

BIOLOGY EXTENDED ESSAY

Title: Effects of varying pH range and light intensity on the growth of *Physarum polycephalum* (Slime mold)

Research Question: How do the varying pH (3, 5, 7, 9, and 12) and varying light intensities (no light: 0 lux, low: 56 lux, moderate: 572 lux, high: 1221 lux) affect the growth of *Physarum polycephalum*, as measured by the length of the longest pseudopod?

TABLE OF CONTENT

Research Question	
1.0 Introduction.....	2
1.1 Background Information.....	3-4
1.2 Hypothesis.....	5-6
1.3.0 Variables	
1.3.1 Independent Variable(s).....	7
1.3.2 Dependent Variable.....	7
1.3.3 Controlled Variables.....	8
2.0 Design and Methodology	
2.1.1 Apparatus Required.....	9
2.1.2 Chemicals and Biological Materials Required.....	10
2.1.3 Method development.....	11
2.2 Procedure.....	12-15
2.3 Risk Assessment	
2.3.1 Safety Considerations.....	16
2.3.2 Environmental Considerations.....	16
2.3.3 Ethical Considerations.....	17
3.0 Results and Data Analysis	
3.1 Raw Data Collection	
3.1.1 Qualitative Data.....	18-19
3.1.2 Quantitative Data.....	20
3.2 Processed Data	
3.2.1 Mathematical calculation for the effect of pH.....	21
3.2.2 Mathematical calculations for the effect of Light Intensity.....	21
3.2.3 Final Processed Data.....	22
3.3 Data Analysis	
3.3.1 pH range.....	24-27
3.3.2 Light Intensities.....	28-31
4.0 Evaluation and Conclusion	
4.1.1 Evaluation	
4.1.2 Limitations of the investigation.....	32
4.1.3 Potential extensions.....	33
4.1.1 Evaluation of sources.....	33
5.0 Conclusion.....	34
6.0 Citations.....	35-37
7.0 Appendices.....	38-47

Research Question

How do the varying pH (3, 5, 7, 9, and 12) and varying light intensities (no light: 0 lux, low: 56 lux, moderate: 572 lux, high: 1221 lux) affect the growth rate (cm/hour) of *Physarum polycephalum*, as measured by the length of the longest pseudopod?

1.0 Introduction

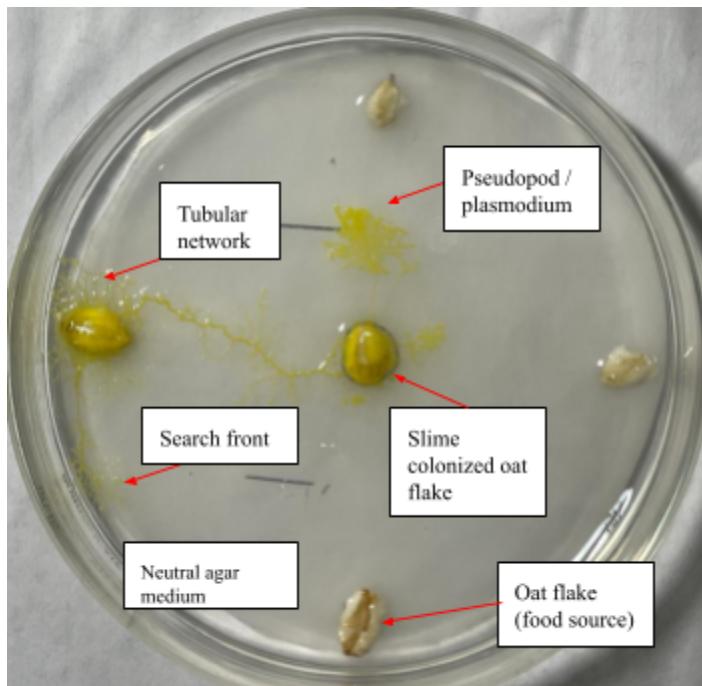


Figure 1. Labeled image of *Physarum polycephalum*

Physarum polycephalum also known as slime mold is a multinucleated (an expectation to the cell theory), giant unicellular organism that is classified as a protist under the class of Myxomycetes (or plasmodial slime molds)¹. The *P.polycephalum* in its vegetative phase usually appears in bright yellow with a network of veins-like projections caused by the extension of its cytoplasm is known as plasmodium (cytoskeleton) seen in **Figure 1**, that allows the transmission of chemical signals and transport of nutrients across the organism. Single veins of the

¹ Reynolds, M. (2019, October 19). All Hail the Blob, the Smart Slime Mold Confounding Science. Retrieved November 25, 2024, from WIRED website: <https://www.wired.com/story/all-hail-the-blob-smart-slime-mold-confounding-science/>

plasmodium are known as pseudopods and are the extensions of the cytoplasm which allows the slime mold to navigate through its environment and search as seen in **Figure 1**.²

The slime mold's extension and movement are oscillatory (contracting and relaxing) and are usually dependent on the stimulus caused by either chemotaxis or phototaxis. Chemotaxis is the primary reason that results in extension and movement in slime molds and it is driven by two proteins, actin and myosin.

While phototaxis is not directly responsible for the slime mold's movement, due to it the slime mold exhibits negative phototaxis when it is exposed to light. When light is sensed by the slime mold through their photoreceptor, it triggers an internal signaling cascade that affects the actin and myosin, where the cytoskeleton at which light is sensed³.

1.1 Background Information

Effect of pH

Environmental factors such as pH and light intensity heavily influence the growth of ***Physarum polycephalum***, because these factors regulate the crucial cellular processes such as the cytoplasmic streaming (pseudopod extension), enzyme activity, and response to oxidative stress. pH plays an important role in maintaining the enzyme structure and its function, which directly impacts the metabolic activity in the slime molds. One of the crucial enzymes affected by pH is **alkaline phosphate**, which is involved in phosphate metabolism and energy transfer. Alkaline phosphate hydrolyses phosphate esters, resulting in releasing of inorganic phosphate that is

² Pseudopods - Definition, Function, Movement and Examples. (n.d.). Retrieved November 25, 2024, from MicroscopeMaster website: <https://www.microscopemaster.com/pseudopods.html>

³ Phototaxis - an overview | ScienceDirect Topics. (n.d.). Retrieved November 25, 2024, from www.sciencedirect.com website: <https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/phototaxis>

essential for ATP synthesis and cell signaling. However, because of extreme pH conditions, alkaline phosphate denatures reduce its efficiency and damage ATP production, eventually inhibiting the cellular process **Furuhasi(2008)**⁴. Another crucial enzyme in slime molds is **calcium-dependent ATP pyrophosphohydrolase**, which is responsible for facilitating the cytoplasmic streaming (pseudopod extensions), which is the primary mode of transporting nutrients and movement in slime molds. Deviation from the optimal pH range results in disruption of enzyme activity, altering ion gradients and eventually inhibiting slime mold's ability to expand and grow.

Effect of light intensities

The ***Physarum polycephalum*** when exposed to light exhibits negative phototaxis (avoiding high-intensity exposure to light sources). Negative phototaxis is a response because of the effect of light on enzyme function and oxidative stress, as high light intensity promotes the formation of reactive oxygen species (ROS), such as superoxide radicals (O_2^-) and hydrogen peroxide (H_2O_2) which causes oxidative damage to lipids, proteins, and DNA (**Reinhardt**)⁵. Additionally, the slime mold depends on superoxide dismutase and catalase enzymes that neutralize oxidative damage. Excessive exposure to high light intensities damages the superoxide dismutase and catalase due to protein degradation and impaired cell metabolism. Moreover, the photoreceptors present in the slime molds regulate actin-myosin interaction. Hence exposure to higher light intensities disrupts this process and slows down the nutrient transport within the organism, inhibiting growth and ATP production.

⁴ Kiyoshi Furuhashi. "Alkaline Phosphatase of *Physarum Polycephalum* Is Insoluble." *Archives of Microbiology*, vol. 189, no. 2, 25 Sept. 2007, pp. 151–156, <https://doi.org/10.1007/s00203-007-0306-x>. Accessed 11 Oct. 2024

⁵ Reinhardt, Donald J. "The Effects of Light on the Development of the Cellular Slime Mold *Acrasis Rosea*." *American Journal of Botany*, vol. 55, no. 1, 1 Jan. 1968, pp. 77–77, <https://doi.org/10.2307/2440496>. Accessed 25 Apr. 2024.

1.2 Hypothesis

The pH range:

Alternate Hypothesis(H₁): I predict that the optimal growth of *Physarum polycephalum*, in terms of the highest extension of the longest pseudopod (measured in cm) over 24 hours, shall occur within the pH range of 5 to 7 because a slightly acidic to neutral environment provides the most favorable (optimal) conditions for the organism's enzyme activity, nutrient uptake, and cellular metabolism. As prior research done in the **American Journal of Botany**⁶, the slime molds exhibit their highest physiological activity and fruiting between the pH range of 5 to 7, however, it has also been deduced that with extreme pH levels such as pH 3 and below, slime molds movement, and nutrient assimilation is restricted and the enzymes are denatured at these extreme pH levels.

On the contrary, I predict significant inhibition of growth in basic(alkaline) conditions at pH 9 and pH 12 because of enzyme denaturation, impaired ion transport, and increased cellular strength. Additionally, highly basic conditions can also disrupt the proton gradient ΔpH, metabolic reaction, and even the cytoplasmic stability, resulting in a significant restriction to the slime mold's ability to extend their pseudopod.

Null Hypothesis(H₀): The pH levels do not affect the growth rate of *Physarum polycephalum*

⁶Gray, W. D. (1939). The Relation of pH and Temperature to the Fruiting of *Physarum polycephalum*. *American Journal of Botany*, 26(9), 709–714. <https://doi.org/10.2307/2437020>

The varying light intensities:

Alternate Hypothesis(H₁): I predict that the growth of ***Physarum polycephalum*** will be optimal under low to moderate light intensity levels because these conditions provide sufficient energy for metabolic and cellular activities to be carried out without causing stress or damage to its cellular structures. On the contrary, I think that the high light intensity should inhibit the slime mold's growth as studies done in an article published in **PubMed⁷** and **Japan Society for Cell Biology⁸**, suggest that intense light influences the slime mold's behavior in terms of searching for the nutrients. The study published on **ScienceDirect⁹** suggests that exposure to high light intensity disrupts the slime mold cellular processes, including the production of ATP and metabolic pathways, leading to slower and inhibited growth. Additionally, high light intensities can also inhibit key metabolic functions, such as reduced nutrient uptake and energy production because of the stress exerted by light.

