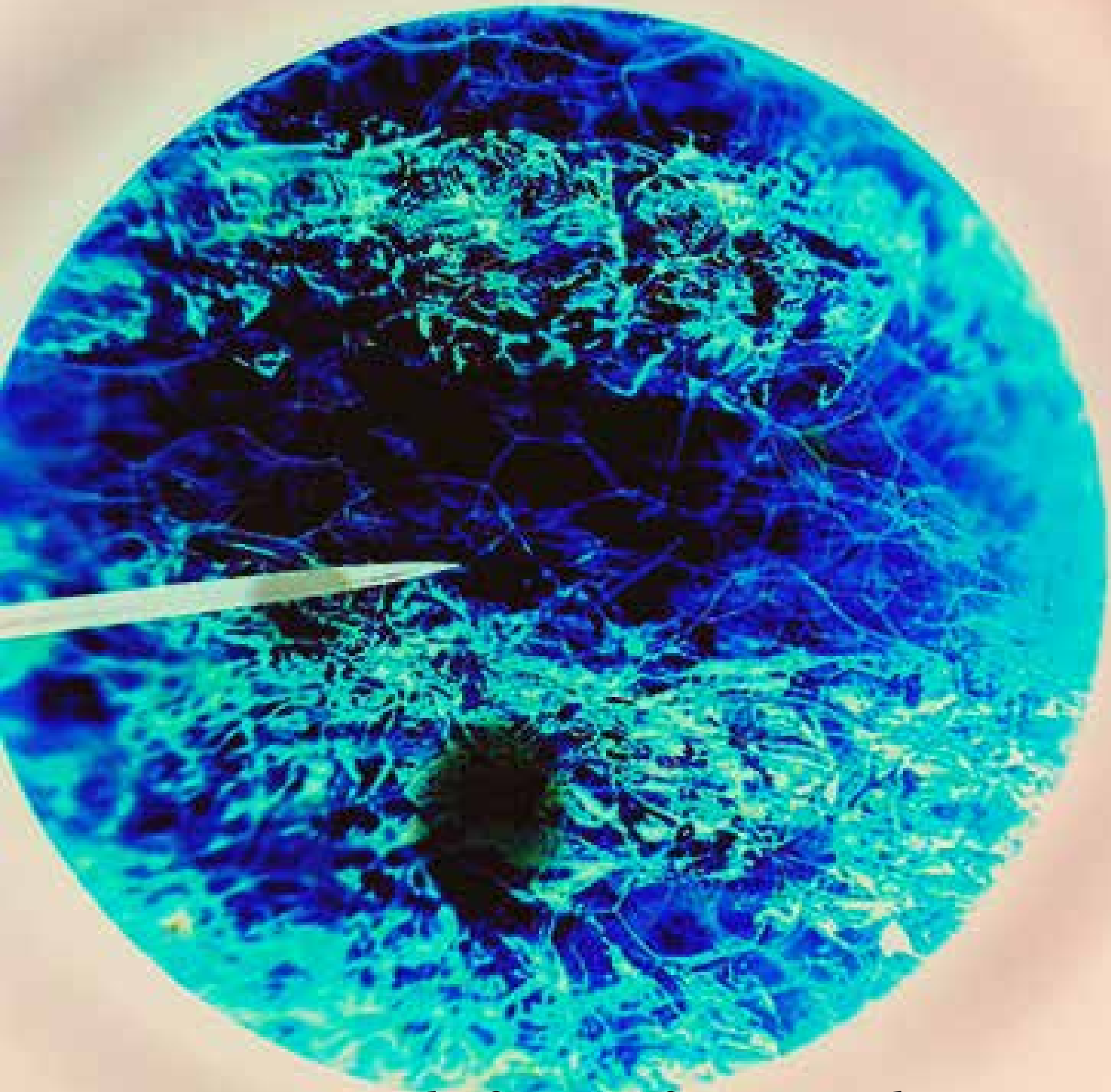
# The Pingry Community Research (PCR) Journal



A Journal of Scientific Research at The Pingry School

*Volume 2: Spring 2014*

# Contents

*Cover photo courtesy Gianna Arata'15*

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

Lucy Miao'16

# Siemens and Intel Winners

By Akash Kumar'17

The iRT, Pingry's own research team, currently has 18 members who have gone through a tough selection process to become part of the team. In order to get onto the team, students must pass a select list of criteria. Those students who actively ask questions at Journal Club are put into a pool of possible candidates. They are allowed to participate as temporary members for a semester in order to see what the iRT is like. As Mentor to Independent Molecular Biology Projects Mr. Luke De said, "If you don't enjoy it, iRT will be torture."

New members are elected to the iRT by the current members. The entry-level position is the "Scrub." Scrubs help clean equipment and watch the Lab Heads and Minions work on the project. They each have a Lab Head who they learn from. The next step in the hierarchy is the "Minion." They are functioning members of the team who are able to conduct experiments in the lab. They have goals for each week and work under the Lab Heads. The highest level is the Lab Head. The Lab Heads have their own projects that they research and work on. The current Lab Heads are Amol Kapoor'14, Avery Vella'14, Avery Hatfield'14, Edward "Teddy" Leithead'14, Derek Hong'14, Brigit Harrison'15, and Abhiram Karuppur'15.

Each research group meets once a week with Mr. De and spends time in the lab conducting experiments and completing weekly lab jobs. They are also obligated to attend Journal Club. According to Mr. De, he is very proud of the iRT members because they show how high school students can dramatically affect the scientific world. "As we get older, our minds become more closed off. As children we are much more creative."

Mr. De puts these creative minds to work in the form of the iRT. The iRT works in collaboration with many universities including Rutgers, Dartmouth, Rockefeller and hopefully Princeton in the future.

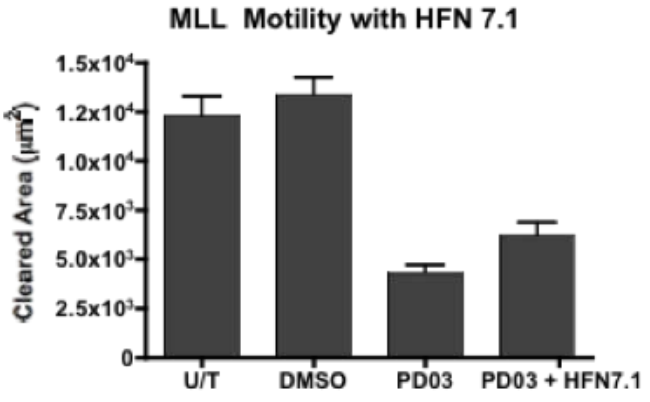
The iRT is currently working on several projects. The first, led by Harrison, is testing a fertilizer, which would mechanically digest food in a liquid form. This project is in collaboration with the University of San Diego and Scotts Fertilizer Company. Another project, led by Hatfield, is using Zebra fish to observe the effects of alcohol addiction on the human brain. The test uses stress levels to determine addiction. A project testing the effects of a particular drug against the motility prostate cancer cells is led by Karuppur in collaboration with the Robert Wood Johnson Medical Center. Another project, led by Kapoor, is testing the Wnt and Notch pathways in fruit flies, in collaboration with New York University. Finally, Leithead is experimenting with salmonella cells and creating different strains.

This year, Pingry had 3 semifinalists and 1 regional finalist in the Siemens Math and Science Competition. The semifinalists were Karuppur, Andrew Verdesca'15, and Kapoor. Peter Shim'15 was the regional finalist.

Shim conducted research along with a student from Choate Rosemary Hall on Fibonacci numbers. He built upon the work of acclaimed mathematician Paul Erdos, who attempted to find the smallest number which could not be expressed as the sum or difference between a Fibonacci and a prime number. Shim discovered a 6-digit number which fit this criterion, making it the smallest such number.

Karuppur conducted an experiment on a drug for prostate cancer at the Robert Wood Johnson Medical Center. The first experiment observed the motility of prostate cancer cells on fluorescent beads. The drug, PD03, was administered to half of the cells. The motility of the cells was quantitatively examined by measuring the average area that each group of cells cleared. When the cells moved, they would ingest the fluorescent beads and leave a black trail behind. After the cells sat on the beads for 12 hours, the areas of the dark sections were measured. The average area of the cells that were administered PD3 was significantly smaller than the area of the untreated cells. This means that PD3 was successfully able to slow the spreading of the prostate cancer cells. PD3 made the cells flatten out by increasing intracellular actin expression and stuck down, which greatly decreased the mobility. The graph below shows the results of the experiment, with PD3 being the drug that decreased mobility the most. This experiment was done in collaboration with Dr. Ramsey Foty at Robert Wood Johnson Medical Center. One problem they faced was that when the fibronectin (the protein that the cells attached to) inhibitor, HFN 7.1, was applied to these cells, they did

not regain the original levels of motility. Only 50% of the motility was recovered. The current task is to find out how to revive the other 50%. Karuppur currently works with Verdesca and Jackson Hoit'16 in order to continue the experiments.




Kapoor also took part in the Intel Competition and is Pingry's first repeat Siemens semifinalist and Intel finalist. His work was on the Notch pathway and Wnt pathway in fruit flies. The Notch pathway controls the creation of several types of cells in the flies' bodies and is also involved in intercellular communication processes. Any mutations in the Notch pathway could result in death, so it was necessary to be extremely careful about the modifications. Many diseases, including cancer, can come from mutations in this pathway. In order to test the results of mutations, Kapoor tried to delete the CG3634 gene. He could see the effects of this deletion in the bristles on the fly. These feelers are part of the nervous system and abnormalities in them show the effects of the gene deletion. In the Wnt experiment, the effects of miRNA 310/13 were tested. These changes were shown through the wing disks of the flies instead of the bristles. Both experiments were conducted with the aid of Professor Ramanuj Dasgupta from New York University's Langone Medical Center. Kapoor conducts the research on the iRT with Sharanya Pulapura'15 and Gaurav Gupta'15. According to Kapoor, the biggest challenge was coming up with a good recipe for fly food. As these flies are accustomed to the laboratory, they are quite sensitive. Many of the earlier versions of the food just killed the flies.

Overall, the students in iRT, Siemens and Intel Competitions are working extremely hard to make a difference in the scientific community. Their research extends from slowing cancer to discovering addiction tests. We should appreciate their work on these topics and the aid they are giving to our society.

## How Naked Mole Rats May Help the War on Cancer

By Brad Hong'16

Naked mole rats have both high life expectancies and a marked resistance to cancer. These rats were the subject of Andrew Verdesca's (V) Journal Club presentation. Published in Nature, the article "High-molecular-mass hyaluronan mediates the cancer resistance of the naked mole rat" identifies the mechanism that provides naked mole rats with cancer resistance. Hyaluronan, known as the "goo" molecule, is found in the connective and epithelial tissues of the body. The connective tissues of the naked mole rat secrete an unusually high amount of hyaluronan, at least five times the amount possessed by humans. Due to a decreased amount of hyaluronan-degrading enzymes and other factors, there is a high accumulation of the molecule in naked mole rat tissues. Scientists compared the effect of activating a pathway, which in mouse fibroblast cells, triggered tumor formation. When hyaluronan was decreased either by overexpressing the degrading enzyme, HYAL2, or by eliminating hyaluronan synthase (the enzyme which produces hyaluronan), the naked mole rat cells became susceptible to malignant transformation and tumor formation. However, unaltered cells, which retained high hyaluronan, were not affected. These in vitro findings assert that the effects of highly-accumulated hyaluronan discourage cancerous growth. By identifying the properties of hyaluronan, this research bears new intelligence on the forefront of cancer, contributing to our understanding of both ourselves and to the disease that comes from within.

