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The Effects of Differing Light Wavelengths and Gravity on

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SENIOR THESIS APPROVAL

This Honors thesis entitled

The Effects of Differing Light Wavelengths and Gravity on *Physarum polycephalum*

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Reese Chesshir

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and has been approved by the undersigned readers.

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The Effects of Differing Light Wavelengths and Gravity on *Physarum polycephalum*

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Abstract

Physarum is a slime mold in the class of Mycetozoan and the family of *Physaraceae*. It is a single cellular, multinuclear organism that is not classified as an animal, plant, or fungi. *Physarum* growth for long-term space travel would be beneficial because it is a decomposer and is able to break down material and waste to recycle. This is important for transferring energy and allowing other organisms to grow. The purpose of this experiment is to study the effect of different light wavelengths and the influence of gravity on *Physarum* growth patterns. Four colors of wavelengths were used: red, green, blue, red and blue mixed light wavelengths were studied with no light being a control. The *Physarum* growth in each wavelength was documented. The possible effects of gravity conditions were produced by a clinostat. A clinostat is an instrument that spins the *Physarum* constantly so that gravity does not play a role in its growth. A microgravity condition was created using the clinostat with the wheel spinning at 1rpm. In all five light conditions, stationary and clinostat conditions were studied. *Physarum* was plated on the middle of a petri dish with oats along the rim to increase growth toward the edge of the dish. One-inch squares were drawn on the back of the dish to determine the amount of growth of the physarum. A line was drawn in the middle of the dish and a black streamer covered half the dish. The black streamer allowed for half of the Petri dish to receive light and the other half to be in the dark, allowing for a side towards the light and a side away from the light on the petri dish. The sides away from light and towards the light of the Petri dish were both studied to conclude whether *Physarum* preferred to grow more towards light or away from light. The findings of the experiment show that a microgravity environment condition did not have an effect on the growth pattern of the *Physarum*. The experiments showed that different wavelengths of light and microgravity environments did not affect the growth of the *Physarum* in

these conditions. The experimental results were analyzed using a single factor ANOVA test; concluding, all p-values showed there was no statistical difference between each condition. Results were concluded by counting up each square that the *Physarum* grew into on each side of the plate. This means that the *Physarum* grew equally in all conditions and areas of the plate. Since the *Physarum* just grew towards its food source, it was recorded that food had more of an impact on the *Physarum* than light and a microgravity condition. In the future, studies can be done with higher intensity of light or different intensity of light to determine at which intensity *Physarum* may start to grow only on the dark (away from light) side of the plate which is the control.

Introduction

Physarum polycephalum is a single-celled slime mold that makes it unique from other forms of life. It is one of the few single-celled organisms that humans can see with the naked eye. *Physarum* is a unicellular slime mold that feeds on protozoa, bacteria, fungal spores, and dead organic matter to make more protoplasm and grow. The life cycle of the *Physarum* is very important. Throughout the *Physarum's* life, it can change into haploid or diploid forms. Most *Physarum* in the lab is in its diploid form, plasmodial form (Youngman, 1981). *Physarum* is one large cell that can have more than 10^{10} nuclei (Paine, 2022). The life cycle of the *Physarum* begins with the germination of a meiospore (Briggs, 2021). During early sporulation, the organism starts to form fruiting bodies. When the spore is forming, a diploid nucleus is in each spore. Once the spore develops, meiosis occurs and the spore contains four haploid nuclei. Once three of the four spores break down, there is a fully developed spore that contains a single haploid nucleus. A Large mass can be made by spores fusing which is called a phaneroplasmodium. Sclerotia can form if *Physarum* is not in the correct environmental conditions (Briggs, 2021). *Physarum* is found in humid, cool, and dark conditions, so the most viable habitat is wet wood, soil, moist dung, and different vegetation (Paine, 2022) (Figure 12). When it comes to foraging, a *Physarum* can sense when it moves closer or farther away from food. This allows for the slime mold to grow in the direction of its food. *Physarum* also creates networks of tubes that drive internal cytoplasmic flows that connect to all different food sources (Figure 11). This allows for more growth to reach towards more food (Alim, 2013). *Physarum* feeds on many different types of protozoa, bacteria, and fungal spores (Paine, 2022). When there isn't any food left, the slime mold thickens, and the networks and mounds turn into sporocarps that produce spores. These spores undergo meiosis and form meiospores, which are eventually

scattered by wind and blow to other sites. The spores are scattered so that the *Physarum* can repopulate and relocate to different areas with more nutrients and food. After the spores relocate the germination stages start again.

