A COMPARISON OF THE EFFECTS OF PHOSPHATE CONCENTRATIONS ON POPULATION GROWTH DYNAMICS OF THE DUCKWEED *LEMNA MINOR* GROWN IN DISTILLED AND SPRING WATER

by

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ABBREVIATIONS, CONSTANTS AND SYMBOLS

K	Carrying capacity
r_{max}	Intrinsic rate of increase
r-value	Per-capita rate of increase
ppm	Parts per million
ANOVA	Analysis of variance
DW	Dry weight
ATP	Adenosine triphosphate

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1. ABSTRACT

In this experiment duckweed *Lemna minor* was grown for 31 days under controlled laboratory conditions. Duckweed was grown in test tubes as control or treatment groups in the following solvents: spring water, lab-distilled water, or commercial-distilled water. Control groups had no additional phosphate. Treatment groups had phosphate added to make various phosphate concentrations. Five tubes were made for every nutrient condition with three-frond duckweed plants placed into each. The number of fronds was counted throughout the experimental period. Carrying capacity and r_{max} were calculated to determine growth levels. Results for spring water showed a significant ($P \leq 0.05$) difference in the carrying capacity for 10 ppm compared to each treatment group. Carrying capacity followed Liebig's law of the minimum and r-max Shelford's law of tolerance. Lab-distilled water showed less growth and lower r_{max} values compared to spring water. The results showed that 50-100 ppm had a significantly higher ($P \leq 0.05$) carrying capacity and r-max compared to 0 ppm. However, trends were not followed while using lab-distilled water. When comparing the controls of each solvent, it was found that lab-distilled water had a significantly lower carrying capacity compared to spring water and commercial-distilled water. It is most likely due to issues with the filtration system or plasticizers from the carboy. These results show the importance of determining phosphate concentrations required for optimal population growth and which concentrations have negative growth effects.

2. INTRODUCTION

2.1 General introduction to population growth dynamics

Duckweed growth is characterized by population growth dynamics. Fluctuations constantly occur in communities as individuals are born and die. Growth defines the changes in size and structure over time. Exponential growth is when populations become greater in proportion to their total growing size, creating a rapid increase with passing time. It occurs when the size of the population increases by the same percentage per unit in time. The growth occurs at the same percentage, not necessarily the same amount. A graph shows an accelerated increase in the size of a population, which appears as a J-shaped curve. Exponential growth depends on the ability of individuals in a population to reproduce and survive under their environmental conditions (Freeman et al., 2017).

Growth can be described using per-capita rate of increase (r-value). It is each individual's contribution to the initial population, compared to the increase of the population. The value is positive when the birth rate is greater than the death rate and is negative when it is the opposite. Exponential growth occurs when the r-value does not change over time. The intrinsic rate of increase (r-max,) is the maximal r-value found in optimal conditions. These conditions are achieved when the birth rates per individual are maximized, and death rates are minimized (Freeman et al., 2017).

Populations cannot grow exponentially indefinitely and will eventually reach their carrying capacity (K) for environmental conditions. Carrying capacity is the maximum number of individuals supported in an environment. It depends on limiting factors such as nutrients, water, light, disease, and space. As a population approaches the carrying capacity, the growth rate slows. Carrying capacity is an important factor in logistic population growth. This growth

begins as exponential growth but levels off at the carrying capacity. It appears as an S-shaped curve on a graph. Exponential growth is density independent, whereas logistic growth is density dependent (Freeman et al., 2017).

2.2. Taxonomic classification and natural history of the duckweed *Lemna minor*

The scientific name for duckweed is *Lemna minor* (*Lemna perpusilla*, 2021). The classification of duckweed is shown in *Table 1.* Duckweed is part of the domain eukarya, the kingdom plantae, the phylum tracheophyta (vascular plant), the class spermatophyta (plants that produce seeds), the order Magnoliopsida (flowering plants), the family araceae (borne on a type of inflorescence called a spadix), the genus lemna and the species *Lemna minor* (Lemna minor, 2021).

Classification	**Classification in *Lemna minor***
Domain	Eukarya
Kingdom	Plantae
Phylum	Tracheophyta
Class	Spermatophyta
Order	Magnoliopsida
Family	Araceae
Genus	Lemna
Species	*Lemna minor*

Table 1. The classification of *Lemna minor* (duckweed), including domain, kingdom, phylum, class, order, family, genus, and species.

Duckweed is a flowering aquatic plant located in quiet waters. It is found in a variety of habitats, including ponds, marshes, lakes, or slow-moving streams. There are nine duckweed species in North America. The common duckweed is the most widespread species, ranging across Canada and the United States. Duckweed is composed of single, flat small oval leaves

called fronds, and roots. The fronds float on the water's surface and roots submerge to absorb nutrients (Fertig, 2021). *Figure 1* shows a diagram of duckweed, with frond, frond buds, and roots labeled.

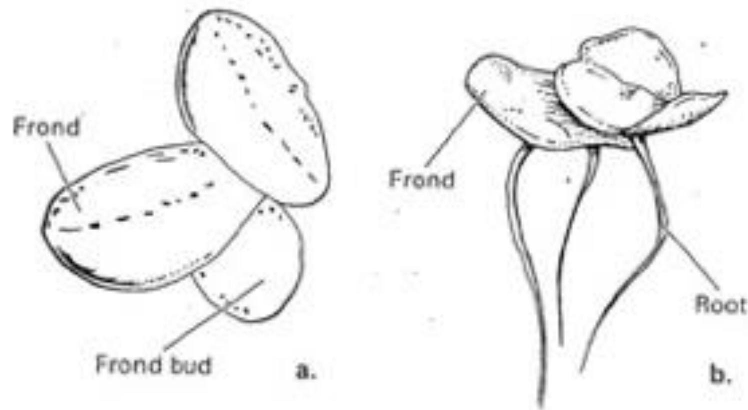

Figure 1. *Lemna minor* (duckweed) consists of small, oval leaves called fronds, new vegetative growth called buds, and roots submerged in water to absorb nutrients (Carolina Biological, 2008).

Duckweed grows in dense floating mats piled up in several layers. The biodiversity in the water under mats is often low. The environment is highly anaerobic and has substantial light competition (Driever et al., 2005). The flowering part of the duckweed consists of two microscopic staminate flowers and one tiny pistillate flower in a pouch-like sac. Duckweed is the world's smallest flowering plant. Their flowing structure is commonly not visible to the naked eye. Most duckweeds reproduce asexually by forming chains of new stems in the form of vegetative buds. Less commonly, duckweed will reproduce sexually through seeding. This process usually occurs during winter when seeds fall from old fronds and sink to the bottom to germinate. (Fertig, 2021). Duckweed can grow at temperatures ranging from 5°C to 35°C (Lasfar et al., 2007). Most species have an optimum growth at 26°C. Duckweed reproduces quickly until all nutrients have been consumed at this temperature. On either side of this optimal value, growth decreases significantly, as viewed in *Figure 2* (Lasfar et al., 2007).

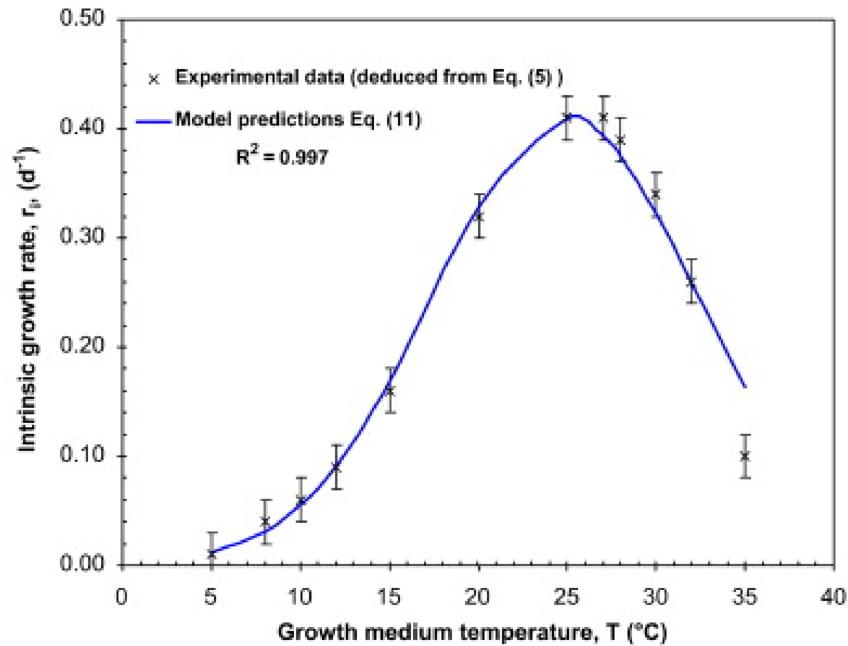

Figure 2. The growth rate of duckweed depends on temperature. Optimal growth is at 26°C. On either side of this value the growth rate decreases. Temperatures lower than 10°C or higher than 35°C strongly inhibits growth (Lasfar et al., 2007).