Null Hypothesis (H₀): There is no significant difference in the growth of ***Physarum polycephalum*** across the varying light intensity levels, as light intensity does not affect the slime mold's growth rate.

⁷ Latty, T., & Beekman, M. (2010). Food quality and the risk of light exposure affect patch-choice decisions in the slime mold *Physarum polycephalum*. *Ecology*, 91(1), 22–27. <https://doi.org/10.1890/09-0358.1>

⁸ Hato, M., Ueda, T., Kurihara, K., & Kobatake, Y. (1976). *CELL STRUCTURE AND FUNCTION I*, 269-278 (1976) C by Japan Society for Cell Biology Phototaxis in True Slime Mold *Physarum polycephalum*. Retrieved from https://www.istage.jst.go.jp/article/csf1975/1/3/1_3_269/_pdf/-char/en

⁹ Phototaxis - an overview | ScienceDirect Topics. (n.d.-b). Retrieved from www.sciencedirect.com website: <https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/phototaxis>

1.3.0 Variables

1.3.1 Independent variable(s)

Independent variable	Why was this changed	How will it be changed
pH range (pH 3, 5, 7, 9 and 12)	The pH will serve as the growth medium for this experiment, hence the pH is being changed to investigate its effect on the growth of the slime mold.	The pH ranges from acidic (pH 3 and 5) to neutral (pH 7) and basic (pH 9 and 12). These pH levels will be prepared using sodium hydroxide for basic pH levels, hydrochloric acid for acidic pH, and distilled water for neutral pH. These pH solutions will be mixed with the agar to prepare the pH-growth medium and will be verified using pH papers.
Light intensities - No light (0 lux), Low light (56 lux), moderate light (572 lux) and high light (1221 lux).	The light intensities were varied using a 6W LED light source, placed 35 cm above the petri dishes, to investigate the effect of different light intensities on the growth of slime mold.	The light intensities were varied with the 6W LED light source's brightness as the LED light lamp had adjustable brightness, which was later verified with a lux meter.

Table 1. Representing the independent variables

Note* For this investigation extreme pH such as 1,2, 13, and 14 were not chosen as they could completely inhibit the slime's growth and cause cellular damage

1.3.2 Dependent variable

Dependent variable	Why is it measured	How it be measured
The growth rate (cm/hour) of <i>Physarum polycephalum</i> , is measured by the mean length of the longest pseudopod (cm).	The growth rate of <i>Physarum polycephalum</i> is measured to determine how the varying pH range and varying light intensities influence the organism's physiological activity and overall growth.	The growth rate will be determined by measuring the length of the longest pseudopod extension (cm) after 24 hours of incubation by using an image analysis software –ImageJ, to determine the effect of the independent variables.

Table 2. representing the dependent variable

Note*The length of the longest pseudopod is being measured because it serves as a quantifiable and direct indicator of growth in **P.polycephalum** as they extend their pseudopods to explore their environment and surroundings in search of nutrients. Hence the extension of pseudopods reflects the physiological response to external conditions, pH, and Light intensities.

1.3.3 Controlled Variables

Controlled variable	Purpose of control	Method for control
Common control (pH and Light intensity)		
Growth medium	Non-nutrient agar was controlled along with the filter paper diameter to provide the slime mold with equal advantage in terms of availability of nutrients and the surface for them to grow.	By using 89mm filter papers and non-nutrient agar as a growth medium for all Petri dishes.
Placement of the slime mold	Placement of the slime mold ensures an equal and sufficient surface for growth and consistent growth measurement.	By placing the oat flake inhabited by slime mold and placing it on the marked point. (mentioned in 2.2).
Length of the oat inhabited by slime mold	To ensure that the starting conditions are consistent and reliable, across all the trials	By weighting the oat flake inhabited by slime mold at 1.5 cm
Humidity	Slime molds showcase optimal growth in humid conditions	By putting wet paper tissue above and below the petri dishes.
Temperature	The temperature between 19°C to 25°C is the optimal range for slime mold growth.	By placing all the petri dishes in the incubator.
Time duration of the experiment	To maintain consistency in growth conditions	The data will be collected after 24 hours of the experiment.
Camera Imaging setup (iPhone 12 Pro)	Maintain consistency in image resolution and camera setting for all trials to minimize the variability or uncertainty in the measurement.	The images for measurement will be captured using the iPhone 12 Pro's camera (12 megapixels) positioned 20 cm above the petri dish at a 90-degree angle across all trials.
pH		
Dark light conditions	To provide all the slime molds with equal advantages and dark light conditions for growth	
Concentration of pH solutions	If the concentration is too high then it may inhibit the growth of slime mold.	The pH solution will consist of a concentration of 0.1 M or 1 M.

Light intensity		
Distance between the light source and Petri dishes	To maintain consistent light exposure for the slime mold.	The distance between the Petri dishes and the light source should be 30 cm and will be verified by using a ruler
LED source	To ensure the consistency of light intensity and avoid any variability that could potentially affect the growth rate of slime molds.	By using the LED source from the Wipro Garnet 6W LED Table Lamp, throughout the experiments of light intensities.

Table 3. Representing the controlled variables.

2.0 Design and Methodology

2.1.1 Apparatus Required

Sr.No	Material - Apparatus	Quantity	Intended use or purpose of use	Uncertainty
1	90 mm diameter Petri dishes	45	For investigating and growing the slime mold	-
2	100 mm diameter Petri dishes	2	For culturing and growing slime mold	-
3	Filter paper sheets	2	Filter paper sheets for preparing the cutouts of 89 mm diameter filter paper.	-
4	A cardboard circle cut out of 89 mm diameter	1	For cutting out an 89 mm diameter of filter paper	-
5	Metal forceps	2	For transferring or placing the specimen	-
6	Spatula	2	For adjusting the specimen	-
8	30 cm ruled scale	1	For keeping a constant distance between the light source and the Petri dishes	±0.5 mm
9	Lux meter	1	To measure the light intensities	±1 lux
10	pH paper strips	2 strips	For measuring the pH of the solution	-
11	100 mL beakers	5	For preparing the pH solutions	-
12	100 mL conical or Erlenmeyer flask	5	For containing and autoclaving non-nutrient agar solution	-
13	Pipettes	1	Used while making the pH solutions	±0.01 mL
14	Pair of scissors	1	For cutting the 89 mm diameter cutouts	-
15	Powdered hand gloves	20	Prevention of contact with contagious organisms or microbes	-
16	PPE kit	1		

17	HB pencil	2	For precise markings and setting the scale bar	-
18	Paper tissue	1 box	To maintain constant humidity	-
19	Incubator	1 unit	To maintain constant temperature and darkness	-
20	Adjustable light intensity lamp 6W LED	1	To provide the source of light intensities at different light levels	-
22	ImageJ software	1	For measuring the length of the slime mold	
23	Sticker Labels	40	For labeling petri dishes.	-
24	Heating plate	1 unit	For the preparation of pH-agar solutions	-

Table 4. Representing the list of materials and apparatus required

2.1.2 Chemicals and Biological Materials Required

Sr.No	Chemicals - Biological Materials	Quantity	Purpose of use
1	<i>Physarum Polycephalum</i> Culture	1 culture	For conducting the investigation/experiment
2	Dried oat flakes	200 grams	For providing nutrients to the slime mold
3	Distilled water	1000 mL	For making pH solutions and maintaining pH (7)
4	Hydrochloric Acid	10 mL	For preparing pH (3 and 5) solutions
5	Sodium Hydroxide pellets	10 grams	For preparing pH (9 and 12) solutions
6	Non-nutrient agar powder	20 grams	For providing a growth medium for the slime molds along with the filter paper

Table 5. Representing the list of chemicals and biological materials required

2.1.3 Method development

Before finalizing the methodology for the experiment, I conducted a pilot test to verify whether the slime molds grew in buffer solutions. Initially, I decided to use buffer solutions such as citrate, acetate, sodium hydroxide,e, and di-sodium hydrogen phosphate buffer to maintain a stable pH level of the growth medium. However during the pilot run, 24 hours after the incubation it was observed that there was no formation of pseudopod extensions, and the yellow color of the slime mold slightly faded. ([Refer to Appendix A](#)). Then I decided to conduct another pilot run with 0.1 M hydrochloric acid for the acidic medium and 0.1 M NaOH for the basic and surprisingly this time growth of the slime mold was present in all four Petri dishes([Refer to Appendix A2](#)). Furthermore, I prepared a slime mold culture which will be used throughout all the trials, ([Refer to Appendix A3](#)).

2.2 Procedure

Lab preparation

1. Label all the Petri dishes accordingly for each pH range (3,5,7,9 and 12) and Light intensities (none, low, moderate, and high) as shown in **Figure**, using sticker labels.



Figure 2. Represents the labeling of Petri dishes

Procedure for preparation of pH solutions

Acidic (pH 3 and 5)

1. **pH 3:** Dilute 1 mL of 1 molar Hydrochloric acid (HCl) with 99 mL of distilled water and confirm the pH of the solution using the pH meter.
2. **pH 5:** Add the required amount of distilled water to adjust the 0.1molar HCl solution and use the pH meter to obtain a solution of pH 5.

Neutral (pH 7)

1. **pH 7:** Distilled water

Basic (pH 9 and 12)

1. **pH 9:** Dilute 10 mL of 1 molar Sodium Hydroxide (NaOH) with 90 mL of distilled water and confirm the pH of the solution using the pH meter.
2. **pH 12:** Add the required volume of NaOH to adjust the pH of 0.1 molar NaOH and confirm the pH of the solution using the pH meter.

Preparing non-nutrient pH agar and filter papers for growth medium

1. Take a fresh sheet of filter paper and draw 40 circles of 89mm diameter using a cardboard cutout and a sharp HB pencil.
2. Using a pair of scissors cut out the 40 circles, drawn in the previous step.
3. After cutting the filter papers, fold them into a cone shape, for obtaining each line and the center of the filter paper.