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# The Role of Alcohol Dosage on HPA Axis Functionality

By Jackson Artis'16, Julia Friend'15, Avery Hatfield'14, and Mr. Luke De


## ABSTRACT

Previous research has shown a connection between alcohol intake and the function of the neuroendocrine stress system, specifically the hypothalamic-pituitary-adrenal (HPA) axis. Studies indicate that acute alcohol self-administration results in up-regulated activation of the HPA axis, and chronic alcohol exposure, indicating dependence, reduces HPA axis functionality in mice. However, the effect of alcohol dosage on HPA axis functionality remains unknown. The experiment uses zebrafish as a model organism to study the dose-dependent effects of alcohol on HPA axis functionality in both acute alcohol administration and chronic alcohol exposure.


## INTRODUCTION

Alcoholism is chronic neurological disease linked with symptoms such as excessive intake of alcohol, loss of control over consumption, and a negative emotional state during withdrawal. Positive reinforcement is one of the behavioral forces that drives the continued intake of alcohol. However, negative reinforcement also plays a role. It manifests as a decline in reward system activation, as well as recruitment of the stress system in the brain. This loss of basal reward function and the desire to escape the negative emotional state drives powerful behavior, the continued use of alcohol, despite severe negative consequences. (4)

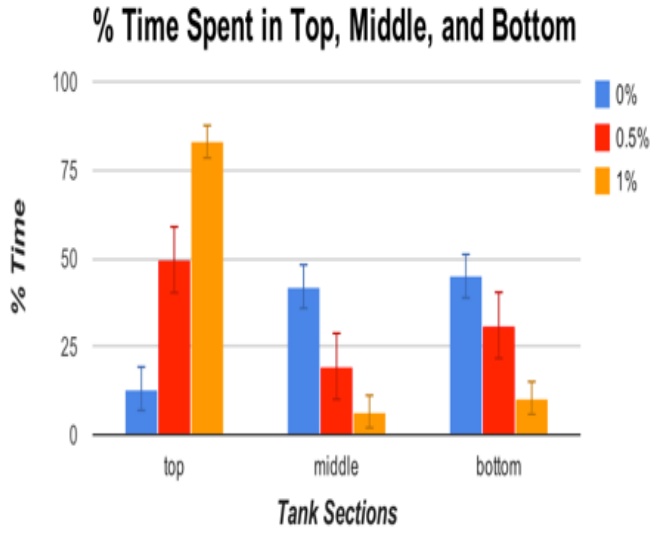
An important element of the stress system is the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis is a pathway that produces glucocorticoid hormones (cortisol) which travel throughout the body to maintain homeostasis in the body. (5) This process begins in the paraventricular nucleus (PVN) of the hypothalamus, which produces corticotropin-releasing-factor (CRF). In response to CRF, the anterior pituitary produces adrenocorticotropic hormone (ACTH), which then binds to the MC2-R receptor on the adrenal cortex resulting in the production of the stress hormone cortisol. (7) Negative reinforcement is characterized by a loss of reward function and a strong negative emotional state, and is therefore associated with the stress system, including the HPA axis. Since negative reinforcement is an important hallmark of addiction, the HPA axis plays a critical role in the transition to alcohol addiction. While acute alcohol consumption has been shown to temporarily increase HPA axis function, addicts experience a loss of function in the HPA axis, upsetting homeostasis and resulting in a negative emotional state. However, while the effect of alcohol and alcohol addiction on the HPA axis has been studied, the effect of alcohol dosage both acutely and chronically in addicts remains unknown.

Zebrafish (Danio rerio) are a tropical freshwater fish breed that are often found in laboratories. This organism is often used in stress research because their physiology includes the hypothalamic-pituitary-interrenal (HPI) axis, which is homologous with the HPA axis in humans. (2) This similarity makes zebrafish an ideal model organism for better understanding the HPA axis in humans. In this experiment we used zebrafish as a model organism to study the effects alcohol dosage on HPA axis functionality. This paper studies the effect of different dosages of acute alcohol concentration on the behavior and cortisol levels of zebrafish. Since previous research has shown that the HPA axis is up-regulated during acute alcohol (6) use, we hypothesize that increased alcohol concentration will directly affect the cortisol levels in the zebrafish. Research has also shown that when zebrafish are stressed and anxious they are prone to spend most of their time at the bottom of the tank. Conversely, when they are less stressed they spend more of their time towards the top of the tank (3). We also believe that the fish in the higher alcohol concentrations will spend less time at the bottom of the tank and more time at the top of the tank.

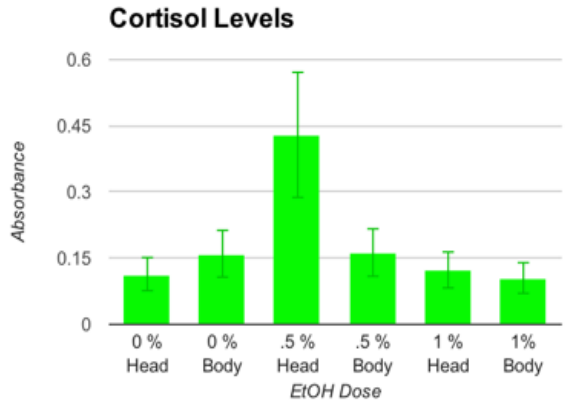
## RESULTS

We confirmed that the group with the least amount of alcohol (0%) spent the least amount of time in the top section of the tank. As the alcohol dosage increased, the amount of time that the fish spent in the

top section of the tank increased. The 0% group spent 13% of the minute at the top, the 0.5% group spent 50% of the minute at the top, and the 1% group spent 83% of the minute at the top. The inverse is true for the middle and bottom sections of the tank. There was a statistically significant increase in the time spent in the top of the tank between each of the groups.




The cortisol levels of the different groups showed no distinctive pattern. The 0.5% head group had significantly higher absorbance and therefore lower cortisol levels than all of the other groups. There was no statistical significance between any of the other groups. This is likely due to experimental error.




## CONCLUSIONS

From the behavioral results we can conclude that since the higher dosage groups spend more time at top of the tank, they are experiencing less stress and anxiety, in agreement with our hypothesis. This signifies that higher alcohol levels decrease stress more than lower alcohol levels. From the data we can also predict that the functionality of the HPA axis is up-regulated with higher alcohol levels because the HPA axis produces cortisol which regulates stress in the body. We hypothesized that the cortisol levels would also be higher in the higher alcohol groups in response to an up-regulated HPA axis. However, the results of the cortisol experiment are inconclusive. While we did achieve results there is no obvious pattern. The only significance is that the 0.5% alcohol head group had significantly lower cortisol levels than all of the other groups. We believe that the reason for inconclusive results is experimental error. Following the experiment we were able to analyze our procedure and recognize the mistakes we need to fix when we repeat the experiment.

While this experiment has helped us understand dose-dependent effects of acute alcohol on the HPA axis functionality, acute and chronic alcohol administration are fundamentally different. We know the effect of acute alcohol dosage on HPA axis function, but we don't know what the effect of chronic alcohol exposure will be. We plan to repeat the experiment, but instead expose the zebrafish to alcohol in a binge-like pattern over a two week period. The purpose of the binge model is to mimic human consumption patterns that are shown to progress into addiction. We also plan on repeating the experiment during acute withdrawal and chronic withdrawal from alcohol to study the functionality of the HPA axis during the withdrawal period.

In order to grow in large quantities, it is necessary to determine under what conditions algae grow, as measured by the amount of $O_2$ released from the best algae sample. Although there are many factors we could test, such as temperature or humidity, we will be testing under which light algae grows best: white light, UV light, or no light. We will collect algae samples from the Pingry pond by the tennis courts and subject them to the three different kinds of light, then measure the amount of oxygen emitted. We hypothesize that the samples receiving no light will produce the least oxygen, the samples receiving UV light will produce the most oxygen, and the samples receiving white light will produce an amount of oxygen less than that of the UV samples but more than that of the no light samples.

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## ACKNOWLEDGEMENTS

We would like to thank the Pingry School, David Maxwell, Morgan D'Ausilio, Chuck Coe, Nat Conard, Irene Morganstern, Nate McKenney, Adedeji Afolalu, and Mary Jane Kreek.

# The Effects of Sleep Deprivation on Anxiety in *Danio rerio*

By Stacy Chen'14, Kathleen Murray'15, and Allison Yu'14

## ABSTRACT

Anxiety is the body's natural response to danger, an automatic alarm that goes off when an individual is feeling threatened, under pressure, or facing a stressful situation. Unfortunately, anxiety is becoming a growing problem throughout the world that interferes with relationships and activities; especially since sleep deprivation is regarded as one of the leading causes of anxiety. Around 50 to 70 million American adults suffer from sleep and wakefulness disorders. The correlation between anxiety and sleep deprivation is still a topic that is being researched today. Using zebrafish (*Danio rerio)*, who have similar circadian rhythms ("body clocks") and are vertebrates like humans, we studied the effects of sleep deprivation on anxiety in zebrafish. The purpose of this experiment was to determine whether or not sleep deprivation has an effect on anxiety. Both humans and zebrafish produce a glucocorticoid known as cortisol that is released from the adrenal cortex in response to stress. Anxiety levels, therefore, can be measured in the test subjects based on the amount of cortisol in much the same way that human cortisol levels can be measured through saliva. We determined that there is no correlation between sleep deprivation and anxiety in our experiment.

## INTRODUCTION

Sleep deprivation is one of the numerous causes of anxiety, often causing anxious thoughts and feelings. This lack of sleep may cause or be caused by anxiety. Zebrafish, like all other organisms, have a "body clock" (known as the circadian rhythm). In humans, the disruption of circadian rhythm has been directly correlated with psychological and mental disorders, including anxiety and depression. This experiment tried to determine if sleep deprivation (using lights to induce insomnia and change the circadian rhythm) stimulates anxiety in Danio rerio (zebrafish).

We determined that zebrafish were the optimal test subjects because sleep in zebrafish can be suppressed by light even if they are already sleep deprived. Zebrafish also have circadian rhythms similar to humans. When zebrafish sleep, they are immobile for at least six seconds, usually at the bottom or the surface of the tank. A mild external stimulus (in this case, fluorescent light) does not have as great an effect on the sleep cycle. These indicators make it easier to observe when the fish are in a sleep-like state. In addition, zebrafish that are exposed to constant light will sleep normally when returned to darkness. This lack of a rebound effect to compensate for the severe sleep deprivation makes these fish better test subjects

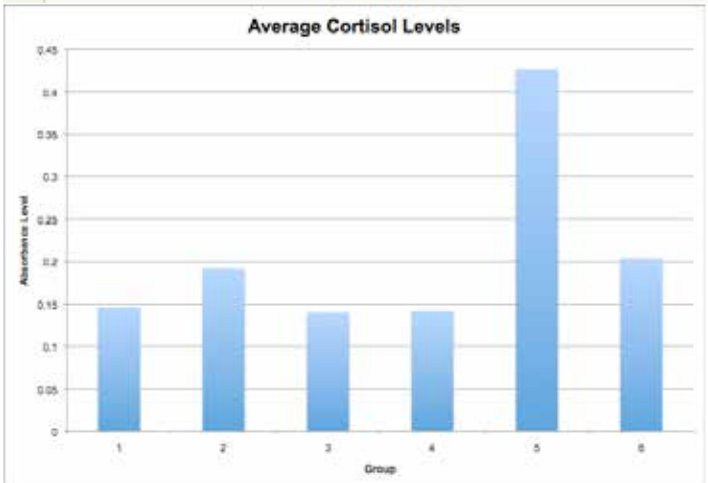
than mammals because they can be easily manipulated without re-percussions. In addition, the zebrafish shares a simi-larly structured central nervous system with mammals. Both humans and zebrafish produce a glucocorticoid known as cortisol (or more formally, hydrocortisone) that is released from the adrenal cortex in response to stress. Anxiety levels, therefore, can be measured in the test subjects based on the amount of cortisol in much the same way that human cortisol levels can be measured through saliva. These parallels allowed us to apply our experiment to humans as well.