Duckweed is a valuable model organism for laboratory work due to its high reproduction rate, small size, and easy maintenance. Fast growth can be used for bioremediation of waterways from excessive agricultural runoff of phosphorus (Fertig, 2021). Duckweed can grow in different conditions such as, dirty, saline, or eutrophic waters, allowing for successful biological treatment of polluted waters (Sivaci et al., 2017). Research is also developing techniques to use genetically modified duckweeds to synthesize commercially valuable proteins, such as insulin (Fertig, 2021).

2.3. The importance of macronutrients on population dynamics, in general, with a particular focus on *Lemna minor*

Nutrients needed in large quantities (exceeding 1.0 mg/L) are macronutrients. (Sharma, 2006). Regular growth, development, fruiting, and blooming of duckweed require macronutrients (Subramanian et al., 2021). Carbon, hydrogen, nitrogen, oxygen, phosphorus, and sulfur are macronutrients required by duckweed. With the exemption of oxygen, all nutrients can be

obtained through fertilizers. Macronutrients are used to produce carbohydrates, lipids, proteins, and nucleic acids. These biological molecules are essential for life (Broyer & Stout, 1959). Carbohydrates are made up of carbon, hydrogen, and oxygen. The covalent bonds connect the macronutrients with frequent polar hydroxyl (-OH) groups (Grindley, 2001). Carbohydrates create duckweed's structure, store energy, and make up the bulk of organic material moving through the phloem. Additionally, glucose formed during photosynthesis is used in cellular respiration to release energy (Wardlaw, 1968). Lipids are a family of molecules composed of carbon, hydrogen, and oxygen. The carbon atom makes a single covalent bond with other carbon and hydrogen atoms to form saturated or unsaturated fatty acids (Meara, 1955). Lipids function in membranes and energy sources for seed germination (Mumtaz et al., 2020). Proteins are composed of carbon, hydrogen, oxygen, and nitrogen. Amino acids are linked creating proteins, and they consist of an amino group ($-NH_2$), a carboxyl group (-COOH), and a variable R-group (Branden & Tooze, 1991). Proteins have several enzymatic, structural, and functional roles in duckweed. They additionally act as storage mediums for the nutritional demands of developing seedlings (Rasheed et al., 2020). Nucleic acids, such as DNA and RNA, contain carbon, hydrogen, oxygen, nitrogen, and phosphorus. Nucleotides consist of a nitrogen-containing aromatic base, pentose five-carbon sugar, and a phosphate group. Nucleic acids make up the genetic material of duckweed (Neidle, 2008).

2.4 Chemical introduction to the phosphate molecule

Phosphate is an important molecule for duckweed physiology. The charged ion ($[PO_4]^{-3}$) has a molar mass of 94.97 g/mol. The structure consists of a central phosphorus atom surrounded by four oxygen atoms in a tetrahedral arrangement, as seen in *Figure 3* (Schirber, 2012).

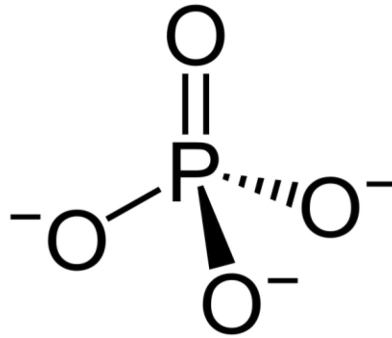

Figure 3. The structure of phosphate ($[PO_4]^{-3}$) with a central phosphate atom surrounded by four oxygen atoms in a tetrahedral shape (Wypych, 2013).

Phosphate has many cellular functions. It makes up the hydrophilic heads of the phospholipid bilayer of cell membranes. Membranes are semi-permeable allowing only select molecules to diffuse across. Small hydrophobic molecules, such as O_2 and CO_2, can cross membranes rapidly. Larger molecules, like glucose; require active transport to pass through. Phosphate also makes up part of the structure of adenosine triphosphate (ATP), the energy carrier of cells. ATP consists of a chain of three phosphate groups, along with a nitrogenous base and ribose sugar. When one of three phosphate groups are removed energy is released. The energy can perform many cellular functions, such as DNA replication, cell division, or protein synthesis. Phosphate is also necessary for the structure of DNA and RNA, which consists of a 5-carbon deoxyribose sugar, nucleotide, and phosphate group. Phosphate is also involved in phosphorylation, which is an important mechanism for altering the activity of proteins after they have been synthesized. In the process, a phosphate group is added to a protein by specific enzymes called kinases. The removal of a phosphate group is called dephosphorylation (Schirber, 2012).

2.5 Phosphate concentrations and population density affect the growth of the duckweed *Lemna minor*

Phosphate is considered a primary nutrient for duckweed growth, development, and reproduction. It is vital for many cellular components (Sivaci et al., 2017). The capacity of phosphate uptake affects biomass, growth, production, and quality of duckweed (Abdolzadeh et al., 2010). Duckweed must receive correct phosphate levels to maintain homeostasis (Razaq et al., 2017). Reviewing the literature on how phosphate and density affect duckweed creates an understanding of growth dynamics (Lasfar et al., 2007).

The article *Intrinsic growth rate: A new approach to evaluate the effects of temperature, photoperiod, and phosphorus–nitrogen concentrations on duckweed growth under controlled eutrophication* determined duckweed intrinsic growth rate as a function of temperature, photoperiod, and phosphorus-nitrogen concentrations. The experiment found optimal growth ranges for each condition. The results for phosphorus showed that intrinsic growth rate was practically constant for phosphorus concentrations ranging from 1 to 20 ppm (parts per million). It decreased rapidly for concentrations less than 1 ppm, and for concentrations more than 20 ppm, it was slightly inhibited. It was hypothesized that concentrations of phosphorus higher than 1 ppm do not significantly influence intrinsic growth. The results are demonstrated in *Figure 4* (Lasfar et al., 2007).

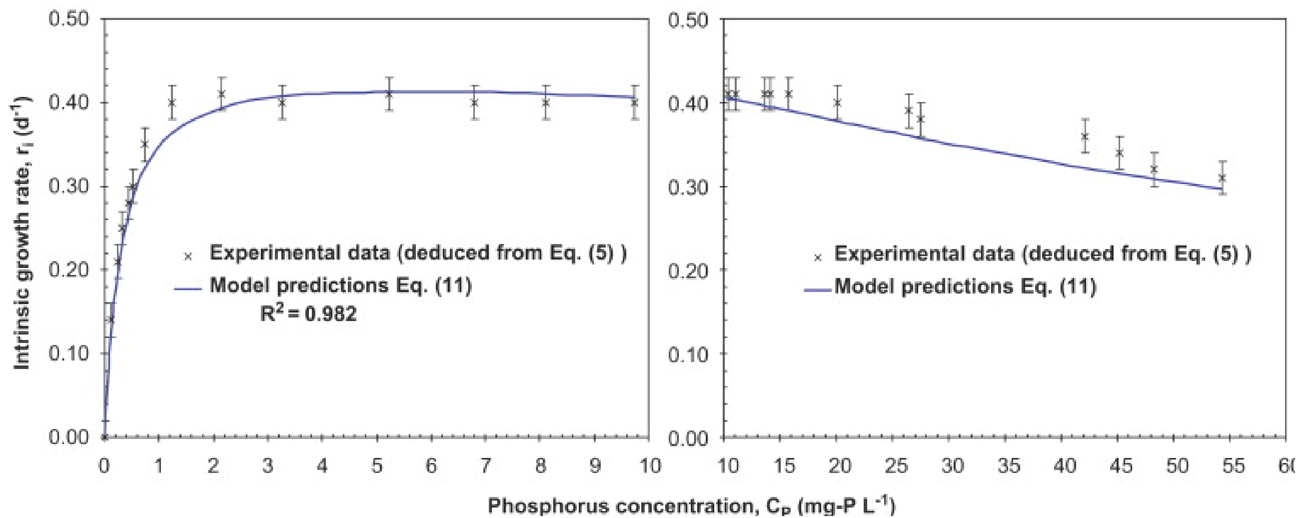

Figure 4. Intrinsic growth rate in dependence of phosphorus concentrations (1 mg phosphorus /L = 1 ppm). It was constant from 1-20 ppm, decreased rapidly at less than 1 ppm, and slightly inhibited at more than 20 ppm (Lasfar et al., 2007).