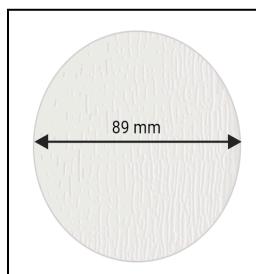


Figure 3. Represents the dimensions and marking of filter paper. Made using (biorender)

4. Store the pre-cut filter papers in a sealed plastic bag and store them until the time of pouring the agar, as **mentioned in the experiment**.
5. Add 1 gram of non-nutrient agar powder into 100 mL of respective boiling pH
6. solution (**made in procedure for pH solutions**) and stir cook for 1 minute, until the agar powder is completely dissolved.
7. Transfer the agar with respective pH solutions into five 100 mL conical flasks and cover it with a cotton plug.
8. Place all five conical flasks into the Autoclave and set the autoclave to 100°C, 15 psi, and autoclave it for 30 mins.

Procedure for Light Intensity Setup

1. For the light intensity part of the experiment, all Petri dishes should contain pH 7 non-nutrient agar as a growth medium for controlling pH 7.

2. Place a table lamp in a dark room, where the distance between the light source of the lamp and the Petri dishes should be constant (30 cm).
3. **No light** (0 lux): Place the Petri dishes containing the set mentioned in the **experiment** step, in complete darkness.
4. **Low light** (56 lux): Place the petri dishes under the table lamp's light source, where the lamp is at the lowest brightness setting.
5. **Moderate light** (572 lux): Place the Petri dishes under the table lamp's light source, where the lamp is at the medium brightness setting.
6. **High light** (1221 lux): Place the petri dishes under the table lamp's light source, where the lamp is at the maximum brightness setting.
7. Before all light intensity trials, use the lux meter to confirm and verify the lux output for each light intensity before placing the petri dish.
8. Experiment with the respective light intensities mentioned above for 24 hours.

Performing the experiment

1. Prepare a separate culture in another petri dish, from where oat flakes inhabited by slime mold would be taken throughout the experiment.
Note*Do not forget to add new fresh oat flakes after taking out existing ones inhabited by slime mold.
2. Place the filter paper in the petri dishes and add about 2 to 4 mm of pH agar solution evenly to the petri dish over the lid of the petri dish and gently swirl it until the agar is evenly distributed and then allow it to cool and thicken up.

3. Once it is cooled down and thickened up, use sterile metal forceps to place the slime mold inhabited oat flake in the center of the filter paper and adjust it if needed.
4. Using another sterile metal forcep then, place 1 fresh oat flake on the 4 marked lines and close the lid of the petri dish.
5. Then place the petri dishes in the thermocol incubation box and add wet paper tissues below and above each petri dish.
6. Capture the image of the petri dish exactly after 24 hours for measuring the length of the longest pseudopod (explained in **measuring the length of slime molds**) Step and also observe any qualitative changes.
7. Repeat the following steps for all pH range and light intensity trials.
8. Check the humidity in 4-hour intervals, if the paper tissues are dried then moist them.

Measuring the length of slime molds

1. Using the line tool in the **ImageJ¹⁰** toolbar, draw a line from one end of the petri dish to the other, across the diameter 4 times from each direction.
2. To measure the number of pixels shown for the line length. On the toolbar click on **Analyze > Measure** (or **Ctrl+M**) to measure the mean pixels across the petri dish diameter.
3. Use the mean pixel length as the **Distance in pixels**, then enter 9 as the known distance and set the unit as centimeters (cm).
4. Use the Line tool to draw a line from the point of contact of the longest pseudopod to of the slime mold to its longest length and then go to **Analyze > Measure** to measure the length of the pseudopod.

¹⁰ ImageJ. (n.d.). Retrieved October 23, 2024, from imagej.net website: <https://imagej.net/ij/index.html>

2.3 Risk Assessment

2.3.1 Safety Considerations

1. Be extremely cautious while handling Hydrochloric acid (HCl) and Sodium hydroxide (NaOH) as they are extremely corrosive and can cause irritation in the respiratory system.
2. Physarum polycephalum is considered non-pathogenic to humans, however, it can potentially cause allergies or irritation in the respiratory system to certain individuals, hence proceed with caution by wearing powdered gloves and avoiding inhaling it.
3. If the slime molds on the skin, then wash the affected area with water.
4. Ensure to sterilize the apparatus such as the forceps or spatula post before the experiment to maintain aseptic conditions during the transfer of the slime molds to minimize bacterial and fungal contamination.

2.3.2 Environmental Considerations

Chemical Disposal

- Sodium hydroxide (NaOH) is harmful to aquatic environments if not disposed of responsibly. To dispose of the sodium hydroxide solution made, firstly neutralize it with an acid strong acid such as hydrochloric acid or sulfuric acid and dispose of it into a designated waste container and hand it over to a lab technician for further disposal concerning local and state regulations ¹¹.

¹¹ New Jersey Department of Health. (2015). *Right to Know Hazardous Substance Fact Sheet Description and Use Hazard Summary Reasons for Citation*. Retrieved from <https://nj.gov/health/eoh/rtkweb/documents/fs/1706.pdf>

Biological Waste

- The biological wastes and agar will be first, autoclaved and sterilized and then disposed of in a designated waste container and handed over to the lab technicians for further treatment and disposal concerning local and state regulations.

Energy Consumption

- The experiment consists of constant running of a light source for 24 hours, which increases the energy consumption, hence LED sources have been used for this experiment because they are energy efficient and overall minimize the energy consumption.

2.3.3 Ethical Considerations

1. The experiment consists of living organisms (*P.polycephalum*), solely for the intent of research — understanding the effect of pH and light intensities on the growth of *P.polycephalum*.
2. The *P.polycephalum* was ethically treated by providing it with optimal environmental conditions and minimizing unnecessary risks. After the experiment, the *Physarum polycephalum* can be either preserved by drying it and using it for future experimental purposes or disposed of ethically by autoclaving and discarding it as biological waste as mentioned in **2.3.2**. At last, this ensures that the living organism was ethically treated and concerning the environmental considerations.
3. Data integrity is maintained by accurately reporting all the quantitative and qualitative data obtained during this experiment, and to ensure the integrity of the experiment's findings.

3.0 Results and Data Analysis

3.1 Raw Data Collection

3.1.1 Qualitative Data

pH range	Qualitative observation				
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
3	Extremely short pseudopod extensions	No growth			Growth of mold at one of the food sources.
5	The yellow pigment appeared extremely bright (refer to Figure 3)	Two pseudopod extensions are already halfway extended toward the food source at the edge of the petri dish	Single pseudopod extension, extending towards the edge		The pseudopod extensions are arborescent (many branches)
7	Three pseudopod extensions, spreading in three different directions		Paper tissue lost its moisture, and remoisturizing was done.		Circular extension of the pseudopod
9	pH was dropped from 9 to 7. Added the pH 9 solution again			Paper tissues were over-moisturized	
12	Single pseudopod extension	Extremely short pseudopod extensions		No growth and mold contamination (refer to Figure 4)	

Table 6. Representing the qualitative observations for pH trials, 12 hours after incubation.



Figure 3. Bright yellow color of the pseudopod extension



Figure 6. Mold contamination

Light intensities	Qualitative observation				
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
No Light (0 ± 1 lux)	Three pseudopod extensions, spreading in three different directions		Paper tissue lost its moisture, and remoisturizing was done.		Circular extension of the pseudopod
Low Light (56 ± 1 lux)	Pseudopod extension is already halfway extended towards the edge of the petri dish.				Paper tissues dried out, remoisturize them
Moderate Light(572 ± 1 lux)		Cloudy white-yellow pseudopod extension (refer to Figure 5)			
High Light (572 ± 1 lux)	Arborescent extensions of the pseudopod with loss of color (refer to Figure 6)		Pseudopods extensions appear to be thinner than usual (refer to Figure 7)		The light source was turned off due to power cut

Table 7. Representing the qualitative observation for Light intensities, 12 hours after incubation

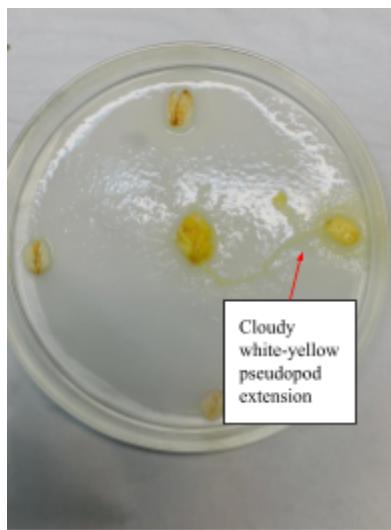


Figure 5. Cloudy white-yellow extension



Figure 6. Color loss of the pseudopod

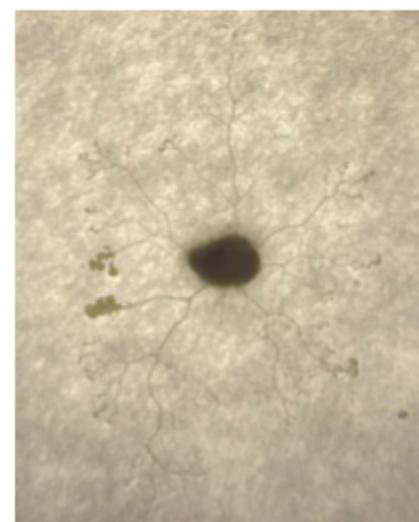


Figure 7. Thinner pseudopod extensions than usual ones

3.1.2 Quantitative Data

pH range	Mean lengths of longest pseudopod per trial ($\pm 0.06\text{cm}$)				
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
3	0.907	0.00	1.289	0.582	1.027
5	3.637	3.426	3.447	3.016	3.377
7	3.248	2.988	2.992	2.858	3.520
9	2.002	1.903	2.230	1.903	2.153
12	0.790	0.367	0.356	0.00	0.827

Table 8. Represents the raw data for pH trials

Light Intensity	Mean lengths of longest pseudopod per trial ($\pm 0.06\text{cm}$)				
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
No Light	3.248	2.988	2.992	2.858	3.520
Low Light	3.814	1.967	1.882	2.393	2.073
Moderate light	2.472	1.971	1.789	2.561	1.732
High Light	1.567	2.047	1.683	2.098	2.536

Table 9. Represents the raw data for light-intensity trials

3.2 Processed Data

(Refer to *Appendix B* for calculation of uncertainty)