## RESULTS

The table below shows the absorbance level measured by the spectrometer using the ELISA kit, which shows the level of cortisol in each test subject. The graph shows the averages for each group (16 hours, 20 hours, and 24 hours) for the 3-week and 6-week period.

The empty slots in the table are due to technical issues while working with the ELISA kit, so we omitted the data for the #6 in the 16 hours/3 weeks and #6 in the 24 hours/6 weeks.

| | 16 hours (3 weeks) | 16 hours (6 weeks) | 20 hours (3 weeks) | 20 hours (6 weeks) | 24 hours (3 weeks) | 24 hours (6 weeks) |
|---|---|---|---|---|---|---|
| #1 | 0.20 | 0.22 | 0.21 | 0.22 | 0.17 | 0.23 |
| #2 | 0.14 | 0.14 | 0.19 | 0.16 | 0.12 | 0.14 |
| #3 | 0.11 | 0.30 | 0.20 | 0.11 | 0.10 | 0.19 |
| #4 | 0.19 | 0.11 | 0.06 | 0.09 | 1.89 | 0.09 |
| #5 | 0.09 | 0.13 | 0.08 | 0.09 | 0.19 | 0.37 |
| #6 | | 0.25 | 0.10 | 0.18 | 0.09 | |




## CONCLUSION

Firstly, we made sure to take the proper steps to ensure humane and ethical treatments of the zebrafish. We used an ice water bath to euthanize the zebrafish, since they are temperature sensitive small tropical fish. We only added a maximum of six fish into an ice bath in order to keep the temperature under 4°C. To check that the fish were indeed deceased, we pinched their tails and checked for any movement; there was no movement in any of the euthanized fish. Decapitation was also another procedure we used in order to ensure death. After running an ANOVA test, the results that our experiment yielded were inconclusive because the overall p-value was 0.592, which was much larger than the acceptable 0.1. In other words, there is no correlation between sleep deprivation and anxiety. This is perhaps because as we were scooping some fish from the tank, they experienced a greater spike in stress than some of their counterparts, which would have produced false cortisol levels not caused by sleep deprivation. And also, there was no guarantee that each fish would react in the same way to its environment; but to counter this variation, we used six fish (greater than three) to get an average. Another possi-ble point of error could have been during the usage of the ELISA kit where minor technicalities such as pipette measurements and time sensitive enzymes could have affected the results. Finally, there is also a possibility that some of the light fixtures were not as constant as we had hoped due to the short life span of the light bulbs.

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# Soil Variations to Maximize *Arabidopsis thaliana* Yield

By Erica Cheung'14, Tammy Gu'14, and Rabia Khan'14

## ABSTRACT

*Arabidopsis thaliana* is a model plant organism used in a variety of experiments due to its quick growth rate and highly successful rate in being genetically modified. Its growth is dependent on a number of factors, from soil type to light exposure to the temperature and humidity of the surrounding environment. Our focus was to determine the best kind of soil to maximize *Arabidopsis thaliana* seed germination and subsequent plant growth. We found that a 1:1:1 combination of sphagnum moss, vermiculite, and perlite produced the greatest yield. This is beneficial for scientific research involving *Arabidopsis thaliana* transformation, as more plants mean more material to work with for future endeavors.

## INTRODUCTION

*Arabidopsis thaliana* is a plant commonly used as a model organism due to its accessibility, its short germination period, and its efficient transformation potential (its capability to being genetically modified) using *Agrobacterium tumefaciens*. Additionally, the majority of the plant's genome has been sequenced. As a result, there are a variety of mutant lines available. All of these factors make *Arabidopsis thaliana* an ideal plant for research (1). Because of its usefulness in the world of research, there are currently a number of growth protocols focused on maximizing *Arabidopsis* growth (2). Like all plants, *Arabidopsis* growth is sensitive to a number of factors: soil type, light exposure, temperature, and humidity.

Our objective is to grow *Arabidopsis* (shown above in Fig. 1 & 2) in varying types of soil in order to figure out which soil medium produces the most *Arabidopsis* growth. Our control for this experiment will be the Miracle-Gro Seed Starting Potting Mix, which lacks vermiculite -- a mineral often used to assist in root growth and the retention of air and moisture (3). The experimental groups will consist of different ratios of vermiculite and perlite – a mineral used for aeration and better retention of moisture (4), moss, and the aforementioned potting soil mix. We will be observing how the presence of vermiculite and perlite in the soil helps maximize Arabidopsis growth.

## RESULTS

Soil 1 (1:1:1 ratio of sphagnum moss, vermiculite, and perlite) produced the greatest plant yield.


**Fig. 3.** Observed sprout growth. Soils 1, 2, and 3 are labeled. Soil 1 visibly has more growth than 2, while soil 3 has less than the other two.

Soil 2 (4:3:2 ratio of potting soil, vermiculite, and perlite) produced less, while soil 3 (potting soil) produced the least (Fig. 3, Table 1).

We calculated our p value to be 0.004622. As this value is less than 0.05, this means that our data is statistically significant.

## DISCUSSION

*Arabidopsis thaliana* experienced the most growth in soil 1 and the least in soil 3, with 132 total sprouts compared to 13. Soil 2, with a total of 60 sprouts, was in the middle. With a p value of 0.004622 (a value less than 0.05), we have found our data to be statistically significant.

The distinguishing feature between soils 1 and 2 vs. soil 3 was the presence of vermiculite: soils

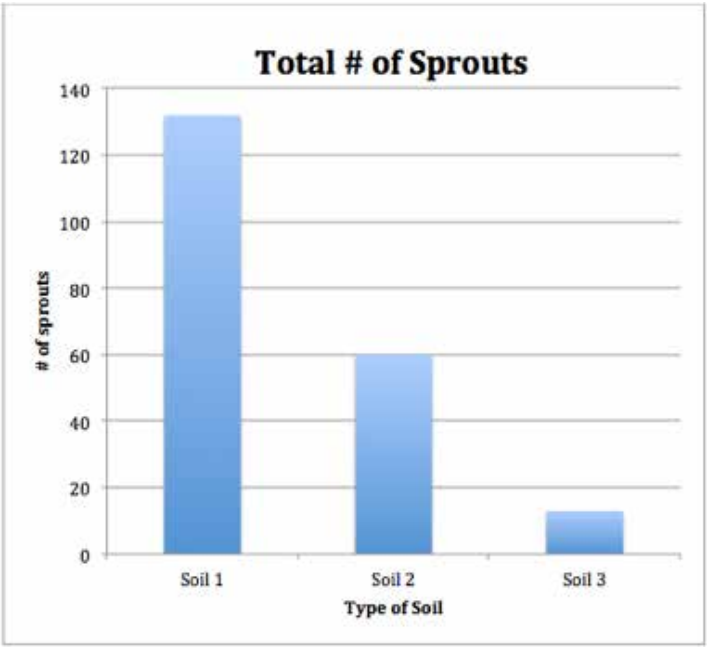


**Table 1 – Total number of sprouts per type of soil.** Soil 1 produced the most, whereas 3 produced the least.

1 and 2 had it, while soil 3 did not. Vermiculite is a mineral that is often used in horticulture to maximize plant growth. Horticultural vermiculite is absorbent, allowing soil to retain maximum air, plant food and moisture. A mixture of vermiculite in a growth medium acts as a fertilizer and also provides growing roots with some anchorage. As soils 1 and 2 produced significantly more growth than did soil 3, we have come to the conclusion that vermiculite is a crucial soil component in *Arabidopsis thaliana* growth.

Additionally, all three soils contained perlite in varying concentrations. Perlite, also a mineral, acts very similarly to vermiculite. An addition of horticultural perlite to a growth medium aerates the medium and keeps it moist. Soil 1, with its 1:1:1 ratio of components, sprouted the most plants. We have come to the conclusion that perlite is best for *Arabidopsis thaliana* growth when it is present in equal amounts to other soil components.

In the end, we have concluded that soil 1, containing equal amounts of sphagnum moss, vermiculite, and perlite, is the most effective soil out of our samples. By completing this experiment, we hoped to have successfully fine-tuned the protocol for growing *Arabidopsis thaliana* in a home environment. We also hoped to have established a standard soil type for future research groups to use.

Although our experiment focused largely on the germination of *Arabidopsis* rather than the adult stages of the plant, finding the most effective soil medium for encouraging *Arabidopsis* growth provides an essential basis for further growth. The growth of *Arabidopsis* thaliana is just the beginning of a slew of incredible opportunities, and we hope that plant transformation and genetic modification are not too far down the road.

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(7) Image: <http://www.carolina.com/plant-genetics/arabidopsis-columbia-col-0-seed/177600.pr>
(8) Image: <https://www.kuleuven-kulak.be/kulakbiocampus/lage%20planten/Arabidopsis%20thaliana%20-%20Zandraket/Arabidopsis_thaliana-zandraket02.jpg>
(9) p value calculated using ANOVA: <http://vassarstats.net/anova1u.html>

## ACKNOWLEDGEMENTS

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# Melanin Protects *Sordaria fimicola* Spores From Ultraviolet Light

By Katherine Curran'14


## ABSTRACT

The purpose of the experiment was to determine whether increased melanin levels and skin pigmentation lessen the chance of developing skin cancer. A UV light box was used to simulate the effects of the sun on the fungus *Sordaria fimicola*. Two types of the fungus were used, wild type to represent more melanin and tan type to represent less melanin. The results of this lab showed that when exposed to the UV light, tan type *Sordaria fimicola* died more rapidly, supporting the theory that more melanin in the skin confers protection against the effects of ultraviolet radiation.


## INTRODUCTION

Skin cancer is the most common form of cancer in the United States, with more than 3.5 million skin cancers in over 2 million people diagnosed annually. If research proves that there is a biological advantage in having more epidermal melanin, then in the future scientists can use this knowledge to help people with lighter skin achieve this advantage as well.

Based on previous scientific research, it is accepted that the degree of melanin in skin, or skin pigmentation, and UV light radiation are implicated in skin carcinogenesis, or the development of skin cancer. UV light causes immediate pigment darkening through the oxidation of melanin in the melanocytes. More simply, when skin cells are exposed to UV light, melanin production is activated, and skin cells become colored as they are drawn to the epidermal, or first layer of the skin. Therefore, the more melanin in the skin, the darker the skin pigmentation. Consequently, scientists are still unsure whether the amount of epidermal pigment melanin has a direct relationship with the development of DNA damage and skin carcinogenesis.