The study *Evaluation of Some Physiological Parameters of Lemna Minor L. Exposed to Different Hypertrophic Phosphate Levels* evaluated several biochemical properties, such as Chlorophyll a, chlorophyll b, carotenoids, phenolic compounds, thiobarbituric acid reactive substances, and protein contents with antioxidant enzyme activities (glutathione reductase and catalase). Duckweed was exposed to different phosphate concentrations for 24, 48, 96, and 144 hours in laboratory conditions. The results showed that an oversupply of phosphorus for long periods of time decreased protein amounts and increased chlorophyll b. These function as adaptive responses to phosphate overexposure (Sivaci et al., 2017).

The experiment *Influence of Nitrogen and Phosphorus on the Growth and Root Morphology of Acer Mono* studied the influence of phosphorus on *Acer mono* (commonly known as maple). The results found that phosphorus fertilization significantly affected the growth and root morphology of *A. mono* seedlings. When the seedlings did not receive sufficient nutrients, they showed lower plant height, root collar diameter, chlorophyll, carotene content, and several root morphology parameters. When seedlings were supplied with optimal phosphorus (8 g plant-

^{1}), maximum values of the parameters were recorded. This study hypothesized that optimal phosphorus levels can be used to ensure the production of healthy *A. mono* seedlings with high levels of growth (Razaq et al., 2017).

The study *Growth Limitation of Lemna Minor Due to High Plant Density* tested growth limitations in *Lemna minor* populations due to high plant density. Results found at high densities (biomass above 180 g DW/m^2) there were slightly negative growth rates. Fronds grow as dense mats piled up in several layers. This piling creates an upper part with nutrient limitations, mostly phosphate and nitrate, and a lower part with light and CO_2 limitations. Negative growth rates are the result of limiting factors of carrying capacity. The experiment also found that at low densities (biomass below 9.5 g DW/m^2) increasing density increased growth rate. The increase was most likely due to higher temperatures caused by solar radiation being trapped in mats at low densities. The experiment proposed growth increase at low densities due to an initial piling of fronds, but as piles reach higher densities there is a decrease due to carrying capacity. This creates a logarithmic model dependent on carrying capacity, displayed in *Figure 5.* The competition for phosphate, nitrate, light, and space also follows this model (Driever et al., 2005).

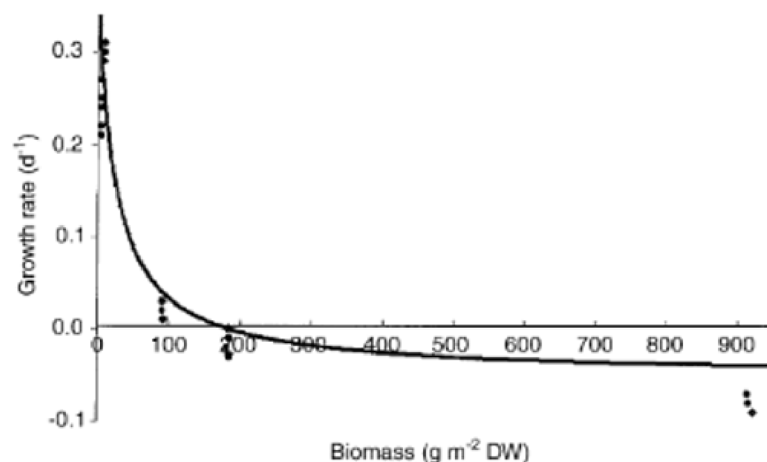

Figure 5. Growth rate as a function of the initial biomass of duckweed. The growth initially increased with increasing density (below 9.5 g DW/m^2), before leveling off at carrying capacity, and eventually at high densities (above 180 g DW/m^2) showed a negative growth rate (Driever et al., 2005)

2.6. The importance of micronutrients (trace elements) on plant growth with a particular focus on *Lemna minor* and phosphate

Micronutrients are important for the life cycle of duckweed. All autotrophic plants require the use of the following micronutrients: potassium, calcium, magnesium, iron, manganese, copper, zinc, molybdenum, boron, and chlorine. These nutrients are specific and cannot be replaced. They are obtained from soil-forming minerals. Micronutrients have many important roles, including structurally in organic compounds, such as membranes. Alterations in plasma membranes have been linked to zinc deficiency. Micronutrients also have a catalytic role as drivers and regulators of enzyme-catalyzed reactions. Another function is electron receptors. Micronutrients assist in controlling the flow of electrons between molecules in enzyme redox reactions. This phenomenon is seen in the mitochondria and chloroplast as part of the electron transport chain. Osmoregulation is affected by the number of micronutrients. Chlorine and potassium fluxes regulate water flow, inducing turgor changes that control the stoma opening. Lastly, micronutrients have an important role in reproduction. Low reproduction rates have been linked to a deficiency in micronutrients. This reduction is due to the lowering of photosynthetic efficiency. There is a quantitative requirement for micronutrients for optimal growth. If there are not enough micronutrients, the structure, function, and development of plants is negatively affected (Sharma, 2006).

Micronutrients and phosphorus together impact duckweed development. The buildup of phosphorus in environments decreases growth or causes death in plants. Excessive soil phosphorus reduces the plant's ability to take up required micronutrients, especially iron and zinc. In high phosphorus conditions, iron and zinc are quickly converted to non-available forms. Iron deficiencies are characterized by yellowing between the leaf veins, and zinc by the bleaching of tissue (Provin & Pitt, 2008).

2.7. Significance of this research

The significance of this research was to empirically show the population growth dynamics of the duckweed *Lemna minor* under experimental conditions. It specifically showed how the concentration of available phosphate affects population growth dynamics (r_{max} and K). It is important to know which phosphate concentrations are required for optimal population growth and which concentrations have negative effects. The study also provided important insights into how storage of distilled water in carboys can potentially inhibit duckweed growth.

3. OBJECTIVES

The research objective was to examine and compare the effects of phosphate on the population growth dynamics of the duckweed *Lemna minor* growing in spring water and distilled waters.

The study idea was to vary the amount of phosphate available while duckweed grew in a controlled environment. Each test tube contained either lab-distilled water, commercial-distilled water, or spring water. Some test tubes were control groups, having no added phosphate. The other test tubes were treatment groups with additional phosphate. A three-frond duckweed was added to each test tube and placed under continuous lighting and temperature. The number of fronds was monitored throughout the experimental period of 31 days.

Growth was characterized by the appearance of a new frond. The number of fronds in tubes was monitored and compared. The results were statistically analyzed for interpretation and graphed on a line plot. The data were used to determine the growth dynamics in terms of r_{max} and K. Statistical analysis was conducted to see if the results were significant.

4. MATERIALS AND METHODS

4.1 Experimental species

Lemna minor duckweed was the experimental species in the experiment. Duckweed was ordered from Carolina Biological Supply Company. It was kept in a finger bowl (seen in *Figure 6*) filled with Poland Spring® 100% Natural Spring Water to mimic the abundance of macronutrients and micronutrients found in their natural environment. The spring water promoted growth producing dense floating mats on the water surface. The bowl was kept in a laboratory at a room temperature of approximately 21°C and under a grow light as described in the experimental setup.

Figure 6. The experimental species *Lemna minor* at room temperature (about 21°C) under a grow light. They grew on top of spring water in the finger bowl.

4.2 Procedure for preparing all experimental frond populations

A long-term experiment was used to collect data on duckweed population growth dynamics. Each time the experiment was conducted it followed the same general procedure with

phosphate, but various solvents were used. The solvent was measured with a graduated cylinder. Phosphate was added using the dropper on the bottle. Phosphate was obtained from Carolina Biological Supply Company. It was a phosphate solution composed of water (99.63%), potassium phosphate monobasic (0.20%), and potassium phosphate dibasic (0.17%). When making the test tubes, phosphate was always added before the solvent for mixing. Five nutrient concentrations were created with varying amounts of solvent and phosphate. Five tubes were made for each nutrient concentration, creating a sample size of $n = 5$. In the control tubes, only 50 mL of solvent was added. There was no phosphate to achieve a baseline. In the next test tubes, 49.5 mL of solvent and 10 drops of phosphate were put into each test tube. The 0.5 mL was added with a pipette. Next, 49 mL of solvent and 20 drops of phosphate were used. The following phosphate environment was created with 40 drops of phosphate and a solvent concentration of 48 mL. Lastly, 47 mL of solvent was added to 60 drops of phosphate. After the nutrient concentrations were created, duckweed was added to each tube. Duckweed was picked from a colony growing in a finger bowl and added to the surface of the water. The collection tool had a slender metal end. Duckweed was chosen which had only three fronds. All three fronds had to be attached, but they could be a variety of sizes, including small buds. A three-frond duckweed selected is observed in *Figure 7*.

