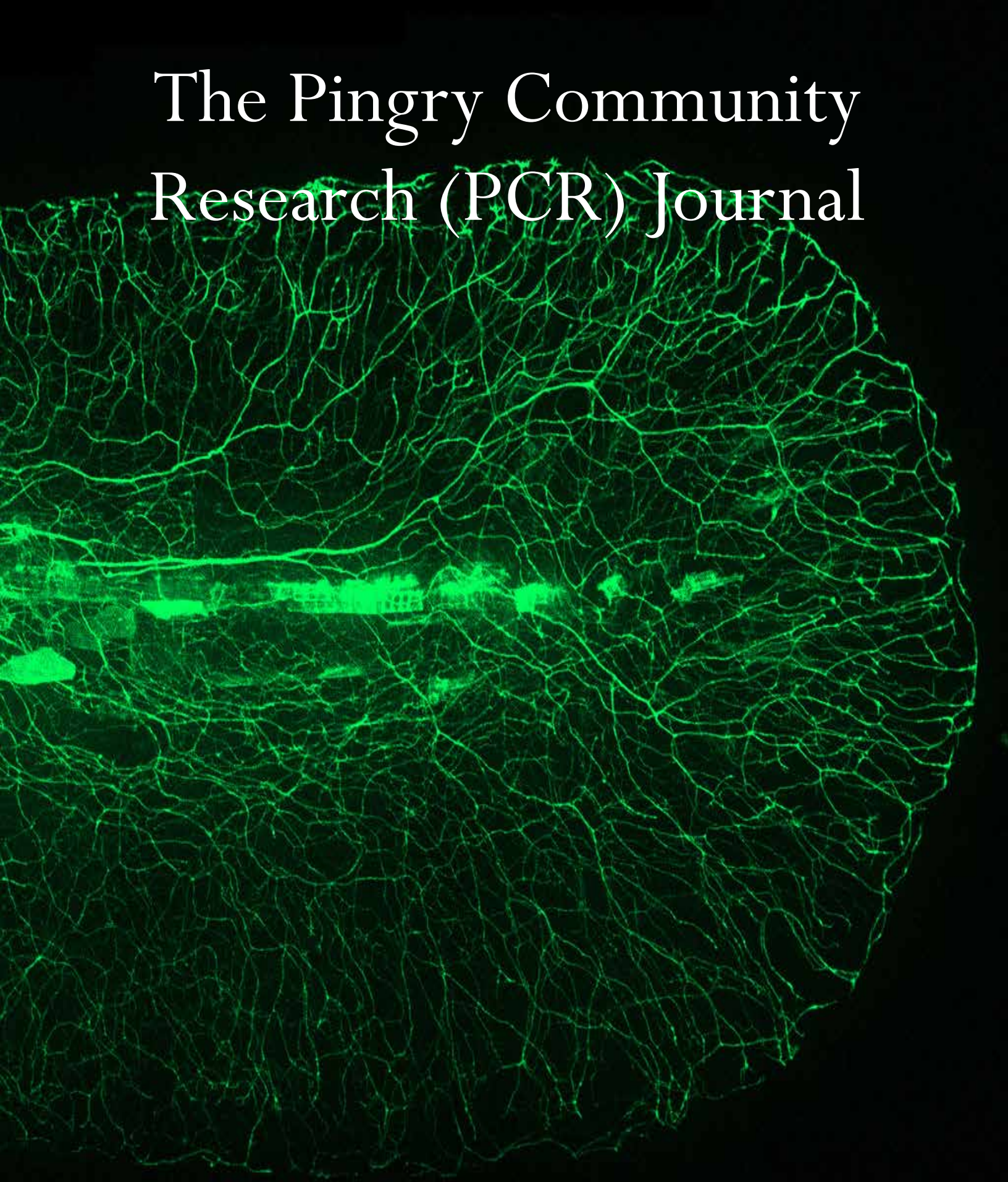
The Pingry Community Research (PCR) Journal



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Contents

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For Materials and Methods sections, please visit www.pingry.org/pcr

The Role of Alcohol Dosage on HPA Axis Functionality

By Julia Friend'15, Gaurav Gupta'15, Sarah Beckmen'15, Lauren McLaughlin'15, and Katia Colon'15

ABSTRACT

The addiction pathway in zebrafish, a model organism for humans, is well documented. The majority of studies regarding zebrafish addiction examine the behavioral effects of harmful drugs, such as cocaine and alcohol; however, the molecular effects of these addictions have also been examined. In such studies, zebrafish are usually force-fed the addictive substance to see its effects on their brain development. However, there has been no previous research conducted that examines self-administration of alcohol in zebrafish. We designed a novel protocol to see if zebrafish who were addicted to alcohol would seek it when given a choice between water and alcohol. Our hypothesis was that if addicted zebrafish were given the choice between water and alcohol, they would self-administer the alcohol. The second part of the experiment, beyond the behavioral analysis, involved isolating the brain tissue from the addicted zebrafish in order to examine any changes in the NMDA and CB1 receptors, which have been shown to play a role in addiction and substance abuse. A prerequisite for examining these receptors is to guarantee that DNA isolation and amplification are possible. After four different behavioral experimental trials, we saw no discernible link between zebrafish addiction and self-administrative tendencies. Additionally, the abridged version of our DNA isolation protocol yielded no results in DNA amplification. Notwithstanding, a few key design inefficiencies were most likely the cause for the negative behavioral data and lack of DNA amplification, which means the experiment needs to be optimized before any conclusive link between alcohol addiction and self-administration can be established.

INTRODUCTION

Alcoholism is a chronic disease that plagues approximately one in every twelve adults in the U.S. It is characterized by a strong craving for alcohol, and/or continued use despite harm or personal injury. Intoxication can impair brain function and motor skills, and heavy use can increase the risk of certain cancers, stroke, and liver disease (1,2).

Drugs of abuse, such as alcohol, have a powerful ability to reinforce behaviors. The molecular and cellular aspects of the reinforcement pathway and its connection to alcohol are not well understood. Conditioned place preference (CPP) is a form of Pavlovian conditioning where a rewarding substance is repeatedly paired with an environmental cue so that the animal associates the cue with the rewarding properties of the substance and thus develops a preference for the cue. This paradigm, which captures the stages of the addiction cycle, has been used in a multitude of past research to study the reinforcing properties of drugs of abuse in rodents (3).

Some of the main issues in curing alcoholism are the high relapse rate and the unknown mechanisms behind the disease. Relapse can be stimulated by stress, re-exposure to a drug, or a cue associated with a drug. Memory of a stimulus, and thus a cue, is a common inducer of relapse in other drugs of addiction such as cocaine (4). The endocannabinoid pathway is a signaling pathway that is present in multiple regions of the brain, specifically those relating to memory. It is composed of lipids that bind to receptors, such as CB1 and CB2, and modulate neurotransmissions involved in pathways relating to addiction. The CB1 receptor has been shown to play an important role in regulating the positive reinforcing properties of alcohol (4). Therefore, the CB1 receptor may be involved in the retrieval of memories and relapse.

The Laboratory of the Neurobiology of Addictive Diseases at The Rockefeller University in New York conducted research on a potential role for the endocannabinoid pathway in cocaine CPP in mice. They looked at how conditioning mice to cocaine affected the activity of the endocannabinoid pathway in the nucleus accumbens and found a trend towards increased protein levels of the CB1 receptor in the NAc of cocaine-conditioned mice after an 8-day cocaine CPP, suggesting increased activity of the pathway. They then administered the CB1 receptor antagonist, AM251, after a 3 day CPP in hopes of blocking the memory created during the conditioning. AM251 ap-

peared to reduce the effects of a 3-day cocaine CPP, suggesting that increased activity of the endocannabinoid pathway is required for the retrieval of memories.

Our team at Pingry wants to find out if the endocannabinoid pathway plays the same sort of role in alcohol addiction in zebrafish (*Danio rerio*) as it does in cocaine addiction in mice. Previous research has shown that it is possible to induce CPP in zebrafish after a single exposure to ethanol (3). There are currently only two published protocols for alcohol CPP in zebrafish. Our study looks at another way to study stages of alcohol addiction, through a model of self-administration in which the zebrafish not only seek alcohol, as in CPP, but also have the option to intake it as well. Alcohol self-administration in zebrafish has never been done before; thus it was our aim this year to develop a novel protocol. We have been learning about the process of experimental design and continue to make modifications to our protocol while also making discoveries about alcohol preference in zebrafish. Lastly, we have begun the process of DNA isolation in the zebrafish brain, using the NMDA receptor as a control, in order to prepare for future isolation of the CB1 receptor.

RESULTS

In the first trial of the self-administration experiment with 2 day alcohol training, the fish with EtOH in the left column spent more time in the right column (Fig 1). The fish spent 41.68% of the time in the column with EtOH (left column) and 58.32% of the time in the column with water (right column). The control fish, without EtOH in the column, spent no time in either column.

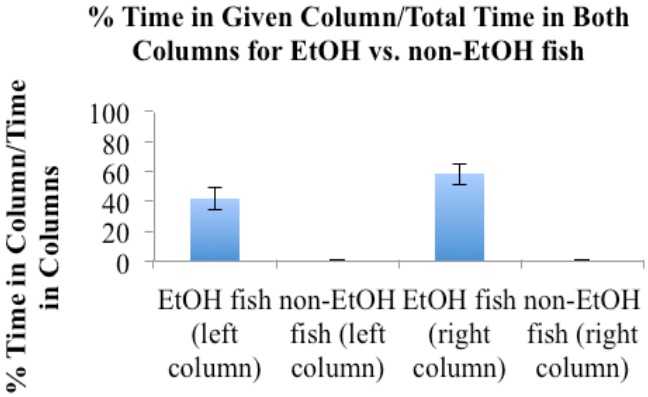

Figure 1. The fish with EtOH in the left column spent more time in the right column. The fish without EtOH in the column spent no time in either column.

EtOH (left) vs. EtOH (right) p-value = .8624416685

Overall, the EtOH fish spent 28.39% of the total time in the columns, while the fish without EtOH spent no time in any of the two columns (Fig 2).

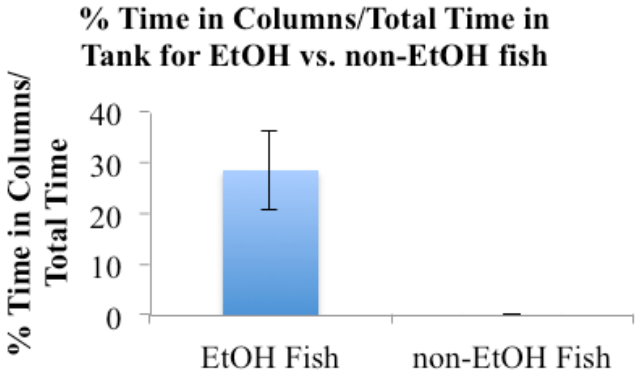

Figure 2. The fish with EtOH in the left column spent 28% of the total time in either column, and the fish without EtOH in the left column spent no time in either column.

In the second trial of the experiment with 2 day alcohol training, the fish with EtOH in the left column spent more time in the right column again (Fig 3). They spent 39.73% of the time in the left column and 60.27% of time in the right column. The fish without EtOH in the left column also spent more time in the right column: 14.36% left and 85.64% right.

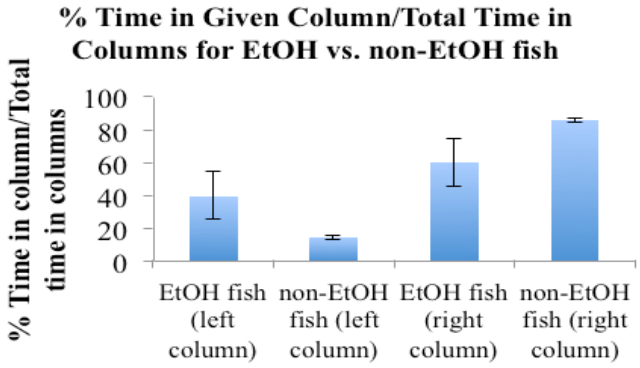

Figure 3. The fish with EtOH in the left column spent more time in the right column. The fish without EtOH in the left column also spent more time in the right column.

EtOH left column > EtOH right column p-value = .9635963191

Overall, the EtOH fish spent less time in the columns than the fish without EtOH (Fig 4). The EtOH fish spent 9.73% of the total time in the columns, whereas the non-EtOH fish spent 13.47%.

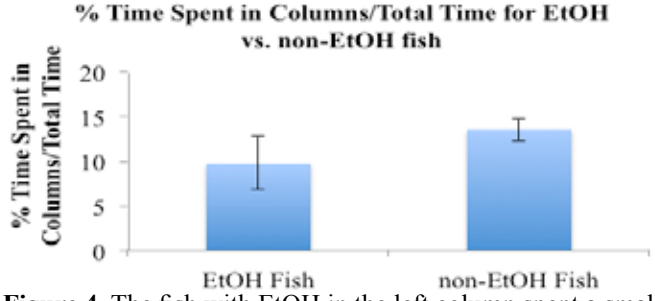

Figure 4. The fish with EtOH in the left column spent a smaller percentage of time in the columns than the fish without EtOH in the left column.

EtOH > non-EtOH p-value = .8195395681

When the self-administration experiment was conducted after the extended 4 day alcohol training, the

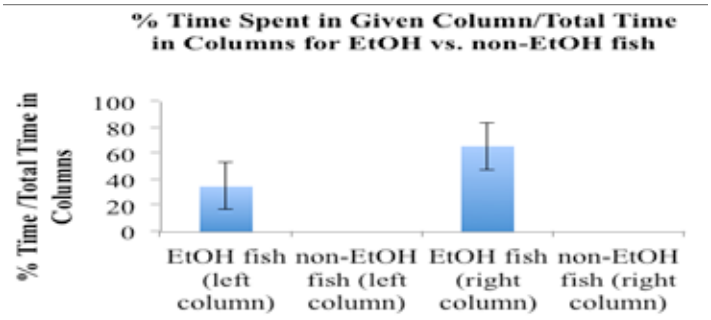

Figure 5. The fish with EtOH in the left column spent more time in the right column than the left column. The fish without EtOH in the column spent no time in either column.

EtOH (left) > EtOH (right) p-value = .1225009887

fish with EtOH in the left column still spent more time in the right column (Fig 5). 34.58% of their time was spent in the left column, and 65.42% of their time was spent in the right column. The fish without EtOH in the left column didn't spend any time in either column. Overall, the EtOH fish spent 23.13% of the total time in the columns, and the fish without EtOH spent no time in either column (Fig 6).

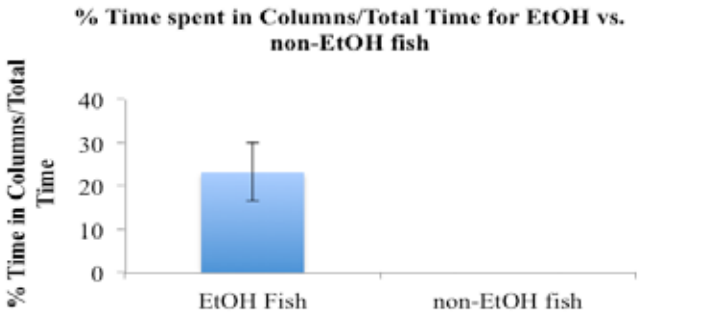

Figure 6. The fish with EtOH in the left column spent 23% of the time in a column, and the fish without EtOH in the left column spent no time in the columns.

CONCLUSION

After running several behavioral experiments, our results did not show a link between alcoholism in fish and their tendency to self-administer alcohol when given a choice between alcohol and water. In fact, the results seem to show that the zebrafish showed a slight affinity to the water when given the choice. Additionally, three different attempts to remove, isolate, and amplify the DNA in the brain tissue of zebrafish yielded no results. However, upon further scrutiny of the experiment, we noticed several design and execution mistakes that could have compromised the integrity of our results. Post-experimental analysis of the DNA isolation protocol revealed that the protocol was executed on the wrong layer of sample. Specifically, the aqueous layer was believed to contain DNA at the time of the experiment, but in hindsight the DNA was actually in the interphase layer. This is a likely cause for our negative DNA isolation results, but further tests with this correction must be completed to know with certainty.