Determining how unicellular organisms behave in microgravity conditions is important for scientists to understand how these organisms will behave in long-term space flight. *Physarum* is a decomposer and breaks down materials by secreting enzymes (Bailey, 1995). *Physarum* could be helpful for space flight because it is able to break down materials and waste and recycle them (Bailey, 1995). This study helps understand how *Physarum* is able to grow under microgravity conditions, which are the conditions that the slime mold would be under in space travel. On Earth, there is no way to perform an experiment without gravity, which is why clinostats were used in past studies. Scientists have been using clinostats to study plant and slime mold gravitropism (Moore, 1998). A clinostat provided circular rotation that allowed for *Physarum* to grow in a hypogravity environment. Past research has also found that *Physarum* has a complex cellular intelligence that only has been known to exist in animals (Oettmeier, 2020). Scientists are now studying *Physarum* intelligence and intelligence and decision-making in other organisms. *Physarum* is able to find short routes through mazes and make decisions on its own. This new discovery is another reason why *Physarum* is important and can be studied to determine how slime mold grows and thinks on its own. More research is to be done on *Physarum* to study how it compares to plants, bacteria, and animals. The environment that enables the best *Physarum* growth should be studied and discovered so that more research can be done on *Physarum* growth and networking.

Gravity and light wavelengths are variables that differentiate the expansion of *Physarum*. Gravity is what attracts a body or object to the center of the earth. In space, there is no gravity so

plant growth is irregular. One way plants detect gravity is by using statoliths. These are packets in gravity-sensing cells filled with starch (Kovo, 2017), These organelles in the cells of the roots filled with starch are called amyloplasts. When the amyloplasts settle at the bottom of the roots, it triggers auxin to move to another area of the plant elongating the roots toward gravity (Blancaflor, 2013). When there isn't gravity, the roots may grow differently. Block and Briegleb found that the mitochondria could be the receptor site for gravity and light in *Physarum.* However, the slime mold, *Physarum,* does not possess any specialized gravireceptors (Block, 1989). This indicates that a microgravity condition will not affect *Physarum* growth any different than *Physarum* growth on Earth. However, different slim molds are affected by gravity in different ways (Kawasaki, 1990). *Dictyostelium discoideum,* a slime mold, had an increase in germination and growth under hypergravity than hypogravity. It was thought that hypergravity would retard cell growth, however, it was found that an increase in the fraction of stalk cells may have increased the growth of *Dictyostelium.* The hypogravity environment resulted in the lowest amount of slime mold growth. Kawasaki and his colleagues also concluded that gravity may have pushed organelles to the cytoskeleton or cell membrane which would have resulted in greater growth. *Dictyostelium discoideum* is a multicellular slime mold and may have a different effect than *Physarum polycephalum* (Kawasaki, 1990).

NASA conducted an experiment on *Physarum polycephalum* in the space shuttle mission STS-42 (Orloff, 2001). *Physarum* was grown to study the way it reacts to microgravity conditions and how this changes its growth. This is studied so that scientists can better understand how gravity, night and day, and the internal biological clock affect different activities and functions in slime molds, plants, and animals. The results of the study showed that the contractions and growth of the *Physarum* were at first shortened but returned to normal growth

because the slime mold adapted to the microgravity. This study shows that *Physarum* growth was not impacted when placed in a microgravity condition (Orloff, 2001). NASA is also conducting experiments on *Physarum* to compare its filamentary networks to the cobweb structure of the galaxy and how all the clusters of galaxies stay together even when expanding over millions of light years long. The galaxies are laced with networks of dark matter and gas. These networks are hard to detect because the gas is so dim. *Physarums* networking was programmed into a computer algorithm and 37,000 galaxy locations were plugged into the algorithm. This computer software allowed scientists to see how gas and dark matter were connecting and interacting between galaxies. *Phyarum* growth is studied for multiple reasons at NASA and for long-term space flight.

In space, there isn't natural light in the atmosphere like on Earth. Light allows for the growth, replication, and photosynthesis of all types of plants and slime molds including *Physarum*. Different wavelengths of light have different effects on the growth and expansion of the *Physarum*. Gravity conditions and light wavelengths can also impact the way that *Physarum* grows. A study found that the longer *Physarum nudum* stayed in the dark before being exposed to the light the more it was able to grow (Rokoczy, 2015). *Physarum* that stayed in the dark for 0-8 days showed the same amount of time for fructifieation was needed. However, *Physarum* which stayed in the dark for 9-16 days extended the fructification period of time for the *Physarum*. This shows that *Physarum* fructification started in the dark, but only when it is able to see the light in a short period afterward. Light still had an impact on *Physarum* growth and has been found that small amounts of light help *Physarum* growth (Rokoczy, 2015). In another study done by Reinhardt, It was found that the slime mold *Acrasis rosea* did not fruit when the slime mold was grown in light environments and dark environments. It was found that *Acrasis rosea*

growth and fruiting happened when stimulating periods of light were administered, followed by periods of darkness (Reinhardt, 1968). So even though high intensities of light can be harmful to *Physarum*, light may play a critical role in its growth.

Past research has shown that light has some effects on *Physarum* but is also needed for growth. Gravity also alters *Physarum* growth but *Physarum* adapts to these different conditions and is able to grow normally. It is predicted that different wavelengths of light and microgravity conditions will have no effect on *Physarum* growth.