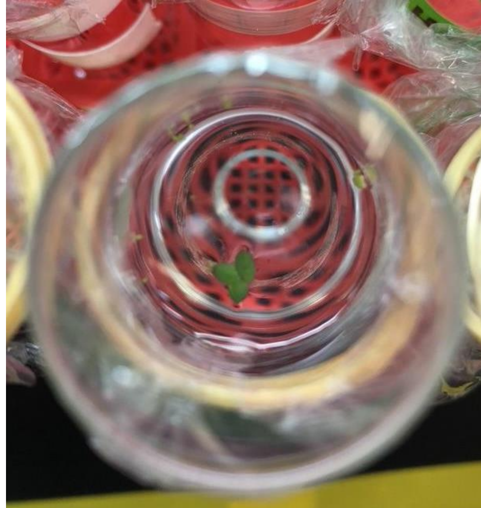

Figure 7. A duckweed chosen from the colony with exactly three fronds. It was added to the surface of a test tube with the desired solvent and phosphate concentration.

After adding duckweed, plastic wrap was placed around the top of the tube and secured with elastic bands. This technique aimed to prevent spillage and evaporation. The last step was to poke eight holes in the plastic wrap to allow gas exchange to occur. Tubes were placed in a test tube rack under a growth light. Grow lights are electric lights used to assist plant growth by providing a light spectrum like the sun. Finalized test tubes under the grow light are seen in *Figure 8*.

Figure 8. The final setup of the duckweed experiment with test tubes in a rack with various phosphate and solvent concentrations, duckweed with three fonds, plastic wrap, and elastic bands sitting under the grow light.

4.3. Solvents used for experimental frond populations

During the experiment three different solvents were used: lab-distilled water, commercial-distilled water, and spring water. Lab-distilled water was purified using a Thermo Fisher Scientific Barnstead Classic Electric Still with a model number A1016-X003 in the Wagner College laboratory. Commercial-distilled water was purchased from ShopRite by the brand Bowl and Basket. It was purified by steam distillation, micron filtration, and UV disinfection to ensure quality. Distilled water removed 99.9% of all minerals found in water. Spring water was Poland Spring® 100% Natural Spring Water. The following nutrients were reported in the 2020 Poland Spring® 100% Natural Spring Water analysis: 0-0.014 ppm of bromide, 3.7-12 ppm of calcium, 0-14 ppm of chloride, 0-0.25 ppm of fluoride, 0.67-1.6 ppm of magnesium, 1.6-9.1 ppm of sodium, 0-0.88 ppm of nitrate, and 0-8.1 ppm of sulfate. All other nutrients tested were not detected (Poland Spring®, 2020).

4.4. Procedure used to measure changes in population size over time

The growth of the duckweed was recorded three days a week: Mondays, Wednesdays, and Fridays. The days since the experiment began were noted every day. The experiment setup was day 0. The daily frond growth was also recorded. On day 0, the frond growth for all tubes was three. The total number of fronds in each tube were counted. Every frond, even small buds, were included. Each frond on duckweed plants were considered an "individual", instead of each duckweed plant being the “individual”. The results were analyzed using the number of counted fronds in each test tube as the population size. When counting fronds, it was noted if they were together or broken apart, the color, and any other significant observations. Magnifying glasses were used to view the small buds. The counting ended when the fronds had fallen apart, became

brown, and ceased to grow. The room temperature was also recorded using a thermometer in a beaker filled with water also placed under the grow light.

Additional results were obtained through light readings. The readings were taken using a LI-COR LI-250A light meter. The sensor used was LI-COR Quantum 47836. The light detector was placed in the front, middle, and back of the grow light station. Three readings were recorded for the front and back, and four were recorded for the middle. The average of the readings determined light activity in each position. The units used were $\mu mol/m^2/s$. Micromoles are the number of photons coming down from the light area per unit of area per second.

Nutrient strips were used to determine the concentration of phosphate in each test tube used in the experiment. The strips were Phosphate 0-100 ppm Test's from the company Bartvation. The nutrient strips were dipped in the test solution for one second, and then after three minutes were held up to the color comparison on the bottle. The results were recorded in ppm phosphate.

4.5. Calculations of r-max and K

To display population growth dynamics carrying capacity and r-max were calculated. Data from each of the five tubes were averaged to create only a singular table with average daily frond growth. Carrying capacity and r-max calculations used the averaged data. Carrying capacity was the maximum amount of growth for a specific nutrient treatment. The r-max values were found through a sequence of calculations. The first step was to subtract the daily frond growth by the prior day's growth. This found new frond growth. Next, days since the previous count was found by subtracting the days since the experiment started by the prior day's number. The change in the number of fronds per day was determined by dividing the new frond growth by the days since the previous count. Lastly, the change in the number per day was divided by

the daily frond growth of the previous day. This value was the change in the number of fronds per day per frond, or the r-values. The r-max value was the highest r-value found for a specific nutrient treatment.

4.6. Statistical analysis of collected data

Statistical analysis was done with a widely used program called IBM® SPSS® (International Business Machines Corporation Statistical Package for the Social Sciences). A comparative mean analysis called One-Way ANOVA (analysis of variance) with four different tests was used. Firstly, descriptive statistics found statistics of location and dispersion for each group. Mean and standard deviation were relevant to the study. Next was a test of homogeneity of variances based on the mean. This tested if the samples had equal variances. If the P-value was greater than 0.05, the variances were not statistically different, and ANOVA test could be conducted without data transformation. ANOVA determined if there were statistical differences between the means of the groups. When P-values were less than 0.05 it indicated that the mean of at least one of the groups significantly differed from the mean of at least one other group. The last analysis was a Tukey test. The purpose of this test was to determine which group significantly differed from which other group. It compared the means of all groups to the mean of every other group to determine if the relationship between two sets of data were statistically significant. When the P-value was less than 0.05 it was statistically significant. Statistical analyses comparing carrying capacities and r-max values were performed for the following groups: spring water, lab-distilled water, and control of all three solvent groups.

5. RESULTS

5.1 Numerical data

The experiment data was organized into spring water, lab-distilled water, and commercial-distilled water results. The data were collected for a total of 31 days. The first 12 days had a sample size of 10 while the remaining days had a sample size of 5. Results were displayed in tables and graphically as line plots.

5.11 Spring water

The data for spring water are displayed in *Table 2*. In the 10 ppm tubes there was a carrying capacity of 9.4 fronds and an r-max of 0.13 per day. The 10-25 ppm showed a carrying capacity of 18.2 fronds and r-max of 0.24 per day. The carrying capacity for 25 ppm was 17.8 fronds with an r-max of 0.27 per day. The tubes which were 50-100 ppm yielded a carrying capacity of 17 fronds and r-max of 0.24 per day. Lastly, >100 ppm had a carrying capacity of 17.2 fronds and r-max of 0.16 per day.

Spring Water: 10 ppm

Days Since Experiment began	Daily Fond Growth	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 (per day)
0	3	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
3	4	1	3	0.33	0.11
5	5	1	2	0.5	0.13
7	6	1	2	0.5	0.1
10	7.2	1.2	3	0.4	0.067
12	7.4	0.2	2	0.1	0.014

14	8	0.6	2	0.3	0.041
17	8.6	0.6	3	0.2	0.025
19	8.6	0	2	0	0
21	8.6	0	2	0	0
24	8.6	0	3	0	0
26	8.6	0	2	0	0
28	9	0.4	2	0.2	0.023
31	9.4	0.4	3	0.14	0.015

Spring Water: 10-25 ppm

Days Since Experiment began	Daily Fond Growth	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 (per day)
0	3	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
3	5.2	2.2	3	0.73	0.24
5	6.6	1.4	2	0.7	0.13
7	8.6	2	2	1	0.15
10	11.6	3	3	1	0.12
12	12.8	1.2	2	0.6	0.052
14	13.2	0.4	2	0.2	0.016
17	14.2	1	3	0.33	0.025
19	15	0.8	2	0.4	0.028
21	15.2	0.2	2	0.1	0.0067
24	15.2	0	3	0	0
26	16.6	1.4	2	0.7	0.046
28	16.8	0.2	2	0.1	0.0060

31	18.2	1.4	3	0.47	0.027

Spring Water: 25 ppm

Days Since Experiment began	Daily Fond Growth	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 (per day)
0	3	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
3	5.4	2.4	3	0.8	0.27
5	6.4	1	2	0.5	0.093
7	8.6	2.2	2	1.1	0.17
10	11.8	3.2	3	1.07	0.12
12	12.8	1	2	0.5	0.04
14	13.2	0.4	2	0.2	0.016
17	13.6	0.4	3	0.13	0.0098
19	15	1.4	2	0.7	0.051
21	15.2	0.2	2	0.1	0.0067
24	16.2	1	3	0.33	0.022
26	16.8	0.6	2	0.3	0.019
28	17.2	0.4	2	0.2	0.012
31	17.8	0.6	3	0.2	0.012