In the future, we will test the concentration of alcohol in the tanks post-experiment by using a spectrophotometer. If there is alcohol in the tanks, we will have to redesign the protocol to ensure that the fish are seeking alcohol instead of being forced into the alcohol. If there is no alcohol in the tanks post-experiment, we will increase the concentration of alcohol during the training days and in the columns to see if that will create more of a preference for alcohol on experiment day. We will also rerun the DNA isolation protocol in an effort to perfect isolation of the NMDA and CB1 receptors and quantify the CB1 levels in the brain after seeking and CPP testing in zebrafish. Once our protocol is perfected, we hope to study relapse and whether, when given the choice between alcohol and water, fish will choose water after being administered a CB1 receptor antagonist to block the addiction pathway. Hopefully, our study of relapse in zebrafish can be extrapolated to humans and work toward ending human addiction and relapse.

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ACKNOWLEDGEMENTS

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Barefoot Running

By Caitlin Mahoney'15, Sydney Streicher'15, and Emma Palmer'15

INTRODUCTION

There are three different types of foot strike: heel strike, midfoot strike, and toe (forefoot) strike. The blue arrows in Image 1 depict the three different angles at which one's foot can strike the ground. A Harvard study has concluded that runners who strike the ground with their toe or midfoot are less prone to injury than those who strike the ground with their heel. Running on the midfoot or toe increases shock absorption while more naturally utilizing the muscles in the foot and calves. In a heel strike, the heel hits the ground first, followed by the rest of the foot, creating a "double" impact per stride that leads to a greater stress on joints and tissues. This can lead to injuries such as shin splints and stress fractures. Approximately 95% of runners are said to be heel strikers.

Our study observed the role sneakers play in foot strike. Sneakers are said to overcorrect any foot imperfections by creating higher arches, more stability, and more cushioning. Therefore, if sneakers cause more runners to heel strike, could they be over-correcting our flaws in an unnatural way? This question was inspired by Christopher McDougall's ethnography, Born To Run, which is about the Tarahumera Indian tribe. The Tarahumera run ultra-marathons and three-day-long races across hills and rugged terrain. These runners run barefoot or with sandals, yet they remain uninjured. The goal of our experiment was to determine if running barefoot can change runners' foot strike from a heel strike to a midfoot or forefoot strike, thus making runners less prone to injuries.

RESULTS

For the carpet surface in Hauser Auditorium, as depicted in Image 4 Chart 2, we observed that 33.334% (~33%) or 5/15 participants' foot strikes changed and 66.667% (~67%) or 10/15 participants' foot strikes did not change. As depicted in Image 4 Chart 5, out of all the heel strikers in sneakers on the carpet, 30.769% (~31%) or 4/13 changed from a heel strike to a midfoot strike. One participant changed from a midfoot strike to a toe strike on the carpet. Image 2 Picture 1 depicts the foot strike of a participant who remained at a constant heel strike with and without sneakers on the carpet. Image 2 Picture 2 depicts a participant whose foot strike changed from heel strike

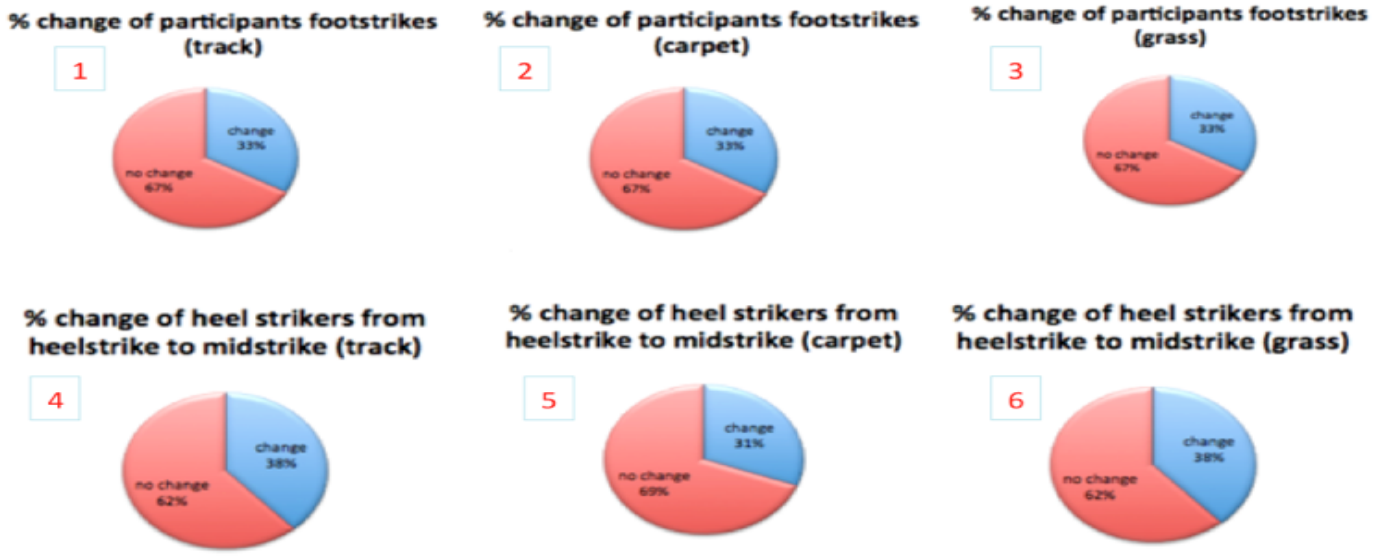

Image 4: This image shows an analyzation of our raw data. 33% of participants footstrikes changed, sneakers vs. no sneakers, on the track (Chart 1). 33% of participants footstrikes changed, sneakers vs. no sneakers, on the carpet (Chart 2). 33% of participants footstrikes changed, sneakers vs. no sneakers, on the grass (Chart 3). 38% of participants with a heel changed from heel to midstrike, sneakers vs. no sneakers, on the track (Chart 4). 31% of participants with a heel changed from heel to midstrike, sneakers vs. no sneakers, on the carpet (Chart 5). 38% of participants with a heel changed from heel to midstrike, sneakers vs. no sneakers, on the carpet (Chart 6).

in sneakers to mid strike without sneakers on the carpet. On the grass, we had similar data. As depicted in Image 4 Chart 3, 33.334% (~33%) or 5/15 participants' foot strikes changed while 66.667% (~67%) or 10/15 did not change. However, on the grass, unlike on the carpet, 5/13 or 38.462% (~38%) of participants with heel strikes while in sneakers changed to a midfoot strike when they were barefoot, as seen in Image 4 Chart 6. Image 2 Picture 3 depicts the foot strike of a participant who remained at a constant heel strike with and without sneakers on the grass, while in Image 2 Picture 4 the participant transitions from a heel strike to midfoot strike on the grass. As depicted in Image 4 Chart 1 and Image 4 Chart 4, our observations on the track provided us with the exact same data as our trials on the grass. Image 2 Picture 5 depicts a participant with a constant heel strike on the track while Image 2 Picture 6 depicts a participant who changes from a heel strike to mid strike on the grass.

DISCUSSION

Our results illustrate that although a small group of runners' foot strikes changed, the majority of runners' foot strikes did not change. Additionally, the surface controls proved that the differences between the track, grass, and carpet do not affect the change in a runner's foot strike. Therefore, while runners with a midfoot strike or forefoot strike might be less prone to injury, running barefoot will most likely not change their strike and therefore will not prevent injury in most runners with a heel strike. Sneakers do not necessarily "overcorrect" flaws, because in the majority of people, wearing sneakers does not make a difference in how their foot strikes the ground. Therefore, many of the Nike campaigns for their "free" sneakers, that are meant to feel like a barefoot running shoe, do not actually make a difference in foot strike and probably have no effect on preventing injuries. However, one important detail to note is that no runner in theory "went backwards" on her foot. For example, a runner who struck the ground with their midfoot while in sneakers did not change to a heel strike when barefoot. Our study was only conducted with only 15 participants and in order to fully determine the impact of barefoot running on foot strike, more studies must be done with more participants, as well as with different ages and genders. Perhaps barefoot running affects men's foot strikes differently than it affects women's. Another factor we did not control was the type of sneaker our participants wore. The girls in our study were wearing a variety of sneakers, ranging from minimalist and very light to moderate and more supportive shoes. More research could be done on how the type of shoe a runner wears affects whether or not their foot strike changes when running barefoot. In addition, each of our runners has been wearing sneakers since they could walk; therefore they are more accustomed to the extra stability that sneakers provide and consequently their heel striking has become permanent and engrained. In contrast, the Tarahumera have been running barefoot or in sandals for generations, and perhaps have adjusted their foot strike accordingly. It would be interesting to study what would happen if our entire society were to get rid of sneakers from birth. Perhaps then new generations might change their foot strike and be less prone to injury. Nonetheless, the results of our own experiment indicate that today's runners should probably keep their sneakers on, as running barefoot does not seem to provide any real preventative injury measure.

Maximizing Biodiesel Production via Oil Extraction and Transesterification

By Matthew Fromm'15 and Sean Fischer'15

ABSTRACT

The demand for biodiesel production has accelerated over the past two decades. While the potential for biodiesel production has been recognized, the means by which the purest diesel fuel should be synthesized from algae -- in the most effective manner -- is still being refined. This experiment focused on optimizing the efficiency of that process, from growing the algae initially to examining, in the oil production stage, whether the lipid content of the various algae strains tested would impact the overall production of biodiesel.

It was found that algae strains with a higher lipid content generally correspond to a higher quantity of biofuel produced. This biofuel was also of a higher quality than the fuel produced by the two algae types with a lower lipid content. It is suggested that future research into biofuel production make use of algae with higher lipid content, as this correlates to more fatty acid cultivation and a higher net quantity of biodiesel produced as a result.

INTRODUCTION

Biodiesel is a newfound energy source that could produce a large supply of diesel fuel for use by the general public. Microalgae can be a sustainable and eco-conscious alternative to fossil fuel, and many in the environmentalism community have looked to natural resources to find a safe way to create fuel without negatively impacting the environment (1). With more research into the process of turning algae into combustible fuel, biodiesel production could potentially overtake fossil fuels as the primary source of the world's power (2).

In this study of two parts, the experimenters examined how to optimize diesel fuel production through microalgae harvesting (from growing the initial algae to producing combustible fuel), which is considered a viable protocol for producing biodiesel after it is treated with several chemical processes. This is the first of two major components in the project. The first tests how to maximize the production of algae oil, and ultimately biodiesel, from microalgae using an olive oil press and filtration technique. After spending 6 months growing and harvesting, the experimenters realized they had not grown enough algae to continue with fuel production. So, they instead obtained three different types of pre-dried algae to test whether the specific plant from which the algae was taken impacts the yield of algal oil (and then biodiesel, which is obtained after transesterification). This is why the experiment was split into two separate sections, due to the results of the first experiment, and a desire by the experimenters to keep the project focused on biodiesel production.

Experiment One involved growing algae to maturity in an environment with direct sunlight and constant temperature, allowing for consistent results. This technique used the least expensive supplies with which algae could be produced in order to determine whether the algae-growing process tested is a practical way of making diesel fuel in a relatively simple and easily reproducible format. If algae can be produced cheaply, then it could become a more widespread energy source and potentially compete with oil as the world's preeminent power supplier. It was hypothesized that a small, but usable, amount of homegrown algae will be produced. A minimum mass of algae - estimated to be 50 grams - was needed in order to continue past the initial growth phase and proceed to the oil extraction and filtration technique that must be carried out to produce biodiesel.

By producing algae on a very small scale and using only low-cost materials, Experiment One intended to replicate what a small-scale algae grower might experience. It is well-known that algae can be produced in large scale cultivation (3), but the aim of this experiment was to assess whether algae growing and cultivation in a person's home, or a small lab, can still produce a viable amount of pure algae. Algae-based biofuels may be particularly suited for the development of small-scale production facilities to provide energy to rural communities or populations residing in areas that are not located near large agricultural regions. Freshwater algae are globally available, and certain species grow prolifically in wastewater, which minimizes costs of providing nutrients.

Experiment Two involved three different types of algae: *Chlorella, Spirulina*, and green algae *Consortia*. These all contain different lipid contents. *Chlorella* has a lipid content of 13.00%, *Spirulina* has 1.05%, and *Consortia* 1.66%. The variable in this study was the respective lipid content of the algae strains. It was estimated that *Chlorella* would have the highest yield of algal oil. Consequently, it was predicted that *Chlorella* would also have the highest yield of oil and biodiesel, and *Spirulina* would have the lowest yield of oil and biodiesel.

RESULTS

The three types of dried algae all produced different volumes of oil extract once they were treated with the methanol. Equal amounts (all having equal mass) of all three types of algae were measured, and the results were as follows.

Treatment with the *Consortia* produced 439 mL of oil extract. Since 0.456 grams of this algae were reacted with the methanol, *Consortia* had a yield rate of 45.794 percent.

Treatment with the *Chlorella* produced 392 mL of oil extract. Since 0.456 grams of this algae were reacted with the methanol, *Chlorella* had a yield rate of 25.243 percent.

Treatment with the *Spirulina* produced 251 mL of oil extract. Since 0.456 grams of this algae were reacted with the methanol, *Spirulina* had a yield rate of 26.182 percent.

Yield Rates

Density = Mass / Volume = 452 g / X L
Yield Proportion = massoil / total mass algae = X /

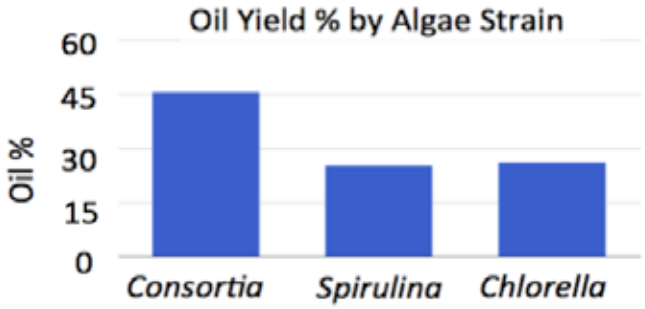

Figure 2. The percent of oil yielded from each algae strain, per unit of volume (mL)

0.456
Yield consortia = 208.82 g / 456.00 g = 45.794% used
Yield chlorella = 115.11 g / 456.00 g = 25.243% used
Yield spirulina = 119.39 g / 456.00 g = 26.182% used

DISCUSSION

To produce a viable amount of algae to transform into biodiesel, growing algae on a scale as small as the one attempted in this study is not feasible. The amount we harvested was not sufficient to convert into biodiesel, since the yield rate for oil extraction and transesterification is not high enough to produce a significant amount of biodiesel. A "significant" amount of biodiesel would be enough to enter into a calorimeter and test for heat production. Algae was harvested over a 7-month period, running the algae samples at the maximum speed -- 6500 rpm -- and maximum time -- 10 minutes -- at which the centrifuge could operate, yet the experiment still could not produce sufficient algae. This suggests the overall yield for producing algae using only sunlight and fertilizer may not be sufficient for industrialized production.