Materials and Methods

Measures

This study measured the growth of *Physarum polycephalum* on 10ml round Petri dishes. The Petri plates were divided into 1.5cm squares to determine the growth of *Physarum* in relation to light and food. The growth of the *Physarum* was measured by the number of boxes that the *Physarum* grew onto. The dish was split into two sides called light and dark sides. The squares were counted on both sides of the dish to determine the growth of the *Physarum* on each side of the dish.

Procedure

A *Physarum polycephalum* culture was maintained for the length of the experiment. Culturing was done once a week or whenever the *Physarum* started to outgrow its environment. When the *Physarum* grew to the ends of the Petri dishes then the culture was replated. The *Physarum* culture was plated on a 70ml petri dish using non-nutrient agar as bedding and autoclaved old-fashioned oats for a food source. The oats were sterilized to prevent fungal or bacterial infection. The previous subculture was harvested by using a sterile micro-lab spatula to scrape the slime mold off of the agar and transfer it to a new Petri dish. After transferring enough

slime to cover a 1cm by 1cm area, oats were spread across the agar for maximum growth. Four 70ml Petri dishes were used each week for keeping the subculture alive.

To conduct the experiment, forty 10ml round Petri dishes were used. Non-nutrient agar was used as bedding in all plates and sterilized oats were used as the food source. The agar was made from 20 grams of bacteriological agar and 1 liter of distilled water. The non-nutrient agar was autoclaved for 20 minutes at 121°c. All transferring was done under a sterile hood using a sterile spatula and tweezers. One culture of *Physarum* was used to start the experiment. *Physarum* from the subculture was placed on all forty plates using a micro-lab spatula. The slime mold was transferred so that it covered a 1cm by 1cm area in the center of each plate. The placement of oats was very specific for the experiment to encourage growth in all areas of the plate. Oats were placed in the middle and in an X shape on the plate (Figure 1). Three oats were placed in the middle, then four oats were placed in between the middle of the plate and the edge, and then four oats were placed at the edge of the plate. This allowed the oats to create an X shape. The oats were pressed into the agar so that rotation from the clinostat would not cause the oats to fall. All forty plates were grouped into ten sets of four. Each set of plates was wrapped in black party streamer with a 1cm by 8cm slit cut out on the light side of the stack (Figure 9). This prevents the reflection of light within the incubator and the control of light entering the plates. This allowed differentiation between light squares (*Physarum* that grew towards the light side of the plate) and dark squares (*Physarum* that grew away from the light side of the plate). After each plate was prepared, they were placed in the experimental incubators. Each set was placed on a clinostat to mimic a microgravity condition or stationed on the ground and placed in their assigned light wavelength (Figure 10). The red light wavelength was 635nm, the blue light wavelength was 460nm, and the green light wavelength was 525nm. The clinostat with the plates

attached rotated at 1rpm counterclockwise. The plasmodia on the clinostat experienced gravity disorientation resembling a microgravity environment. Five clinostat groups (red light, blue light, green light, red/blue light, and dark) and five stationary groups (red light, blue light, green light, red/blue light, and dark) were tested for *Physarum* growth. The incubators had 3 specific light wavelengths (Red- 635nm, Green- 525nm, Blue- 460nm, and Red/Blue being a mixture of 635nm and 460nm) (Figures 6, 7, and 8).

The plates remained in the incubators for 72 hours during each test. The *Physarum* covered the plates at 72 hours (Figure 2). Every 24 hours the stationary plates were photographed. The clinostat plates were kept in the incubator because the microgravity condition would be disturbed. At the end of the 72 hours, all plates were taken out and counted. The *Physarum* growth was counted by placing a 1.5cm by 1.5cm grid paper under the plate and counting the squares in which the *Physarum* grew towards the light and away from the light. Each square with active *Physarum* was counted as occupied. If a square did not have any active *Physarum*, it was not counted. A total of three experiments were conducted for each wavelength and gravity condition giving a total of 120 plates. A single-factor ANOVA test was used to compare the difference between light and dark growth, the difference between light wavelengths, and the difference between the clinostat and stationary conditions. This information was then analyzed and graphed.