Spring Water: 50-100 ppm

Days Since Experiment began	Daily Fond Growth	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 (per day)
0	3	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
3	5.2	2.2	3	0.73	0.24

5	7	1.8	2	0.9	0.17
7	10	3	2	1.5	0.21
10	12.4	2.4	3	0.8	0.08
12	12.8	0.4	2	0.2	0.016
14	13.4	0.6	2	0.3	0.023
17	14.4	1	3	0.33	0.025
19	14.8	0.4	2	0.2	0.014
21	15	0.2	2	0.1	0.0068
24	15.6	0.6	3	0.2	0.013
26	15.6	0	2	0	0
28	15.8	0.2	2	0.1	0.0064
31	17	1.2	3	0.4	0.025

Spring Water: >100 ppm

Days Since Experiment began	Daily Fond Growth	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 (per day)
0	3	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
3	5.2	2.2	3	0.73	0.024
5	6.4	1.2	2	0.6	0.12
7	8.4	2	2	1	0.16
10	12.4	4	3	1.33	0.16
12	12.4	0	2	0	0
14	12.6	0.2	2	0.1	0.0081
17	13.6	1	3	0.33	0.026

19	13.8	0.2	2	0.1	0.0074
21	14.6	0.8	2	0.4	0.029
24	14.8	0.2	3	0.067	0.0046
26	15.6	0.8	2	0.4	0.027
28	15.6	0	2	0	0
31	17.2	1.6	3	0.53	0.034

Table 2. Results of duckweed grown in spring water with various phosphate concentrations. Carrying capacity is the maximum daily frond growth and r-max the maximum change in the number of fronds per day per frond.

The line plot in *Figure 10* displays the daily frond growth in spring water for each phosphate concentration. Each line shows an upward trend as growth occurred continuously. The y-axis goes up to 20 fronds. At the concentration of 0 ppm there was significantly lower growth than the treatment groups. The 10 ppm, 25 ppm, 50-100 ppm, and >100 ppm tubes all had similar growth levels.

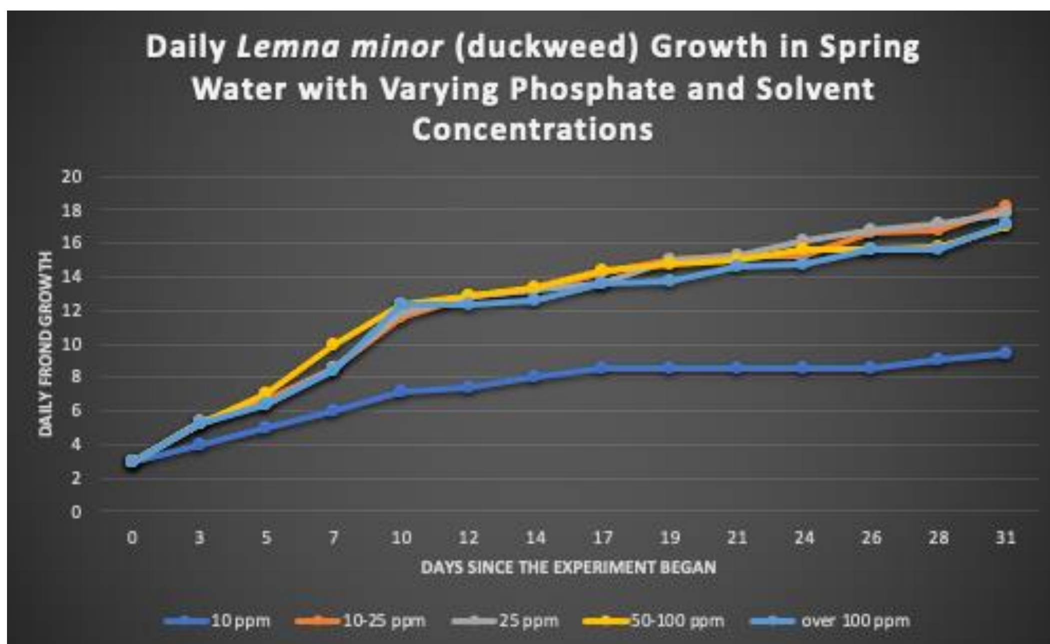

Figure 9. Line plot of the average daily frond growth of duckweed grown in spring water with varied phosphate concentrations.

5.12 Lab-distilled water

Results for lab-distilled water are displayed in *Table 3*. The 0 ppm tubes had a carrying capacity of 4.6 fronds and an r-max of 0.057 per day. At 10 ppm it yielded a carrying capacity of 4.8 fronds and r-max of 0.14 per day. The carrying capacity for 10-25 ppm was 5.6 fronds with an r-max of 0.11 per day. At 50 ppm the carrying capacity was 4.8 fronds and r-max was 0.085 per day. Lastly, 50-100 ppm had a carrying capacity of 5.6 fronds with an r-max of 0.15 per day.

Lab-Distilled Water: 0 ppm

Days Since Experiment began	Daily Fond Growth	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 (per day)
0	3	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
3	3.5	0.5	3	0.17	0.057
5	3.6	0.1	2	0.05	0.014
7	3.9	0.3	2	0.15	0.042
10	3.7	0	3	0	0
12	3.9	0.2	2	0.1	0.027
14	4.2	0.3	2	0	0
17	4.4	0.2	3	0.067	0.016
19	4.6	0.2	2	0.1	0.023
21	4.6	0	2	0	0
24	4.4	0	3	0	0
26	4.6	0.2	2	0.1	0.023
28	4.6	0	2	0	0
31	4.6	0	3	0	0

Lab-Distilled Water: 10 ppm

Days Since Experiment began	Daily Fond Growth	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 (per day)
0	3	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
3	3.6	0.6	3	0.2	0.067
5	4.6	1	2	0.5	0.14
7	4.5	0	2	0	0
10	4.7	0.2	3	0.067	0.015
12	4.8	0.1	2	0.05	0.011
14	4.6	0	2	0	0
17	4.6	0	3	0	0
19	4.6	0	2	0	0
21	5	0.4	2	0.2	0.043
24	5	0	3	0	0
26	5	0	2	0	0
28	5	0	2	0	0
31	4.6	0	3	0	0

Lab-Distilled Water: 10-25 ppm

Days Since Experiment began	Daily Fond Growth	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 (per day)
0	3	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
3	4	1	3	0.33	0.11
5	4.6	0.6	2	0.3	0.075
7	4.7	0.1	2	0.05	0.011

10	4.7	0	3	0	0
12	4.9	0.2	2	0.1	0.021
14	5.6	0.7	2	0.35	0.071
17	5.4	0	3	0	0
19	5.4	0	2	0	0
21	5.4	0	2	0	0
24	5.4	0	3	0	0
26	5.4	0	2	0	0
28	5.4	0	2	0	0
31	5.4	0	3	0	0

Lab-Distilled Water: 50 ppm

Days Since Experiment began	Daily Fond Growth	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 (per day)
0	3	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
3	3.5	0.5	3	0.17	0.057
5	3.8	0.3	2	0.15	0.043
7	4.1	0.3	2	0.15	0.039
10	4	0	3	0	0
12	4.1	0.1	2	0.05	0.013
14	4.8	0.7	2	0.35	0.085
17	4.6	0	3	0	0
19	4.6	0	2	0	0
21	4.6	0	2	0	0
24	4.8	0.2	3	0.067	0.015

26	4.8	0	2	0	0
28	4.6	0	2	0	0
31	4.6	0	3	0	0

Lab-Distilled Water: 50-100 ppm

Days Since Experiment began	Daily Fond Growth	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 (per day)
0	3	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
3	3.5	0.5	3	0.17	0.057
5	4.3	0.8	2	0.4	0.15
7	5.4	1.1	2	0.55	0.13
10	5.5	0.1	3	0.033	0.0061
12	5.6	0.1	2	0.05	0.0091
14	4.8	0	2	0	0
17	5.2	0.4	3	0.13	0.043
19	5.4	0.2	2	0.1	0.05
21	5	0	2	0	0
24	5.4	0.4	3	0.13	0.043
26	5.4	0	2	0	0
28	5.4	0	2	0	0
31	5.2	0	3	0	0

Table 3. Results of duckweed grown in lab-distilled water with various phosphate concentrations. Carrying capacity is the maximum daily frond growth and r-max the maximum change in the number of fronds per day per frond.

The line plot in *Figure 11* displays the daily frond growth in lab-distilled water. The line plot shows that each nutrient concentration had similar growth levels, but the growth was

sporadic as the lines were not constantly increasing. The y-axis only went up to 6 fronds. Tubes at 0 ppm, 10 ppm, and 50 ppm all had the exact same growth, whereas 10-25 ppm and 50-100 ppm had slightly greater growth.