In this experiment, I attempted to provide evidence showing that since epidermal pigment melanin absorbs UV rays, the amount of protection from UV-induced DNA damage that causes skin cancer is directly correlated with the presence of melanin in the skin. I used two forms of the fungus *Sordaria fimicola* to grow and mate spores and then exposed the two types to various intervals of UV light.

The tan type of *Sordaria fimicola* was used as a vehicle to represent skin with less melanin and each type was exposed to UV light for various time intervals. Spore counts were used to assess what type can live longer in the presence of UV light

A second hypothesis explored was that after a certain amount of exposure to UV light, both tan mutant type and wild type are affected or damaged equally. Another hypothesis tested was that if the longest-surviving examples of both the wild type and tan type were selected for proliferation, this would result in an adaptation of the fungus to withstand even longer exposure to UV light radiation.

## RESULTS

Results showed that tan type produce more colonies in the 100uml solution than wild type. But when exposed to UV light, tan also lost colonies more quickly than wild type, as seen in the graph.

Results also showed that at 5 minutes of exposure to UV light, the tan type of *Sordaria fimicola* lost the most colonies. Wild type lost the greatest amount of colonies at 20 minutes. These results demonstrate that wild type *Sordaria fimicola* can endure longer exposure to UV light than tan.

Results for the second part of the experiment, re-growing spores or creating an F1 generation, were inconclusive. While trying to re-grow the wild type *Sordaria fimicola,* contamination occurred in all 3 tries.

## DISCUSSION

These results confirm my first hypothesis that tan type and wild type *Sodaria fimicola* are analogous to humans with more melanin, as they are less likely to suffer DNA damage from UV exposure. As seen in the graph, the tan type of Sordaria fimicola died much more rapidly after UV exposure as compared to the wild type. The death of these colonies is comparable to DNA damage in humans. Having more melanin is advantageous and protects the epidermal layer of skin, preventing DNA damage from occurring.

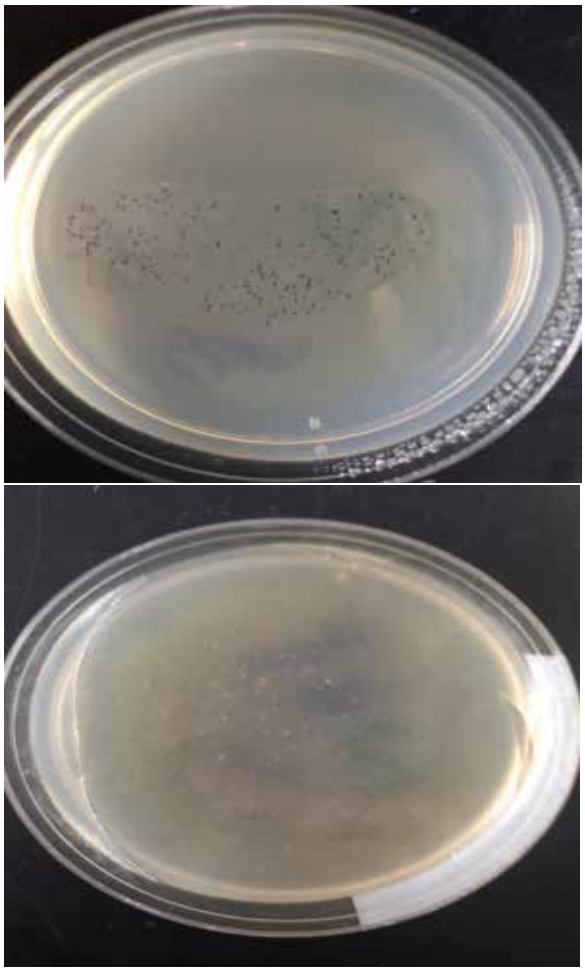

**Figure 1: Tan type before (above) and after sun exposure (below)**

The results also showed that as time of exposure increased for wild type Sordaria fimicola, the amount of colonies lost generally increased. Tan type Sordaria fimicola, on the other hand, seemed to plateau in colonies lost after hitting a peak after 5 minutes. This partially supports the hypothesis that after a certain amount of UV exposure, DNA damage in both the wild type and tan type is essentially equal. In other words, people will both light skin and dark skin (less melanin and more melanin) are equally at risk for DNA damage after an extended period of UV exposure. In a new experiment, I would like to further explore the hypothesis that if both tan and wild type are exposed to UV long enough (past the 20 minutes tested in this experiment), they will lose the same amount of colonies. This would prove that after a certain amount of exposure to UV light and DNA damage, there is no biological advantage for humans to have more melanin in their skin. Another experiment I would like to test is continuous short period of UV exposure to study whether or not a biological advantage still exists for constant exposure.

The second part of this experiment was not

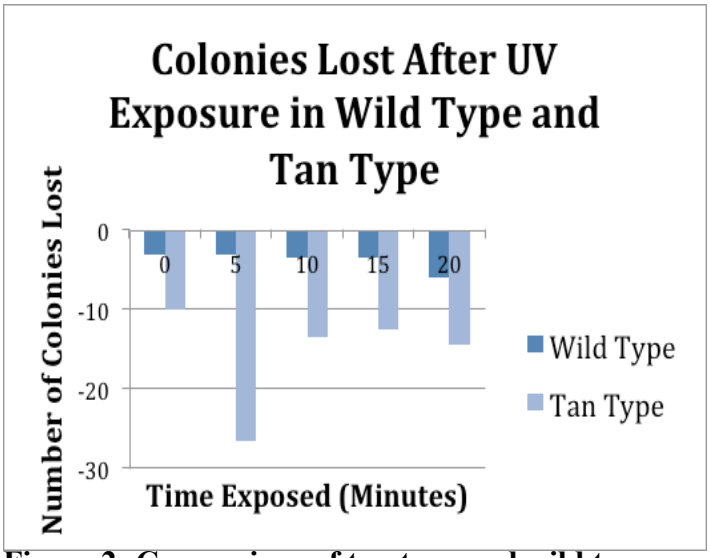


**Figure 2: Comparison of tan type and wild type colonies lost over time.**

completed for results to be reported because the wild type agar plate was subject to contamination. Research is still being conducted. However, new agar plates with both wild type and tan type were exposed to 20 minutes of UV light, stored for 24 hours, and then transferred to a crossing agar plate to grow. Since the wild type would not re-grow on the crossing agar plate all 3 times this was tried, this may mean that there is DNA damage from the UV exposure in the F1 generation of wild type, but not in the F1 generation of tan type. The F1 generation of tan type re-grows, though it re-grows considerably less spores than the parental generation. To summarize, the offspring of UV-exposed wild type may be more seriously damaged than that of tan type. In a new experiment, new tubes of fungus should be drawn from and new plates made to completely rule out contamination as the cause of wild type's failure to re-grow.

Hopefully, these results and results from my next experiments can help scientists in the fight against skin cancer. My research helps to confirm that more melanin is a biological advantage, but only to an extent. As my graphs show, there may be a point, after a certain amount of UV exposure, where there essentially is no biological advantage and damage from UV exposure impacts both pale and tan humans alike. These results can helps scientists determine whether or not melanin can be used to help prevent the onset of skin cancer, or whether adjusting melanin levels would be useless in preventing the disease.

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Special thanks to Mr. Maxwell.

# The Effects of Various Iron Concentrations on Algal Growth

By Rachel Davis'14 and Melanie Naratil'14

## ABSTRACT

Global climate change is a major issue in today's world that results in melting ice caps, changing habitats, extinct organisms, rising sea levels since glaciers are melting, and ocean acidification. Algae absorbs carbon dioxide (CO2), a greenhouse gas, and releases oxygen (O2). Absorbing carbon dioxide is essential to slowing down global climate change. The purpose of our experiment is to figure out how different amounts of iron affect the rate of algae growth and to observe the amount of time the effects last for. 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

We are interested in observing the effects of different iron concentrations on the growth of algae because of the effects of global climate change. The world is heating up and as a result, ice caps are melting, habitats are changing and being lost, organisms are becoming extinct, and the sea level is rising as glaciers are melting. The cause of global climate destabilization is the rise of greenhouse gases (which absorb infrared radiation) in the atmosphere. The earth cools by giving off infrared radiation. Ocean acidification, for example, results from rising CO2 in the atmosphere. In recent years, the pH of the ocean has declined from a pH of about 8.3 to a pH of around 8.1. As pH decreases, carbonate becomes less available, because hydrogen atoms bond with carbonate to produce bicarbonate. Carbonate is important to organisms because they use calcium and carbonate to form calcium carbonate shells or skeletons.

As algae grows, it absorbs CO2 and releases O2. This is important because organisms need oxygen to breathe, and if we can increase the amount of oxygen, all life will benefit. In addition, the absorption of carbon dioxide, a greenhouse gas, is critical to slow down climate change, the greenhouse effect, and ocean acidification. Higher levels of phytoplankton will take more carbon out of the atmosphere.

In 2000, through results of the IronEx II experiment, it was observed that phytoplankton productivity increased as a response to iron fertilization in the eastern equatorial Pacific Ocean (1). Although an increase of algal growth in the ocean would be a positive effect, iron may also have negative effects, which is a concern. Iron can produce harmful algal blooms, which may alter other marine life negatively. Harmful algal blooms may include blooms of particular species that produce toxins that can kill marine life, mammals, and birds, and cause illnesses in humans. Other harmful algal blooms include nontoxic algae that clog fish gills and smother corals. Some algae discolor the water, smell, or cause drinking water and fish to taste unpleasant. Additionally, in the Iron Ex II experiment, there was a noticeable grazing mortality, which balanced out the growth of the experiment up to Day 6.

Our prediction is that as iron concentrations increase, algal growth will also increase. However, we must take into consideration the amount of iron concentration used and its duration. Similarly, the "carbon export ratio did not increase with the iron-induced diatom boom" (1). However, in Southern Ocean deepwater carbon export enhanced by natural iron fertilization, there was a noticeable increase in CO2 uptake from the ocean that was purposely fertilized by iron compared to adjacent ocean without iron (2). This may have something to do with the salt concentration of the different oceans and different iron concentrations.

We will be performing our experiment using Instant Ocean, which contains the amount of salt in the water of a home aquarium and presumably, the amount of salt in the ocean with reef fish. We will obtain algae from a phytoplankton solution.

Our study will advance our knowledge about the precise concentration of iron that induces algae growth and how long the positive effects will last. Our study will provide a time frame for when the positive effects wear off and the iron starts to affect the algae and the surrounding ecosystem differently. We predict that algal growth will increase as the iron concentrations increase, and that around the middle of the second week of the experiment, the algae will not continue to grow at the same increased rate as it did at the beginning of the experiment.