It was then determined that the study should continue towards the goal of producing biofuel; algae

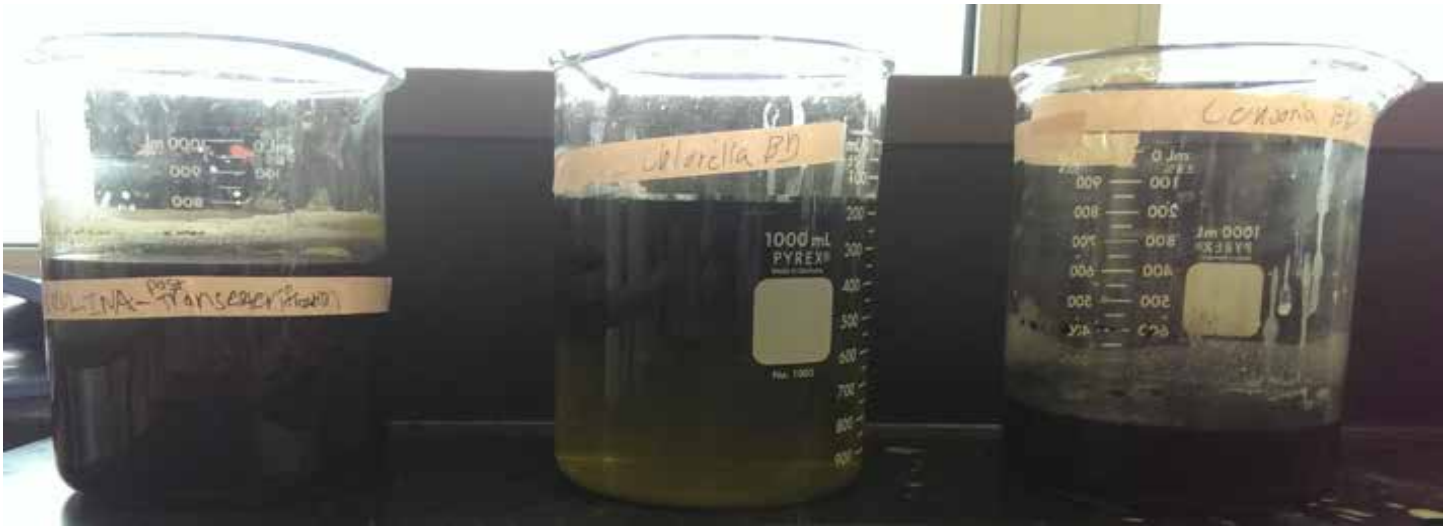

Figure 1. The three types of algae after transesterification was performed. From left to right: *Spirulina, Chlorella, Consortia.*

was obtained from a lab, and the variable in our experiment was changed.

The central point of Experiment Two, oil extraction for biodiesel production, was to analyze which type of algae would produce the most biodiesel. The chemical purity of the fuel created was not tested, although would be a logical next step for future research of the material discussed herein.

The *Consortia* did not produce any biodiesel, and there was no separation between layers after transesterification.

Spirulina was the second best result. Initially, it separated into three distinct layers, but these layers were formed oddly and not separated along uniform lines in the beaker. After this sample was allowed to sit overnight in the open air, the three initial layers homogenized and consolidated into one indistinct layer. No biodiesel could be separated from this solution. It is considered a possibility for future fuel cultivation but at this time it was not believed to be useful for this study.

Chlorella had the best result of all algae types. After transesterification there were 3 entirely distinct layers, which signified a success. It is suggested that future investigation of transesterification should make use of *Chlorella* algae for optimal biodiesel results. *Chlorella* had the highest lipid content of all three algae types tested, and it is believed that there is a correlation between high lipid content and potential for biodiesel cultivation.

After transesterification was complete, 300 mL of biodiesel had been produced. The next step of the process would be to use bomb calorimetry to discover the heat of combustion of this substance. If the heat at which the solution combusted was high enough, it could be used to power an engine and function as a tangible source of energy.

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Thank you to The Pingry School, Mr. David Maxwell, Mr. Jeffrey Jenkins, and Mr. Drew Burns for their assistance in the project.

The Effects of Different Agar Media on Sporulation of *Sordaria fimicola*

By David Braverman'15 and Diana Masch'15

ABSTRACT

We used three strains of *Sordaria fimicola* (a popularly used 8-spored species of fungi that produces ascospores) -- black, tan and gray -- to determine the most effective sporulation agar. We expected sodium acetate and potassium acetate agar to be the most effective in inducing sporulation. However, we found that growth agar and crossing agar were significantly more effective for all strains of *S. fimicola*. The amount of original phytoplankton was calculated and a spectrophotometer was used to calculate the growth of algae each week. The amount of absorbance fluctuated, even for the containers with no iron present. Overall, more growth was present in the containers with higher concentrations of iron. However, throughout the five weeks of the experiment, the amount of growth changed for each concentration of iron.

INTRODUCTION

Determining the most efficient agar for the harvesting of spores for *Sordaria fimicola (S. fimicola)* is a crucial step for the future of fungal experiments at Pingry. Following the protocols we develop, Pingry students will be able to use S. fimicola for future experiments (for example, experiments on the effects of UV Radiation on melanin leading to cancer). Ascospores can be produced on many different types of agar; we were trying to determine which agar produces the most easily harvested spores. *S.fimicola*, in particular, is a homothallic pyrenomycete which, like 8-spored species of Neurospora, produces asci. Each asci has eight dark ascospores in a single orderly series (2). Ascospores produced are visible microscopically (4).The features by which most fungi are identified are related to the mode of sporulation and the arrangement of the spores produced (1).

In the case of some fungi, sexual reproduction results in the production of sexual spores in a structure called an ascocarp. This formation contains smaller sacs called asci, each of which contains ascospores.

In this experiment, we attempted to provide evidence that shows which agar induces the most sporulation in *S. fimicola.* We used three forms of the fungus Sordaria fimicola and four different types of agar. We are comparing cornmeal agar (growth agar), mating agar (crossing agar), potassium acetate agar, and sodium acetate agar. Potassium agar has been found to be superior to sodium acetate in its ability to enhance the production of ascospores, and we are trying to confirm this hypothesis (1).

RESULTS

Although we expected the sodium acetate agar and especially the potassium acetate agar, to cultivate the most spores, the *S.fimicola* growth agars and crossing agars displayed the most sporulation. Many plates of both growth and crossing agar showed signs of sporulation, but these spores would not detach from the agar when immersed in water and scraped with an inoculating loop.

All types of *S.fimicola* plated on the growth agar released clearly visible black spores that easily detached from the agar when immersed in water. Similarly, the gray *S.fimicola* released dark, visible spores on the crossing agar. However, the tan *S.fimicola* sporulation appeared darker on the crossing agar and some spores dislodged from the agar. The agar seemed weak and would often break off when it was being scraped by the inoculating loop. Although the spores of the black *S.fimicola* on crossing agar appeared very black, no spores actually detached from the agar. The spores on the sodium acetate agar from the black and tan *S.fimicola* appeared very light brown or white. The potassium acetate plates appeared the same as before the *S.fimicola* was plated and no spores were visible. After counting the amount of spores on each plate, we averaged the spore counts by type of *S.fimicola* for every plate type and used Microsoft Excel to plot the data in the bar graph shown below.

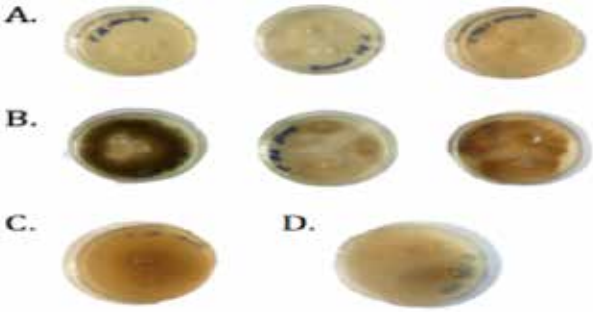

Figure 2. A) Black, Gray, Tan *S.fimicola* (left to right) dark, visible spores on plates with growth agar. B) Black, Gray, Tan *S.fimicola* (left to right) on plates with crossing agar. C) Black *S.fimicola* on Sodium Acetate agar with minimal spores. D) Black *S.fimicola* on Potassium Acetate agar with minimal spores.

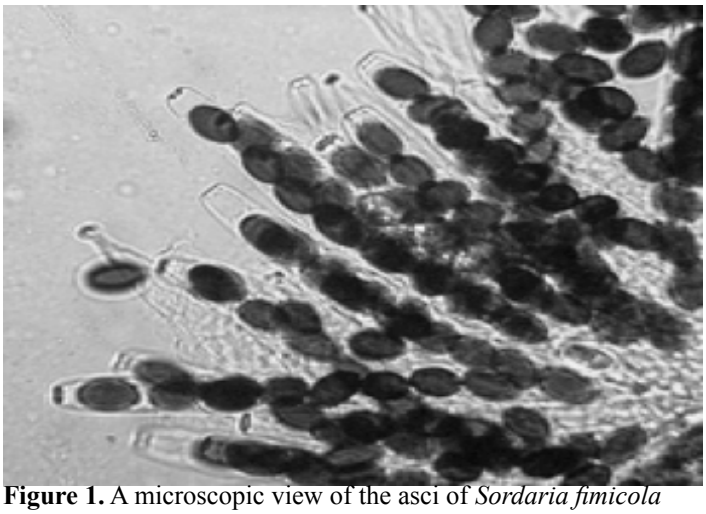

Figure 1. A microscopic view of the asci of *Sordaria fimicola* containing the round, darker ascospores.

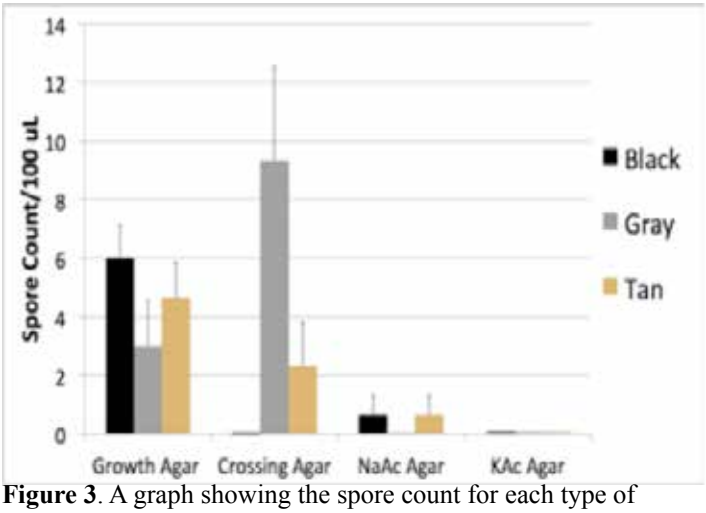

Figure 3. A graph showing the spore count for each type of *S.fimicola* on each type of agar. Statistical analysis shows the type of agar and type of *S.fimicola* that have an effect on the spore count.

DISCUSSION

The statistical analysis we conducted on our research has allowed us to come to the conclusion that growth agar and crossing agar are the most effective in producing sporulation of *S.fimicola*. This conclusion does not support our original hypothesis that the potassium acetate agar would produce the most sporulation. Pingry students can use our research and protocols to utilize *S. fimicola* sporulation for any type of experiment. Additionally, although the growth and crossing agar produced sporulation, they did not produce the large amounts of sporulation most scientists expect from *S.fimicola*, which may lead to more experimentation on how to increase sporulation.

ACKNOWLEDGEMENTS:

A special thanks to Mr. Maxwell, Dr. D'Ausilio, and Katherine Curran for all of their help and advising.

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Feasibility Study of the Aquaponics Home System

By Rachel Noone'15

ABSTRACT

Aquaponics is not a new concept. The process of cultivating crops in water shared with fish was first implemented around 1150–1350 CE by the Aztecs in fertile Mexican lakebeds (5). Aquaponics, in the modern sense, necessitates a body of water for the fish environment, from which water is typically pumped to a growing site where plants are raised in a growing medium bereft of soil or fertilizers. Aquaponics is more productive than traditional farming, conserves resources, and promotes sustainability by efficiently recycling water and waste products to nourish the symbiosis between growing fish and plants (2, 3). In this system, fish produce excrement that fertilizes crops and the crops, in turn, filter and purify the water for the fish. In a world confronted with the epidemic of dwindling resources and global hunger, the need to implement innovative and sustainable food cultivation methods is crucial (6).

INTRODUCTION

Aquaponics is promising for its productivity and efficiency. Traditional farming methods exhibit incredible waste, but the duo of protein and produce that could be grown using this agricultural technique would be hugely beneficial for farming crowded areas, environmentally conscious nations, and developing countries with constricted resources. However, differing opinions exist on the subject of aquaponic feasibility. Several factors such as market, scale, and labor greatly affect the degree of profitability (1, 2). Aquaponics can yield abundant and nutritious crops and depending on water pH, temperature, feedings, and crop density, the system can be modeled to yield more protein or produce, depending on demand (4). Economic prosperity may be expected from well-run and established systems (3, 6). Aquaponics is a monitoring-intensive cultivation technique that requires a deep understanding of the system and how to immediately respond to complications when they arise. If a careful balance is not maintained within the system, net economic loss may be expected, or even complete failure of the system (1). The major potential challenge of this study is the difficulty in concretely measuring labor input and skill in managing the system and resolving problems. Previous studies focused on relatively large scale/strictly commercial farms, and therefore data on a small-scale system remains a bit ambiguous. According to other research, a small-scale operation in a home would be feasible (1). Because aquaponics demands a large upfront investment and continuous monitoring, a small-scale, indoor aquaponic unit was built to investigate if this sustainable method of agriculture could realistically be introduced to the masses as a viable option to grow their own produce and protein. Through this study, I hope to prove that the inputs would be repaid over time by the products grown by the system. Documenting the experience and recording measurements weekly will offer a definitive answer.

RESULTS

Plant growth was very strong once transplanted. While the tomatoes prospered, however, the spinach grew very spindly (Fig. 4). Fish were fed twice a day. Food quantities were based on the kind of behavior they demonstrated. The first problem encountered was a dangerously toxic 5ppm nitrite spike that began on 12/2/14 (Fig. 5). A 30-gallon hospital tank was established to house the fish until the spike, caused by an undersized population of nitrifying bacteria, was brought under control. Water test kits, stabilizing solutions from the pet store, and pH reducing solution were implemented to help establish bacteria. When ammonia, nitrite, and nitrate levels all returned to 0ppm, the eight remaining fish were immediately returned to the system on 12/3/14 (Fig. 6). The system was functioning beautifully for the following two months, abundantly growing tomatoes and fish. The unsuccessful, spindly spinach plants were extracted on 2/26/15 to make more room for the 9 large tomato plants (Fig. 7). Two days later, a pathogen appeared to

have entered the system as one fish unexplainably died (Fig. 8). By 3/13/15, all of the fish, which had grown about 4" in length, were found dead only two weeks after the first tomato fruits had sprouted (Fig. 9).

Figure 5a.

Figure.5b.

Figure 6.

Figure 7.

Figure 8.

Figure 9.

CONCLUSION

Considering the failed outcome of the aquaponics investment, this study did not find that such a small-scale system would be feasible in homes. Although great success was experienced for weeks with fish and tomato growth, the death of the entire fish crop could not allow this study to be deemed feasible, as there was no substantive payoff. Any fish or crop loss on such a small scale amplifies the financial and resource costs to enormous extents. Home aquaponics has definite hopes for future success, but ensuring that the system is fully settled, protected from extraneous variables, and attended to daily is absolutely crucial to minimizing risk.

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Use of Percentage Body Fat to Assess Body Health in Children

By Kevin Chow'16

ABSTRACT

Body Mass Index (BMI) is the most widely accepted method of measuring body health (1). The exact accuracy of assessing body health using BMI methods is questionable. Measurements that determine Percent Body Fat (PBF) may be more accurate at determining body health, especially in specific demographics (4,5). This study included 25 boys and 29 girls varying from ages 9-11. Their heights, weights, and PBFs were measured in May 2014 and September 2014. The results showed mean BMI in boys following a positive trend, becoming unhealthier, while PBF showed the boys loosing fat and gaining muscle. In girls, both systems reflected similar numbers. The results showed the use of PBF methods in boys is more accurate. In girls, further research must be done with additional parameters incorporating the body characteristics unique to young pubescent girls.