Equation 1
$$\text{Overall mean length} = \frac{\text{Total mean length per trial}}{\text{Total number of trials}}$$

Equation 2
$$\text{Growth Rate} = \frac{\text{overall mean length}}{\text{Time}}$$

3.2.1 Mathematical calculations for the effect of pH

Calculation for overall mean length for pH 7

$$= \frac{0.907 \pm 0.06\text{cm} + 0.00 \pm 0.06\text{cm} + 1.289 \pm 0.06\text{cm} + 0.582 \pm 0.06\text{cm} + 1.027 \pm 0.06\text{cm}}{5}$$
$$= 0.761 \pm 0.3\text{cm}$$

Calculation for growth rate of P. Polycephalum at pH 7

$$= \frac{0.761 \pm 0.3\text{cm}}{24 \text{ hours}}$$
$$= 0.031 \text{ cm/hour}$$

3.2.2 Mathematical calculations for the effect of Light Intensity

Calculation for overall mean length for Moderate light intensity

$$= \frac{2.472 \pm 0.06\text{cm} + 1.971 \pm 0.06\text{cm} + 1.789 \pm 0.06\text{cm} + 2.561 \pm 0.06\text{cm} + 1.732 \pm 0.06\text{cm}}{5}$$
$$= 2.105 \pm 0.3\text{cm}$$

Calculation for growth rate of P. Polycephalum at moderate light intensity

$$= \frac{2.105 \pm 0.3\text{cm}}{24 \text{ hours}}$$
$$= 0.087 \text{ cm/hour}$$

3.2.3 Final Processed Data

pH	Overall mean length of longest pseudopod($\pm 0.3\text{cm}$)	Growth rate of P.polycephalum (cm/hour)
3	0.761	0.0317
5	3.380	0.140
7	3.121	0.130
9	2.098	0.0874
12	0.468	0.0195

Table 10. Represents the final processed data for the pH range

Light intensities	Overall mean length of longest pseudopod($\pm 0.3\text{cm}$)	Growth rate of P.polycephalum (cm/hour)
No Light ($0\pm 1\text{ lux}$)	3.121	0.130
Low Light ($56\pm 1\text{ lux}$)	2.425	0.101
Moderate Light($572\pm 1\text{ lux}$)	2.105	0.0877
High Light ($572\pm 1\text{ lux}$)	1.986	0.0827

Table 11. Represents the final processed data for light intensities

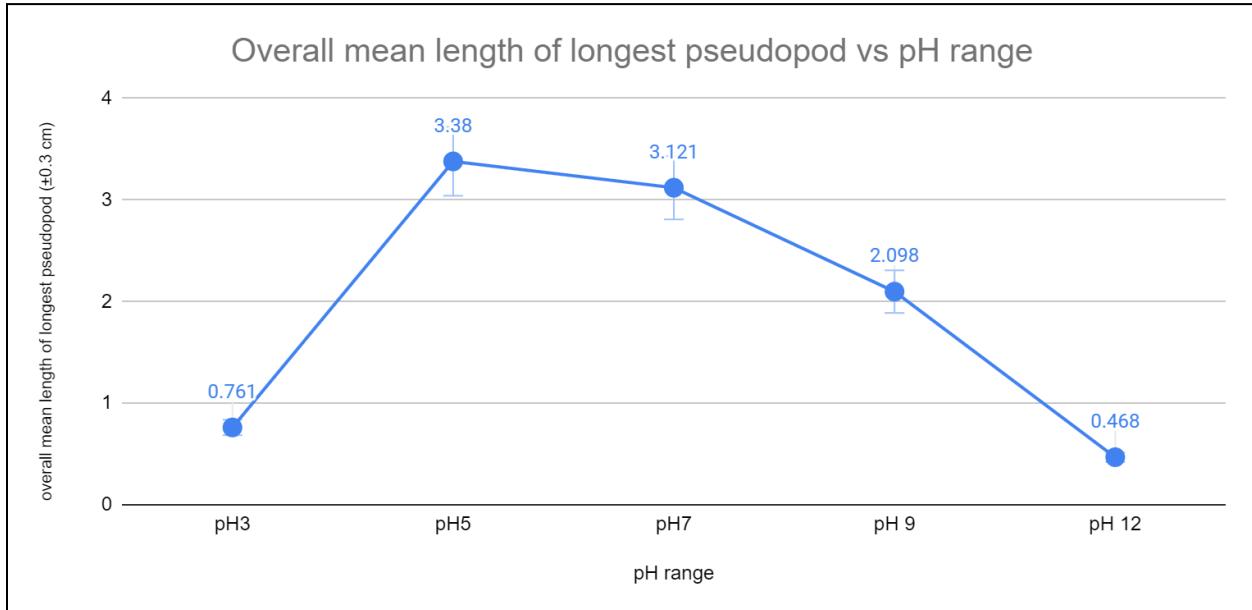
3.3 Data Analysis

Statistical analysis using Chi-Square (χ^2) Test

(Refer to *Appendix E* for Chi-square tables and calculations)

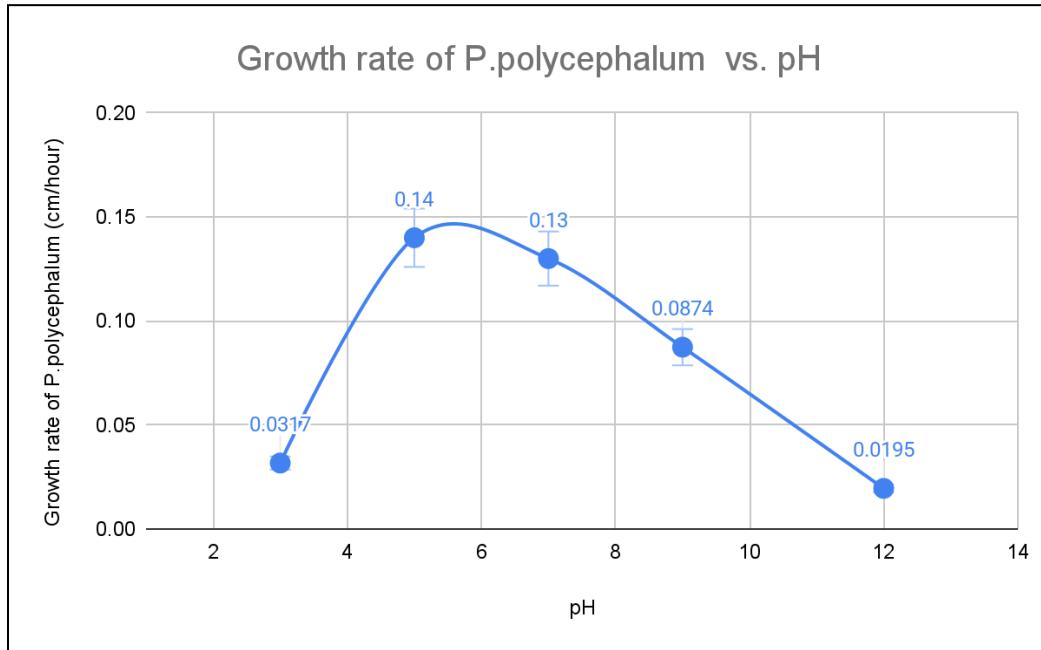
The chi-square test is utilized to assess the relationship between two categorical variables. This study aims to explore the correlation between the independent variables—pH range and light intensities—and the dependent variable, the growth rate of *Physarum polycephalum*, measured by the lengths of its longest pseudopods across various trials. The pseudopod lengths were categorized into three defined growth categories, facilitating the transformation of raw data into frequency counts for each category corresponding to the pH and light conditions. Subsequently, chi-square values for both independent variables were calculated using the chi-square distribution table at a 0.05 significance level and appropriate degrees of freedom.

3.3.1 pH range



Graph 1. Representing the overall mean length of the longest pseudopod against the pH range
(Refer to Appendix D to see the length of the longest pseudopod for each trial)

As evident in the data presented in **Graph 1**, we can see that pH 5 corresponds to the greatest value of the mean length of the longest pseudopod, which is $3.380 \pm 0.3\text{cm}$. Additionally, pH 7 has a prominent mean length of $3.121 \pm 0.3\text{cm}$, which is quite close to the mean length observed in pH 5, suggesting that the slime molds can even grow. Conversely, the mean length starts to decline with extreme pH levels such as pH 3 and pH 12 with the mean length of $0.761 \pm 0.3\text{cm}$ and $0.468 \pm 0.3\text{cm}$ respectively. However, the mean length at pH 9 is $2.098 \pm 0.3\text{cm}$, suggesting that *Physarum polycephalum* can tolerate stress and grow in basic mediums with lower pH levels.



Graph 2. Representing the growth rate of *P.polycephalum* against the pH range

The data presented in **Graph 2** shows that the highest growth rate occurs between pH 5 and pH 5.5, which is from the 0.140-0.145 cm/hour range, suggesting the optimal pH level for the slime molds lies between pH 5 to 5.5. Additionally from the graph, it has also been observed that the growth rate at pH 7 (0.130 cm/hour) is quite close to that of pH 5. Hence, this suggests that slime molds should have a faster growth rate between pH 5 and 7, which verifies with the study done in **MYCOLOGIA, (1973)¹²** on optimum pH conditions for *P.polycephalum*. Additionally, as the pH extends towards extreme pH levels (pH 3 and pH 12), the growth rate of slime sharply drops to 0.0317 cm/hour and 0.0195 cm/hour, respectively. The sharp decrease in the growth rate is due to enzyme denature of cellular homeostasis caused by either highly acidic or

¹² Collins, O'Neil Ray, and Hsi-chang Tang. "Physarum Polycephalum: PH and Plasmodium Formation." *Mycologia*, vol. 65, no. 1, Jan. 1973, p. 232, <https://doi.org/10.2307/3757810>. Accessed 16 Apr. 2022.

alkaline(basic) conditions, as studies suggest that extreme pH levels can cause severe damage to the slime mold's cell membrane, nutrient absorption, ion exchange that significantly inhibits its growth (Collins and Tang). Moreover, the growth rate corresponding at pH 9 is quite mediocre, however, it shows the ability of *P.polycephalum* to manage stress to a certain extent as seen in **Graph 2**, the growth rate at pH 9 is 0.0873 cm/hour, which is still significantly higher growth rate observed at pH 3 and pH 12.