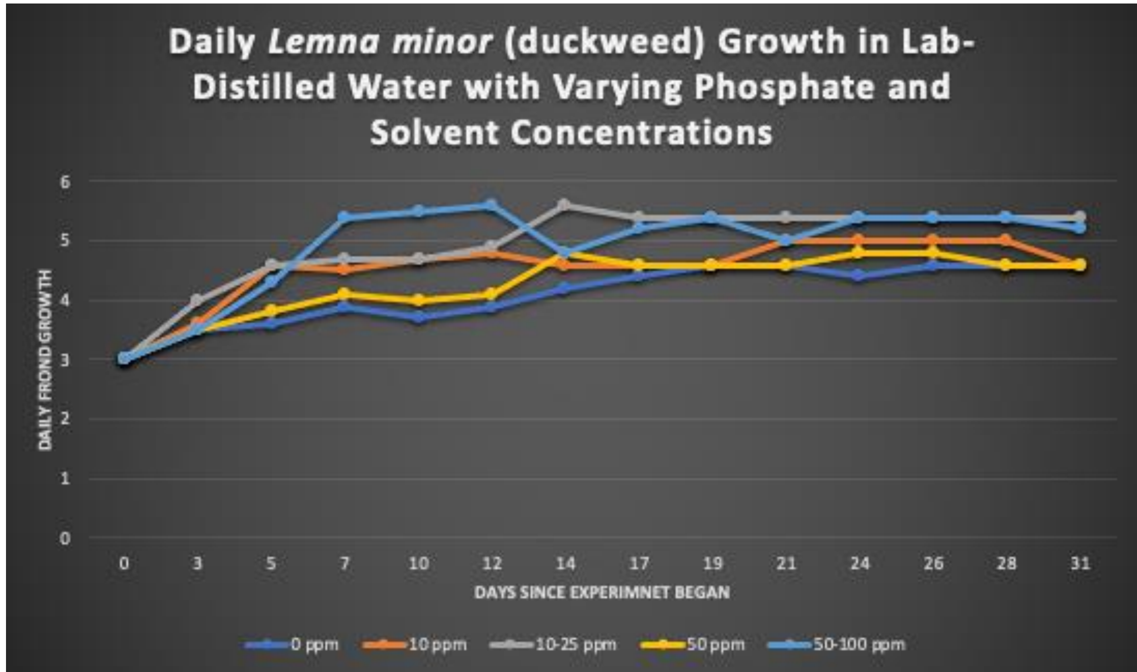

Figure 10. Line plot of the average daily frond growth of duckweed grown in lab-distilled water with varied phosphate concentrations.

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5.13 Commercial-distilled water

Lastly is the data from duckweed grown in commercial-distilled water. Results are viewed in *Table 4*. The carrying capacity for 0 ppm was 9.4 fronds with an r-max of 0.14 per day.

Commercial-Distilled Water: 0 ppm

Days Since Experiment began	Daily Fond Growth	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 (per day)
0	3	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
3	3.6	0.6	3	0.2	0.067

5	4.6	1	2	0.5	0.14
7	5.4	0.8	2	0.4	0.087
10	6	0.6	3	0.2	0.037
12	6.2	0.2	2	0.1	0.017
14	7	0.8	2	0.4	0.016
17	8	1	3	0.33	0.047
19	8.6	0.6	2	0.3	0.038
21	8.6	0	2	0	0
24	8.8	0.2	3	0.067	0.077
26	9	0.2	2	0.1	0.011
28	9	0	2	0	0
31	9.4	0.4	3	0.13	0.014

Table 4. Results of duckweed grown in commercial-distilled water with various phosphate concentrations. Carrying capacity is the maximum daily frond growth and r-max the maximum change in the number of fronds per day per frond.

The line plot in *Figure 12* displays commercial-distilled water duckweed growth. It included only 0 ppm. The graph displays growth increasing steadily upward.

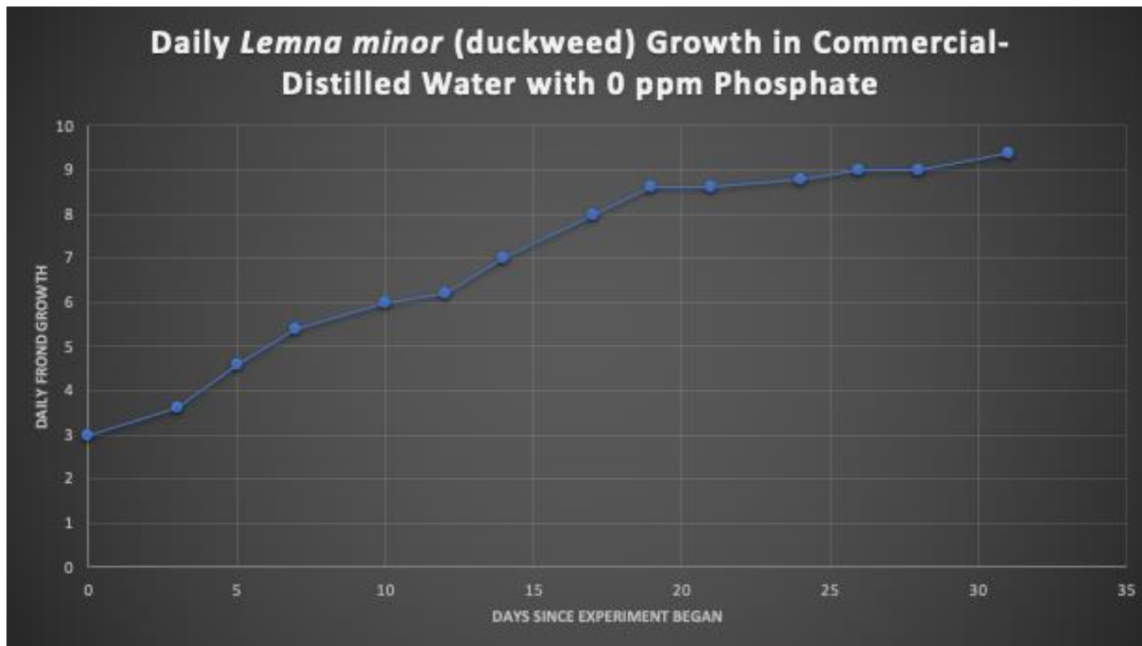

Figure 11. Line plot of the average daily frond growth of duckweed grown in commercial-distilled water at 0 ppm.

5.14 Light Readings

The last results were light readings taken under the grow light in multiple positions. The front had an average reading of 25.9 μmol/m^2/s. The back's average reading was 28.28 μmol/m^2/s. Lastly, the middle had an average reading of 31.33 μmol/m^2/s.

Position	Readings (μmol/m^2/s)	Average (μmol/m^2/s)
Front	26.0, 26.0, 25.7	25.9
Back	28.4, 28.0, 30.0, 26.7	28.28
Middle	31.3, 31.2 , 31.5	31.33

Table 5. Average light readings under the experimental grow light. Readings were taken three times in the front and back, four times in the middle, and averaged.

5.2 Statistical analysis data

5.21 Spring water

Statistical analysis was performed to determine significance in the data. The first analysis used carrying capacity values for spring water. Descriptive statistics yielded standard deviations of: 1.517 for 10 ppm, 1.304 for 10-25 ppm, 3.564 for 25 ppm, 2.280 for 50-100 ppm, and 3.564 for > 100 ppm. The following were the mean values: 9.4 fronds for 10 ppm, 16.8 fronds for 10-25 ppm, 17.8 fronds for 25 ppm, 17.2 fronds for 50-100 ppm, and 17.2 fronds for > 100 ppm. The homogeneity of variance had a Levene statistic of 2.488 and a P-value of 0.076. Since $P \geq 0.05$, the variances were not significantly different from each other. This condition must be met before ANOVA can be performed. The ANOVA test found a F-value of 8.997 for 4 and 20 degrees of freedom. The P-value was 0.001 meaning at least one group was significantly ($P \leq 0.05$) different from at least one other group. Lastly, the Tukey test found that all four phosphate treatments had significantly ($P \leq 0.05$) higher carrying capacities compared to the control group at 10 ppm. The four treatment groups did not significantly differ from each other.