INTRODUCTION

Obesity and body health have become increasingly important issues, and recent statistics show that about one in three children in the US is either overweight or obese (2). These children are more prone to health issues in both adolescence and adulthood, leading to an overall lower quality of life. In children with eating disorders, many of whom develop disorders during puberty, monitoring health to the most accurate level is also extremely important. In genetic diseases such as cystic fibrosis, malnutrition and stunted growth are common. In order to properly address these issues, the most accurate methods must be used. BMI is the most prominent and commonly used method for measuring a patient's body health. This method provides an estimate for total body fat. However, this system has drawbacks. Overestimations of body fat in those that have more muscular builds occur frequently,

while people with smaller physiques, especially the elderly, often have their body fat underestimated. These discrepancies are due to the fact that BMI does not take into account or measure the actual amount of Fat Mass (FM) and Lean Body Mass (LBM). As a result, BMI data may significantly differ from PBF data. Therefore, the aim of this study is to compare BMI and PBF data and determine how accurately they reflect body health.

Different demographics, especially those separated by age, show great variability in body health standards and measurements (3). Previous research concluded that it was most important to distinguish gender and age when screening for the presence of obesity (4,5). In this study, children of both genders, ages 9-11, were examined. This age group includes children on the verge of entering puberty, when body growth starts in many children, and the amounts of FM and LBM start to change at rapid rates. During this time period, body image becomes more important, thus making eating disorders more common (6). The duration of this study spans from May 2014 to September 2014. The expectation is to find significant differences between the two systems of measurement, supporting PBF as the more accurate system.

RESULTS

The mean BMIs reflect a gain of .03 in boys and a gain of 0.2 for girls. Both of these are positive trends. For boys, PBF numbers decreased significantly by 2%, contrasting the increasing trend shown by BMI. The mean PBF for girls showed an increase of 0.3%, a smaller increase when compared to BMI. Overall, the measurements for girls show similar trends. Examination of the mean body compositions in boys shows a definite drop in FM of 1.069 pounds and a rise in LBM of 2.7655 pounds. For girls, both FM and LBM increased, by 0.804 pounds and 3.12 pounds

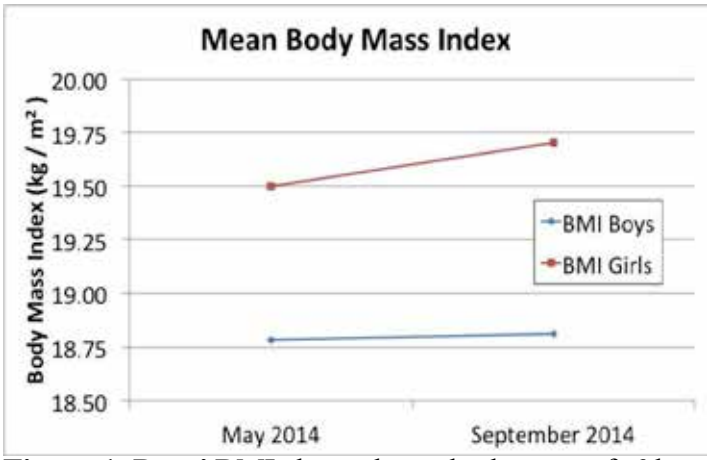

Figure 1. Boys' BMI showed standard errors of .6 before and .71 after. Girls' BMI showed standard errors of .87 and .88.

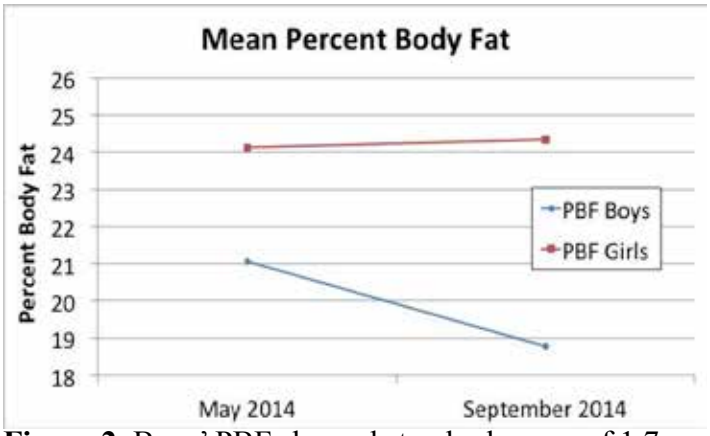

Figure 2. Boys' PBF showed standard errors of 1.7 before and 1.9 after. Girls' PBF showed standard errors of 2.1 and 2.

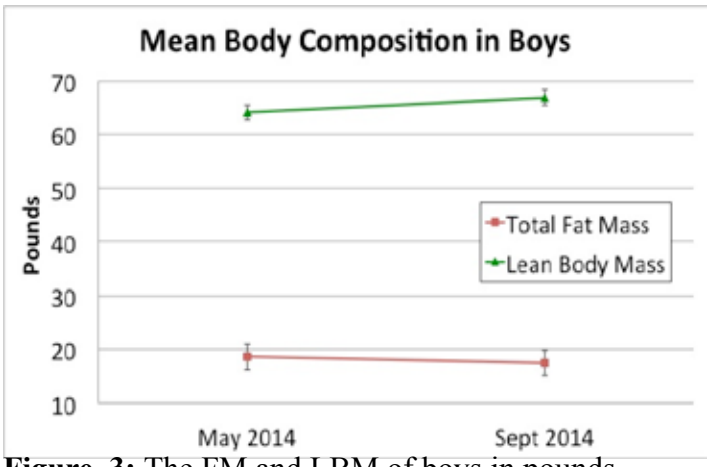

Figure. 3: The FM and LBM of boys in pounds.

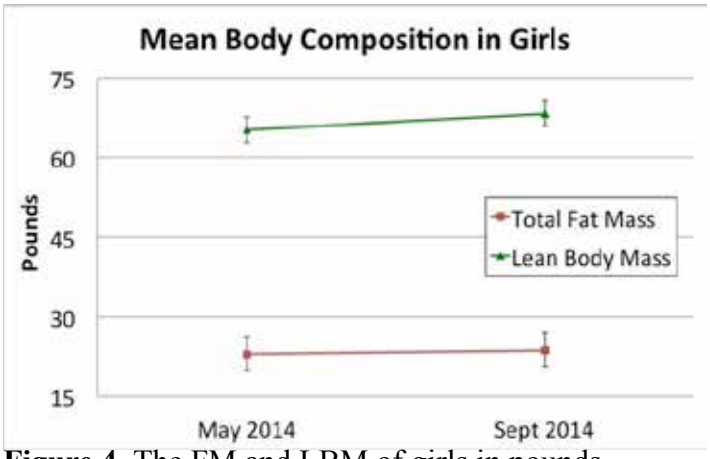

Figure 4. The FM and LBM of girls in pounds.

respectively.

DISCUSSION

While BMI measurements reflected an overall increase in boys, PBF measurements showed that the amount of fat in the boys decreased. The increase in BMI can be attributed to gains in LBM. This finding supports PBF as a more accurate system of measurement, because unlike BMI, PBF takes LBM into account. With girls, the measurements of BMI and PBF showed no significant difference. This is attributable to the simultaneous increases in both FM and LBM. These results show the importance of creating adolescent and gender-specific recommendations. For adolescent boys, statistics show that using BMI to assess their health can be ineffective in ensuring proper diagnosis and medical advice. For adolescent girls, the onset of puberty provides a unique challenge in assessing their health with either system, and further research should be done to explore this area. Additional studies should look at both genders individually. In addition, the studies should include larger sample sizes and varying increments of time. Such studies should also look into how puberty in teenagers affects the efficacy of these measuring systems in comparison to children aged 9-11.

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The Effect of Polluted Plastic on Plant Growth

By Dana Wang'15, Adriana Savettiere'15, and Nicole Korogodsky'15

ABSTRACT

Plastic pollution not only affects aquatic animal life, but vegetation as well. We tested the effects of polluted plastic on lettuce growth. We did this by comparing various concentrations of plastic soaked in distilled water and polluted water. Our hypothesis was that the plants that were seeded with polluted plastic would grow less than the plants with plastic soaked in distilled water, and that the plants with a higher concentration of plastic would have less growth. Our results were inconclusive. This could be due to the fact that having only 4 plants skewed the average height grown.

INTRODUCTION

Plastic is slow to break down in the environment. Eventually, plastic decomposes into smaller particles that absorb toxic chemicals, and then is ingested by animals, contaminating the food chain.

Plastic in the ocean breaks down into pieces that have been found to contain toxins (PCB and DDT) at levels up to 1,000,000 times the level found in natural seawater (2). Studies have found that species like zooplankton, seabirds, cetaceans, and sea turtles ingest the nonnutritive, indigestible, and possibly toxic plastic (3). The United Nations Joint Experts on the Scientific

Aspects of Marine Pollution (GESAMP) estimated that land-based sources account for 80% of marine pollution, and 60-95% of this pollution is from plastic (4). A significant portion of the plastic floating around our oceans consists of plastic bags. During the 2011 International Coastal cleanup, volunteers collected 120,450 lbs of bags in the U.S. (2). The effects of plastic pollutants in marine life are well known, and since most of the plastic originates from land, we tested how plastic pollution (bags) affects plant growth. The goal was to see if polluted plastic has any effect on lettuce growth.

RESULTS

This chart shows the averaged height of the four plants of each concentration measured roughly every 10 days. The bar graphs compare the growth of the distilled and the polluted plants at each concentration. The polluted and distilled measurements for a concentration of zero (no plastic) are the same.

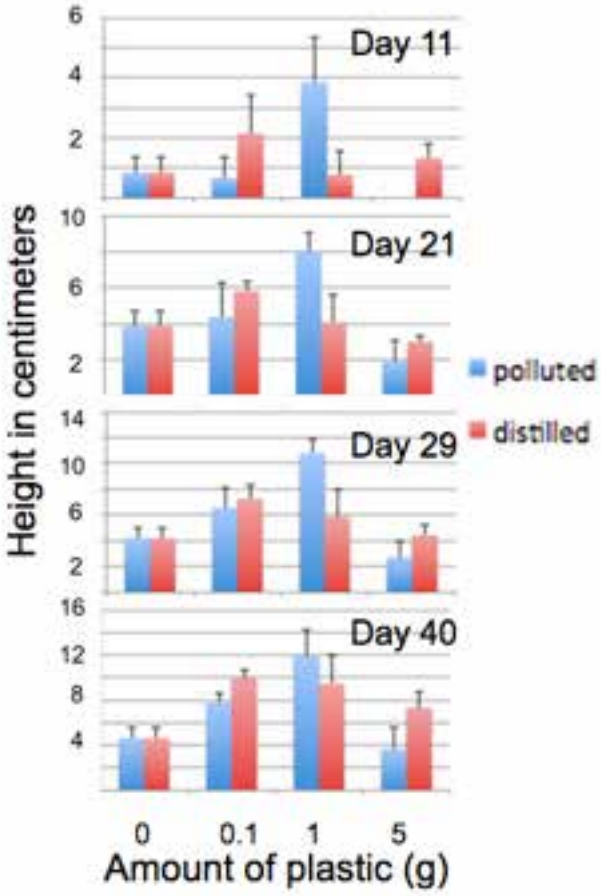

Figure 1. Polluted Plants on Day 40

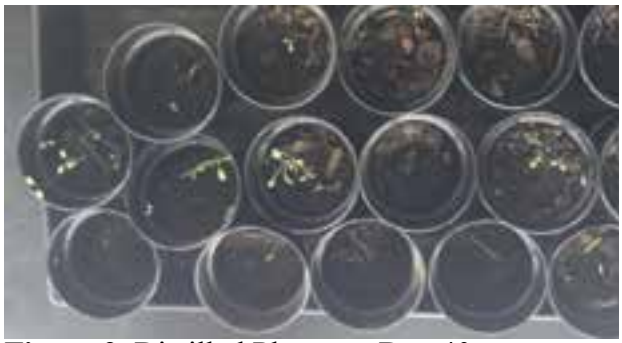

Figure 2. Distilled Plants on Day 40

DISCUSSION

Our experiment produced varied results. By Day 40 all concentrations of distilled, except for 1g ($p = 0.16$), grew significantly more than their respective polluted plants.

Overall, the least growth came from the 5g plants, then the 0g, then the 0.1g. Surprisingly, the 1g plants had the most growth. The fact that the highest overall growth came from polluted 1g plants can be attributed to the fact that only 4 plants of each concentration were grown, and then averaged. This could be due to the fact that only 4 plants for each concentration were grown, and one plant not sprouting, or one

plant growing a lot could skew the results.
Since our results were so varied, we cannot say definitively the effects of plastic pollution on lettuce growth. If we were to redo this experiment, we would like to plant more plants per concentration to negate the effect of a single plant on the average growth.

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Purification of *Agrobacterium tumefaciens* from Grapevines

By Claire Putman'15 and Lily Graff'15

ABSTRACT

Agrobacterium tumefaciens is a strain of bacteria that is known to infect and create tumors at the site of infection with its Ti-plasmid in many plants, such as grape vines and many common vegetable plants. These specific tumors are known as crown galls and severely weaken the host plant. By using a selective Biovar 3 Media, we were able to purify the bacteria from a sample tumor and inoculate a new host plant. By being able to purify *Agrobacterium tumefaciens*, more studies can now be conducted to test possible treatment.

INTRODUCTION

Crown gall disease infects and destroys grape populations across the globe. Each year vineyards and plant nurseries lose thousands of plants to crown gall and as a result, have lost huge amounts of business in the wine industry. For our project, we wanted to isolate the bacteria that causes crown gall disease, known as *Agrobacterium tumefaciens*, so that it can be used in researching possible cures for the disease. A. tumefaciens is a gram-negative bacteria, which means that the "unique nature of their cell wall makes them resistant to several classes of antibiotics" (4). It is known to infect many plants, such as grape vines and many common vegetable plants, with its Ti-plasmid, and create tumors, or crown galls, at the site of infection. *A. tumefaciens* is especially lethal because it has "flagella that allow it to swim through the soil towards photoassimilates that accumulate in the rhizosphere around roots" (1). "Severely galled young plants are weakened, stunted, and unproductive and occasionally die due to an inferior root system" (3). By isolating the cause of these tumors and being able to recreate them on other surfaces, it is beneficial in the opportunity to find a cure of this growing problem in the wine industry.
After entering the plant cells, T-DNA programs host cells to grow and divide rapidly, generating crown galls on the stems and roots of grapevines. "In essence, the metabolism of the plant cell is diverted to satisfy the highly distinctive appetite of the intruder. Tumor-inducing plasmids (Ti plasmids) that are carried by *Agrobacterium* carry instructions for the switch to the tumor state and the synthesis of opines" (2). This causes crown galls caused by *Agrobacterium*

Figure 1. Crown gall

tumefaciens to grow very rapidly, forming almost a full tumor in less than two weeks. The fast-paced growth of these tumors makes *A. tumefaciens* ideal to work with in the lab.

Our goal was to purify *Agrobacterium tumefaciens*, induce crown gall growth on vegetable plants, and then test if any common human tumor methods are effective on crown galls. We hypothesize that there will be growth on our plates using a highly selective Agrobacterium agar, Biovar 3 media. Later, when the bacterium is then placed onto carrots, there should be crown gall growth.

After obtaining a vinet grapevine infected with crown galls, samples of the tumor were taken and placed onto Biovar 3 agar. Since the crown galls did in fact contain *Agrobacterium tumefaciens*, the colonies had the appearance of a white dot with a red center. Our next step was to place a colony onto a sample of carrot to see if a tumor would grow.