Independent Variable	Calculated CHI SQUARE (X^2) value	p-value	Critical Value
pH range	41.964	1.37×10^{-6}	15.51

Figure 8. Chi-square values and critical values for the pH range

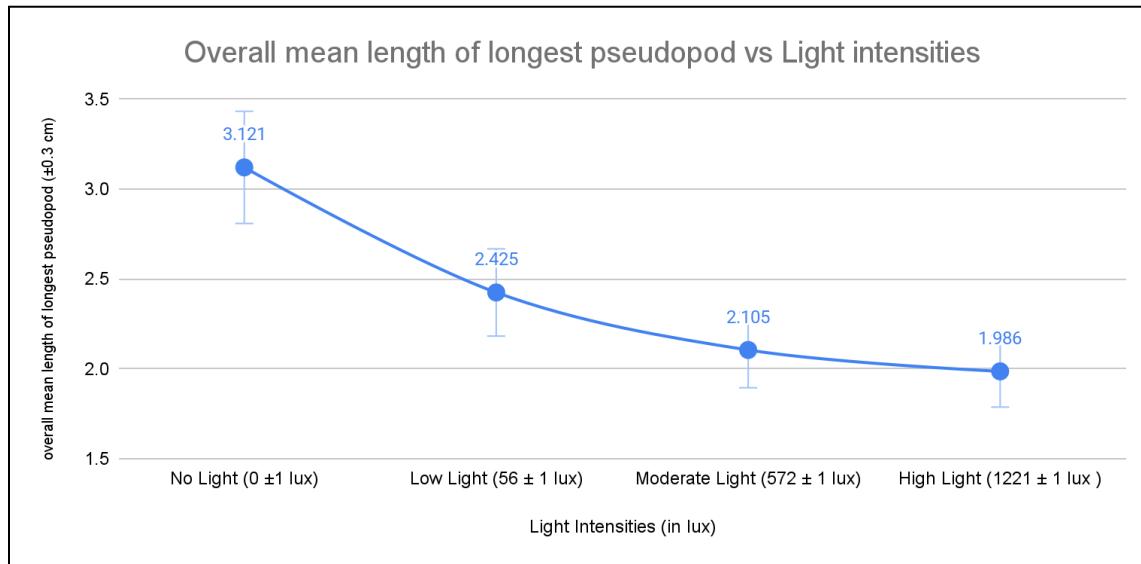
The relationship between the pH range and the growth of *P.polycephalum* was further verified using Pearson's Chi-square test, where I compared the **Null Hypothesis**, H_0 (The pH levels do not affect the growth rate of Physarum polycephalum) with the **Alternate Hypothesis**, H_1 (There is a significant relationship between pH and the growth rate of *P.polycephalum*). After conducting the chi-square test, as seen in **Figure 8**, the X^2 value (41.964) significantly exceeds the critical value (15.51), and the **p-value** (1.37×10^{-6}) is far less than the significance level (0.05). Therefore the **Null Hypothesis** H_0 , is rejected and the **Alternate Hypothesis** H_1 is accepted, indicating that pH has a significant relationship with the growth rate of the slime molds. Hence the results of the chi-square validate my hypothesis, which was framed in section **1.2 Hypothesis**. From the results of the experiment, it was observed that the relationship between the pH ranges and growth rate shows a clear trend for growth across the different pH levels, where it exhibits optimum growth between pH 5 to pH 5.5 corresponding to the highest growth rate that was observed (0.140 -0.145 cm/hour) and as mentioned earlier that pH 7 is also quite favorable

for the slime molds growth. This confirms that the optimum pH for the enzymatic activity and cellular processes of *P.polycephalum* is between pH 5 and 7, as mentioned in the findings from Kawamura & Nagano (1975)¹³ and mentioned in section 1.1, the enzyme **pyrophosphohydrolase** exhibits peak activity in slightly acidic to neutral environment, allowing the ATP hydrolysis, which has a crucial role in the cytoplasmic streaming (pseudopod extension), by which the organelles and nutrients are transported throughout the organism. Additionally, at the optimum pH, the availability of the calcium ion enhances the enzymatic interactions, thus stabilizing the actin-myosin cytoskeletal interactions that are responsible for cytoplasmic streaming. In contrast at extreme pH levels, such as pH 3 and 12 the growth of *P.polycephalum* is significantly inhibited due to the denaturation and insolubility of another crucial enzyme, also mentioned in section (). As mentioned in the study by Furuhashi(2008)¹⁴, the enzyme **alkaline phosphatase** in the slime molds becomes insoluble under extreme conditions, and it becomes ineffective in performing its biochemical functions, as when exposed to extremely acidic or basic(alkaline) environments, the enzyme's tertiary structure destabilizes which eventually leads to denaturation and loss of activity. Additionally due to the denaturation of the alkaline phosphatase the processes like the cytoplasmic streaming are damaged, resulting in restriction to the organism's ability to acquire nutrients and grow. Therefore this explains the reduced growth rates of the slime molds, observed in **Graph 2**, at pH 3 and 12, as the enzymatic efficiency is extremely crucial for retaining and maintaining their physiological functions.

¹³ Kawamura, M, and K Nagano. "A Calcium Ion-Dependent Atp Pyrophosphohydrolase in Physarum Polycephalum." *Biochimica et Biophysica Acta*, vol. 397, no. 1, 1975, pp. 207–19. pubmed.ncbi.nlm.nih.gov/238634/[https://doi.org/10.1016/0005-2744\(75\)901941](https://doi.org/10.1016/0005-2744(75)901941).

¹⁴ Kiyoshi Furuhashi. "Alkaline Phosphatase of Physarum Polycephalum Is Insoluble." *Archives of Microbiology*, vol. 189, no. 2, 25 Sept. 2007, pp. 151–156, <https://doi.org/10.1007/s00203-007-0306-x>. Accessed 11 Oct. 2024.

3.3.2 Light Intensities

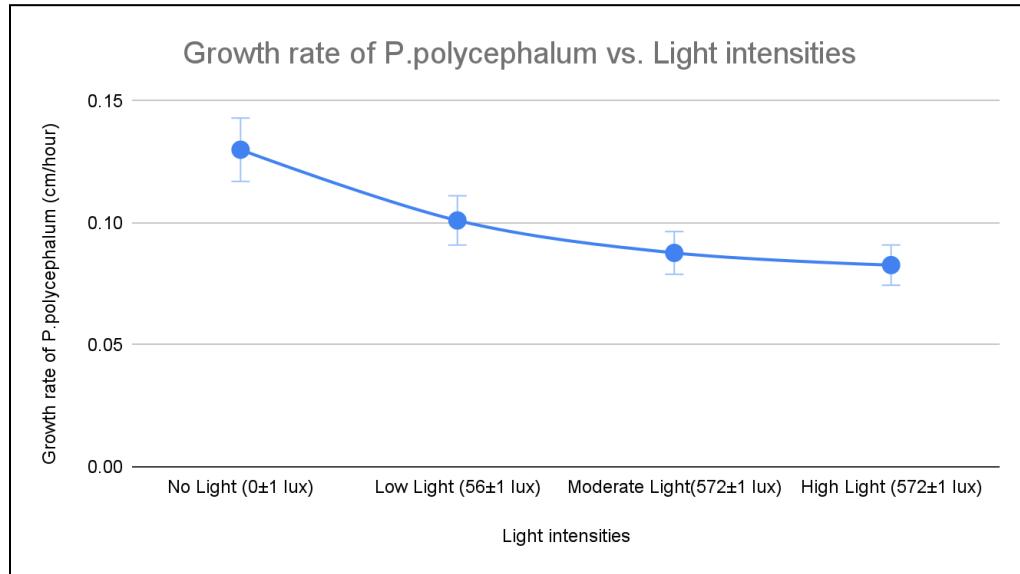


Graph 3. Representing the overall mean length of the longest pseudopod against different light intensities

(Refer to [Appendix D](#) to see the length of the longest pseudopod for each trial)

As evident in the data presented in **Graph 3**, we can see that as the light intensity increases, hence the length of the longest pseudopod decreases, suggesting a negative relationship between light intensities and the length of the longest pseudopod. The greatest value of the mean length of the longest pseudopod, corresponding to No light intensity (0 ± 1 Lux) is 3.121 ± 0.3 cm. Moving on, as the light intensity increases, the length of the longest pseudopod tends to decrease significantly, as evident in the graph that at No light (0 ± 1 Lux) the length was 3.121 ± 0.3 cm, however at Low Light intensity (56 ± 1 Lux) the length to 2.425 ± 0.3 cm, which is a difference of 0.696 cm, shows that just exposure to even low light affects the slime molds. Additionally, moving to Moderate light intensity (572 ± 1 Lux), and High light intensity (1221 ± 1 Lux), the length further decreases with the increasing light intensities,

from 2.105 ± 0.3 cm and 1.986 ± 0.3 cm. Although the pseudopod extensions were observed with high light intensities, with the loss of yellow pigment color and the thickness of the extensions, as seen in **3.1.1 Qualitative Data, Figures 6 and 7.**



Graph 4. Representing the growth rate of *Ppolycephalum* against different light intensities

The data presented in **Graph 4** shows that the optimum growth rate of *Ppolycephalum* occurs with No light intensity (0 ± 1 Lux), which is 0.130 cm/hour, however, the growth rate declines with increasing light intensity, as in a study done by **Latty and Beekman (2010)**¹⁵, they found that slime molds generally avoids light because the organism experience stresses that triggers a negative phototaxis, in where the slime molds move away from light sources, hence due to which the slime molds change their physiological behavior and redirect their energy towards avoiding

¹⁵ Reinhardt, Donald J. "The Effects of Light on the Development of the Cellular Slime Mold *Acrasis Rosea*." American Journal of Botany, vol. 55, no. 1, 1 Jan. 1968, pp. 77–77, <https://doi.org/10.2307/2440496>. Accessed 25 Apr. 2024.

the light exposure rather than growth as light causes stress that may result in cellular damage and eventually inhibiting the growth.