Results and Discussion

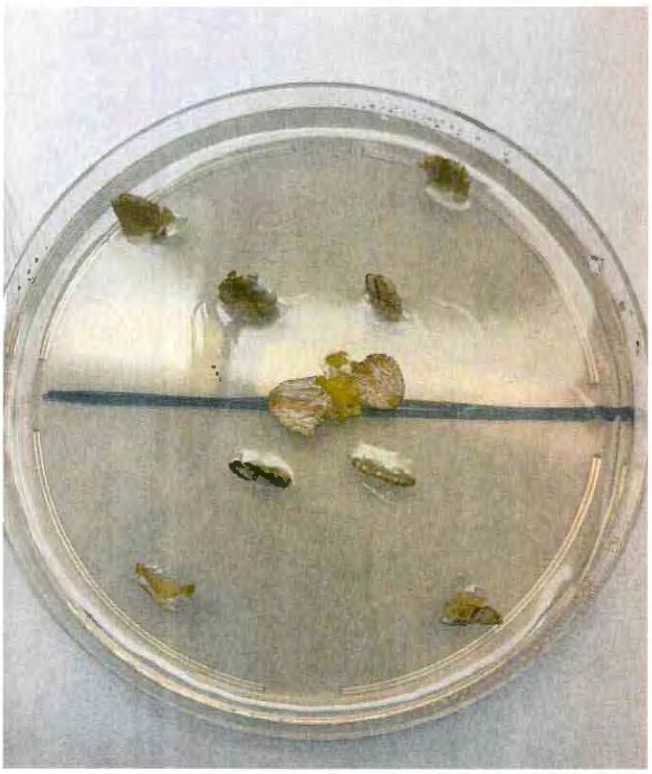

Figure 1: *Physarum* plated on Petri dish at day 0.

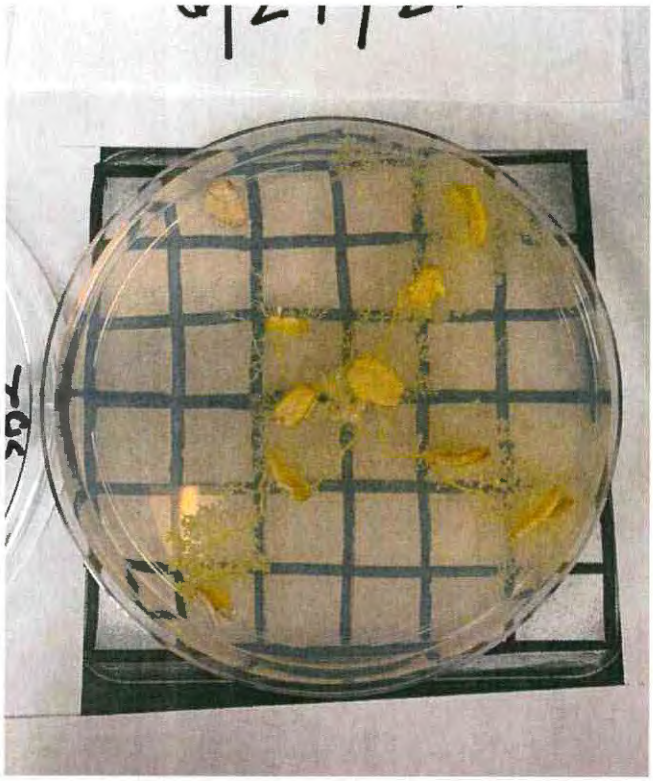

Figure 2: *Physarum* growth on Petri dish after 72 hours

The first figure shows when the *Physarum* and pressed oats were just plated under the hood. The second figure shows the plate after it was taken out of the incubator after 72 hours and about to be counted. Both images show stationary plates. These two figures show the rapid growth of *Physarum* in just three days.