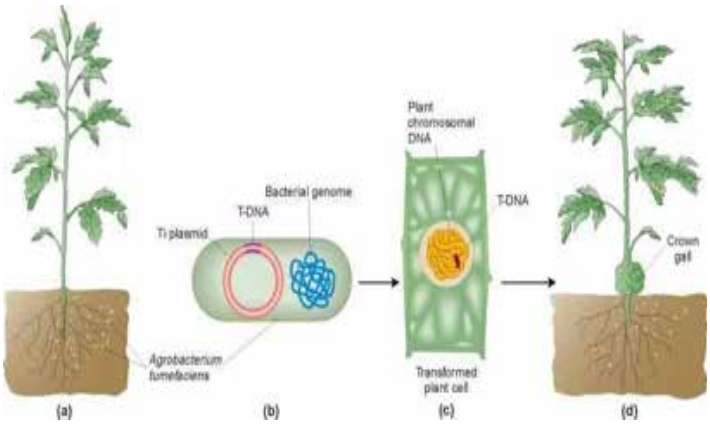

Figure 1. The progression of *A. tumefaciens* infection

RESULTS

After placing the tumors onto Biovar 3 Media, we were able to successfully purify and grow *A. tumefaciens*. *A. tumefaciens* colonies grow as white circles with a red center and can be seen in the picture on the right, signalling that we did in fact grow the correct bacteria.

In order to recreate the tumor, we inoculated carrots with the grown bacteria to see if there would be tumor growth. Figure 5 is an image of our first carrot inoculation where there is a lot of mold. We realized that instead of inoculating carrots in selective media, we needed to inoculate them in a water agar solution. Figure 6 clearly shows one carrot with the bacteria on it and one without. The carrot disks in water agar clearly show the beginnings of a tumor.

DISCUSSION

After completing our experiments, we were

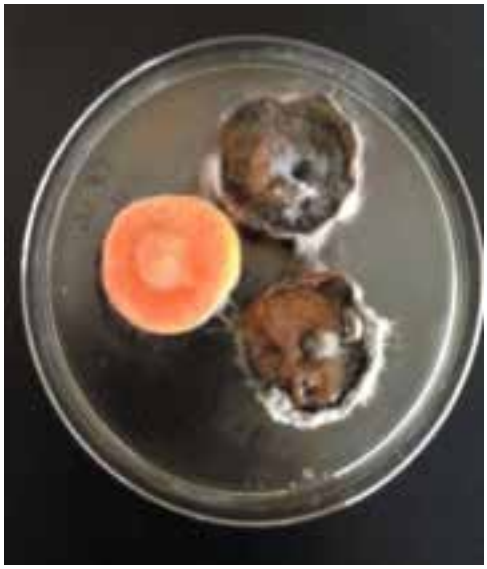

Figure 5.

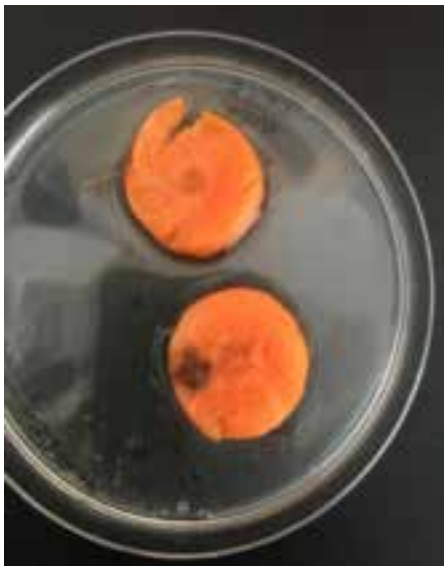

Figure 6.

able to successfully purify *A. tumefaciens* from a crown gall tumor and inoculate a host plant. The carrot disk that had *A. tumefaciens* placed on it began to grow a crown gall. However, there were some points of improvement that we could have made. We recognized that during our first trial on the Biovar 3 plate, two of the inoculated carrot disks began to grow mold. Using more sterile lab techniques can prevent some of these complications in the future.

FUTURE STEPS

Due to the timing of our project, we were unable to grow a full tumor and perform tests on it. In the future, many studies can be conducted to try and prevent crown gall disease from infecting grapevines. Studies should be done both on fully grown tumors to see if plants can be saved after being infected, as well as on isolated colonies of *Agrobacterium tumefaciens*

to see if the bacteria can be killed prior to infection. In the case of treating fully grown tumors, it is very difficult to reverse the symptoms of crown gall disease. Research has been done with *Agrobacterium radiobacter* strain K84 and has been shown to "completely prevent disease when added to wound sites at a 1:1 ratio with cells of *A. tumefaciens*" (5). With further research, other methods of treatment can be tested on both crown gall tumors and isolated bacteria in the hopes of solving the huge problem that crown gall disease poses to vineyards today.

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ACKNOWLEDGEMENTS

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The effect of food concentration on *Physarum polycephalum*

By Jacob Robinson'16

ABSTRACT

I tested the ability of a slime mold, *Physarum polycephalum*, to find the shortest path between two food sources in a maze, with varying concentrations of food. The molds grew to fill up a triangular maze, and then were fed with a certain concentration of food. Once they were fed, the molds were photographed hourly in order to determine when the mazes were solved. I found that molds with more food navigated the maze more quickly than molds with less food. These results indicate that an increased concentration of food does improve the mold's ability to find the shortest path through the maze quickly and reliably.

INTRODUCTION

The design of transport networks that efficiently and effectively transport resources and information is essential for the development of modern-day infrastructure and technology. However, in a rapidly advancing world, it is increasingly difficult to design networks that are easily adaptable and still as efficient as possible. Instead of looking to computers to find solutions to these increasingly complicated problems, it may be more effective to look to biology. Many species of fungi and protists have evolved to grow in networks of cells that connect and transport resources in their environment effectively. After millions of years of evolution, these organisms have developed the ability to design and grow efficient transport networks better than any computer program.

Previous research has shown the ability of *Physarum polycephalum*, a species of slime mold shown in Figure 1, to form the most efficient connections between resource nodes. Slime molds are types of protists that spend most of their lives as single cells, but have the ability to form large multicellular networks in order to reproduce or find food. Experiments with these multicellular networks have shown that they are capable of replicating the Tokyo rail network when placed on a map of the area with food sources in the place of train stations, and finding the shortest route between two food sources at opposite ends of a maze. The goal of this project is to determine whether or not the amount of food available in one of these mazes affects the slime mold's ability to find the shortest route. The surface area of the food was kept constant, but the

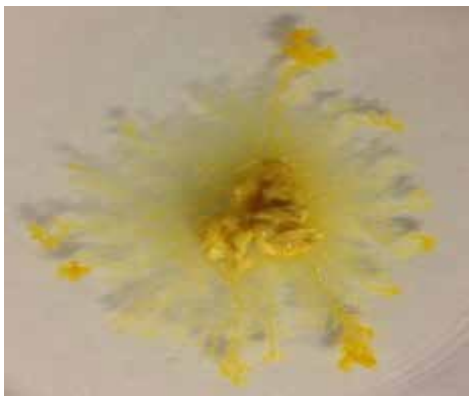

Figure 1.

concentration of nutrients was varied. This could offer new insight into how "intelligent" the slime molds really are, and how much information about the outside world they are able to sense. Understanding this about the slime molds will help future researchers better manipulate them to design transport networks.

RESULTS

Figure 2 shows a single maze with 5% oat powder agar demonstrating maze solving. The maze was photographed at 0 hours, 3 hours, 6 hours, 9 hours, and 12 hours after being fed. The lower row of images has been altered to make the mold, in black, more clearly visible.

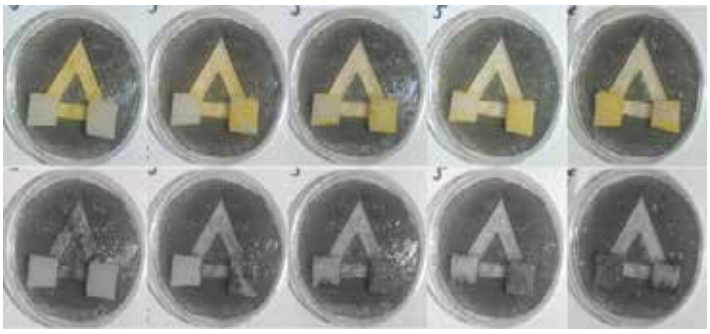

Figure 2.

Figure 3 shows the average number of connections between the two food sources over time for each different food concentration from five different trials. Each line represents the average number of connections at each hour for a particular food concentration. Two connections means the mold has not yet solved the maze, and it is still growing in both pathways. One connection means that the mold has completely solved its maze. Some molds retracted even further and broke the connection between the food sources, resulting in zero connections.

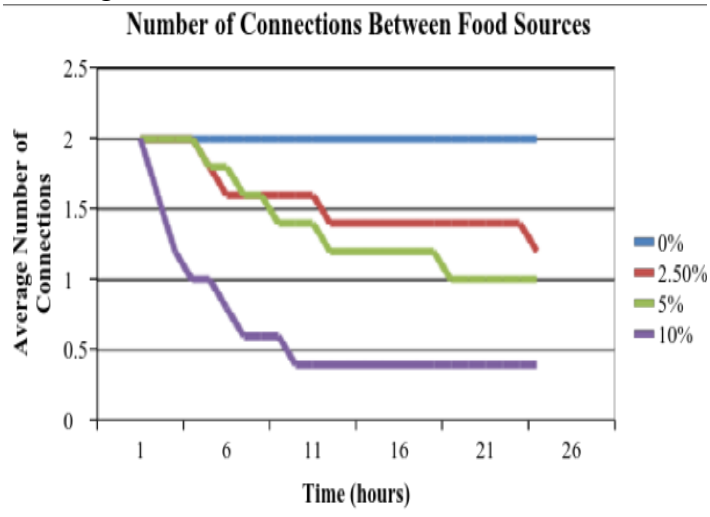

Figure 3.

DISCUSSION

Although the molds fed 2.5% and 5% oat agar blocks did solve the maze in approximately the same time, it is possible that this is just because there is only a difference of 2.5% between the food concentrations of these two groups. There is a difference of 5% between the food concentrations of the groups fed 5% and 10% oat agar blocks, and there is a significant difference in the times these two groups were able to solve their mazes. Based on these results, it seems that there is a direct correlation between the quantity of food sources and the slime mold's ability to efficiently connect these food sources, although it is still unclear why this happens. Previous research has shown that slime molds use chemical signals to communicate information about their environment, so a similar signaling mechanism might communicate information about food sources the mold has encountered (3). However, further research must be done in order to determine if such a mechanism truly exists, or if the mold has some other system to communicate information. If slime molds are used in the future as a tool for designing efficient transport networks, it will be important to determine exactly what information they communicate and how they communicate that information. Understanding this will help future researchers manipulate the molds to solve various kinds of problems (6).

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Computational Modeling of Malapposition in Coronary Stents

By Sharanya Pulapura'15, Jackson Artis'16, and Sean Wang'17

ABSTRACT

Being the number one killer of humans worldwide, Coronary Artery Disease affects over 7.4 million people each year. The disease is characterized by the buildup of atherosclerotic plaques in the coronary arteries, which disrupts the flow of blood out of the heart. The most common and least invasive treatment for Coronary Artery Disease is stenting, the process of inserting a metal mesh tube into the artery to keep it open. However, stented patients often experience in-stent restenosis, wherein an excess of plaque reforms within the walls of the stent. Our team designed an experiment to test the effects of mechanical stresses undergone by the stented artery on the development of these biological complications. In particular, we studied the effects of stent malapposition on the development of in-stent restenosis. Using the optical coherence tomography (OCT) imaging modality, we were able to reconstruct a three-dimensional geometry of a malapposed stent using computer-aided design (CAD) software. After correcting intersecting and unrealistic geometries using CAD software, we used the computational fluid dynamics solver ANSYS to calculate the wall shear stress along the surface of the stent. A finite volume analysis yielded moderately elevated wall shear stress along malapposed regions of the stent.

INTRODUCTION

Coronary artery disease accounts for over 7.4 million deaths every year, making it the most deadly disease worldwide [1, 2]. This cardiovascular disease develops when atherosclerotic plaques build up inside of coronary arteries, which narrows or "stenoses" the vessels and blocks blood flow from the heart. Symptoms of coronary artery disease include angina, shortness of breath, and fatigue. However, it is often asymptomatic until the artery is completely blocked, which results in a heart attack.

Although several methods exist for treating coronary artery disease, including coronary artery bypass grafting (CABG), the most common and least invasive procedure is percutaneous coronary intervention (PCI) followed by stenting [3]. A metal mesh tube called a stent is placed onto a balloon catheter and is inserted into the stenosed coronary artery via the femoral artery. When the balloon is inflated with water, the stent expands and holds open the stenosed artery, allowing blood flow to resume. However, between 20 and 25% of all stented patients experience in-stent restenosis, wherein the stented artery is once again blocked off by plaque buildup within the stent. In-stent restenosis can be a dangerous complication because, like the original stenosis, it is often asymptomatic until a heart attack occurs. Other complications include in-stent thrombosis, or the development of frequently fatal blood clots inside the stent [4].

Several methods have been proposed to eliminate in-stent restenosis and thrombosis such as drug eluting stents, but these treatments are often ineffective. Therefore, we sought to determine what biomechanical factors contribute to the development of in-stent complications in order to later devise a better mechanism for designing and deploying stents and ultimately reduce the risk of these complications [5, 6].

The biomechanical factor that we examined in this study was stent malapposition. Stent malapposition occurs when the balloon used to deploy the stent does not expand fully; as a result, the stent does not align with the inner wall or lumen of the artery [7]. It has been hypothesized that stent malapposition could interrupt the flow of blood through the arteries, altering the forces that act on the artery. Mechanical factors potentially affected by the disruption of blood flow include wall shear stress (force per unit area coplanar with the cross section of the artery) and strain rate (rate at which the artery is deformed with respect to time).

For this experiment, we will use optical coher-

ence tomography pullback frames to reconstruct a three-dimensional model of a stent malapposed within a rabbit artery. Optical coherence tomography is an imaging modality that uses long wavelength, near-infrared light to penetrate through tissue and create high resolution images [8]. The OCT frames will be fused together into the full geometry of the stent and repaired in the CAD software Rhinoceros. Using the finite volumes solver ANSYS Fluent, the wall shear stress and strain rate will be calculated at a series of points along the surface of the artery. If increased stress in malapposed regions of the stent correlates to regions of in-stent restenosis in the rabbits, the mechanical disturbances caused by stent malapposition could potentially contribute to the development of restenosis.

RESULTS

As is evident from the contour plot output from ANSYS (Figure 3), stent malapposition does seem to have an effect on the forces acting upon the artery. In the malapposed regions of the stent— that is, the regions in which the stent is protruding from the artery— the wall shear stress ranges from 2 to 10 pascals. Conversely, in the regions where the stent is not malapposed, the wall shear stress ranges from 0 to 2 pascals. However, we are unable to conclusively state if these results are significant or not due to the limitations of the ANSYS output received from the laboratory at MIT. Since numerical data was not able to be distributed publically at this time, no significance tests were carried out at this time. However, based solely on the contour plot, there seems to be evidence supporting our hypothesis that disruption in blood flow caused by malapposed stents can contribute to an increased level of wall shear stress, as demonstrated by the elevated levels of wall shear stress that our model predicts in regions of high malapposition.

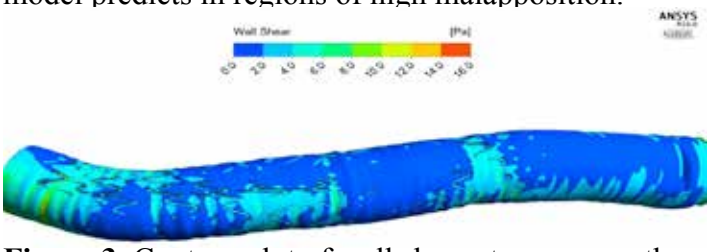

Figure 3. Contour plot of wall shear stress across the surface of the artery. Regions of high wall shear stress are shown in light blue and green. Regions of low wall shear stress are shown in dark blue. In regions where the stent is visibly protruding from the artery, wall shear stress is apparently elevated.