Independent Variable	Calculated CHI SQUARE (X^2) value	p-value	Critical Value
Light intensities	21.98	1.21×10^{-3}	12.59

Figure 9. Chi-square values and critical values for light intensities

The relationship between the light intensities and the growth of *P.polycephalum* was also tested and verified using Pearson's Chi-square test, where the **Null Hypothesis** H_0 (There is no significant difference in the growth of *P. polycephalum* across the varying light intensity levels, as light intensity does not affect the slime mold's growth rate) was compared with the **Alternate Hypothesis**, H_1 (There is a significant difference in the growth of *P.polycephalum* across the varying light intensity levels, as light intensity affects the slime molds's growth rate). As seen in **Figure 9**, the X^2 value (21.98) is greater and exceeds the critical value (12.59), with the **p-value** (1.21×10^{-3}) being less than the significance level (0.05). Therefore the **Null Hypothesis** H_0 , is rejected and the **Alternate Hypothesis** H_1 , will be accepted, indicating that varying light intensity level has a significant relationship with the growth rate of the slime molds, therefore the results of the chi-square validate my hypothesis, which also framed in section **1.2 Hypothesis**. Through the results of the experiment, a clear trend is observed that indicates, with increasing light intensity, the growth rate of the slime molds decreases. The optimal growth occurred with No light (0 ± 1 Lux) intensity, that is 0.130 cm/hour. Moving on, as the light intensity increases from Low (56 ± 1 lux)) to Moderate(572 ± 1 lux) to High (572 ± 1 lux) light intensities, the growth rate exponentially decreases from 0.101 cm/hour, 0.0877 cm/hour, and 0.0827 cm/hour respectively. Hence, this confirms that *P.polycephalum* prefers and exhibits the best growth in darker

conditions with no light exposure, also mentioned in a study by **Ueda, T.,& Terayama, K. (1975)**¹⁶ that in darkness the enzymatic processes such as the calcium-dependent ATP pyrophosphohydrolase efficiently functions, allowing the organism to efficiently extend pseudopod and nutrient transport. However, upon exposure to light, the slime molds alter their cytoplasmic streaming direction to migrate away from light as this is a phototactic response for its survival because the organism will experience oxidative stress due to the formation of reactive oxygen species (ROS), that disrupts the ATPase activities, resulting in inhibition of pseudopod extension and the overall growth (**Reinhardt, 1968**)¹⁷.

¹⁶ Hato, M., Ueda, T., Kurihara, K., & Kobatake, Y. (n.d.). *CELL STRUCTURE AND FUNCTION 1, 269-278 (1976) C by Japan Society for Cell Biology Phototaxis in True Slime Mold *Physarum polycephalum**. Retrieved from https://www.jstage.jst.go.jp/article/csf1975/1/3/1_3_269/_pdf

¹⁷ Reinhardt, D. J. (1968). The Effects of Light on the Development of the Cellular Slime Mold *Acrasis rosea*. *American Journal of Botany*, 55(1), 77–77. <https://doi.org/10.2307/2440496>

4.0 Evaluation and Conclusion

4.1.1 Evaluation

4.1.2 Limitations of the investigation

Source of limitations/errors	The possible impact on data	Potential improvement
Length of the oat flake inhabited by slime mold	There may be variations in the amount of the slime mold present on the oat flake that could lead to an inconsistent growth rate and potentially affect the reliability of the results.	By standardizing the initial colonization or inhibition of the oat flakes, meaning allowing the slime mold to colonize equal sized or mass of oat flakes and verifying whether there is a consistent amount of slime mold on the oat flakes across all the trials.
Inconsistency in pH solution (pH 9)	During the trials of pH 9, it was observed that the pH level dropped from pH 9 to pH 6 potentially due to the atmospheric carbon dioxide dissolving in the aqueous solution and forming carbonic acid, hence lowering the pH. Incorrect pH may misrepresent the impact of pH 9 conditions on the slime mold's growth.	By using a well-buffered pH 9 solution, minimizing the exposure to air, and using a calibrated pH meter or pH strips to verify the pH of the growth medium in timely intervals.
Light source fluctuation	Inconsistent light intensity caused by electricity cuts and fluctuations may potentially lead to variability in growth patterns.	Using a stable regulated light source by connecting it to a voltage stabilizer, reduces the fluctuations in the light source and ensures consistent intensity.
Single culture used throughout all trials	Throughout all the experiments the slime mold was used from a single culture, therefore using the same culture may reduce genetic variability and potentially limit the generalizability of the results because all results are dependent on the organism, hence these results may be inconsistent with other slime molds.	Performing the experiments using different cultures to account for this biological variability and minimize errors.
Potential contamination of the Petri dishes	Contamination of the petri dishes by either bacteria or fungi may inhibit or slow down the slime mold's growth, potentially because of competition between the organisms.	By maintaining the aseptic, and sterile condition and by using a Parafilm to seal the petri dish to minimize contamination via air.

Limited Data collection (due to time constraints)	The data was collected only after 24 hours of incubation, which may not fully capture the long-term growth trends of the slime molds. (refer to Section 3.1.1, Figure 6)	Extending the observation/incubation period to collect data at multiple time intervals extending, that could potentially extend to a week, allowing a better understanding of long-term growth trends in the slime molds.
---	--	---

Table 15.Represents the potential source of errors or limitations, their impact on the results, and potential solutions

4.1.2 Evaluation of sources

The majority of the sources used in my investigation were from highly credible and trusted platforms such as JSTOR, the National Institutes of Health, reputable scientific journals, and academic books. However, there were not many direct sources that talked about the effect of pH on the slime molds implicitly, while a few other sources were less formal web articles lacked rigorous peer review and were slightly less credible.

4.1.3 Potential Extensions

1. Studying the effect of pH range and light intensities on various other slime mold species, such as ***Badhamia utricularis, Acrasida, and Plasmodiophomycetes***.
- 2 . Studying how growth is influenced by exposure to stress-inducing chemicals such as salts and heavy metals.
3. Investigating the effect of combined pH and light intensity to analyze the combined effect on the growth rate of ***Physarum polycephalum***
- 4 . Investigating the long-term growth and adaptation of the slime mold over several days to weeks under their pH and light conditions.
5. Investigating the effect of different wavelengths of light such as infrared, blue, and UV, influence the growth rate in comparison to white light

5.0 Conclusion

In conclusion, the results from my investigation validate the hypotheses framed in section **1.2**, regarding the pH range and light intensities, where my results suggest that the optimal pH level for the ***Physarum polycephalum*** lies between pH 5-5.5 with the greatest growth rate.

Additionally, as I hypothesized for the light intensities, exposure to increasing light intensity resulted in the decline of the growth rate, whereas the greatest growth rate occurred with no light intensity. The validity of the data was further verified or validated with Pearson's chi-square test, where the chi-square values for both the independent variables (pH range and light intensities) were significantly greater than the critical value, suggesting a strong relationship between the independent variables and the dependent variable (growth rate). However, I relied on a technological tool, ImageJ, for collecting the data, which can be quite precise and uncertain at the same time.

6.0 Citations

Briard, L., et al. "Stress Signalling in Acellular Slime Moulds and Its Detection by Conspecifics." *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 375, no. 1802, 18 May 2020, p. 20190470, <https://doi.org/10.1098/rstb.2019.0470>.

Accessed 30 Mar. 2021.

Briard, L., et al. "Stress Signalling in Acellular Slime Moulds and Its Detection by Conspecifics." *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 375, no. 1802, 18 May 2020, p. 20190470, <https://doi.org/10.1098/rstb.2019.0470>.

Accessed 30 Mar. 2021.

Collins, O'Neil Ray, and Hsi-chang Tang. "Physarum Polycephalum: PH and Plasmodium Formation." *Mycologia*, vol. 65, no. 1, Jan. 1973, p. 232, <https://doi.org/10.2307/3757810>. Accessed 16 Apr. 2022.

Boussard, A., Fessel, A., Oettmeier, C., Briard, L., Döbereiner, H.-G., & Dussutour, A. (2021). Adaptive behaviour and learning in slime moulds: the role of oscillations. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 376(1820), 20190757.
<https://doi.org/10.1098/rstb.2019.0757>

Gray, W. D. (1939). The Relation of pH and Temperature to the Fruiting of Physarum polycephalum. *American Journal of Botany*, 26(9), 709–714.
<https://doi.org/10.2307/2437020>

Hato, M., Ueda, T., Kurihara, K., & Kobatake, Y. (1976). *CELL STRUCTURE AND FUNCTION I*, 269-278 (1976) C by Japan Society for Cell Biology Phototaxis in True Slime Mold

Physarum polycephalum. Retrieved from

https://www.jstage.jst.go.jp/article/csf1975/1/3/1_3_269/_pdf/-char/en

ImageJ. (n.d.). Retrieved October 23, 2024, from imagej.net website:

<https://imagej.net/ij/index.html>

Kawamura, M, and K Nagano. “A Calcium Ion-Dependent Atp Pyrophosphohydrolase in *Physarum Polycephalum*.” *Biochimica et Biophysica Acta*, vol. 397, no. 1, 1975, pp. 207–19, pubmed.ncbi.nlm.nih.gov/238634/,
[https://doi.org/10.1016/0005-2744\(75\)90194-1](https://doi.org/10.1016/0005-2744(75)90194-1).

Kiyoshi Furuhashi. “Alkaline Phosphatase of *Physarum Polycephalum* Is Insoluble.” *Archives of Microbiology*, vol. 189, no. 2, 25 Sept. 2007, pp. 151–156,
<https://doi.org/10.1007/s00203-007-0306-x>. Accessed 11 Oct. 2024.

Latty, T., & Beekman, M. (2010). Food quality and the risk of light exposure affect patch-choice decisions in the slime mold *Physarum polycephalum*. *Ecology*, 91(1), 22–27.

<https://doi.org/10.1890/09-0358.1>

Phototaxis - an overview | ScienceDirect Topics. (n.d.-a). Retrieved November 25, 2024, from www.sciencedirect.com website:

<https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/phototaxis>

Pseudopods - Definition, Function, Movement and Examples. (n.d.). Retrieved November 25, 2024, from MicroscopeMaster website:

<https://www.microscopemaster.com/pseudopods.html>

Reinhardt, D. J. (1968). The Effects of Light on the Development of the Cellular Slime Mold Acrasis rosea. *American Journal of Botany*, 55(1), 77–77.

