test tube rack, 4 pipettes, 50 mL graduated cylinder, 9 test tubes, flask, ~450 mL of springwater, parafilm, china markers, masking tape

Procedure:

You will prepare and label nine test tubes – three replicates of three different treatment types. Use a china marker to label the test tubes. Label three test tubes with the name of each treatment type, and label the three replications of each treatment (e.g. R1, R2, R3) so that you can tell the replicates apart.

Table 1: Test Tube Preparation

Group and Test Tube Type	Medium (Springwater)	Treatment and Label
Group Saline	48 mL 47 mL 46 mL	2 mL saline, *Low Salinity* 3 mL saline, *Medium Salinity* 4 mL saline, *High Salinity*
Group Phosphate	49 mL 48 mL 47 mL	1 mL phosphate, *Low Phosphate* 2 mL phosphate, *Medium Phosphate* 3 mL phosphate, *High Phosphate*
Group Nitrate	49 mL 48 mL 47 mL	1 mL nitrate, *Low Nitrate* 2 mL nitrate, *Medium Nitrate* 3 mL nitrate, *High Nitrate*
Group Shade	50 mL 50 mL 50 mL	4 screens, *Full Shade* 2 screens, *Partial Shade* 1 screen, *Light Shade*
Group pH	40 mL 40 mL 40 mL	10 mL pH 5 buffer, *pH 5* 10 mL pH 6 buffer, *pH 6* 10 mL pH 7 buffer, *pH 7*
Group pH/Control	40 mL 40 mL 50 mL	10 mL pH 8 buffer, *pH 8* 10 mL pH 9 buffer, *pH 9* Nothing added, *Control*

1. Test tube preparation: Add the solutions as directed in the following steps for the treatment assigned to your group.

   Group ___: Saline

   Measure 48 mL of springwater in a graduated cylinder and pour into a test tube. Add 2 mL of saline solution to the test tube with a pipette. Label the test tube Low Salinity. Repeat this process two times. Next, measure 47 mL of springwater in a graduated cylinder and pour into a test tube. Add 3 mL of saline solution to the test tube with a pipette. Label the test tube Medium Salinity. Repeat this process two times. Finally, measure 46 mL of springwater in a graduated cylinder and pour into a test tube. Add 4 mL of saline solution to the test tube with a pipette. Label the test tube High Salinity. You should now have three test tubes for every salinity level assigned to your group.

Group ___: Phosphate

Measure 49 mL of springwater in a graduated cylinder and pour into a test tube. Add 1 mL of phosphate solution to the test tube with a pipette. Label the test tube Low Phosphate. Repeat this process two times. Next, measure 48 mL of springwater in a graduated cylinder and pour into a test tube. Add 2 mL of phosphate solution to the test tube with a pipette. Label the test tube Medium Phosphate. Repeat this process two times. Finally, measure 47 mL of springwater in a graduated cylinder and pour into a test tube. Add 3 mL of phosphate solution to the test tube with a pipette. Label the test tube High Phosphate. Repeat this process two times. You should now have three test tubes for every phosphate level assigned to your group.

Group ___: Nitrate

Measure 49 mL of springwater in a graduated cylinder and pour into a test tube. Add 1 mL of nitrate solution to the test tube with a pipette. Label the test tube Low Nitrate. Repeat this process two times. Next, measure 48 mL of springwater in a graduated cylinder and pour into a test tube. Add 2 mL of nitrate solution to the test tube with a pipette. Label the test tube Medium Nitrate. Repeat this process two times. Finally, measure 47 mL of springwater in a graduated cylinder and pour into a test tube. Add, 3 mL of nitrate solution to the test tube with a pipette. Label the test tube High Nitrate. Repeat this process two times. You should now have three test tubes for every nitrate level assigned to your group.

Group ___: Shade

Measure 50 mL of springwater in a graduated cylinder and pour into a test tube. Add a three-frond colony that has no discoloration. Cover the top of the test tube with four (4) layers of screens. Secure the screens with a rubber band. Label the test tube Full Shade. Repeat the 4-screen setup two more times. Measure 50 mL of springwater in a graduated cylinder and pour into a test tube. Add a three-frond colony that has no discoloration. Cover the top of the test tube with two (2) layers of screens. Secure the screens with a rubber band. Label the test tube Partial Shade. Repeat the 2-screen setup two more times. Finally, measure 50 mL of springwater in a graduated cylinder and pour into a test tube. Add a three-frond colony that has no discoloration. Cover the top of the test tube with one (1) screen. Secure the screen with a rubber band. Label the test tube Light Shade. Repeat the I-screen setup two more times. You should now have three test tubes for every shade level assigned to your group. Proceed to Step 5.

Group ___: pH 5, pH 6, and pH 7

Measure 40 mL of springwater in a graduated cylinder and pour into a test tube. Add 10 mL of pH 5 solution to the test tube. Label the test tube pH 5. Repeat the process two more times. Measure 40 mL of springwater in a graduated cylinder and pour into a test tube. Add 10 mL of pH 6 solution to the test tube. Label the test tube pH 6. Repeat the process two more times. Measure 40 mL of springwater in a graduated cylinder and pour into a test tube. Add 10 mL of pH 7 solution to the test tube. Label the test tube pH 7. Repeat the process two more times. You should now have three test tubes for every pH level assigned to your group.