DISCUSSION

There is a great deal of future research to be done before our computational model can be deemed effective in predicting whether or not a stented artery is at risk for restenosis. The first step would be to confirm the accuracy of the model's predictions by correlating the computational data to the in vivo data. If these computational models calculate elevated levels of wall shear stress in a region of the stent that later develops in-stent restenosis in the rabbit artery, then it is possible that we might be able to use this model to accurately predict when a stented artery will develop in-stent restenosis. Furthermore, a far greater sample size is necessary to determine the accuracy of the model. We are currently in the process of reconstructing the geometries of several more stented rabbit arteries. In addition to the right coronary artery that was examined in this paper, we also are creating models of stents implanted into the other two major coronary arteries, namely the left anterior descending artery and the left circumflex artery. If the wall shear stress is also elevated in malapposed regions within a large number of a variety of types of stented arteries, we would be far more confident in saying that the malapposition is causing or contributing to the increased wall shear stress and subsequent development of in-stent restenosis. Another improvement we could include in our model would be to account for not only mechanical factors but also the biological and chemical factors that could possibly contribute to stent malfunction.

Once the accuracy of our computational model has been confirmed, the ultimate goal of our research would be to use the model to design idealized treatments for each individual case of coronary artery disease. We would combine our work on the mechanical factors contributing to in-stent restenosis with other work being performed in the Edelman lab on chemical, biological and physiological factors such as immune response to the foreign body (i.e. the stent) and the effects of drug-eluting stents. Ultimately, we will create a more complete computational model that can accurately predict most of the future complications of implanting a stent in an individual person. That way, we can run the simulations before the stent has actually been implanted into the patient and determine what complications the patient might experience in the future. If a great deal of complications are predicted, the doctor would be able to choose a different type of stent or mechanism of deployment to minimize

the risk to the patient. Rather than simply observing the stenosed artery and attempting to guess how and where to deploy the stent, as is the current practice, doctors would be able to use this computational model to know with relative certainty which treatment will be the safest and most effective.

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Investigating the eIF4E-4EBP1 Signaling Axis

By Andrew Verdesca'15, Bridget Harrison'15, Sofia Briones'17, and Katie Coyne'16

ABSTRACT

Breast cancer is one of the most pervasive and deadly forms of cancer, with over 200,000 diagnosed cases in the United States in 2013 [5]. Approximately 70% of breast cancers can be classified as estrogen-receptor positive, characterized by the presence of the estrogen receptor (ER+) [3] [6]. Although the drug tamoxifen has been developed specifically to treat ER+ breast cancer, approximately 33% of patients develop resistance to tamoxifen during the five-year course of treatment. [4]. Previous studies have implicated the proteins eIF4E and 4EBP1 in the development of this resistance. In this paper, we seek to further investigate and test the relationship between these two proteins, and the larger implications of this interaction in tamoxifen resistant breast cancer.

INTRODUCTION

Eukaryotic Initiation Factor 4E (eIF4E) is a key regulator of selective translation in mammalian cells. In tamoxifen-resistant breast cancer cells, eIF4E is believed to be hyperactive, and is responsible for the selective translation of certain oncogenic mRNAs (such as c-myc and cyclin D1) by binding to the m7GTP (5') cap of these mRNAs and recruiting the small subunit of the ribosome [1] [2] [8] (Topisirovic & Sonenberg, 2011). The protein eukaryotic Initiation Factor 4E Binding Protein 1 (4EBP1) sequesters eIF4E (Figure 1). When sequestered by 4EBP1, eIF4E is unable to translate these oncogenic factors. [8] 4EBP1 is itself regulated by mTORC1 (molecular Target of Rapamycin Complex 1). [8] In vivo, mTORC1 phosphorylates 4EBP1's 114th phenylalanine residue, which is believed to result in a conformational change that decreases its binding affinity for eIF4E, preventing it from being properly sequestered by 4EBP1.

In this paper, we aim to further characterize the relationship between eIF4E, 4EBP1, and mTORC1. We hypothesize that mutating the 114th phenylalanine residue of 4EBP1 into an alanine (which should prevent phosphorylation by mTORC1) will decrease relative binding affinity of 4EBP1 for eIF4E. We further hypothesize that mammalian cells expressing 4EBP1 F114A will undergo less cell proliferation relative to cells expressing wild-type 4EBP1.

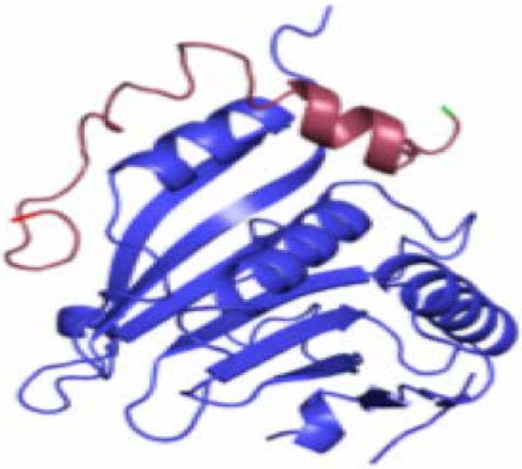

Figure 1. [7] Cartoon model of a crystal structure of the quaternary structure of eIF4E (blue) in complex with a fragment of 4EBP1 (red). When 4EBP1 binds to eIF4E in such a manner, eIF4E is sequestered, preventing it from initiating selective translation of oncogenic mRNAs. Note that the 114th phenylalanine residue is not actually present in this figure; it is further downstream of the N-terminus (marked here in red).

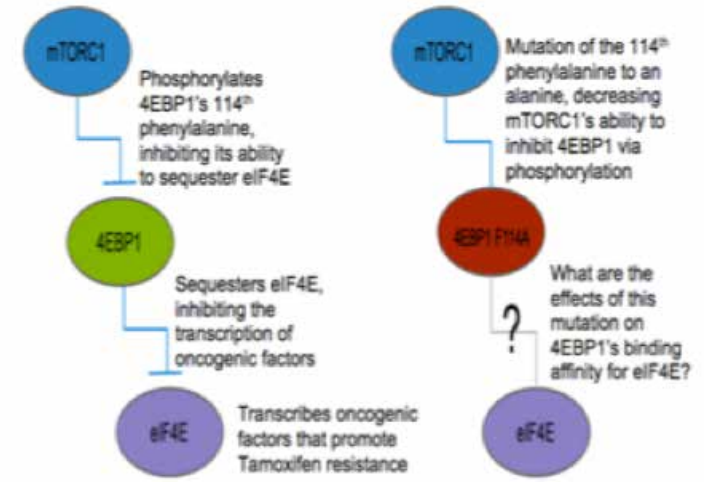

Figure 2. A representation of the cytoplasmic interaction between the protein complex mTORC1, wild-type 4EBP1, and eIF4E (left) and the predicted interaction between mTORC1, 4EBP1 F114A, and eIF4E (right).

RESULTS

Gel electrophoresis was performed on the resulting PCR product of eIF4E to determine if the reaction had succeeded and we had obtained purified eIF4E, as well as to determine the optimal concentration of initial template for the reaction. (Figure 3) The results of the gel show consistent banding at approximately 650 BP. This implies that we created sufficiently high levels of DNA, demonstrating that we have successfully purified eIF4E out of PTXB1 using PCR.

Additionally, a similar gel was performed on

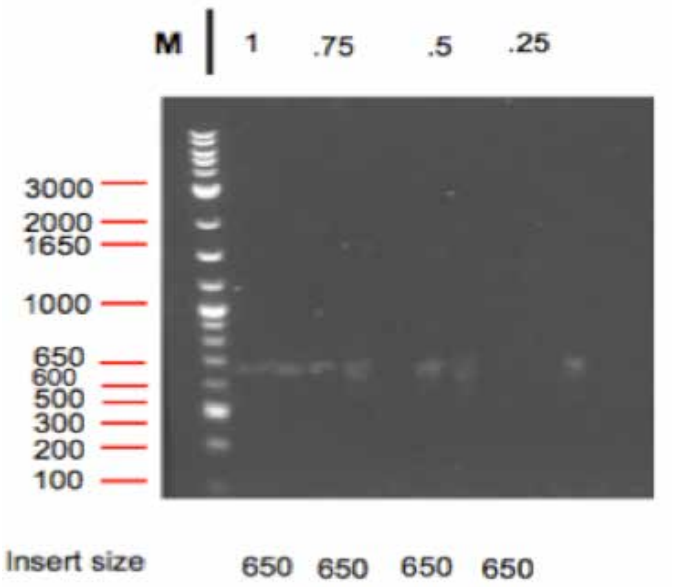

Figure 3. The results of gel electrophoresis performed on eIF4E after removing it from the PTXB1 vector using PCR. In the leftmost lane is a two-log ladder, consisting of dye and pieces of DNA of known lengths, used to estimate the lengths of the sample fragments. Lanes labeled "1", ".75", ".5", and ".25" represent the results of PCR reactions performed using various concentrations of eIF4E as template DNA. Although each of the reactions succeeded at least once, producing a band of DNA with length of 650 BP (approximately the same length as eIF4E), the 1x concentration of template was most effective in generating these results.

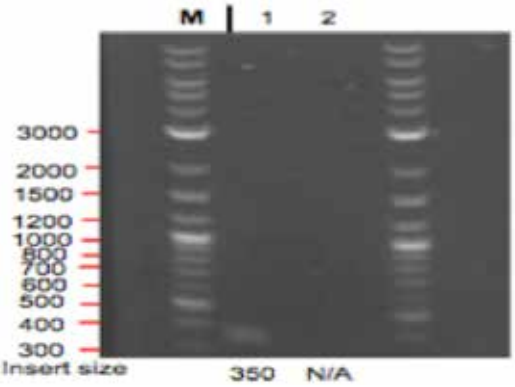

Fig. 4: The results of gel electrophoresis performed on 4EBP1 after removing it from the pMUD vector using PCR. In the leftmost lane is a two-log ladder, consisting of dye and pieces of DNA of known lengths, used to estimate the lengths of the sample fragments. Lanes labeled "1"and "2" represent the results of two separate PCR reactions performed using 4EBP1 as template DNA. Although the second reaction was unsuccessful, the first reaction worked, producing a band of DNA with length of 305 BP (approximately the same length as 4EBP1).

the product resulting from the PCR performed on the pMUD+4EBP1 construct. The results of this procedure can be found in Figure 4. Although the second reaction failed, the first reaction produced DNA fragments of around 350 base pairs in length, which is approximately the size of 4EBP1, implying that the reaction was a success.

FUTURE EXPERIMENTS

We have thus far been able to successfully obtain, clone, and purify the genes coding for eIF4E and 4EBP1. In the future, we will need to successfully perform PCR purification on these two genes. Once purified, these genes will be digested with restriction enzymes and ligated into pBABE, a bacterial expression vector. Naïve competent DH5α *Escherichia coli* Cells will be transformed with either the pBABE+eIF4E or the pBABE+4EBP1 constructs, and glycerol stocks of those cells will be created. The construct will be cloned, and the resulting miniprep product will be subjected to a mutagenic PCR using specially designed primers (Figure 4) that contain the desired mutation: a substitution of the codon for the 114th phenylalanine residue (TTT) for an alanine (GCT). Once purified, the resulting mutated product will be digested with Dpn1, a restriction enzyme that strictly degrades methylated DNA. Since DNA is only methylated in vivo, only the original, unmutated template DNA will be degraded. The resulting product will be purified and amplified by transformation into competent DH5α *Escherichia coli* cells. Then, eIF4E, wild-type 4EBP1, and 4EBP1 F114A will be expressed separately in bacteria, harvested, and purified, before relative binding affinity will be assessed in vitro between 4EBP1 F114A and eIF4E and wild-type 4EBP1 and eIF4E. Additionally, we may express these constructs in Chinese Hamster Ovary (CHO) Cells and observe their effect on cell proliferation in the presence and absence of tamoxifen, to observe the effects of these mutations on cancer proliferation in vivo.

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Social Defeat in Zebrafish

By Gabrielle Stern’15 and Peter Rothpletz’15

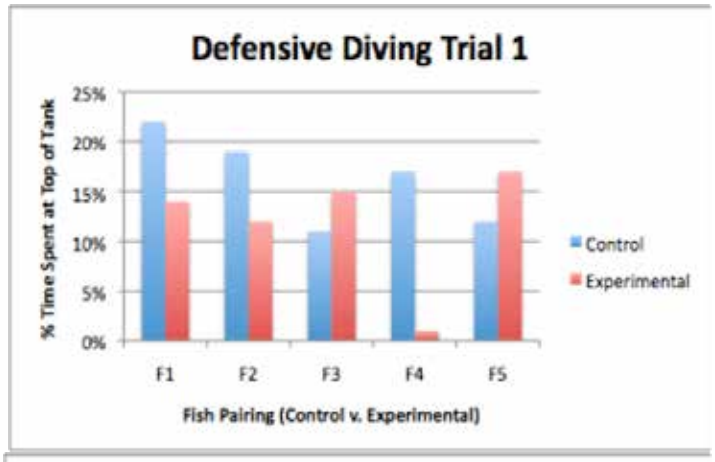
ABSTRACT

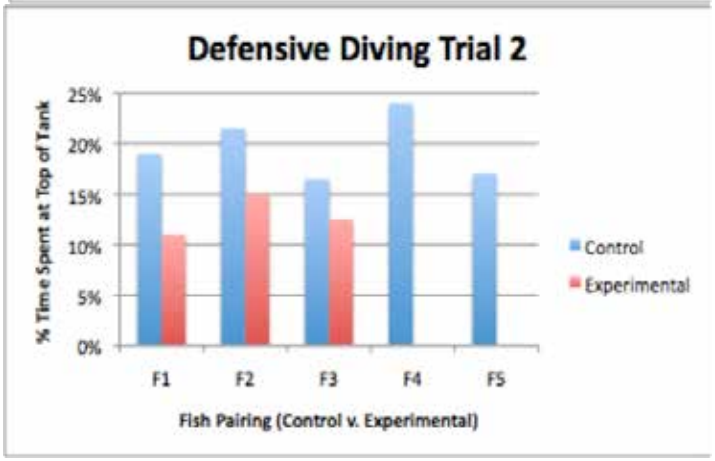
Research indicates that animals’ responses to social defeat can serve as a model for the behavior of human beings under duress. Our goal was to further explore this notion by studying how zebrafish react when exposed to a form of social defeat known as the “resident-intruder” paradigm. We accomplished this by comparing the defensive diving of an experimental group of fish exposed to social defeat and a second group serving as a control. Results of the investigation were mixed, but they do correlate with the established notions regarding social defeat in animal models.

INTRODUCTION

Social defeat is the term used to refer to repeatedly losing a confrontation, either in a dyadic or a group-individual context. In humans, this is more colloquially known as "being bullied." Studies have shown that when exposed to social defeat over an extended period of time, many adolescents experience a variety of effects, from anxiety, to depression, to aggressive behavior later in life. If this is the case, can these same effects be replicated in an animal model in order to better understand the mechanism of social defeat? Previous studies have investigated the potential of zebrafish (*Danio rerio*) as a model organism for behavioral and biological psychiatry research, as certain stimuli have been shown to evoke a robust anxiety response in the species. These reactions have been well cataloged over the last decade, and can now be easily quantified through automated video tracking technologies that provide standardized, unbiased observation. With this information in mind, a zebrafish model is ideal for an analysis of social defeat where observing alterations in behavior is critical. While animal behavioral assays may seem inconsequential, in reality, studying experimental models of neurobehavioral disorders is the foundation of research for future approaches and studies in the field. The trauma resulting from bullying is a problem; in order to fix the problem, we need to first understand why it is occurring. With further research and understanding, perhaps the bullying cycle can be broken.