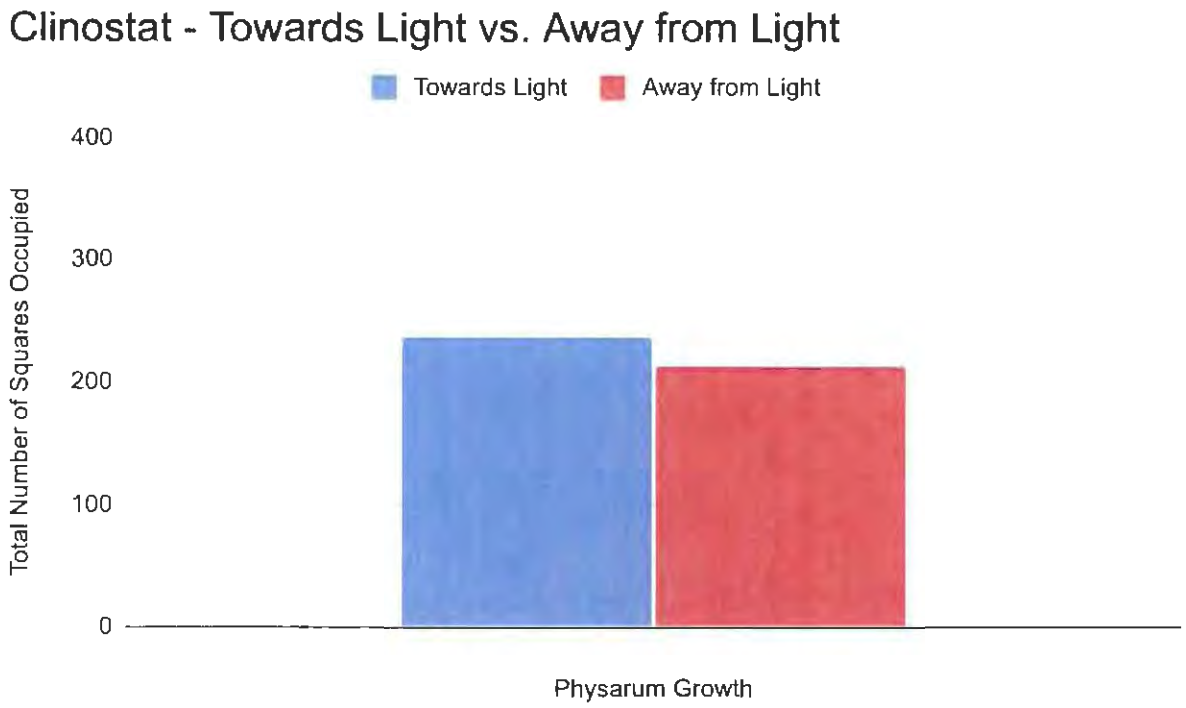

Figure 3: The mean amount of squares found in the light compared to the dark for the clinostat plates.

Plates that experienced a microgravity condition were on clinostats during the experiment. A clinostat is an apparatus that has a slowly revolving plate that skews gravity. The clinostat rotates at 1 rotation per minute (rpm) counterclockwise to allow for no gravitational pull. The plasmodia in the slime mold are still active and respond through the small slit created at the light side of the plates. The total growth for the clinostat condition in all wavelengths of light did not differ when comparing the number of occupied squares in the light to the number of

occupied squares away from light. The clinostat plates for every wavelength had a p-value of 0.483 indicating that the null hypothesis (no statistical difference) for the clinostat experiment cannot be rejected (Figure 3).

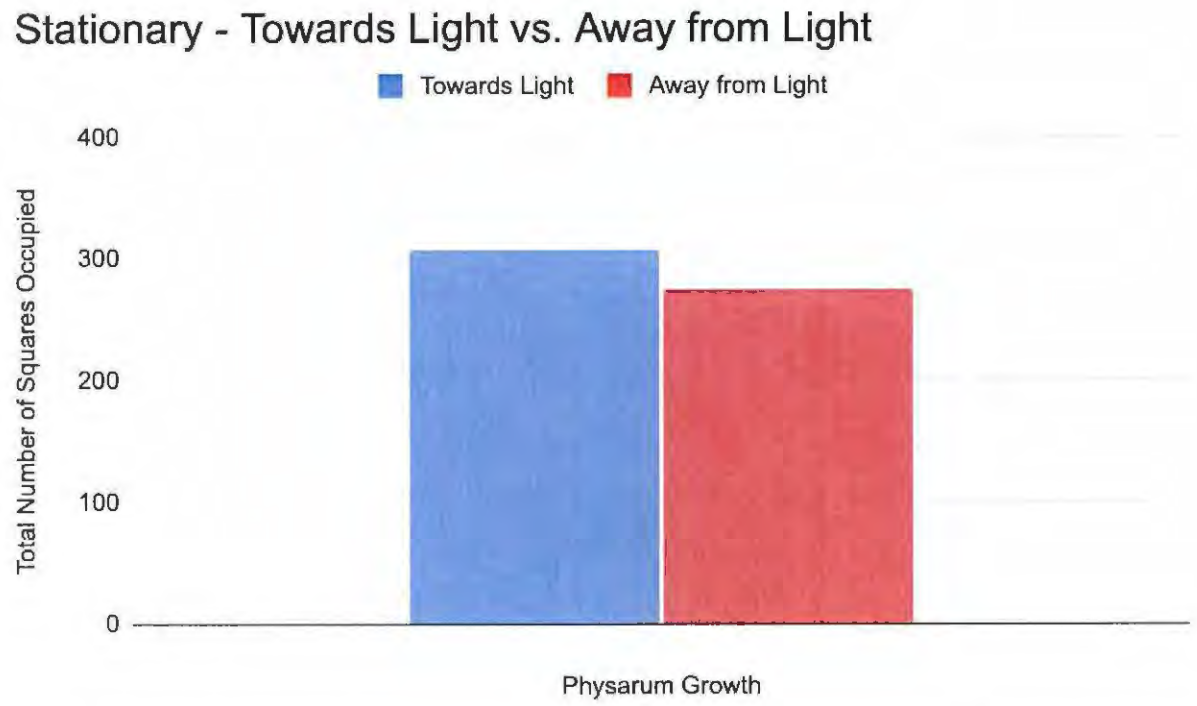

Figure 4: The mean amount of squares found in the light compared to the dark for the stationary plates.

Stationary plates were positioned on their sides with the 1cm by 8cm slit on the light side pointed north. Each stack was placed in the incubator and not attached to the clinostat. This supposedly allowed for gravity to have an impact on the *Physarum.* However, the difference in *Physarum* growth in the towards-light squares and the away-from-light squares was statistically not significant. The stationary plates for every wavelength had a p-value of 0.425 indicating that the same null hypothesis for the stationary experiment can not be rejected (Figure 4).