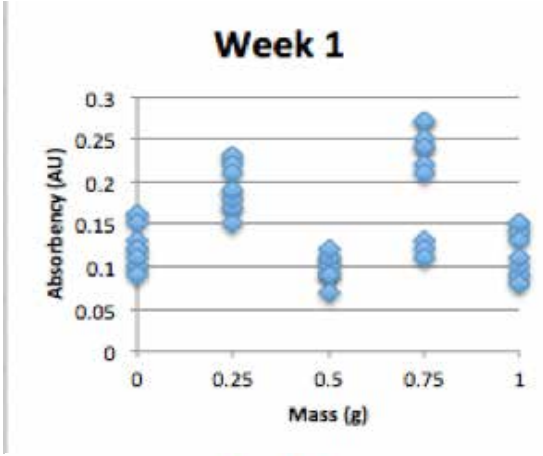
## RESULTS

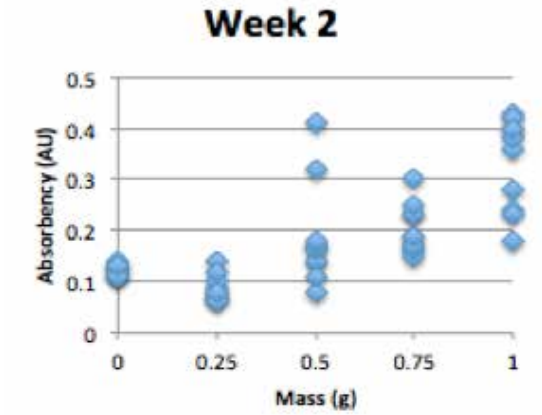
| | | | |
|---|---|---|---|
| 9 algae | 9 algae | 15 algae | 17 algae |
| 17 algae | 17 algae | 17 algae | 21 algae |
| 18 algae | 17 algae | 19 algae | 14 algae |
| 16 algae | 12 algae | 24 algae | 16 algae |

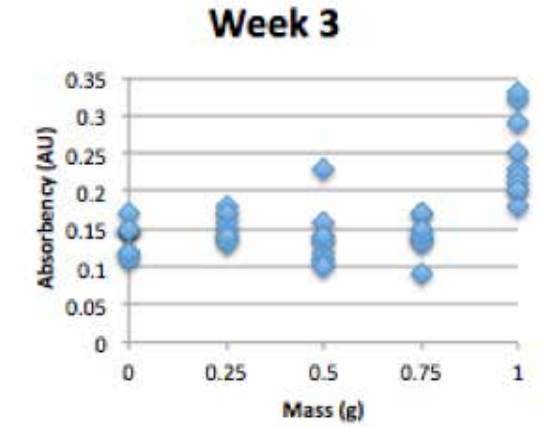
First the amount of phytoplankton was counted. The chart above represents the grid on the microscope slide used with the amount of phytoplankton in each section. The following is the formula used to calculate the total amount of phytoplankton in one tube: **Erythocytes Counted * Dilution * 4000/(Number of squares counted). There were a total of 60 squares and 250 phytoplankton in one tube.**

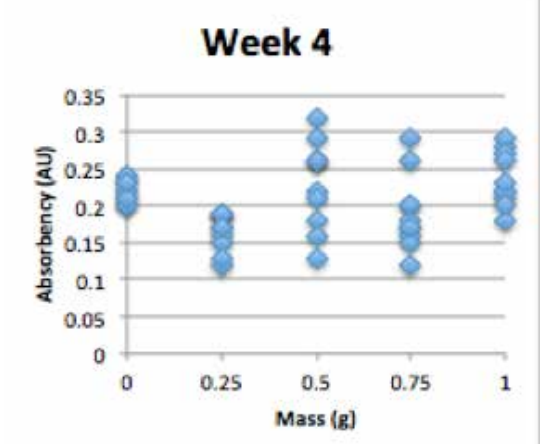
The original absorbance for each amount of iron was measured when inserted into the solution. These measurements were 0.10 for 0 grams, 0.10 for 0.25 grams, 0.10 for 0.5 grams, 0.11 for 0.75 grams, and 0.15 for 1 gram. The graphs display the absorbance for all the samples throughout the whole experiment.

Below are graphs that represent the data collected from weeks of measuring absorbance (AU). Absorbance can help us determine the amount of algae growth that occurred.




## DISCUSSION

Our prediction was that as the iron concentration in each sample increased, the rate of algae growth would increase. This was our hypothesis based on the results from the IronEx II experiment in 2000 in which it was observed that phytoplankton productivity increased in the eastern equatorial Pacific Ocean as a

result of iron fertilization in that area. We also predicted that the algal growth rate would decrease around the middle of the second week of our experiment, as the effect of the iron would possibly wear off.
The results displayed some fluctuation of algal growth between samples with different amounts of iron and even samples with the same amount of iron. Overall, the samples with 1 gram of iron grew the most algae during the four-week period. The average amount of growth increased as the amount of iron in the samples increased. However, there was no significant decrease in growth after the second week of the experiment. Our hypothesis proved to be partially correct as iron increased algal growth and since as the amount of iron increased so did the growth slightly. The p-value was 9.49. We proved to be incorrect in our prediction that the increased growth rate would start to decrease after the second week. These results are important for determining what can be done about global climate change. Adding iron to the ocean may slow down global warming by increasing the amount of algae present in the ocean. This algae takes carbon dioxide out of the atmosphere and releases oxygen. In the future, an experiment could be done using a large tank of salt water with iron and algae and adding marine life to it to observe how iron affects other life in the ocean. It would be beneficial to observe if the iron causes harmful algal blooms. Additionally, the length of the experiment could be extended beyond four weeks to observe more long-term effects of iron in the ocean.

**ACKNOWLEDGEMENTS**:
Mr. Maxwell, Mr. and Mrs. Davis, Mr. and Mrs. Naratil

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# Recycling Waste $CO_2$ to Grow Algae

By Sofia Deak'14, Alli Dorneo'14, and Ben Kaminoff'14

## ABSTRACT

In accordance with Pingry's "Go Green" initiative, our research project tested the growth rate of algae utilizing both natural air and waste carbon dioxide emitted from the building in order to determine which produced better growth. In the search for sustainable fuel sources, algal fuel has emerged as a promising source for the future. An initial concentration of algae was inoculated in a bioreactor and was measured with a Spectrometer 200. One bioreactor received an influx of natural air from the environment, while the other bioreactor was aerated with the exhaust fumes. After a period of several days, samples from the bioreactors were collected and re-measured with the spectrometer. Our findings showed no difference in growth between the group aerated with natural air and the group aerated with waste CO2.

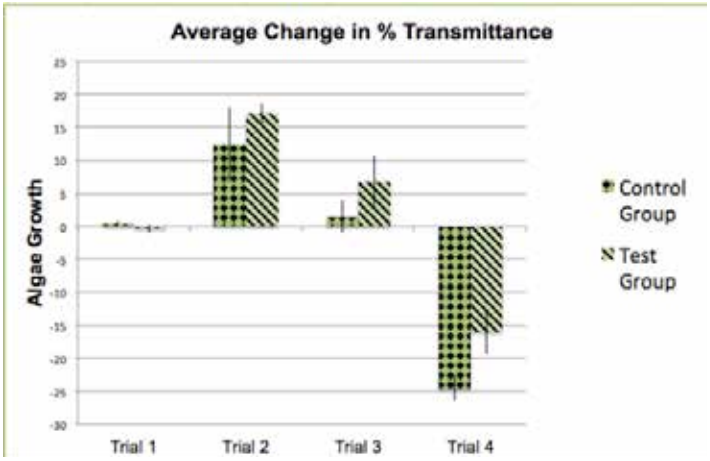
## INTRODUCTION

The purpose of our project is to identify the effect of waste carbon dioxide on algae production in a freshwater environment. In the growing search for renewable energy sources to replace a depleting amount of fossil fuels, many people have focused on biodiesel as an alternative source of energy. Biodiesel is made up of monoalkyl esters of fatty acids from vegetable oils and other animal fats (1). A common biodiesel used today is corn-based ethanol, but we contend that algae biodiesel is a much more efficient option. Algae is non-toxic, environmentally friendly, and biodegradable. Unlike making corn-based ethanol, producing algae biodiesel does not take away from crop production, which can drive food costs up. Algae, according to the US Department of Energy, are capable of yielding thirty times more energy per acre from land crops like corn (1). However, the current cost for algae production is more expensive than obtaining the petroleum based fuels we typically use (4). The biggest obstacle algae biofuel faces is finding an efficient and affordable method of producing usable algae on a large-scale basis. However, through our experiment, we hope to find possible solutions to bring us closer to an algae-fueled society as well as further Pingry's green initiative.

Microalgae is a unicellular species that is typically found in freshwater environments like lakes and

ponds. Similar to plants, algae contain light-absorbing chloroplasts that enable them to turn sunlight into energy through photosynthesis. Algae's simple structure, photosynthetic qualities, and fast rate of growth make algae a perfect candidate for biodiesel production. In a process called transesterification, the lipids from the algae are extracted and then converted into oil that we can extract energy from (1). In our experiment, however, we focused on growing the algae instead of the extraction and transesterification process. To create a large and functional amount of algae, we took into account the various factors of photosynthesis such as light, water, and carbon dioxide. Recent studies have shown that algae respond well to increasing concentrations of CO2. When there is a higher amount of CO2 concentration, the algae grow faster and there is more productivity (3). Considering the high CO2 emission from fossil fuels, the mere production of algae will reduce our carbon footprint and help the environment. In our efforts to help promote a green environment at Pingry, our group developed a bioremediation experiment to see how we could utilize the carbon emissions from the Carriage House on campus to increase the growth rate of algae obtained from the pond behind the school. Not only are the Pingry pond algae readily available and free, but the algae also demonstrate how powerful our local and natural resources can be.

## RESULTS




Based on the data, none of our results is statistically significant. The average P value, or percent error for each trial, ranged from 0.07295 to 0.45774. However, the acceptable P Value for an experiment is 0.05. We were able to observe some algal growth in some trials, but other trials displayed either minimal growth or a decline in algal concentration.

## DISCUSSION

A variety of factors may have led to our failure to see significant algal growth in our test group. Our leading hypothesis to explain our results is the weather conditions during our trials. The average temperature during each trial was very low, but fluctuated significantly over the course of the twenty-day experiment period. Cold (and even occasionally frozen) water does not create an environment conducive for algae growth, explaining our minimal overall observed growth. If we were to do anything different, we would test this experiment anywhere in between the months of April-August, when temperatures are at least 60 degrees Fahrenheit. Something else we could have done differently was finding a better design for our bioreactor that could thrive in any climate or environment. Another area of possible error was the airflow differences between the test group and the control group. The control group had a constant influx of atmospheric air over the course of each trial, while the test group only received the waste emissions. The heat was running sporadically in the Carriage House, which could have affected our results. This means that there was not a consistent and comparable amount of air flowing through the bioreactor for the entirety of the trial. In addition, we do not rule out the possibility that some of the gases emitted from the Carriage House are noxious and detrimental to plant growth. Finally, we suspect the presence of Miracle-Gro in the solution had an effect on our Percent Transmittance readings when tested by the Spectrometer 200.

## ACKNOWLEDGEMENTS:

We'd like to thank D. Maxwell, M. Virzi, M. Waelz, J. Chilmonik, G. Deemer, and the rest of the Pingry Maintenance Staff for all their help.