RESULTS





DISCUSSION

Our study produced results predominantly consistent with both the established notions of social defeat in animal models and our own hypothesis. The fish exposed to the "resident-intruder" paradigm exhibited higher levels of anxiety than those of the control group with limited exception.

In the first trial, we observed that the control group fish spent an average of 18.4% of the assessed time at the top portion of the tank; in comparison, the experimental group fish spent an average of 6.4% of the assessed time at the top portion.

In the second trial, our results showed that the control group spent an average of 17.4% of the assessed time at the top portion; meanwhile, the experimental group spent an average of 13.1% of the assessed time at the top portion of the tank.

This data indicates that the fish in the experimental group exhibited a greater degree of anxiety as compared to the fish in the control group when placed in a novel tank. Since both groups were exposed to all the same elements and internal stresses, with the only variable being the "resident-intruder" paradigm subjected to the experimental group, the results suggest a direct link between stress response and social defeat in

zebrafish.

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Surveying Biology Knowledge in Pingry Students

By Keerthi Kotha'15

ABSTRACT

This research measures the levels of retention of basic biology among high school students at Pingry. Under the assumption that there would be a negative trend in retention as students got further from the course in time, a survey was distributed to all four grades of high school. The respondents were factored into the research, though the small sample size, probability of the respondents being more biology inclined, and possibility of seniors and juniors being in AP Biology were all noted. The results did not support the hypothesis by showing the seniors achieving the best results, followed by the sophomores, then freshmen, and last juniors.

INTRODUCTION

Do students actually retain the biology material they learn as freshmen at Pingry? More than ever, today's students need a strong foundation in biology as they prepare not only to enter college, but also to become members of an informed citizenry debating current scientific breakthroughs, such as stem cell research and genetically modified organisms. Therefore, in order to test whether or not students actually retain what they learned in freshman biology, a survey of basic biology knowledge was administered to the Upper School. Results were projected to reflect a negative trend in retention as high school students moved on in years. It must be noted that these results are based on the number of surveys returned and that they do not encompass the entirety of the Pingry biology curriculum; however, they do measure retention of material covered in Biology I.

RESULTS

Out of a total of 64 respondents, 16 were from Form III, 18 from Form IV, 14 from Form V and 12 from Form VI, with 4 respondents not specifying a form. Only those responses from students who answered what form they were in were factored into the results.

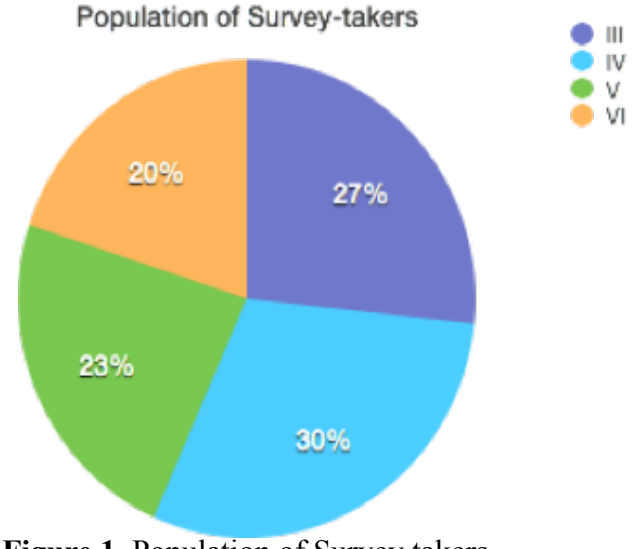

Figure 1. Population of Survey takers

The seniors achieved the highest mean score at 5, followed by the sophomores at 4.28, then the freshmen at 3.75, and lastly the juniors at 3.29 out of a total of 8 possible points.

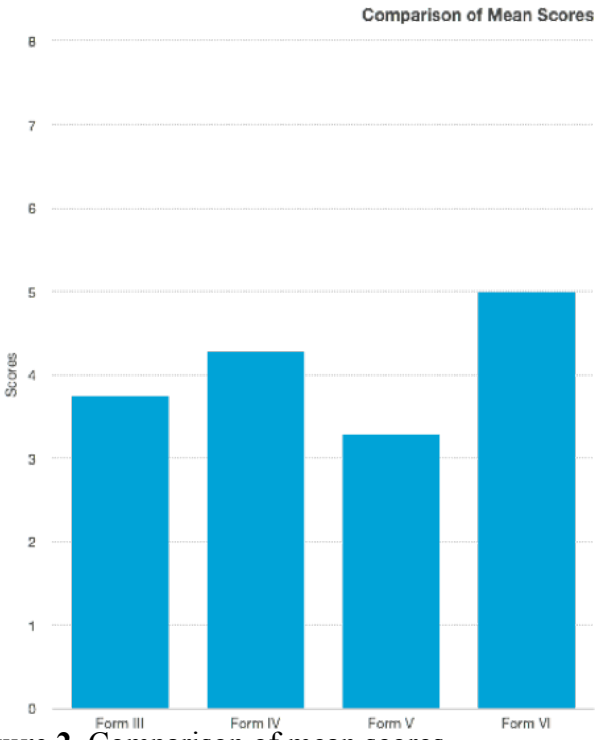

Figure 2. Comparison of mean scores

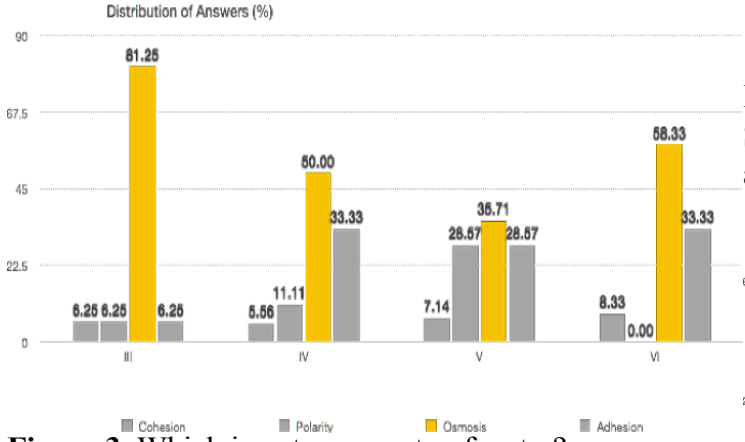

Figure 3. Which is not a property of water?
The first question garnered the most correct results with 81.25% of freshmen responding correctly, 50% of sophomores responding correctly, followed by 35.71% of juniors and 58.33% of seniors.

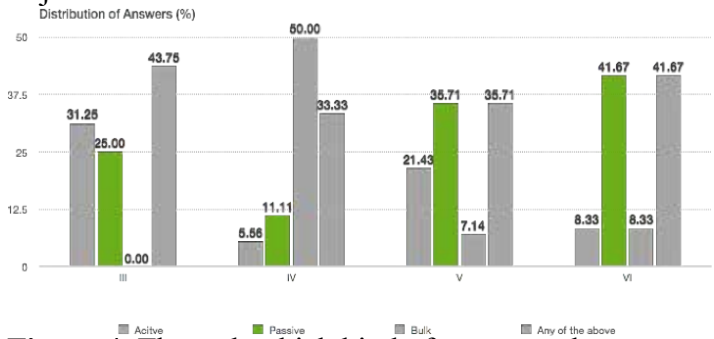

Figure 4. Through which kind of transport does a water molecule enter a cell?
The seniors performed the best on the second question with 41.87% of seniors answering correctly, then the juniors with 35.71%, next the freshmen with 25%, and then the sophomores with 11.11%.

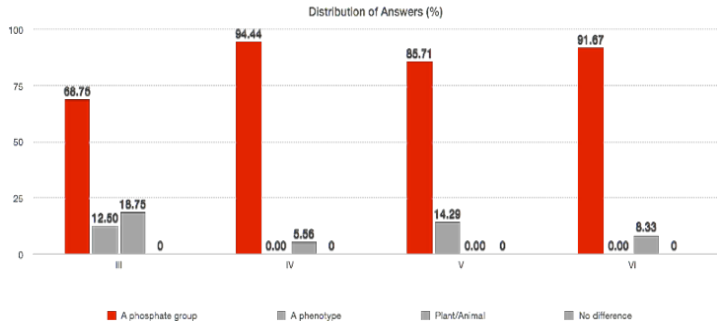

Figure 5. What is the difference between ATP and ADP?
The sophomores answered the most correctly with 94.44% of answers correct, followed by 91.87% in the senior class, 88.71% in the junior class, and 68.75% in the freshmen class.

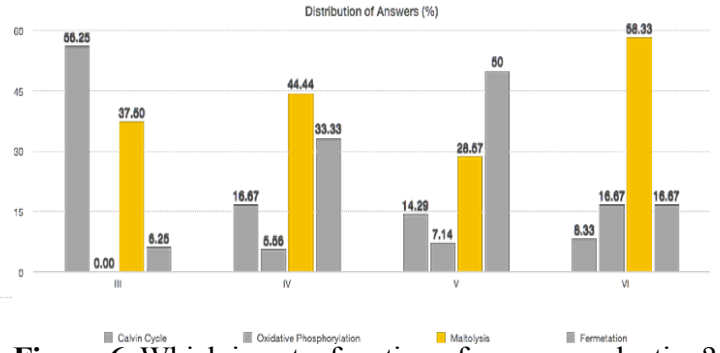

Figure 6. Which is not a function of energy production?
Seniors (58.33%) followed by sophomores (44.44%) and then freshmen (37.5%) and juniors (28.57%).

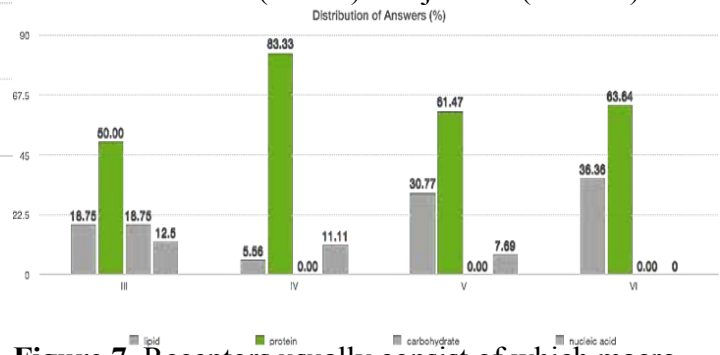

Figure 7. Receptors usually consist of which macro-moleculue?

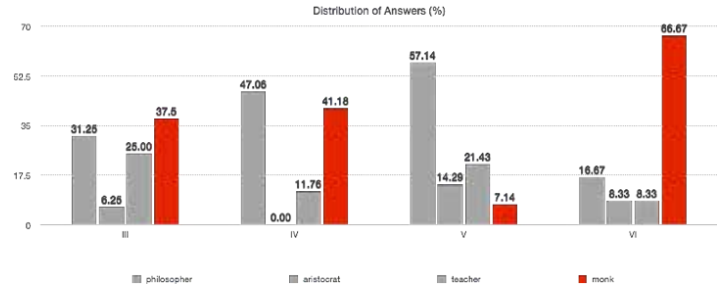

Figure 8: Gregor Mendel was a(n)

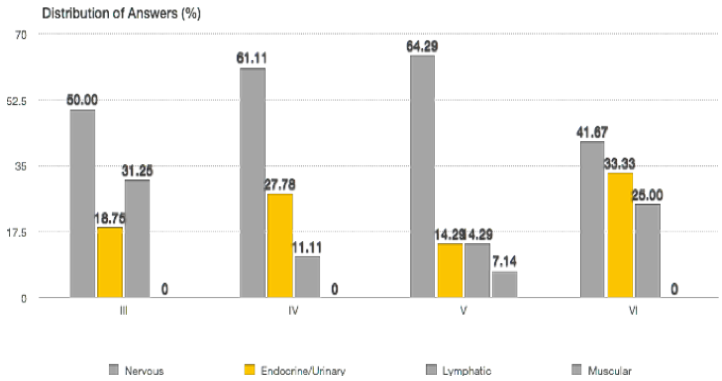

Figure 9. Nephrons are a component of which physiological system?

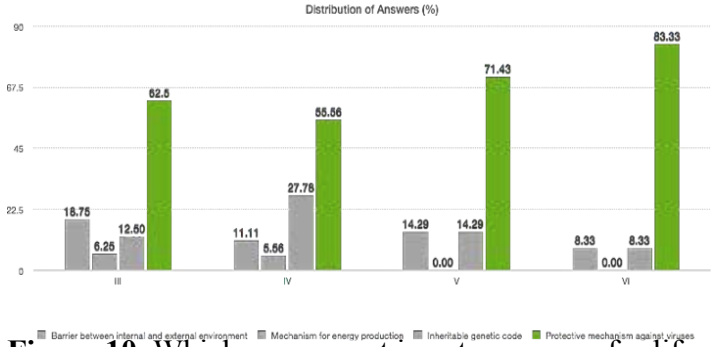

Figure 10. Which component is not necessary for life on a cellular level?

DISCUSSION

Since only a small group participated in this survey, it might be assumed that the respondents include those who are seriously interested in Biology. There are two outliers with unusually high scores who may have skewed the results in favor of the seniors, who maintained the highest mean score. Also, the small sample size does not allow for a comprehensive study of the entirety of Pingry students' biology knowledge.

Since Biology is taken for one semester during Form III and for a second semester during Form IV, the results show a higher mean score for sophomores who have completed Biology I and II than for freshmen who have only completed Biology I. As expected, retention of material drops between sophomore and junior year, since those students have not taken Biology for a whole year. At that point, however, the trend reverses, as seniors, some of whom take AP Biology, show the highest mean score of all four years on the survey.

For the first question, all grades had the highest number of correct responses. This result can be accounted for by the fact that the material is covered in the first unit taught in Biology I, not only at the beginning of the school year when both teachers and students are energized and motivated, but also at the start of a new marking period when time is not a concern. Additionally, the basic nature of the information allows for easy retention and feedback. Conversely, question number seven covers material from the last unit of the year, a unit many teachers are unable to complete. Consequently, only 18.75% of freshmen answered correctly. These results remain consistent for the following two grades, with a slightly positive increase for seniors, probably due to a number of them taking AP Biology.

For the most part, this trend, showing a higher retention rate among freshmen, remains constant, especially with questions that require less memorization and more deduction, like the last question. This trend may be attributed to the time between completing Biology I and taking the survey.

The highest mean, however, is awarded to the seniors, suggesting that biology knowledge is merely dormant during junior year until the class is taken again and the students' memory is triggered. It is nevertheless impossible to test the validity of the postulation.

In the future, perhaps teachers can use the results of this survey to design more efficient alignment of learning and assessment, to evaluate new teaching methodologies, and to assess which units need more attention over time.

Of course, the survey measures a limited population and material, but one can assume that it demonstrates a trend in terms of retention. Allowances must also be made for the seniors taking AP Biology whose knowledge would skew the results.

The next logical step in improving teaching methodology would be to expand the survey mechanism beyond biology into other sciences and eventually other subjects based on whether or not this system proved useful in the assessment of current teaching tactics.

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Purification of *Salmonella typhimurium* Transcription Factors HilC and HilD

By Kaelea Composto'15, Elizabeth Kraeutler'15, Arjun Patel'16, Jessica Day, F. John Kull, Morgan D'Ausilio

ABSTRACT

Salmonella enterica serovar Typhimurium is a bacterium that causes salmonellosis, or specifically mammalian gastroenteritis. In general, nontyphoidal salmonellosis alone kills over a hundred thousand people each year, leading scientists to more closely examine the virulence cascade of *S. enterica*. The virulence cascade of *S. enterica* includes two AraC superfamily transcription factors, HilC and HilD, which regulate the primary toxicity factors. Structural analysis carried out in the Kull Laboratory at Dartmouth College revealed the regulatory mechanism of ToxT, an AraC family member involved in the virulence cascade of *V. cholerae*. The crystal structure of ToxT, a transcriptional activator of cholera toxin production, revealed that DNA binding is inhibited by a small fatty acid. A comparison of ToxT and *S. enterica* transcription factor sequences suggests that lipid binding may also inhibit transcription of HilC and HilD. To investigate whether or not HilC and HilD can be inhibited in a manner similar to ToxT inhibition, we are working to express, purify and ultimately, solve the three dimensional structures of HilC and HilD. Solving the structures of HilC and HilD would better the understanding of transcriptional regulation in S. enterica and in turn could lead to development of novel treatments for Salmonella related illnesses.

