STEM Project Grade 8

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Physarum Polycephalum Physarum Cultures

### Title

Investigating Chemotaxis by Analyzing the Effect of Glucose/Caffeine on Physarum Polycephalum cultures.

### Abstract

How will Physarum Polycephalum respond towards varying concentrations of glucose and caffeine? My hypothesis stated that the Physarum Polycephalum will grow towards the higher concentrations of glucose, and away from coffee. Following the online procedure from [Science Buddies/Studying Chemotaxis in Physarum](https://www.sciencebuddies.org/science-fair-projects/project-ideas/MicroBio_p028/microbiology/chemotaxis-slime-mold#procedure), Physarum plasmodium cultures were grown in petri dishes and then added as 0.5cm x 0.5cm blocks (total of 4 blocks) onto the junction of 2 filter strips, one soaked in distilled water and the other soaked in various concentrations of glucose, per petri dish(\*5 for 5 petri dishes); the same process was repeated for various concentrations of coffee. For various concentrations of glucose, the results of trial 1 and trial 2 were not consistent. Trial 1 results showed positive growth (growth towards the glucose strip) for glucose cocentrations > 1mM (milliMolar), while Trial 2 results showed negative growth (Growth away from the glucose strip) for concentrations > 1Mm. Trial results for coffee showed no growth (positive, negative or neutral) after a 2 day observation. Based on the results from both the glucose and coffee trials, determining chemotaxis on Physarum Polycephalum cultures per my hypothesis is inconsistent and therefore inconclusive.

### Introduction

I wanted to do a project that involved observations in nature and apply statistics on those observations. We take our 10-lb dog Prince on a walk everyday around the Jordan Woods Natural Area, and I’ve noticed slime molds on fallen trees…etc…This got me interested in learning more about slime molds. Per this article <https://www.quantamagazine.org/slime-molds-remember-but-do-they-learn-20180709/>, there are more than 900 different species of slime molds, and a particular fascinating read (also cited in “Background Information”), titled “Slime molds crack 3 of the biggest issues in the U.S” by Robby Berman, that summarizes the results of the experiments by Jonathon Keats using slime molds to crack those 3 biggest issues (Destruction of our climate, The opioid epidemic, and immigration) motivated me to further understand these problem-solving, habituated-learning and smart “neuron-less and brain-less” Physarum Polycephalum via a project.

### Questions

* How will Physarum Polycephalum respond towards varying concentrations of glucose?
  + Is there a correlation we can establish between varying concentrations of glucose and PP’s chemotaxis?
  + How will it respond when we substitute caffeine or salt for glucose?
* How long does it take a P. Polycephalum to find the glucose?
* What are the different ways Physarum Polycephalum responds to its environment/chemical stimuli?
* What are the various stages of growth of Physarum Plycephalum?
* Is Physarum Polycephalum capable of optimally finding food sources and avoiding harmful stimuli?

### Terms and Concepts (cited from Science Buddies; please see Background Information)

* Physarum: Physarum is a genus of mycetozoan(grouping of slime molds) slime molds and Physarum Polycephalum is one of its species.
* Eukarytotic: Organisms whose cells have a nucleus enclosed within membranes.
* Slime mold: An informal name given to several kinds of unrelated eukaryotic orgranisms. Physarum Polycephalum is a member of slime mold.
* Protist: Any eukaryotic organism that is not an animal, plant or fungus. The slime mold is classified as a protist.
* Plasmodium: The vegetative phase of a two part life-cycle of a slime mold. It consists of networks of protoplasmic veins, many nuclei, and lives as a shapeless, growing blog during moist, warm weather. It’s color varies from gray, cream, colourless, to bright yellow or orange. It is during this stage that it searches for food.
* Fruiting body: During the second phase of a slime mold, when the environment is dry, the shapeless, growing blob transforms into small stock-like fruiting bodies that are full of dust like spores.
* Environmental cue: The information a physarum plasmodium has to rely on in search of food. It uses the information to decide whether to move towards a food source or away from the harmful substances.
* Chemical cue: The chemical information that a plasmodium uses to decide whether to move towards or away from the chemical in question.
* Chemotaxis: When an organism moves in repsonse to a chemical stimulus. Movement toward a higher concentration of chemical in question is termed as positive chemotaxis, while a movement in the opposite direction is negative chemotaxis.
* Physarum polycephalum: One of the slime molds that literally translates to “many-headed slime”. It is a unicellular organism that lives in dark, cool and humid areas such as leaf litter and other organic debris.
* Millimolar concentration: Measurement unit for the concentration of an ingredient in solution.