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# The Effect of UV Radiation on Bacterial Tolerance in *Escherichia coli*

By Natalie Gilbert'14, Stephanie Yeh'14, and Aigner Mizzelle'14

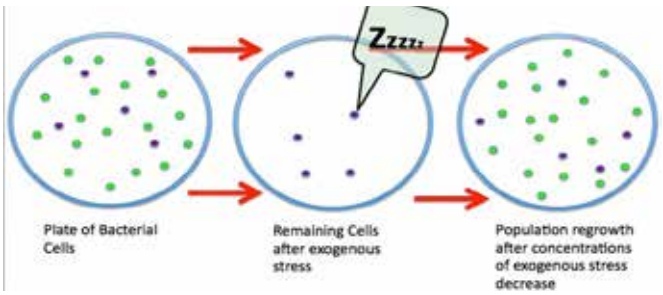
## ABSTRACT

Over the past century, studies have indicated that persister cells, bacteria tolerant to exogenous stress, are implicated in the chronicity of virulent bacterial infections. Research shows that bacteria have the ability to enter a dormant state in response to exogenous stress (like antimicrobial agents) and then reawaken and replicate once concentrations of the agent have diminished. The mechanisms behind persister dormancy are still unknown. Using *Escherichia coli* as the model organism for this project, we were able to observe the extent to which bacteria will die through UV exposure. Understanding the effect of UV radiation on bacterial persistence will undoubtedly open doors to new treatments for patients suffering interminable bacterial infections.

## INTRODUCTION

Bacterial persisters constitute a small subpopulation of cells that are generally tolerant to lethal doses of antibiotics. Persisters, unlike resistant bacteria are tolerant to antibiotics due to physiological processes (1). Characterized by a biphasic killing pattern, persisters exhibit a slower death rate than those of normal cells (4). This atypical response to antibiotics is not in fact influenced by genetic modification, but rather by the persisters' ability to become dormant in response to exogenous stress. Consequently, once antibiotic concentrations have decreased, these cells will then reactivate and reproduce a new population of bacterial cells (5). This phenotype portrays the basis for typical chronic infections.

In 1944, Joseph Bigger conducted one of the first major studies on persister cells. After discovering that penicillin did not sterilize a culture of *Staphylococcus*, he finally concluded that persister cells are present in massive numbers in bacterial biofilms (5,6). This groundbreaking study opened new doors to biofilm research, allowing subsequent scientists to understand why biofilms are tolerant to various antibiotics.

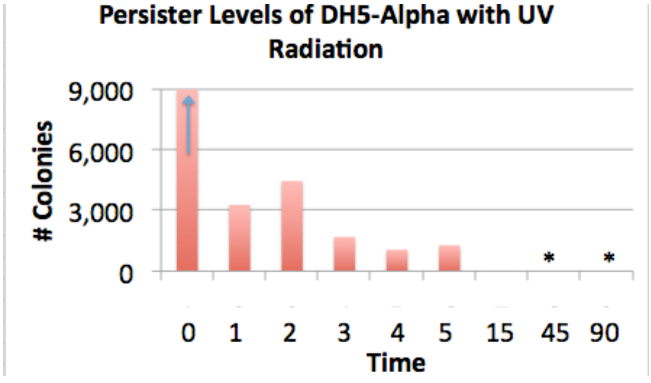



Biofilms are bacteria that adhere to damaged tissue and encase themselves in an extracellular polymeric substance matrix (2, 7). Their ability to adhere to damaged tissue and create a slimy protective layer over their mediums is attributed to their resistance to antibiotics. In addition, biofilms are associated with several chronic infections that include Cystic Fibrosis, Tuberculosis, Urinary Tract infections, and Pneumonia. Unfortunately, our current lack of knowledge on persister functions prevents researchers from generating effective treatments for these bacterial infections.

Urinary tract infections, specifically cystitis, are among the more common bacterial infections found in the United States. Emanating from Escherichia coli, Cystitis accounts for more than 36,000 deaths per year

## RESULTS

After exposing the plates with DH5-Alpha to UV light, we discovered that, even after 5 seconds



of exposure, approximately 1,200 persister colonies remained on the petri plate. We then discovered that, after 45 seconds, no persisters remained on the plate.

## DISCUSSION

Our main goal was to formulate the most effective treatment plan that could eradicate bacterial persisters without harmful side effects, nationwide. Fluoroquinolones, particularly Ciprofloxacin, are the primary antibiotics used to treat Cystitis because of their ability to target slow-growing planktonic cells.

For our project, we would like to understand the response of pathogenic strains of *E. coli* to UV radiation. We plan to grow and expose colonies of *E. coli* DH 5-Alpha to UV radiation over various time intervals and evaluate the changes in persister levels. If successful, this discovery could open doors to new drug therapies and potentially delay the onset of chronic bacterial infections.

Although, for some reason, fewer persisters appeared in t=1 than in t=2, we nonetheless discovered that more bacteria died when exposed to UV light for longer periods of time. When we graphed our data, we observed an exponential curve pattern within the results. We also concluded that there must be a certain threshold that exists between t=15 and t=45 in which all the bacteria are killed off. We can also conclude that even 1 second of exposure to UV radiation will cause an extreme effect on the bacteria's ability to survive.

Our data, which indicated that longer exposure times led to fewer bacteria, allows us to conclude that UV radiation is an effective treatment option for patients with bacterial infections.

When we collected our data for time intervals 1-5 seconds long, we noticed that, as time increased, the number of persisters did not continue to decrease tremendously, but rather wavered. This observation may have been a technical human error. Since the times were so short, it is possible that our timing was not entirely perfect, and the dishes were exposed for more time than desired. Nevertheless, in the future we could expose plates of bacteria with different antibiotics, with UV radiation, or with both forms of exogenous stress to come up with the most effective and efficient way of killing the bacteria. Since UV radiation technology and equipment can be reused, unlike individual doses of medicine, creating novel treatments using UV radiation can be especially advantageous to healthcare in third world countries. Hospitals will only need to purchase a few prototypes of the UV machinery rather than continuously stocking up on single doses of antibiotics, thereby saving money while saving lives.

## ACKNOWLEDGEMENTS:

Thank you to Chris Fazen and Dr. Mark Brynildsen (Princeton University) for being amazing mentors and for introducing Natalie to bacterial infections. We'd also like to thank Mr. Maxwell, Mr. De, Dr. D'Ausilio, Mr. Weinkopff, Teddy Leitheid, and Mikaela Lewis for guiding us through the process and for helping us whenever needed. Lastly, we'd like to thank our families for being unbelievably supportive of our endeavors.

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# Are We As Smart As We Think We Are?

By Lauren Graves'14

## ABSTRACT

We explored various conditions that could potentially affect how a high school student performs on an IQ tests, including gender, grade, average hours of sleep, hours of sleep before test taking, concussions, caffeine consumption, exercise, and time spent on homework. The average Pingry high school student has an IQ of 133. We found a negative correlation between IQ and number of concussions, and a positive correlation between IQ and grade. No correlation was found with other factors.

## INTRODUCTION

The Pingry School is known for recruiting some of the most academically promising students in all of the United States. Each year, Pingry students win countless national academic awards, excel on standardized tests, and enter the most prestigious universities. However, are we as smart as we think we are? We aimed to answer this question, and look into what sort of factors might affect our intelligence.

First, however, what is intelligence? We chose to test intelligence through an IQ test that was most closely related to the Cattell–Horn–Carroll theory, which tests several different mental processes. Our test examined the participants' memory, perception, language comprehension, visual and spatial recognition, logical reasoning, creativity, and numerical understanding, covering most of the factors in the Cattell–Horn–Carroll theory. The Cattell–Horn–Carroll theory is relatively new, however, having first been developed in 1999. Intelligence testing is an ongoing science, and intelligence is still subjective and very difficult to test.

As interest in intelligence testing has increased, many researchers have looked into what conditions influence performance and development. We chose to look at any potential correlation between IQ and sleep, caffeine consumption, concussions, exercise, and time spent on academic work. According to research done by Annette Sterr, 29% of mild traumatic brain injury subjects were found to perform poorly on neuropsychological tests as compared to their healthy counterparts (3). Caffeine intake was also shown to increase performance according to a study done by A. Smith, which stated that, "caffeine improves performance on vigilance tasks and simple tasks that require sustained response," such as an IQ test (2). On the contrary, sleep deficiency has not been shown to affect intelligence performance, as shown in research done by Manos Alchanatis, who stated that "the relationship between OSA (obstructed sleep apnea) severity and cognitive deficits is usually weak," (1). Research by Phillip D. Tomporowski also claims that exercise does not have an effect on intelligence either, showing that regulated exercise in mentally retarded adults showed no increase in IQ (4).

It is our hypothesis, therefore, that we see a positive correlation between caffeine and IQ, a negative correlation between concussions and IQ, and no correlation for the other factors.

## RESULTS

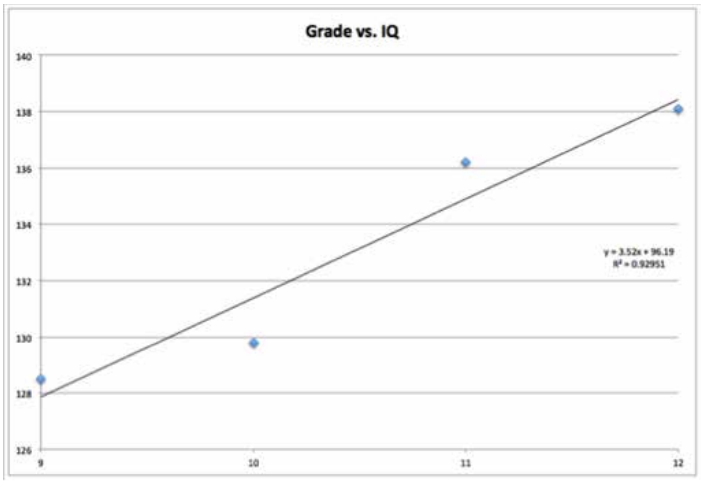


**Figure 1: The data indicate that there is a strong positive correlation between grade and IQ. Students perform better on IQ tests during their senior year compared to their freshman year.**

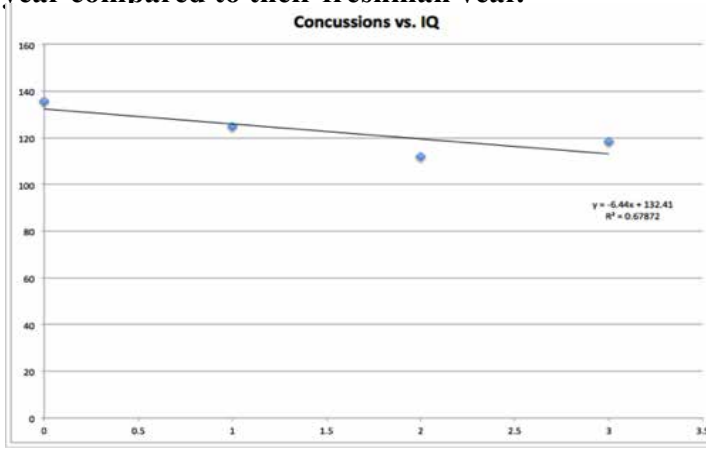


**Figure 2: The data indicate that there is a decent negative correlation between number of concussions and IQ. Students do not test as well on IQ tests after having one or more concussions.**

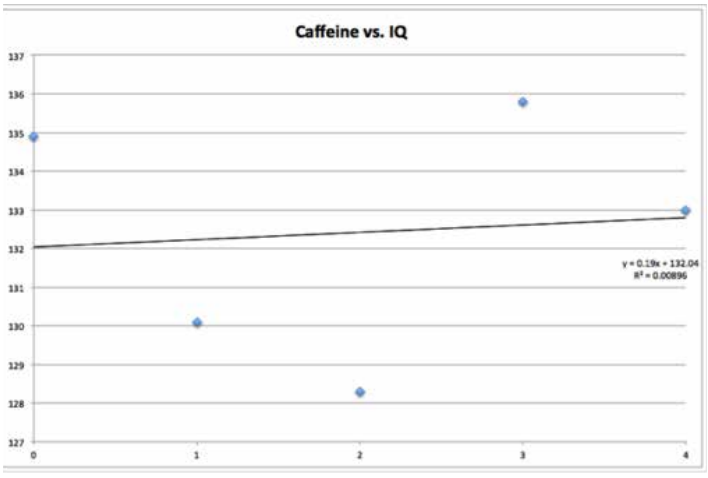


**Figure 3: The data indicate that there is no correlation between amount of caffeine consumed and IQ. Students who have consumed caffeine on the day of testing were not shown to perform better than those who did not.**

## DISCUSSION

Our study has shown a strong positive correlation between grade and IQ, and a moderately negative correlation between number of concussions and IQ. Other topics that were studied showed an insignificant correlation, and therefore a relationship between these factors and IQ was dismissed. These correlations are shown in the graphs above. An R-squared value higher than .5 shows moderate to strong correlation. A value under .5 indicates a weak correlation or lack of correlation. This would agree with our hypothesis with the exception of the relationship between caffeine and IQ, which showed no correlation in our study.