<https://doi.org/10.2307/2440496>

Reynolds, M. (2019, October 19). All Hail the Blob, the Smart Slime Mold Confounding

Science. Retrieved November 25, 2024, from WIRED website:

<https://www.wired.com/story/all-hail-the-blob-smart-slime-mold-confounding-science/>

7.0 Appendices

Appendix A

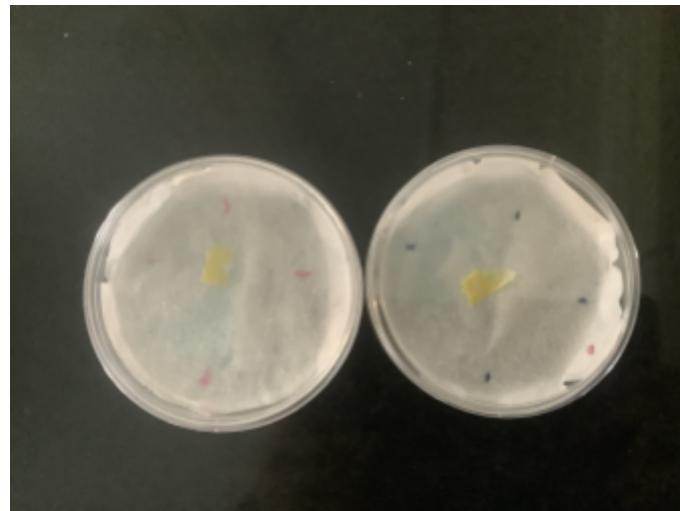


Figure A1. Grown using a buffer solution

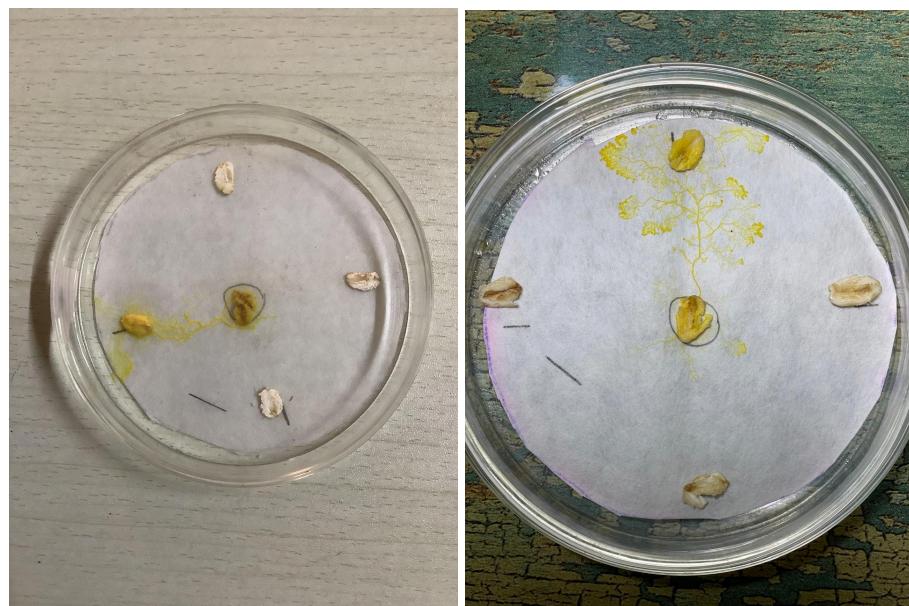


Figure A2. Grown using NaOH and HCl solutions



Figure A3. Slime mold culture

Appendix B

Calculating uncertainties in measurement

$$\begin{aligned} \text{Mean pixels of Petri Dish} &= \frac{\text{pixels of petri dish diameter}}{\text{Number of measurements}} \\ &= \frac{832.101+837.289+828.005+823.00+823.00}{5} \\ &= 828.679 \text{ pixels} \end{aligned}$$

$$\text{Standard deviation of pixels (SD)} = 5.56 \text{ pixels}$$

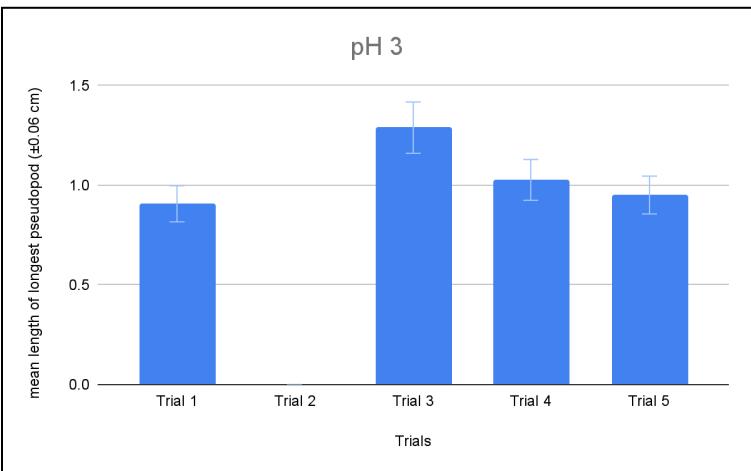
$$\begin{aligned} \text{Converting pixel to cm} &= \frac{\text{Diameter of petri dish (in cm)}}{\text{Mean pixels}} \\ &= \frac{9 \text{ cm}}{828.679} \\ &= 0.0108 \text{ cm /pixel} \end{aligned}$$

$$\begin{aligned} \text{uncertainty (in cm)} &= \text{Standard deviation of pixels in petri dish} \times \text{cm/pixel} \\ &= 5.56 \times 0.0108 \\ &= \pm 0.06 \text{ cm} \end{aligned}$$

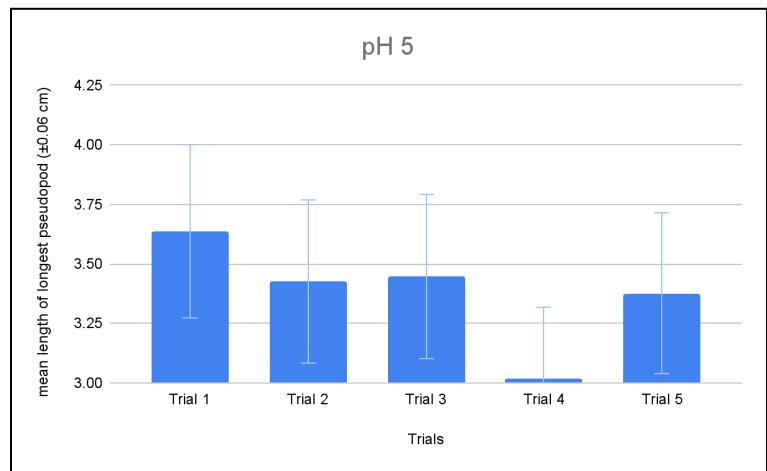
Appendix C

Graphical representation of data from each trial for pH and Light intensities

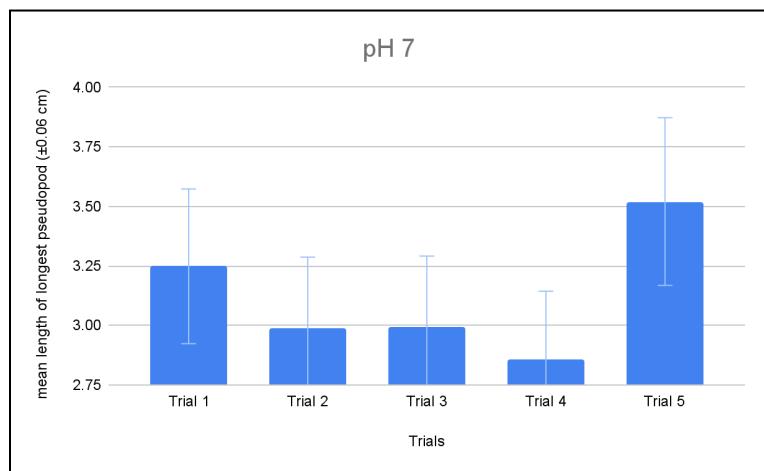
Graphs C1.1 to C1.5 represent the length of the longest pseudopod for each trial in the pH variable.



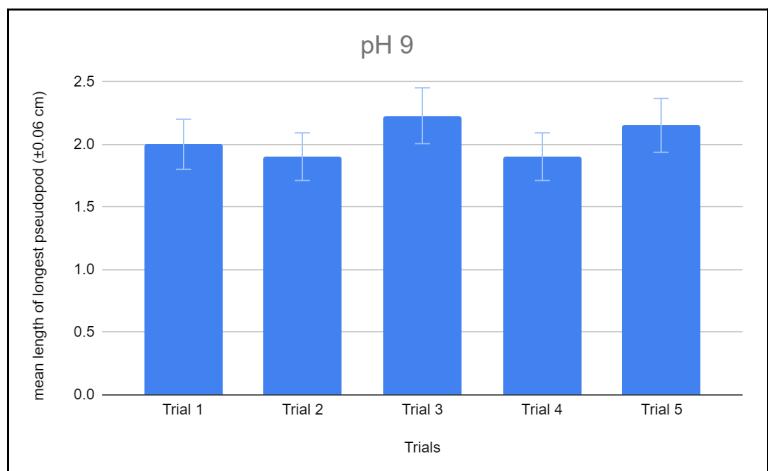
Graph C1.1. Represents the length of pseudopod at different trials at pH 3