To compare the plates in the stationary condition with the plates in the microgravity condition an ANOVA test was also conducted. The *Phyasrum* growth did not differ between clinostat and stationary settings ($p = 0.287$) which concludes that there is not a significant difference between *Physarum* growth when comparing the clinostat and stationary settings.

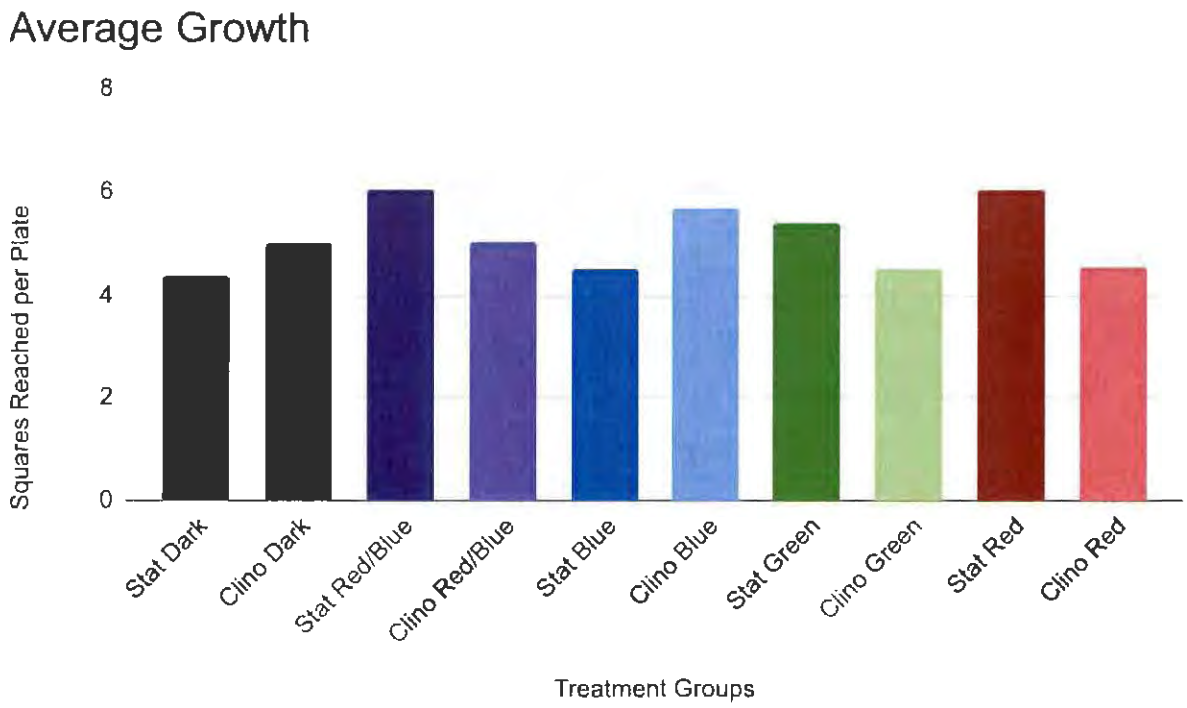

Figure 5: *Physarum* growth for each different wavelength.

Each wavelength and condition was analyzed by comparing the total *Physarum* growth. The graph shows the average amount of squares that the *Physarum* growth reached for each plate. An ANOVA test was then used to test all wavelengths and environment conditions with each other, resulting in a value greater than ($p>0.05$). Indicating that different wavelengths of light and microgravity conditions had no statistical effect on *Physarum* growth.

Conclusion

Physarum polycephalum showed no difference in growth when introduced to a microgravity condition and under different wavelengths of light. In its normal environment, *Physarum* tends to be found in the dark, wet places of the woods. This was not seen in the experiment because the growth was indifferent when comparing how *Physarum* grew towards the light and away from the light sides of the dish. One reason behind this could be the risk factor of whether the light was more harmful than the food beneficial. *Physarum* grew on all portions of the Perti dishes suggesting that food source was more important than light and gravity conditions. It has been found that *Physarum* performs risk-management tasks and considers which options are better (Oettmeier, et al., 2017). This shows that *Physarum* is more or less able to determine the risks of which way to grow. Latty and colleagues, in 2009, studied the risks *Physarum* would take for food. They found that *Physarum* would typically go for the higher concentration of food even if the light risk was high (Latty, et al., 2010). This can conclude that the wavelength intensity for this experiment, 10 μM, was not high enough for the *Physarum* to grow away from the light instead of towards the light. In further studies, enhancing the light intensity could possibly show an effect of different wavelengths and how *Physarum* grows differently in the dark rather than the light. A second study could be done to determine if reaching food is the most important thing for *Physarum* or if a specific light intensity is too harmful to *Physarum* for it to grow. Another subject to look forward to would be to study the genetics of *Physarum*. Advancements in the genetics of *Physarum* would shed some light on what membrane-bound sensors and signaling pathways are used to allow for the growth of the slime mold. This would be beneficial to see how *Physarum* grows not just when and where (Oettmeier, et al., 2017). Clinostats are used to mimic how gravity would be in long-term space flight. *Physarum* growth was not different when comparing clinostat and stationary plates,

indicating that *Physarum* will be able to grow normally in long-term space flight. This is important because growth can be an indicator that all activities and functions are working properly and *Physarum* needs to be able to grow networks and decompose material to recycle in space. Overall, light wavelength and gravity in this experiment did not affect the growth of *Physarum* Polycephalum.