### Background Information:

* Robby Berman(March 17th, 2018). Slime molds crack 3 of the biggest issues in the U.S. Retrieved October 31st, 2018, from <https://bigthink.com/robby-berman/slime-molds-crack-3-of-the-biggest-issues-in-the-us>. “At an event on March 2, their secretary, experimental philosopher and conceptual artist Jonathon Keats, announced the group has cracked three of America’s most vexing and controversial problems: the destruction of our climate, the opioid epidemic, and immigration. The Consortium’s secret? It brings a unique perspective to these issues. “They’re all slime molds,” says Keats. This is the first analysis of these issues by non-human scholars, he notes, asserting, “Their advice is objective, and transcends our polarized political environment because they don’t belong to our species.” (A slime mold was previously appointed to the Hampshire faculty.)”
* Katia Moskvitch, Contributing Writer, Cognitive Science (2018, July 9). Slime Molds Remember, but do they learn? Retrieved November 15th, 2018, from <https://www.quantamagazine.org/slime-molds-remember-but-do-they-learn-20180709/>. “In experiments conducted by Dussutour’s team, disks of yellow slime mold (at bottom) can eat plates of oatmeal (at top) — but only if they cross gelatinous bridges (at center) laced with noxious but harmless compounds. Here, the middle slime mold sample has learned to disregard the chemicals, a process called habituation.”
* Science Buddies Staff. (2017, July 28). Slimey Likes It! Studying Chemotaxis in Physarum Polycephalum . Retrieved October 31st, 2018, from <https://www.sciencebuddies.org/science-fair-projects/project-ideas/MicroBio_p028/microbiology/chemotaxis-slime-mold>. Details summary, background, materials and procedures required for the study of chemotaxis in Physarum Polycephalum with the objective of exploring chemotactic responses of P.polycephalum in culture exposed to various amounts of glucose.
* Ferris Jabr, Nature|Scientific American (2012, November 13) How brainless slime molds redefine intelligence. Retrieved November 18th, 2018, from <https://www.nature.com/news/how-brainless-slime-molds-redefine-intelligence-1.11811>. “Something scientists have come to understand is that slime molds are much smarter than they look. One species in particular, the SpongeBob SquarePants–yellow Physarum polycephalum, can solve mazes, mimic the layout of man-made transportation networks and choose the healthiest food from a diverse menu—and all this without a brain or nervous system.”Slime molds are redefining what you need to have to qualify as intelligent," Reid says.”
* Elizabeth Paine, Product Developer, retrieved November 12th, 2018, from <https://www.carolina.com/teacher-resources/Interactive/think-single-celled-organisms-are-simple-think-again-the-slime-mold-physarum-polycephalum-a-single-celled-organism-for-student-investigations/tr41405.tr>
* Wkipedia: <https://en.wikipedia.org/wiki/Chemotaxis>

### My Hypothesis

* The physarum polycephalum will grow towards the higher concentrations of glucose and away from caffeine (coffee)

### Methods:

**Materials**

All required items except for Coffee are listed at [Science Buddies/Studying Chemotaxis in Physarum](https://www.sciencebuddies.org/science-fair-projects/project-ideas/MicroBio_p028/microbiology/chemotaxis-slime-mold#materials). The Physarum polycephalum kit we purchased from [Carolina Biological Supply Company](https://www.carolina.com/science-buddies-project-materials/science-buddies-slimey-likes-it-studying-chemotaxis-physarum-polycephalum--project-materials/P_SB_FAM_CHEMOTAX.pr?s_cid=ptnr_scibuddies) contained the following:

* Physarum culture, as plasmodium on agar plate
* Petri dishes
* Non-nutrient agar
* Oatmeal flakes (Physarum “food”)
* Filter paper
* Aluminum foil
* Beaker, 250 mL
* Graduated cylinder, 10 mL
* Graduated cylinder, 100 mL
* Disposable gloves.