We hypothesize that the significant jump in average IQ between the 10th grade and the 11th grade is as a result of standardized test preparation. The negative correlation between IQ and number of concussions agrees with Sterr's research, which states that those who have suffered from minor traumatic brain injury continue to experience "symptoms and difficulties in everyday situations [which are] related to objectively measurable parameters in neurocognitive function." (3)

If we were to do our project again, we would use a more reputable, professionally made test in order to garner more accurate results. We would also use a randomized sample. Not only was it impossible to be sure that all of the data we accumulated was entirely valid, but the test itself could have been done improperly. Given the setup we used, there was no way to be sure that all the students took the test in an environment that would promote the most accurate results. To make the study more applicable to a greater high school population, we could have used students from many different high schools rather than just from Pingry. We would suggest that further research be done to prove causation, rather than simply correlation that was shown in this study. We would also suggest that further research isolate individual categories in order to show the most accurate results.

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# CG-3634 Notch Phenotype Screen

By Amol Kapoor'14

## ABSTRACT

The Notch signaling pathway is found in most eukaryotic organisms, and is responsible for intercellular communication. Notch has highly complex regulation mechanisms involving many proteins. Mis-regulation of Notch regulation genes has been implicated in multiple diseases. Identifying genes that interact with Notch is therefore important to better understand the signaling pathway. We suspect that the gene CG3634 may interact with Notch. Using transposase/P-element genetic modification, we will introduce a CG3634 knockout mutation in order to observe any Notch related phenotypes. Researchers have found that Notch activity is involved with the bristle formation in *Drosophila melanogaster*, with Notch up-regulation resulting in increased bristles, and Notch down-regulation resulting in decreased bristles. By examining the bristle formation after mutating the CG-3634 protein, we can determine whether CG-3634 plays a role in the Notch pathway. Currently, our data is inconclusive, with mutated flies showing either no visible mutation or sterility phenotypes.

## INTRODUCTION

The Notch signaling pathway is a highly conserved cell signaling system that plays a key role in the development of most multicellular organisms, including all metazoans. Notch is essential for proper embryogenesis (1, 11, 12), neuronal development (12, 13, 5), cardiovascular development (10), and organogenesis (12, 4). On a cellular level, Notch is responsible for lateral inhibition, coordinated differentiation, and boundary formation - key functions in the development of complex cellular structures (12, 5, 9, 7). Because of the key role Notch plays in organism development, Notch mis-regulation has been implicated in the development of cancers, multiple sclerosis, CADASIL, and numerous other cardiovascular, neurological, and cellular disorders (5). Understanding the complete functionality of the Notch signaling pathway is vital for a complete understanding of human development and for the eventual creation of treatments for the numerous Notch related diseases.

The Notch signalling pathway was first discovered in *Drosophila melanogaster* (3), or fruit flies. Due to the high evolutionary conservation of Notch, the pathway in Drosophila is very similar to the pathway in humans (3), making Drosophila a good model to study the effects of Notch. The canonical Notch pathway functions through the Notch signaling receptor, a trans-membrane protein (3). The Notch receptor binds to the Delta/Serrate/LAG-2 (DSL) family of proteins (3). In *Drosophila,* Notch binds to Delta and Serrate; in mammals, the corresponding homologs are Delta-like and Jagged (3). Ligand binding to the extracellular domain of the Notch receptors results in proteolytic cleavage and the release of the intracellular domain to the cell nucleus as a transcription factor (3). Notch is in this way both a trans-membrane receptor and a transcriptional mediator. More importantly, Notch ligands are also trans-membrane proteins; Notch signaling is normally triggered through cell-to-cell contact, allowing groups of cells to organize through signals transferred across cell membranes (3). In recent years, research on the Notch pathway has uncovered numerous variations of the canonical proteins, as well as binding and transcriptional regulation, resulting in a very complex pathway with many missing links (3).

In Drosophila, Notch directly regulates sensory organ formation through bristle development (2). Down-regulated or mis-regulated Notch signaling affects the segregation of epidermal and sensory organ cell lineages, resulting in inhibition of bristle formation (2). Knockout of target genes resulting in bristle deformation indicates a possible Notch protein component (2). Using P-element insertions and transposase, it is possible to quickly develop multiple gene disruption genotypes from a single stock (8). Together, it is possible to screen gene knockouts for potential Notch activity.

The sequenced gene CG3634 is uncharacterized in Drosophila, and may play a role in the Notch pathway. Previous expression data reported peak expression within 0-18 hour embryonic stages, early pupa stages, and in adult female stages (6). CG3634 is expressed in the *Drosophila* adult head, eye, CNS, crop, salivary and accessory gland, trachea, and ovary. Due to its presence during embryonic development and in sensory and reproductive organs, we suspect CG3634 may be a component of the Notch signaling pathway. In order to examine the relationship CG3634 may have on the Notch pathway, we ran a gene interruption phenotype screen. We hypothesize that interruption of CG3634 will result in deformed bristle formation in adult *Drosophila.*

## RESULTS

The F1 generation contains the CG3634 P-element and the transposase (Figure 1). The P-element codes for a red eye (wt) marker, while the fly has a white-eye marker on its first chromosome. Transposase constantly adds and removes the P-element from the genetic code. Transposase causes expression of various shades of orange in the eye - the mosaic phenotype - due to various expression of red eye marker (Figure 1, C). Transposase is also marked by curly wings (Figure 1, B). The presence of both mosaic eyes and curly wings indicates successful cross of transposase and P-element.

The F2 generation removes the CG3634 P-element and the transposase in order to prevent future mutation (Figure 2). Removal of both would result in white eyes and straight wings (Figure 2, B-C). Males of this generation may exhibit abnormal bristle development and Notch related mutations if CG3634 is related to Notch and if the transposase correctly removed the targeted P-element.

Seven stocks were made from single F2 generation males by crossing white-eyed males with double balancer females (Figure 3, A). Interestingly, five of the seven stocks seemed to be sterile (Figure 3, B-C). There seemed to be a large number of eggs, but there were no larvae or pupae present at any point (Figure

3, B-C). The other two stocks displayed no visible phenotypic mutations.

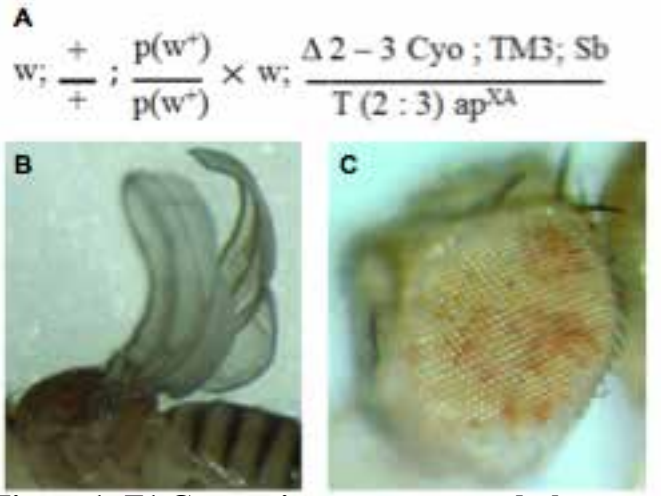


**Figure 1: F1 Generation genotypes and phenotypes. (A) The genotype of the parents of the F1 generation. Males contained P-element binding sites around CG3634, linked to a red-eye phenotype. Females contained the gene encoding for transposase, linked to a curly wing phenotype. Males and females also have a white-eye mutation. (B) Curly wing phenotype in the F1 generation. (C) Mosaic eye phenotype in the F1 generation. The Mosaic pattern indicates successful introduction of the CG3634 P-element and transposase to the fly.**

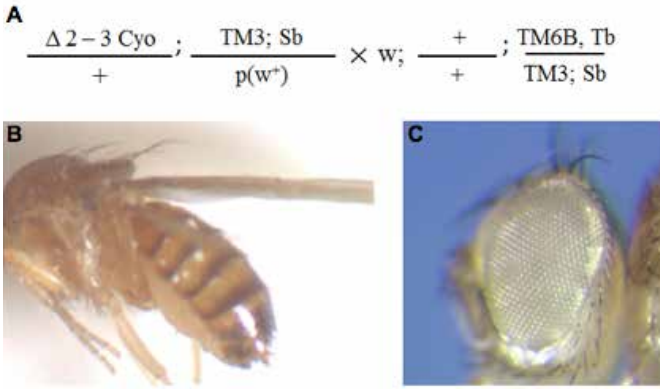


**Figure 2: F2 Generation genotypes and phenotypes. (A) The genotype of the parents of the F2 generation. Males contained the gene encoding for transposase, linked to the curly wing phenotype, and P-element binding sites around the CG3634 gene, linked to the red-eye phenotype. Females contained a third chromosome double balancer. Males and females also have a white-eye mutation. (B) Straight wing (wt) phenotype. Straight wings, or lack of curly wings, indicate the removal of the transposase protein. (C) White-eye phenotype. White eyes, or lack of red eyes, indicate the removal of the CG3634 P-element.**

## DISCUSSION

The uncontrollable nature of transposase requires an extremely large sample size (n > 100) to make any conclusive statements about the nature of CG3634. Due to uncontrollable delays caused by inability to procure fly food, snow, and lab closings, the breeding and crossing schedule for the Drosophila is only now reaching stability. We currently have ten stocks of the final phenotype, none of which display irregular bristle growth or other Notch related abnormalities. Of those ten, seven of them are not viable, displaying some form of sterility. This is not statistically significant. With the exponential nature of fly stock development, we expect to have enough stocks for conclusive data within the next month. For now, however, the data is inconclusive. We cannot confirm or reject the hypothesis at this time.