INTRODUCTION

Our research originated from the Kull Lab at Dartmouth College, which studied the AraC superfamily protein ToxT. ToxT is a transcription factor which is known to regulate the virulence cascade of *V. cholerae*. The Kull Lab expressed, purified and crystallized ToxT using traditional methods. They collected their data through x-ray crystallography, a technique that involves blasting the crystals with x-rays and running the diffraction patterns through a computer to create an electron density map and then an atomic model to solve the structure. After solving the structure, the researchers ran experiments to determine that a certain small unsaturated fatty acid could bind to ToxT in the ligand binding pocket and prevent ToxT from binding to DNA, thus inhibiting the virulence cascade of *V. cholerae.*

Expression of the virulence cascade of *S. typhimurium*, as opposed to just the colonization of the Salmonella bacteria in the gut (often as a result of ingestion of bad food or water) is the cause of salmonellosis. Inhibition of the virulence cascade of S. typhimurium would prevent the bacteria from producing the symptoms of illness discussed earlier, rendering *S. typhimurium* relatively harmless. The transcription factor HilA is the major regulator of the Salmonella toxin and is located on Salmonella Pathogenicity Island 1 (SPI1). SPI1 is a 40 kb stretch of DNA that encodes for a needle-like mechanism that injects proteins into the cytosol of the host cells in the intestine, which is responsible for beginning the process of Salmonella toxin release. HilA is regulated by three AraC superfamily transcription factors known as HilC, HilD and RtsA and the genes that code for these proteins are also located on SPI1. (HilA, however, is not part of the AraC superfamily.) HilA in turn regulates the transcriptional activator InvF.

To inhibit HilA, there is evidence to suggest that two out of the three AraC superfamily transcription factors (HilC, HilD, RtsA) regulating HilA must be inhibited. (Even though HilD has by far the greatest influence on transcription of HilA, the pathway of infection will not work with HilD alone regulating HilA.) HilC and HilD increase expression of HilA by opposing downregulating activity. Inhibiting HilA would in turn inhibit (or at least, dramatically decrease the symptoms) of the *S. typhimurium* virulence cascade. Based on the research done by the Kull Lab on the inhibition of the transcriptional regulator ToxT by a fatty acid (in *V. cholerae*), we hypothesize that HilC and HilD could be inhibited similarly, as all three transcription factors are AraC superfamily proteins.

RESULTS

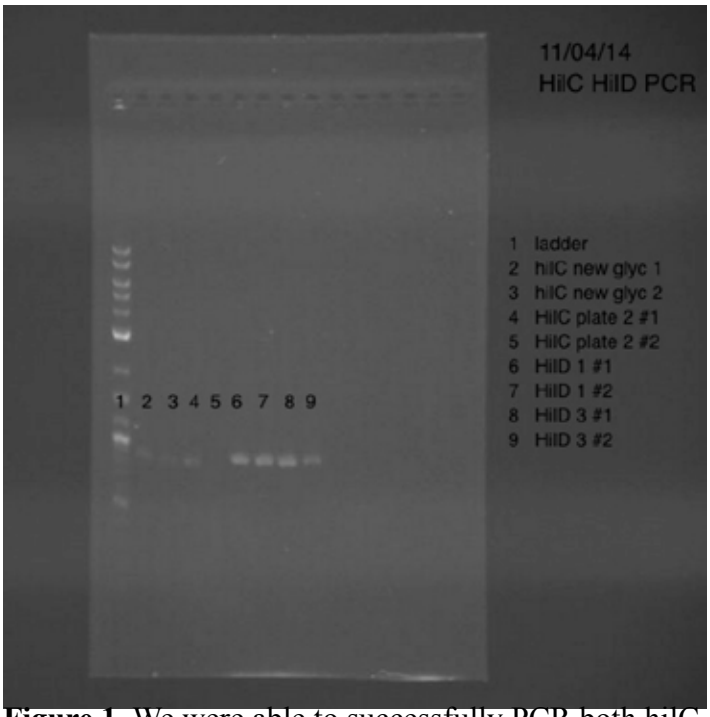

Figure 1. We were able to successfully PCR both hilC and hilD using our designed primers. We are able to see the DNA because the DNA had Sybrsafe added which allows it to be seen under an ultraviolet light. Both hilC and hilD are running at 1 kb, proving that the DNA successfully replicated our genes of interest.

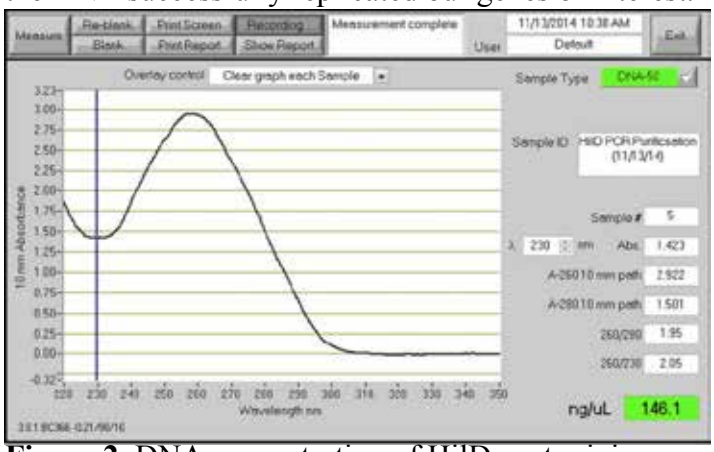

Figure 2. DNA concentration of HilD post miniprep shown on a nanodrop curve.

DISCUSSION

Unfortunately, we were unable to successfully ligate hilC and hilD respectively into pTXB1 this year. Our test digests were not cutting pTXB1 (with either hilC or hilD) at the correct lengths. From our test digests, we expected to see two bands in each well, one at roughly 6.7 kb (pTXB1) and another at roughly 1 kb (hilC or hilD). This could be due to contamination, wrong initial construct, denatured enzymes or human error. After the respective constructs of hilC and hilD are purified in pTXB1, expression trials will be run in order to find the optimal temperature and incubation time for the DNA to be transcribed and translated into protein. Once the protein is synthesized, it will be run through a protein purification column with a chitin resin because pTXB1 contains a chitin-binding domain (chosen because ToxT was purified in the same manner). After the protein is purified, it is concentrated in a conical tube with a filter until the protein is at a high enough concentration in order to crystallize, which varies from protein to protein. On a 96 well plate using the sitting drop vapor diffusion method, the protein will be combined with crystallization solutions under different crystal conditions. The crystals would be taken to a synchrotron, where they would be blasted with x-ray beams and the diffraction patterns analyzed to create an electron density map and from there, an atomic structure. From the structure, we hope to discover an inhibitory mechanism similar to the lipid inhibitory mechanism found in ToxT. Beyond that, future research could examine practical methods of creating and delivering a drug or preventative treatment from the inhibitory mechanism.

CONCLUSION

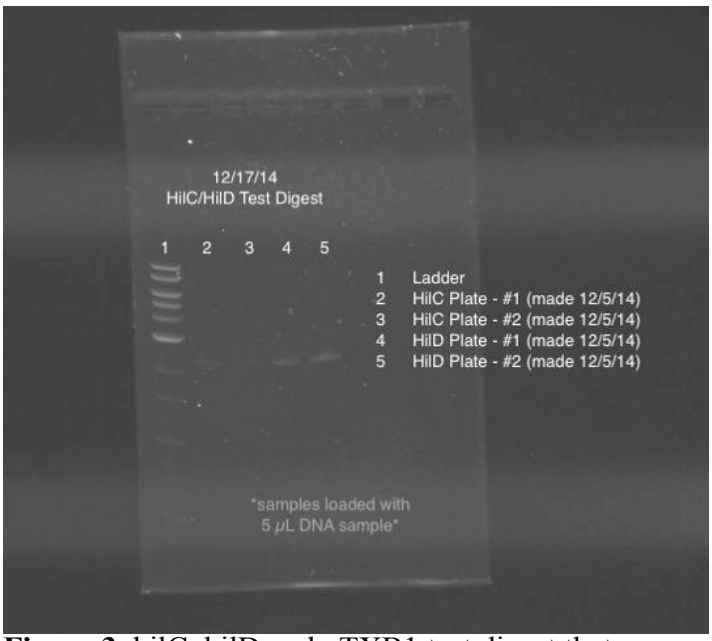

Figure 3. hilC, hilD and pTXB1 test digest that are showing only one DNA band under ultraviolet light. Ideally two bands (one the insert and the other the vector) should appear on these gels.

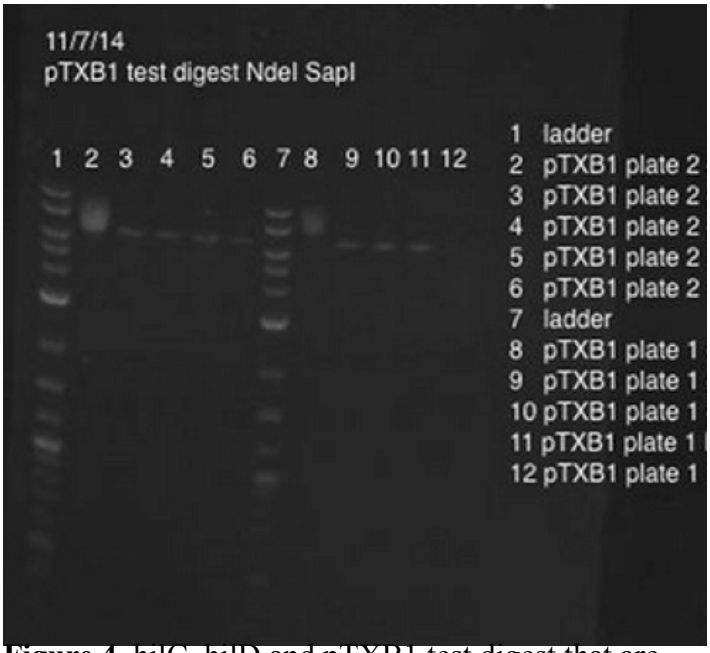

Figure 4. hilC, hilD and pTXB1 test digest that are showing only one DNA band under ultraviolet light. Ideally two bands (one the insert and the other the vector) should appear on these gels.

In conclusion, the overall goal of this project is to create a successful hilC/hilD pTXB1 construct, express, purify and crystallize HilC and HilD protein. Failed test digests, gel electrophoresis with bands at the incorrect weights, and unexpected DNA sequencing results suggest that our DNA construct for gene hilC is incorrect. Our test digests often resulted in a single band rather than two bands, meaning that the restriction enzymes did not cut the plasmid into two separate strands of DNA. The construct for hilD, while we have not yet been able to successfully ligate hilD into pTXB1, appears to be correct after examining sequencing results. In the future, in order to successfully create the hilC/hilD-pTXB1 construct, some troubleshooting strategies of note would be: varying ligation ratios, different temperature gradients for PCR and in general, sterilization and proper storage. While there have been many problems this year, the results we gathered and the changes in protocol and technique we decided to make will aid future work on the end goal of solving the crystal structures of HilC and HilD.

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Effect of Coffee Grounds on Lettuce Growth

By Ingrid Shu'16 and Sophia Cortazzo'16

ABSTRACT

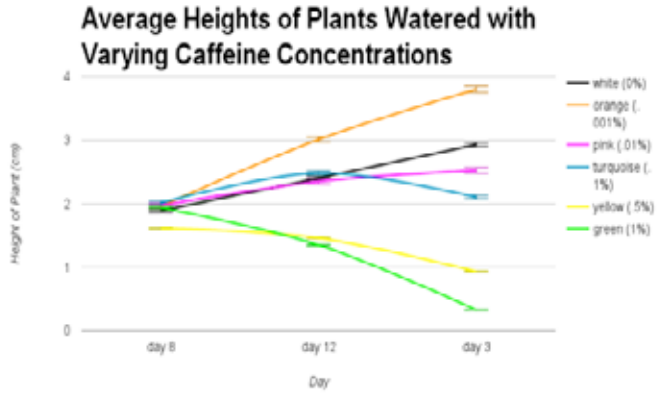
Our experiment tested for a correlation between plant growth and caffeine concentration. We germinated lettuce seeds, watered them with solutions containing different percentages of caffeine, and tracked their growth. Ultimately, the plants watered with a 0.001% caffeine solution were the only group to grow larger in height and width than the control (0% caffeine) group. Overall, our trial supports our hypothesis; the rate of lettuce plant growth will increase until caffeine concentrations become too saturated.

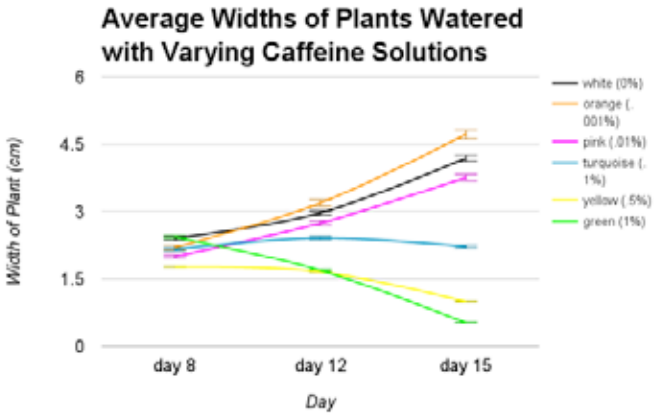
INTRODUCTION

The rate of plant growth, which is essential to a productive harvest, can partially be attributed to minerals in the soil and their interactions with plants. In their paper "Effect of Coffee Grounds on Lettuce Growth," Pingry students found a positive correlation between coffee grounds in the soil of lettuce plants and the rate of plant growth, but the increase was attributed to key nutrients such as phosphorus, potassium, and nitrogen present in coffee grounds (1). However, because 1g coffee grounds contain 3.59 - 8.04 mg of caffeine, we believe that caffeine may also have an affect on plant growth rate (2). If the presence of caffeine and plant growth rate have a positive correlation, consumers and farmers would be able to utilize caffeine as an inexpensive fertilizer.

Our objective was to test the effect of caffeine on lettuce plant growth. We tested this by watering germinated lettuce plants with solutions containing varying concentrations of caffeine. We hypothesize that with increasing caffeine concentrations, the rate of lettuce plant growth will increase until growth plateaus due to caffeine saturation.

RESULTS





The height and width of the 0%, 0.001%, 0.01%, and 0.1% caffeine concentration plants increased once watered with caffeine solutions. Plants watered with caffeine concentrations of 0.5% and 1% decreased in size and did not surpass their original heights or widths. Plants watered with 0.001% caffeine solutions were the only group that consistently had a higher growth rate than the control group plants, and in both height and width.

Significance: ANOVA, a statistical analysis, calculated a p-value of 0.0000, proving that our data was statistically significant. It was also used to calculate the standard deviation used for the error bars on the graphs.

DISCUSSION

The 0.001% caffeine concentration plants were consistently larger in height and width than the white (0%) control plants. These 0.001% plants had a higher growth rate than the control group. Based on these findings, further research can be done with concentrations between 0.01% and 0% in order to find the concentration at which plant growth rate is highest. Once found, this concentration of caffeine could be utilized by farmers and gardeners in the future to grow lettuce faster. Additional research could also be done on other commonly grown plants to see if any concentrations of caffeine increase their respective growth rates.

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