Other required materials as stated on [Science Buddies/Studying Chemotaxis in Physarum](https://www.sciencebuddies.org/science-fair-projects/project-ideas/MicroBio_p028/microbiology/chemotaxis-slime-mold#materials).

**Procedure:**

Follow procedure from [Science Buddies/Studying Chemotaxis in Physarum](https://www.sciencebuddies.org/science-fair-projects/project-ideas/MicroBio_p028/microbiology/chemotaxis-slime-mold#procedure).

**Variables:**

* Independent: Food Source:
  + Different concentrations of glucose: 100-mM(millimolar), 10-mM, 1.0-mM, 0.1-mM, No \* Glucose
  + Different concentrations of caffeine: TBD, No Caffeine
* Dependent: Number of PP cultures that show:
  + Positive Growth: Towards Glucose/Caffeine
  + Negative Growth: Away from Glucose/Caffeine
  + Neutral Growth: Equal growth in Glucose/Caffeine or No Glucose/No Caffeine
* Control: PP cultures, Various concentrations of Glucose/Caffeine The experiment will be conducted using plasmodium cultures on filter paper strips soaked with the food source (as defined under Variables: Independent) in petri-dishes (5 in total with different concetrations of the food source) in our garage with minimal sunlight. The experiment will be repeated twice for the two food sources (Glucose, Caffeine). Observations(numerical and digital pictures) will be recorded. The analysis of the observations will be done using the statistical programming language R.

### Results

I’ll be using programming language R to create data tables and plots:

The first trial1 supports my hypothesis of positive growth at glucose concentration of 1mM, 10mM and 100mM, and no growth at 0mM, does not support my hypothesis with neutral growth and negative growth at 0.1 and 1 mM. The second trial2 does not support my hypothesis at all levels of concentration, except for 2 observations at concentrations 1mM and 10mM.

### My Hypothesis

* Physarum Polycephalum will grow towards the higher concentrations of glucose and grow away from coffee.

#load required library  
library(tidyverse)

Recording and graphing the observations from trial 1 and trial 2 of my experiment which is understanding chemotaxis in Physarum Polycephalum

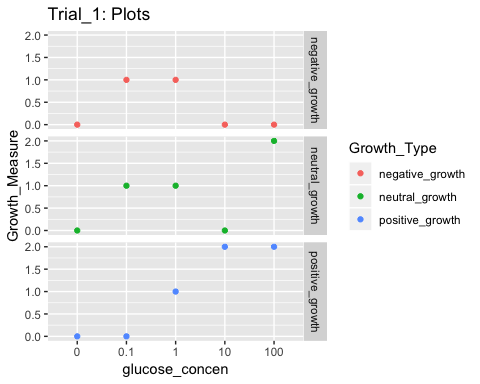
#construct graphs from the experiment  
glucose\_concen <- c(100, 10, 1, .1, 0)  
positive\_growth <- c(2, 2, 1, 0, 0)  
negative\_growth <- c(0, 0, 1, 1, 0)  
neutral\_growth <- c(2, 0, 1, 1, 0)  
  
Physarum\_Growth <- tibble(glucose\_concen,positive\_growth, negative\_growth, neutral\_growth) %>%   
 mutate(glucose\_concen = as.factor(glucose\_concen))  
  
Trial\_1\_Data <- Physarum\_Growth %>% knitr::kable(., caption = "Trial\_1 Data")  
  