Next, statistical analysis was determined between spring water r-max values. The descriptive statistics found the following standard deviations: 0.0850 for 10 ppm, 0.0508 for 10-25 ppm, 0.0677 for 25 ppm, 0.0492 for 50-100 ppm, and 0.0966 for > 100 ppm. The mean values were: 0.212 per day for 10 ppm, 0.276 per day for 10-25 ppm, 0.336 per day for 25 ppm, 0.242 per day for 50-100 ppm, and 0.274 per day for > 100 ppm. The homogeneity of variance had a Levene statistic of 1.264 and a P-value of 0.317, meaning the variances were not significantly ($P \geq 0.05$) different from each other. ANOVA yielded an F-value of 2.042 with 4 and 20 degrees of freedom. The P-value was 0.127, meaning that none of the groups were statistically different from any other group.

5.22 Lab-distilled water

Statistical analysis was performed for the carrying capacities in lab-distilled water. Descriptive statistics yielded the following standard deviations: 0.548 for 0 ppm, 1.140 for 10 ppm, 1.140 for 10-25 ppm, 0.0894 for 50 ppm, and 1.304 for 50-100 ppm. The mean values were as follows: 4.6 fronds for 0 ppm, 5.4 fronds for 10 ppm, 5.4 fronds for 10-25 ppm, 4.4 fronds for 50 ppm, and 7.2 fronds for 50-100 ppm. The homogeneity of variance found a Levene statistic of 0.939 and a P-value of 0.462, meaning the variances were not significantly ($P \geq 0.05$) different from each other. ANOVA yielded an F-value of 5.648 for 4 and 20 degrees of freedom. The P-value was 0.003, meaning at least one group was significantly ($P \leq 0.05$) different from at least one other group. The Tukey test concluded that there were no significant differences between 0 ppm, 10 ppm, 10-25 ppm, and 50 ppm phosphate groups. Only at 50-100 ppm was there a significantly ($P \leq 0.05$) higher carrying capacity compared with 0 ppm.

The statistical analysis was also conducted on r-max values. The descriptive statistics found the following standard deviations: 0.0696 for 0 ppm, 0.0654 for 10 ppm, 0.0423 for 10-25 ppm, 0.0333 for 50 ppm, and 0.0482 for 50-100 ppm. The mean values were as follows: 0.0972 per day for 0 ppm, 0.158 per day for 10 ppm, 0.423 per day for 10-25 ppm, 0.0920 per day for 50 ppm, and 0.222 per day for 50-100 ppm. The homogeneity of variance had a Levene statistic of 0.472 and a P-value of 0.755, meaning the variances were not significantly ($P \geq 0.05$) different from each other. ANOVA yielded an F-value of 4.905 with 4 and 20 degrees of freedom. The P-value was 0.006 showing that at least one group was significantly ($P \leq 0.05$) different from at least one other group. Lastly, the Tukey test concluded that 50-100 ppm had a significantly ($P \leq 0.05$) higher r-max compared with the 0 ppm. There were no significant differences between any other groups.

5.23 Control groups of spring water, lab-distilled water, and commercial-distilled water

The control groups of each solvent were analyzed for statistical significance. The control groups had no added phosphate. For spring water, the phosphate concentration was 10 ppm, and lab-distilled water and commercial-distilled water were both 0 ppm. When analyzing the carrying capacities, the descriptive statistics yielded the following standard deviations: spring water was 1.517, lab-distilled water was 0.548, and commercial-distilled water was 1.517. The following were the mean values: spring water was 9.4 fronds, lab-distilled water was 4.6 fronds, and commercial-distilled water was 9.4 fronds. The homogeneity of variance had a Levene statistic of 2.699 and a P-value of 0.108 meaning the variances were not significantly ($P \geq 0.05$) different from each other. ANOVA yielded an F-value of 23.510 for 2 and 12 degrees of freedom, and a P-value was 0.001, meaning at least one group was significantly ($P \leq 0.05$) different from at least one other group. Lastly, the Tukey test showed that lab-distilled water control had a significantly ($P \leq 0.05$) lower carrying capacity compared to spring water and commercial-distilled water controls. The spring water and commercial-distilled water controls did not significantly differ from each other.

The last statistical analysis compared the r-max of the solvent controls. The descriptive statistics found the following standard deviations: spring water was 0.0850, lab-distilled water was 0.0690, and commercial-distilled water was 0.100. The following were the means: spring water was 0.212 per day, lab-distilled water was 0.0972 per day, and commercial-distilled water was 0.234 per day. The homogeneity of variance had a Levene statistic of 0.856 and a P-value of 0.449, meaning the variances were not significantly ($P \geq 0.05$) different from each other. ANOVA yielded an F-value of 3.656 with 4 and 20 degrees of freedom. The P-value was 0.058, meaning that none of the groups were significantly different from any other group.

6. DISCUSSION

6.1 Methodological aspects

It is possible that this experiment could contain errors due to the scientific methods used. Inconsistencies arise when working with model organisms in laboratories. Plants are temperamental to growing in non-ideal conditions, and the experimental laboratory did not have controlled air circulation, light, or temperature. Changing conditions in the lab were not optimal for duckweed temperament.

Another possible oversight in the procedure was the number of days allowed for duckweed growth. According to research in the literary overview of the introduction, there is a growth inhibition at high phosphate concentrations (Lasfar et al., 2007). If the experiment continued for longer than 31 days, the inhibition could have occurred.

A limitation of the study was the lack of data on the commercial-distilled water. Representatives at ShopRite would not release their chemical composition of Bowl and Basket distilled water. The absence of nutrient information limits the certainty of conclusions drawn on differences between controls of distilled water.

Errors in measurement could have occurred. Counting large amounts of drops had significant capacity for mistakes. Variation in drop amounts would lead to growth level inconsistencies. A more accurate measurement for counting drops should have been used. There also could have been a greater number of groups per nutrient concentration. Increasing the sample size would create less chance for outliers to skew the data.

6.2 Spring water

Spring water yielded a statistically significant ($P \leq 0.05$) difference in carrying capacities for the 10 ppm control compared to each of the phosphate treated groups. The control had a

carrying capacity of 9.4 fronds, while the treatment groups had 18.2 fronds (10-25 ppm), 17.8 fronds (25 ppm), 17 fronds (50-100 ppm), and 17.2 fronds (> 100 ppm). The treatment groups were not statistically different, but each were approximately double the 10 ppm carrying capacity. This phenomenon follows Liebig's law of the minimum. The law states that adding more of an abundant nutrient does not increase growth. Only adding the limiting nutrient will increase growth (Tang & Riley, 2021). Since phosphate was provided in abundance, it was not the limiting nutrient. Further additions of phosphate did not affect the carrying capacity. These results correlate with past research on the effects of phosphate on intrinsic growth rate. In this study, concentrations of phosphorus higher than 1 ppm did not significantly influence growth (Lasfar et al., 2007). The results in this study had a similar conclusion. It found that only phosphate concentrations less than 10 ppm affected growth. Concentrations greater than 10 ppm had no significant influence. The difference in the concentrations which affected growth can be attributed to variations in the experimental setup, solvents, length of the experiment, and experimental laboratory conditions.

The r-max showed no significance in the statistical analysis; however, a general trend can be observed. The results showed a maximum with decreasing values on either side. The highest value was at 0.27 per day at 25 ppm, and the lowest values were at 0.13 per day at 10 ppm and 0.16 per day at >100 ppm. This trend follows Shelford's law of tolerance. The law states that growth relies on a variety of complex conditions creating minimum, maximum and a tolerance range for growth. The tolerance range is the area between minimal and maximal values within which species can survive. A Shelford’s law curve can be seen in *Figure 12* (Erofeeval, 2021).