Graph C1.2. Represents the length of pseudopod at different trials at pH 5

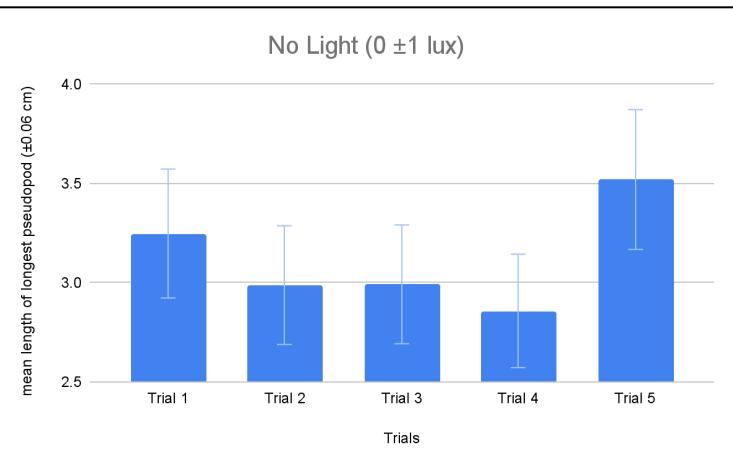


Graph C1.3. Represents the length of pseudopod at different trials at pH 7

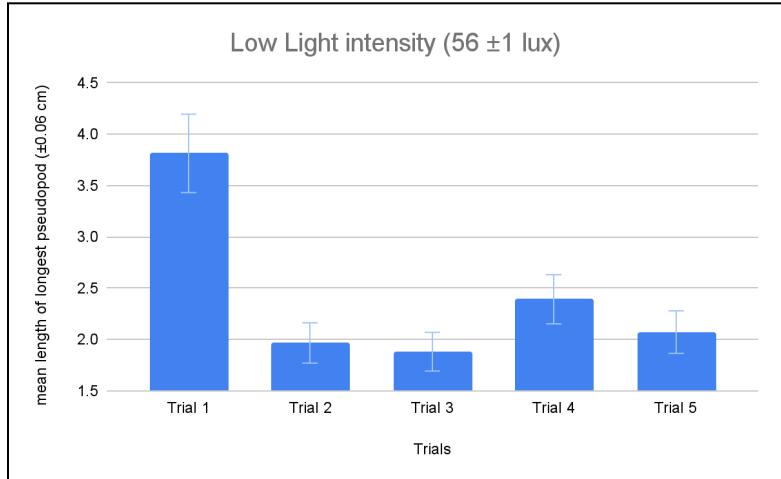


Graph C1.4. Represents the length of pseudopod at different trials at pH 9

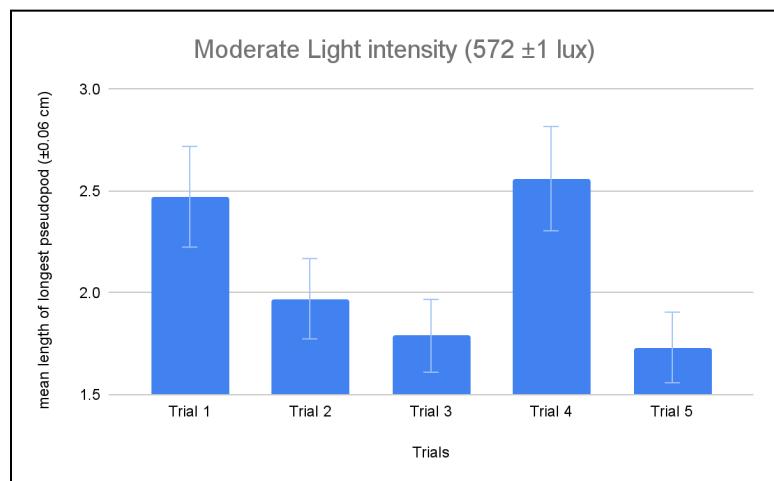
Graphs C2.1 to C2.4 represent the length of the longest pseudopod for each trial in the light intensity variable.



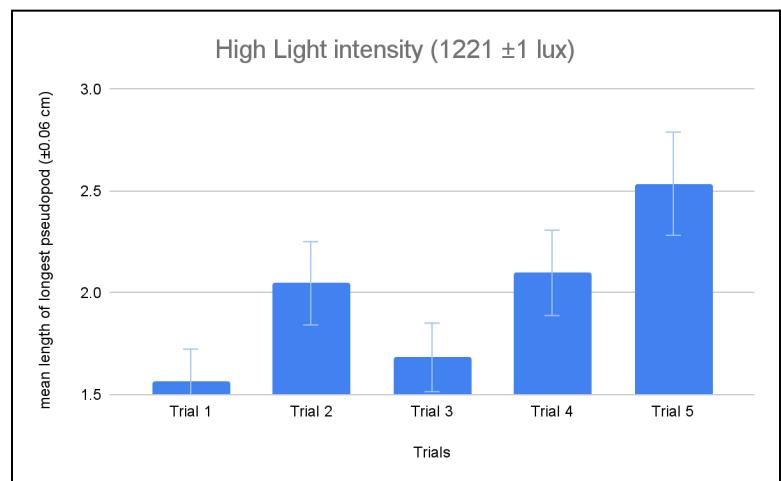
Graph C2.1. Represents the length of pseudopod at different trials at no light



Graph C2.2. Represents the length of pseudopod at different trials at low light



Graph C2.3. Represents the length of pseudopod at different trials at moderate



Graph C2.4. Represents the length of pseudopod at different trials at high light

Appendix D

Growth Categorisation Key based on length of longest pseudopods observed per trial (cm) for pH	
Low Growth	≤ 1.5 cm
Medium Growth	>1.5 and ≤ 3.00 cm
High Growth	>3.00 cm

Table 12 .Growth categorisation for pH

Growth Categorisation Key based on length of longest pseudopods observed per trial (cm) for light intensities	
Low Growth	> 1.8 cm
Medium Growth	≤ 2.8 cm and <1.8
High Growth	>2.8 cm

Table 13.Growth categorisation for Light intensities

pH	3	5	7	9	12	Total
Growth						
Low Growth	5.00	0.00	0.00	0.00	5.00	10.00
Moderate Growth	0.00	0.00	3.00	5.00	0.00	8.00
High Growth	0.00	5.00	2.00	0.00	0.00	7.00
Total	5.00	5.00	5.00	5.00	5.00	25.00

Table D3.Observed value

pH	3	5	7	9	12
Growth					
Low Growth	2.0	2.0	2.0	2.0	2.0
Moderate Growth	1.6	1.6	1.6	1.6	1.6
High Growth	1.4	1.4	1.4	1.4	1.5

Table D4.Expected value

O	E	O - E	(O - E) ²	$\frac{(O-E)^2}{E}$
5.00	2.00	3.00	9.00	4.5
0.00	2.00	-2.00	4.00	2.00
0.00	2.00	-2.00	4.00	2.00
0.00	2.00	-2.00	4.00	2.00
5.00	2.00	3.00	9.00	4.5
0.00	1.6	-1.6	2.56	1.6
0.00	1.6	-1.6	2.56	1.6
3.00	1.6	1.4	1.96	1.225
5.00	1.6	3.4	11.56	7.225
0.00	1.6	-1.6	2.56	1.6
0.00	1.4	-1.4	1.96	1.4
5.00	1.4	3.6	12.96	9.257
2.00	1.4	0.6	0.36	0.257
0.00	1.4	-1.4	1.96	1.4
0.00	1.4	-1.4	1.96	1.4
Total X² Calculated value				41 .964

Table D5. X² Calculation

Degree of Freedom = (columns - 1) × (rows - 1)

Degree of Freedom = (5 - 1) × (3 - 1) = 8

Light intensities	No Light	Low Light	Moderate Light	High Light	Total
Growth					
Low Growth	0.00	0.00	0.00	2	2
Moderate Growth	0.00	4	5	3	12
High Growth	5	1	0	0	6
Total	5	5	5	5	20

Table D6. Observed value

Light intensities	No Light	Low Light	Moderate Light	High Light
Growth				
Low Growth	0.5	0.5	0.5	0.5
Moderate Growth	3	3	3	3
High Growth	1.5	1.5	1.5	1.5

Table D7. Expected value

O	E	O - E	(O - E) ²	$\frac{(O - E)^2}{E}$
0.00	0.5	-0.5	0.25	0.5
0.00	0.5	-0.5	0.25	0.5
0.00	0.5	-0.5	0.25	0.5
2	0.5	1.5	2.25	4.5
0.00	3	-3	9	3
4	3	1	1	0.33
5	3	2	4	1.33
3	3	0.00	0.00	0.00
3	1.5	3.5	12.25	8.16
1	1.5	-0.5	0.25	0.16
0.00	1.5	-1.5	2.25	1.5
0.00	1.5	-1.5	2.25	1.5
Total X² Calculated value				21.98

Table D8. X² Calculation

Degree of Freedom = (columns – 1) × (rows – 1)

Degree of Freedom = (4 – 1) × (3 – 1) = 6

Percentage Points of the Chi-Square Distribution									
Degrees of Freedom	Probability of a larger value of χ^2								
	0.99	0.95	0.90	0.75	0.50	0.25	0.10	0.05	0.01
1	0.000	0.004	0.016	0.102	0.455	1.32	2.71	3.84	6.63
2	0.020	0.103	0.211	0.575	1.386	2.77	4.61	5.99	9.21
3	0.115	0.352	0.584	1.212	2.366	4.11	6.25	7.81	11.34
4	0.297	0.711	1.064	1.923	3.357	5.39	7.78	9.49	13.28
5	0.554	1.145	1.610	2.675	4.351	6.63	9.24	11.07	15.09
6	0.872	1.635	2.204	3.455	5.348	7.84	10.64	12.59	16.81
7	1.239	2.167	2.833	4.255	6.346	9.04	12.02	14.07	18.48
8	1.647	2.733	3.490	5.071	7.344	10.22	13.36	15.51	20.09
9	2.088	3.325	4.168	5.899	8.343	11.39	14.68	16.92	21.67
10	2.558	3.940	4.865	6.737	9.342	12.55	15.99	18.31	23.21

Figure D1. Chi-square distribution table

Percentage Points of the Chi-Square Distribution									
Degrees of Freedom	Probability of a larger value of χ^2								
	0.99	0.95	0.90	0.75	0.50	0.25	0.10	0.05	0.01
1	0.000	0.004	0.016	0.102	0.455	1.32	2.71	3.84	6.63
2	0.020	0.103	0.211	0.575	1.386	2.77	4.61	5.99	9.21
3	0.115	0.352	0.584	1.212	2.366	4.11	6.25	7.81	11.34
4	0.297	0.711	1.064	1.923	3.357	5.39	7.78	9.49	13.28
5	0.554	1.145	1.610	2.675	4.351	6.63	9.24	11.07	15.09
6	0.872	1.635	2.204	3.455	5.348	7.84	10.64	12.59	16.81
7	1.239	2.167	2.833	4.255	6.346	9.04	12.02	14.07	18.48
8	1.647	2.733	3.490	5.071	7.344	10.22	13.36	15.51	20.09
9	2.088	3.325	4.168	5.899	8.343	11.39	14.68	16.92	21.67
10	2.558	3.940	4.865	6.737	9.342	12.55	15.99	18.31	23.21

Figure D2. Chi-square distribution table