Trial\_1\_Data

Trial\_1 Data

|  |  |  |  |
| --- | --- | --- | --- |
| glucose\_concen | positive\_growth | negative\_growth | neutral\_growth |
| 100 | 2 | 0 | 2 |
| 10 | 2 | 0 | 0 |
| 1 | 1 | 1 | 1 |
| 0.1 | 0 | 1 | 1 |
| 0 | 0 | 0 | 0 |

#Taking a wide data frame and making it a long data frame for graphing purposes by using the function gather()   
Physarum\_Growth\_longformat <- gather(Physarum\_Growth, Growth\_Type, Growth\_Measure, positive\_growth:neutral\_growth)  
#Physarum\_Growth\_longformat %>% knitr::kable(.)

#ggplot(Physarum\_Growth\_longformat, aes(x = as.factor(glucose\_concen), y = Growth\_Measure)) + geom\_point()  
#I used the function facet\_grid() to enable graphs in one visual.  
  
Trial\_plot\_1 <- Physarum\_Growth\_longformat %>% group\_by(Growth\_Type) %>%   
 ggplot(aes(x = glucose\_concen, y = Growth\_Measure, col = Growth\_Type)) + geom\_point() + facet\_grid(Growth\_Type ~.) + ggtitle("Trial\_1: Plots")  
  
Trial\_plot\_1



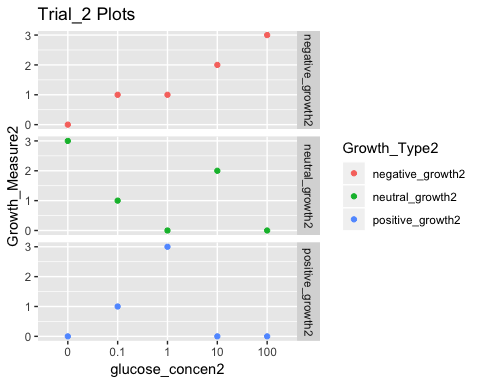
Trial 2

#construct graphs from the experiment  
glucose\_concen2 <- c(100, 10, 1, .1, 0)  
positive\_growth2 <- c(0, 0, 3, 1, 0)  
negative\_growth2 <- c(3, 2, 1, 1, 0)  
neutral\_growth2 <- c(0, 2, 0, 1, 3)  
  
Physarum\_Growth2 <- tibble(glucose\_concen2,positive\_growth2, negative\_growth2, neutral\_growth2) %>%   
 mutate(glucose\_concen2 = as.factor(glucose\_concen2))  
  
Trial\_2\_Data <- Physarum\_Growth2 %>% knitr::kable(., caption = "Trial\_2 Data")  
Trial\_2\_Data

Trial\_2 Data

|  |  |  |  |
| --- | --- | --- | --- |
| glucose\_concen2 | positive\_growth2 | negative\_growth2 | neutral\_growth2 |
| 100 | 0 | 3 | 0 |
| 10 | 0 | 2 | 2 |
| 1 | 3 | 1 | 0 |
| 0.1 | 1 | 1 | 1 |
| 0 | 0 | 0 | 3 |

#ggplot(Physarum\_Growth\_longformat, aes(x = as.factor(glucose\_concen), y = Growth\_Measure)) + geom\_point()  
#I used the function facet\_grid() to enable graphs in one visual.  
  
# Physarum\_Growth\_longformat %>% group\_by(Growth\_Type) %>%   
 #ggplot(aes(x = glucose\_concen, y = Growth\_Measure, col = Growth\_Type)) + geom\_point() + facet\_grid(.~Growth\_Type)  
  
Trial\_2\_Plot <- Physarum\_Growth\_longformat2 %>% group\_by(Growth\_Type2) %>%   
 ggplot(aes(x = glucose\_concen2, y = Growth\_Measure2, col = Growth\_Type2)) + geom\_point() + facet\_grid(Growth\_Type2 ~.) + ggtitle("Trial\_2 Plots")  
  