Group ___: ph 8, pH 9, and Control

Measure 50 mL of springwater in a graduated cylinder. Pour the springwater into a test tube. Label the test tube Control. Repeat the process two times. Next, measure 40 mL of springwater in a graduated cylinder and pour into a test tube. Add 10 mL of pH 8 solution to the test tube. Label the test tube pH 8. Repeat the process two more times. Measure 40 mL of springwater in a graduated cylinder and pour into a test tube. Add 10 mL of pH 9 solution to the test tube. Label the test tube pH 9. Repeat the process two more times. You should now have three control test tubes and three test tubes for each pH level assigned to your group.

2. Select a *Lemna minor* colony with three fronds. Using a spatula, gently transfer the colony to a test tube. To avoid cross-contamination of solutions, rinse the spatula thoroughly after placing the plants in each test tube.
3. Cover all of the test tubes with parafilm.
4. To allow transpiration, poke eight holes the size of a sharpened pencil tip in the parafilm.
5. Put the test tubes in a test tube rack and label the rack with your period, group number, and treatment using masking tape.
6. Place the rack under a fluorescent light source. Leave the light on continuously for the duration of the project.

Data Collection

1. In your lab notebook, in the format of Table 2, construct 9 data tables – one for each replicate for each treatment tested. Each table should have 6 columns and at least 16 rows.

Table 2

Treatment: _____ **Starting Date:** _____

Replicate: _____

Day Number	Daily Frond Count	New Frond Growth	Days Since Previous Count	Change in the Number of Fronds per Day	Change in the Number of Fronds per Day per Frond
0	3				

2. According to your teacher's instructions, count the number of adult fronds and frond buds daily during the first week, and every second or third day thereafter.
3. Record the data of each test tube in a table. On the day the fronds are counted, write down the day number since the start of the project and the number of fronds counted.
4. Calculate the data for the remaining columns (refer to "Estimating Biotic Potential" below).
5. Add notes beside each day's record regarding any changes in the health of the fronds. This information can aid later discussions about *Lemna* population growth.
6. Estimating Biotic Potential:
   The biotic potential of a population (or the maximum, unrestricted growth rate) is determined by finding the interval of time when the population growth rate is highest. Use Table 2 to identify the time interval (i.e., the day when the number of new fronds per day per frond is greatest) for each treatment.

   Count the number of fronds in the test tube and record this as the "Daily Frond Count" for the day. Then, calculate the difference between the current "Daily Fond Count" and the previous count. Record the result as "New Frond Growth." Divide the number in the "New Frond Growth" column by the number of days since the previous count. Record the result in the column, "Change in the Number of Fronds per Day." Divide the number in the "Change in the Number of Fronds per Day" column by the "Daily Frond Count" total from the previous day. Record this data in the column, "Change in the Number of Fronds per Day per Frond." Use the data in this column to determine the time interval when the population growth rate is highest.

7. Use the equation $N_t = N_0 e^{rt}$ to determine the population's intrinsic rate of increase, which is represented by "r" in the equation. You should do this for each of your treatments separately. Then calculate the average value for "r" for each of your treatments.
8. When the experiment is concluded, collect the data produced by each of the groups and record them in your notebook. For each treatment level, average the values obtained for carrying capacity and intrinsic rate of increase. Use these replicate averages to create a whole-class results data table in your lab notebook. Collect data for the control and for each treatment investigated: saline, phosphate, nitrate, shade, and pH. Include columns for carrying capacity and intrinsic rate of increase.

Graph

Plot the frond count over time for each treatment level and replicate. Ideally, the curves will closely resemble each other in slope and height. To distinguish between the treatments, draw the lines with three different colors or line styles.

Determine the carrying capacity for each test tube using the graph. Draw a horizontal line to indicate the value of the carrying capacity and label the line with "*K* = ." Include the *r* value for each curve. Label each curve with its *K* value and *r* value.

Results & Analysis

On a separate sheet of paper, answer the following questions thoroughly using complete sentences. You may complete your work on the computer. Staple your work, including the graph(s) to the back of this packet.

1. What can you conclude from your evidence about the effect of the treatment on population growth rate?
2. Label the point on a line curve where the population growth rate is
    i. exponential.
    ii. increasing at a decreasing rate.
3. Look at the class data table.
    a. Which habitat treatment is most conducive to fast population growth?
    b. Which habitat treatment is most conducive to high carrying capacity?
4. Based on the class results, choose the treatment levels for each environmental parameter that is optimal for *Lemna minor*.
5. Is there a relationship between the number of screens on a test tube and carrying capacity? Is there a relationship between the number of screens on a test tube and duckweed's intrinsic rate of increase?
6. Does a population's intrinsic rate of increase change with habitat, or is it an inherent characteristic?
7. Support your conclusion with references to your data.
8. Compare and contrast the effects of nitrate and phosphate on duckweed. Does one nutrient have a greater impact on duckweed growth and/or carrying capacity? Be sure to use the calculated data in your explanation.
9. How are birth and death rates accounted for in the exponential population growth equation?
10. What assumptions are made about the plants in this experiment?
11. What are some possible sources of error in this experiment?