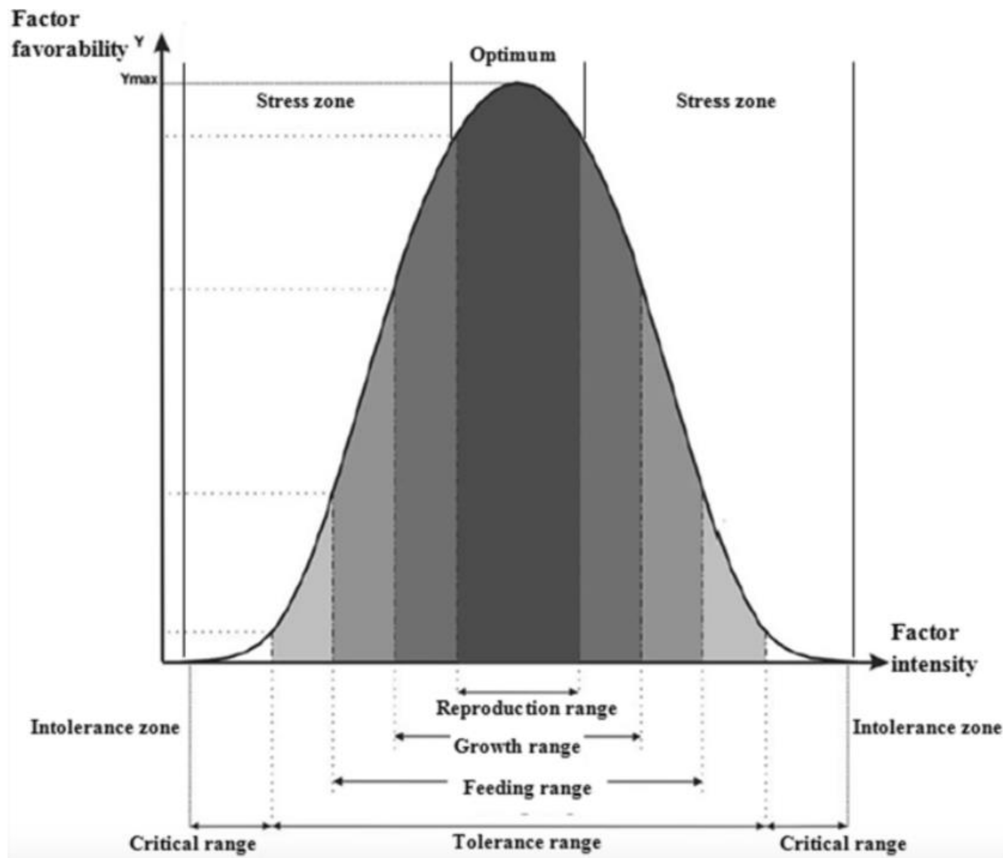

Figure 12. Shelford's law of tolerance curve, showing minimum, maximum and a tolerance range for growth (Erofeeval, 2021).

The r-max at 10 ppm was a minimum value, 25 ppm was the maximum value, and >100 ppm was another minimum value. These values create a tolerance range. Theoretically, when a certain phosphate concentration under 10 ppm and over 100 ppm is added, duckweed would be unable to survive in the conditions due to being out of the tolerance range. Past studies support the results. In a study on the effects of phosphorus on the growth and root morphology of *A. mono* seedlings, when supplied with optimal phosphorus (8 g plant^{-1}), maximum values of the growth parameters were recorded (Razaq et al., 2017). Another study showed that an oversupply of phosphorus, for long periods of time, caused decreases in cellular components (Sivaci et al., 2017). Sufficient phosphate is needed for healthy duckweed, but too much has negative effects.

It is important to avoid an oversupply or undersupply of phosphate. These conditions cause low r-max values leading to growth inhibition.

6.3 Lab-distilled water

Lab-distilled water results showed that 50-100 ppm had a significantly ($P \leq 0.05$) greater carrying capacity and r-max compared to the 0 ppm control group. While there was statistical significance in the data, overall trends are not consistent. The r-max values initially increase, before decreasing, and then increasing again. This does not follow Shelford's law of tolerance as the spring water results did. It is opposite, as it has a minimum value with increasing values on each side. The carrying capacity results also do not follow a trend. The values began by having a slow increase; however, they had a decrease at 50 ppm before increasing again. Adding more of the limiting nutrient should not increase the growth rate. There is also no logical reason for a decrease in growth at 50 ppm. The r-max and carrying capacity results are inconsistent, leading to the hypothesis that there may be something in the lab-distilled water leading to growth inhibition. Comparing the control groups of each solvent provides insight into this hypothesis.

6.4 Control groups of spring water, lab-distilled water, and commercial-distilled water

In the control results, lab-distilled water (0 ppm) had a significantly ($P \leq 0.05$) lower carrying capacity compared to spring water (10 ppm) and commercial-distilled water (0 ppm). The spring water and commercial-distilled water controls did not significantly differ from each other. Both distilled water solvents contained 0 ppm phosphate and grew in the same conditions. As distilled water should theoretically lack macronutrients and micronutrients outside of the added phosphate, the carrying capacities are expected to be the same. However, lab-distilled water had significantly less growth compared to commercial-distilled water. The carrying capacity of commercial-distilled water was 9.4 fronds, while lab-distilled water was only 4.6

fronds. The growth was more than halved. There could be two conclusions based on this finding: lab-distilled water is inhibiting growth or commercial-distilled water is promoting growth.

The hypothesis drawn from the data is that lab-distilled water is inhibiting growth. Commercial-distilled water and spring water both had the exact same carrying capacity of 9.4 fronds. Since both carrying capacities were the same, the growth increase cannot be due to micronutrients in spring water. Commercial-distilled water had to go through rigorous testing to be sold as distilled water, so there are most likely no additional nutrients in the water. Another contributor is the appearance of duckweed during the experiment. Duckweed in lab-distilled water tubes fell apart, turned yellow or brown, had a smaller size, and occasionally sank to the bottom of the tube within days of the experiment starting. These characteristics were rarely seen in both spring water and commercial-distilled water. If they were seen, it was within the last days when the carrying capacity had already been reached. The quick degradation in lab-distilled tubes shows that growth inhibition is occurring. A comparison of duckweed on day 10 at 0 ppm between lab-distilled water (left) and commercial-distilled water (right) is shown in *Figure 13*.

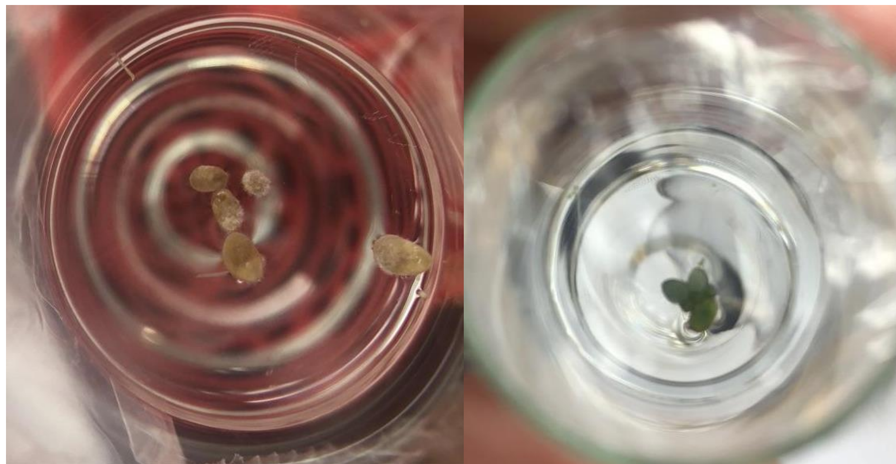

Figure 13. Comparison of experimental duckweed in lab-distilled water (left) and commercial-distilled water (right) both on day 10 at 0 ppn. Lab-distilled water appeared brown and fallen apart, while commercial distilled water was green and together.

The hypothesis is also supported when comparing spring water and lab-distilled water at the same phosphate concentrations. When both solvents were at 10 ppm, spring water had a carrying capacity of 9.4 fronds and lab-distilled water 4.8 fronds. At 10-25 ppm, spring water had a carrying capacity of 18.2 fronds and lab-distilled water 5.6 fronds, and at 50-100 ppm spring water had a carrying capacity of 17 fronds and lab-distilled water 5.6 fronds There are substantial decreases in growth considering each had the same amount of phosphate available. Additionally, when looking at the graphs in *Figure 9* and *10*, the y-axis goes up to 20 fronds in spring water but only 6 fronds in lab-distilled water. The lines are also consistently increasing in spring water, but sporadic in lab-distilled water. The data suggests that lab-distilled water is inhibiting growth.

The inhibition of growth in lab-distilled water could be due to the distillation system in the laboratory or storage in carboys. After contacting representatives at Fisher Scientific, they could not communicate the exact nutrient concentration after distillation using their system. It depended on resistivity, dissolved ions, volatile organics, bacteria levels, and pH of the added water. There are many factors, meaning the distillation in the laboratory may not be a reliable distilled water source. The storage of distilled water is carboys could potentially leak chemicals. Carboys are soft bottles which hold water under unpredictable laboratory conditions for long periods of time. They contain plasticizers, which are substances added to a polymer solution to promote plasticity and flexibility. Studies have been conducted to examine water contamination from soft bottle materials and plasticizers. The study reported 66 elements contaminating bottled waters. Another study by the University of Copenhagen found more than 400 different substances in water from plastic material, with the toxicity of at least 70% being unknown (Reimann et.al, 2010). The decrease of carrying capacity could be due to toxic chemicals from the plastic in the carboy inhibiting duckweed growth.

6.5 Summary

The results of this research show that spring water or commercial-distilled water were better as experimental solvents. Issues arise while using lab-distilled water. This could have been due to the laboratory filtration system or storage of the water in carboys. When duckweed is grown in spring water the carrying capacity follows Liebig's law of the minimum and r-max Shelford's law of tolerance. Future research should focus on the causes behind growth inhibition in lab-distilled water. Additionally, it is important to learn more about duckweed growth for applications such as bioremediation or synthesizing proteins.

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