Future Directions

Physarum grows naturally in a dark environment and it has been found that harsh light and UV can cause a hindrance in *Physarum* growth and cell death. Past research shows that light can break up the *Physarum* network (Kakiuchi, et al., 2001). An experiment could be done that determines what light intensity causes harm to *Physarum* cell growth. This can be done by using all the same materials as this experiment but with different protocols. Plating *Physarum* would be the same for this new experiment. Sterilized equipment, a *Physarum* culture, oats, agar, and Petri dishes will all be used to make the plates for the experiment. The plates could be wrapped in black crepe to also compare how the *Physarum* grows towards and away from light with different intensities. The light boxes for this study would not have different wavelengths of light but different light intensities. The control lightbox would stay the same and still be kept dark. The other light boxes could have white light with increasing light intensities (10, 50, 75, and 100 watts). The Petri dishes would not have to be placed onto clinostats for this study since light intensity is the only variable being tested. The *Physarum* growth could be counted the same way with a 1.5cm grid sheet and a one-way ANOVA could be conducted to compare the *Physarum* growth for the different light intensities. It is predicted that the higher intensity of light the less *Physarum* growth there will be.

After the study to determine at what light intensity harms *Physarum* growth, another study could be done to consider if food or survival was the most important aspect of growth. Larger Perti plates and an abundance of oats could be used so that *Physarum* growth would be able to grow at its maximum rate and surface area. One *Physarum* plate would be put into the dark light box to be a control and the other plate would be put into the lightbox with the minimum intensity that *Physarum* growth is harmed. After the *Physarum* grows for 5 days the growth could be determined with the 1.5cm grid. If the *Physarum* growth in the lightbox is more or equal than in the dark box then food is shown to be the most important growth factor to *Physarum*. This could mean that *Physarum* would typically go for food and risk the high light intensity. However, if *Physarum* grows better in the dark environment rather than the light environment then it shows that survival is more important to *Physarum*. This study would conclude what aspect of *Physarum* growth is most prevalent in the life cycle of *Physarum*.