Trial\_2\_Plot



**Trial 1 Data Observation:**

* For glucose concentrations greater than 1mM, 3 observations showed positive growth - Supports hypothesis for the 3 observations.
* For glucose concentration’s 0.1mM, 1mM, and 100mM, 3 observations showed neutral growth - Refutes hypothesis for the 3 observations.
* For glucose concentration’s 0.1mM and 1mM, 2 observations showed negative growth - Refutes hypothesis

**Trial 2 Data Observation:**

* For glucose concentrations 1mM and 10mM, 2 observations showed positive growth - Supports hypothesis for the 2 observations.
* For glucose concentrations 0.1mM, 10mM and 100mM, 3 observations showed neutral growth - Refutes hypothesis.
* For glucose concentrations less than 100, 4 observations showed negative growth - Refutes hypothesis.

### Discussion

My hypothesis was partially correct and incorrect as proved by positive and negative growths in Trials 1 & 2 (for the glucose solutions). This could suggest variations in the control variables like room temp, preparation of various concentrations of glucose, and health of Physarum culture. For example, the glucose we used was grape flavored and this could have affected the results seeing as the artificial flavor could have repelled the Physarum. The room temperature may have been different when the separate trials were conducted. The health of the Physarum Culture may have varied with the trials. The trials with the various concentrations of coffee solution was interesting, as we observed no growth in any of the filter strips in 5 petri dishes; one could interpret this as the Physarum avoiding toxic substance or the Physarum culture was unhealthy. If I were to repeat this experiment in the future, I would try to find unflavored glucose or make my own glucose solution, conduct trial 1 & 2 on the same day at the same temperature, use cultures grown on a single petri dish. If I were to expand on this project, I would experiment chemotaxis on Physarum Polycephalum by placing glucose/oats (positive chemotaxis) and coffee/chilli pepper (negative chemotaxis) at the end of the maze, and see if the Physarum can find the quickest way to the glucose/oats or away from coffee/chilli pepper.

### Conclusion

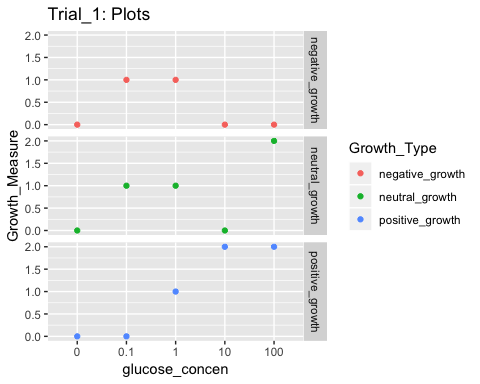
My hypothesis that the Physarum Polycephalum will grow towards the higher concentrations of glucose was partially supported and partially refuted as proved by positive and negative growths in Trials 1 & 2 (for various glucose concentrations; please see data/plots below). Trial results for coffee showed no growth (positive, negative or neutral) after a 2 day observation. Based on the results from both the glucose and coffee trials, determining chemotaxis on Physarum Polycephalum cultures per my hypothesis is inconsistent and therefore inconclusive.

Trial\_1\_Data

Trial\_1 Data

|  |  |  |  |
| --- | --- | --- | --- |
| glucose\_concen | positive\_growth | negative\_growth | neutral\_growth |
| 100 | 2 | 0 | 2 |
| 10 | 2 | 0 | 0 |
| 1 | 1 | 1 | 1 |
| 0.1 | 0 | 1 | 1 |
| 0 | 0 | 0 | 0 |

Trial\_plot\_1



Trial\_2\_Data

Trial\_2 Data

|  |  |  |  |
| --- | --- | --- | --- |
| glucose\_concen2 | positive\_growth2 | negative\_growth2 | neutral\_growth2 |
| 100 | 0 | 3 | 0 |
| 10 | 0 | 2 | 2 |
| 1 | 3 | 1 | 0 |
| 0.1 | 1 | 1 | 1 |
| 0 | 0 | 0 | 3 |

Trial\_2\_Plot