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Appendix

Figure 6: Blue light (460nm) incubator and clinostat

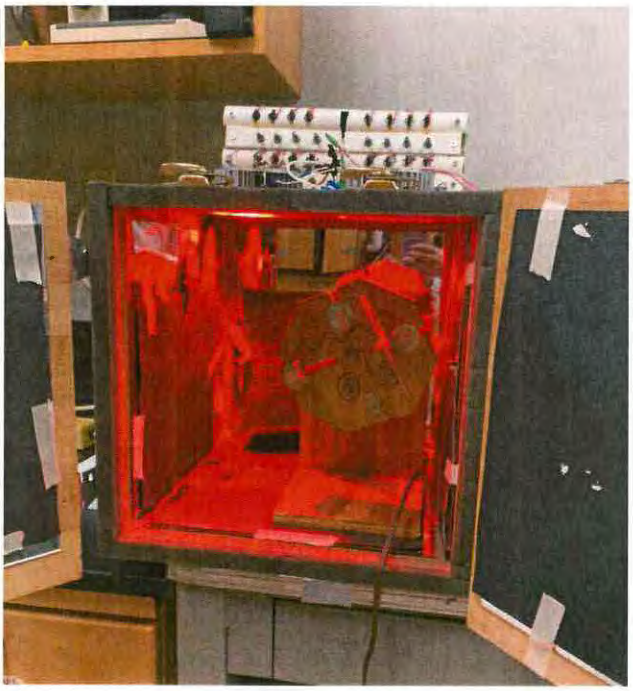

Figure 7: Red light (635nm) incubator and clinostat

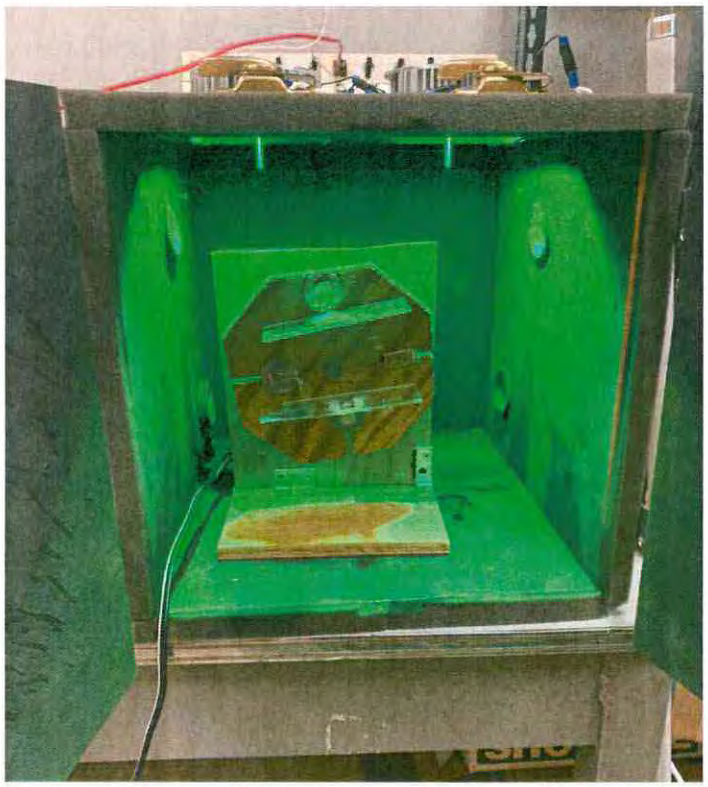

Figure 8: Green light (525nm) incubator and clinostat

Figure 9: Plates wrapped in crepe streamers with a slit cut onto the light side of the plate

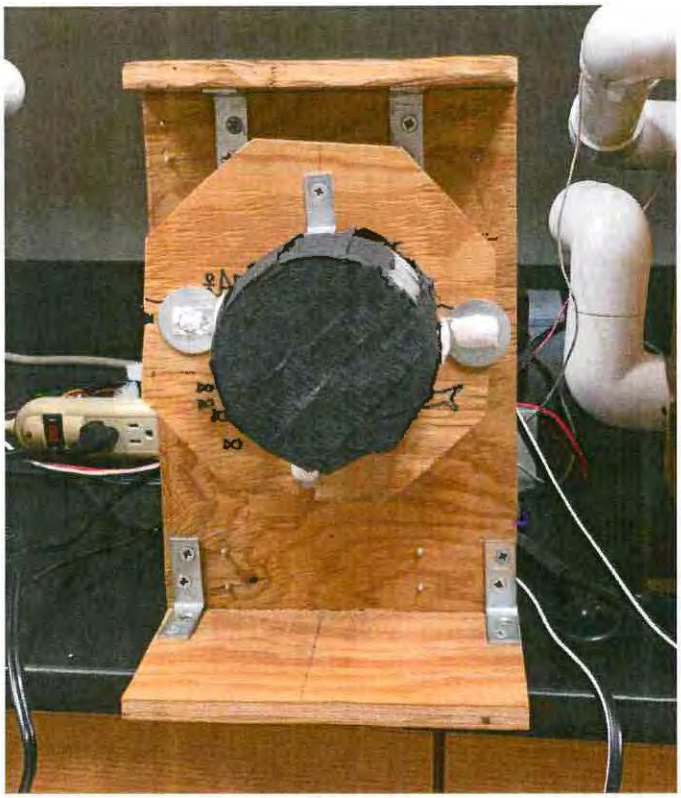

Figure 10: Plates on the clinostat

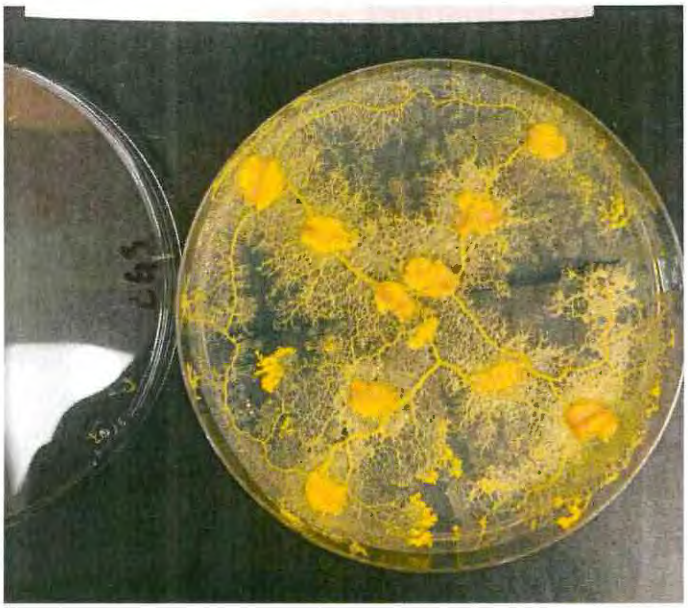

Figure 11: Regular *Physarum* growth

Figure 12: *Physarum* in the environment

Disclaimer

For this research study, not all of the statistics are present. The p-values for the ANOVA tests are presented, however, the statistical F-values, means, and standard deviations are missing. The results of the study are written as expected but to analyze all the statistical values, the study would need to be repeated.