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# Purification of *Salmonella* Transcription Factors HilD and HilC

By Teddy Leithead'14, Elizabeth Kraeutler'15, Jessica Day,
F. John Kull, and Morgan D'Ausilio

## ABSTRACT

Typhoid fever is an acute enterotoxic disease caused by *Salmonella* bacteria. For the *Salmonella enterica serovar typhimurium* (*S. typhimurium*) to cause typhoid fever, it must produce the primary toxicity regulating transcription factor, HilA. HilA expression is regulated by two secondary transcription factors, HilC and HilD, both of which are members of the AraC regulatory protein superfamily. Recently, research conducted in Dr. John Kull's structural biology laboratory at Dartmouth College revealed that an AraC regulatory protein, ToxT, which is responsible for initiating production of toxicity factors in Vibrio cholerae, binds the fatty acid cis-palmitoleate, causing inhibition of ToxT function (ii). The goal of this project is to clone, express, and purify the HilC and HilD proteins for crystallization and subsequent determination, in hopes of identifying a similar inhibitory mechanism of *S. typhimurium* virulence.

## INTRODUCTION

*S. typhimurium* is a Gram-negative enterobacterium found worldwide. It is most commonly known for its proclivity to infect humans through consumption of uncooked contaminated foods. Every year approximately 42,000 cases of *Salmonella* infection are reported in the US alone.[i] While it is most often not fatal when treated swiftly, countries that lack basic health care suffer greatly from this disease. Due to these factors, research is currently being conducted to elicit a greater understanding of the molecular pathways that contribute to *Salmonella* toxicity.

A complex transcriptional cascade regulates the expression of *S. typhimurium* toxicity genes contained on the *Salmonella* Pathogenicity Island (SPI1). This cascade directly regulates the primary transcription factor, HilA, which facilitates expression of the *Salmonella* toxin. Two central proteins in the cascade, HilC and HilD, are critical for initiating HilA activity. (Figure 1) Both HilC and HilD are members of the regulatory protein AraC superfamily. Recently, an AraC transcription factor implicated in *Vibrio cholerae* toxicity was shown to bind unsaturated fatty acids that regulated protein function. [ii] Theoretically, such a mechanism could be exploited to prevent toxin production and subsequent cholera disease symptoms. We hope to find this regulatory mechanism strongly conserved across many species of enterotoxic bacteria, allowing the possibility of a new line of antibiotic drugs targeting the pathogens' toxin production pathways.

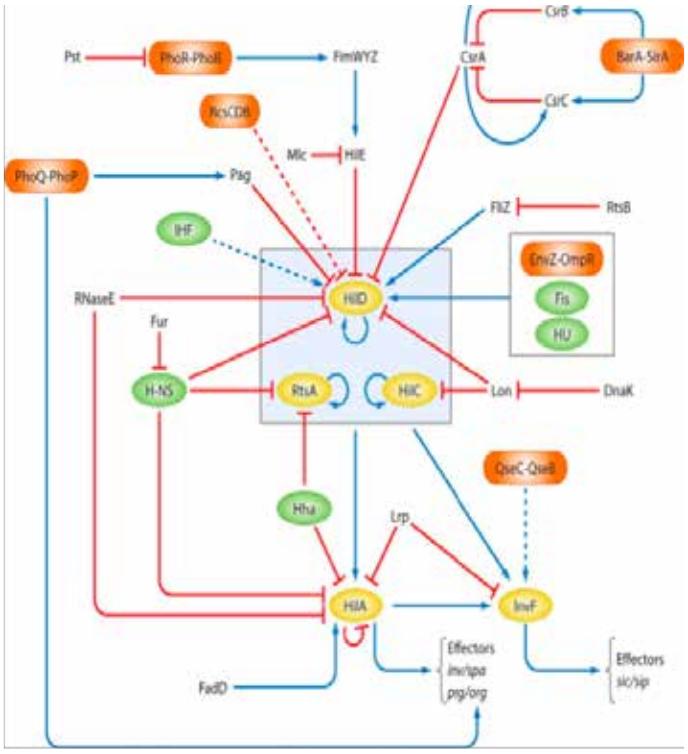


**Figure 1. This image displays the proteins and complex mechanisms involved in the regulation of the Salmonella toxicity pathway.**

## RESULTS

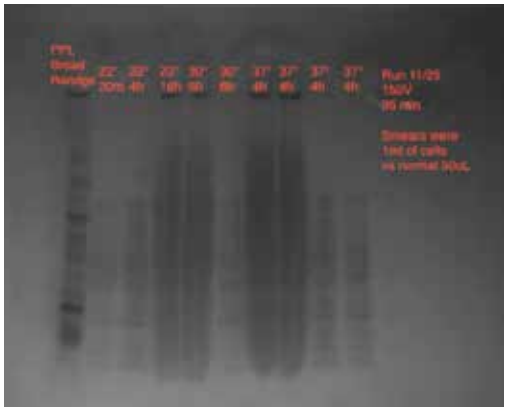

**Figure 2. A protein gel of BL21 cells post expression test protocol. Cells were induced at 22, 30, and 37 degrees for varying times. It is expected that successful protein expression would show a distinct band at 68 kD.**

After cells produce protein, they are scaled up to a 1 liter volume and prepped using gravity column flow with chitin beads to associate with the protein of interest. A Bradford test confirms high levels of the protein, and a protein gel indicates the purified protein was of the correct length, allowing us to move on to crystallization.

## DISCUSSION

With successful purification of the HilC and HilD transcription factors, further steps can be taken to crystallize the proteins with the ultimate goal of determining their structures. Crystals are generated using the hanging drop vapor diffusion method, then imaged with the Synchrotron at Brookhaven National Laboratories. By taking these steps, we hope to discover a regulatory binding site similar to the site observed on ToxT in *Vibrio cholerae* that would allow us to pursue future research in the development of a new antibiotic drug targeting the toxicity pathway of *Salmonella typhimurium.*

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# Modeling ToxT to Explain How Cholera Toxicity can be Regulated by Fatty Acids: The 2014 Pingry SMART Team project

By Emily Kwon'14 and Rachel Wu'14

## ABSTRACT

*Vibrio cholerae* (*V. cholerae*) is an enteric bacterium and the causative agent of the acute intestinal infection, cholera. *V. cholerae* is regulated by the expression of two virulence factors: toxin-coregulated pilus (TCP) and cholera toxin (CT). The expression of these gene products is controlled by a transcriptional cascade culminating with the expression of ToxT, a protein of the AraC-family. The solved crystal structure of ToxT reveals the unexpected presence of a buried 16-carbon fatty acid, cis-palmitoleate. Analysis has demonstrated a direct link between the presence of cis-palmitoleic acid and the reduction of TCP and CT expression, preventing the binding of ToxT to DNA. The ability of cis-palmitoleic acid to decrease interaction of ToxT with DNA presents potential opportunities to prevent and/or treat cholera.

## INTRODUCTION

Cholera is caused by infection of the intestine by *V. cholerae*. Infection leads to severe dehydration for those who ingest water contaminated with the bacterium. When *V. cholerae* enters the small intestine, the high concentration of fatty acids present within the middle of the intestine prevents the expression of the virulence cascade. When *V. cholerae* reaches the villi of the small intestine, the decreased concentration of fatty acids close to the intestinal walls allows the bacteria to release CT. The expression of CT triggers an uncontrolled release of water and electrolytes from the host cells. This rapid loss of fluids causes severe dehydration through watery diarrhea or vomiting, and the afflicted person can die within hours. The production of both TCP and CT is regulated via a transcriptional cascade involving the master regulator ToxT, a member of the AraC family of transcription factors.

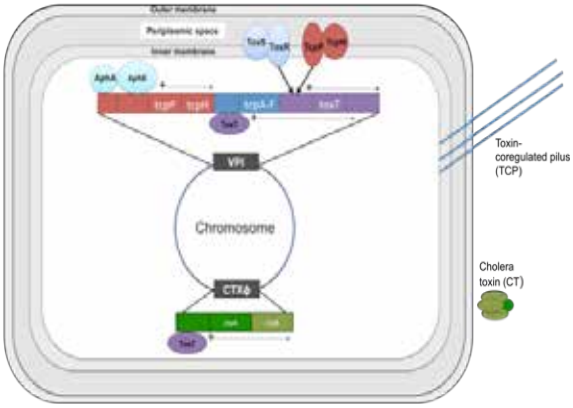


**Figure 1. Schematic of the V. cholerae virulence cascade (2).**

An X-ray crystallographic study of ToxT revealed a 16-carbon fatty acid, cis-palmitoleate, in the ligand-binding pocket of the ToxT regulatory domain. Further analysis indicated that expression of both TCP and CT was reduced in the presence of cis-palmitoleate. The binding of the fatty acid to ToxT prevents DNA binding and leads to decreased virulence factor expression. Using the structure determined in this study, The Pingry School S.M.A.R.T. (Students Modeling A Research Topic) Team used a 3D-printer from the Milwaukee School of Engineering (MSOE) to model the interactions between ToxT and cis-palmitoleate. Ligand binding by the regulatory domain of the ToxT changes the conformation of the DNA-binding domain, resulting in decreased interaction with DNA. Fatty acid regulation of ToxT-DNA binding provides insight into the mechanism of controlling virulence gene expression in *V. cholerae*. Studies based on this research are currently investigating the structure and function of other AraC-family members from a variety of infectious bacteria, seeking to determine if fatty acids may also be involved in regulation of virulence factor expression in other organisms.

## STRUCTURE

As a member of the AraC family of transcriptional regulators, ToxT has two characteristic domains: a conserved C-terminal helix-turn-helix DNA binding domain and a variable N-terminal ligand binding and dimerization domain. The N-terminal domain is comprised of three alpha helices and a nine-stranded beta sheet. These conserved portions contain a binding pocket enclosed by residues Y12, Y20, F22, L25, I27, K31, F33, L61, F69, L71, V81, and V83 from the N-terminal domain, and residues I226, K230, M259, V261, Y266, and M269 from the C-terminal domain. The interface between the two domains is necessary for binding to DNA (1).

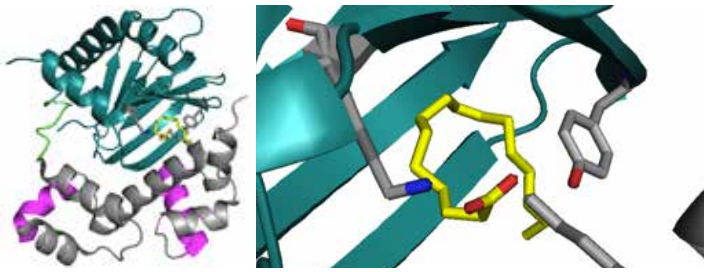

**Figure 2. A) Ribbon diagram of ToxT. B) Close up of the cis- palmitoleic-binding region.**

An unsaturated fatty acid ligand, cis-palmitoleate, is bound to the regulatory domain of ToxT. In the binding pocket formed by the C-terminal and the N-terminal domain, Lys31, Lys230, and Tyr12 interact with the polar head of the fatty acid, allowing the ligand to attach. Cis-palmitoleic acid is able to inhibit the function of ToxT by forming a bridge between these lysine residues; thus, ToxT is locked into a closed conformation that is unable to bind to DNA.

## PROPOSED MECHANISM

In order to determine whether cis-palmitoleate was able to prevent ToxT from binding to DNA, different unsaturated and saturated fatty acids were added to cultures of *V. cholerae* strains. It was found that the expression of the tcp and ctx operons was reduced between 6-8 fold with cis-palmitoleic acid and between 10-15 fold with oleic acid, while only a twofold reduction was observed with palmitic acid (1).

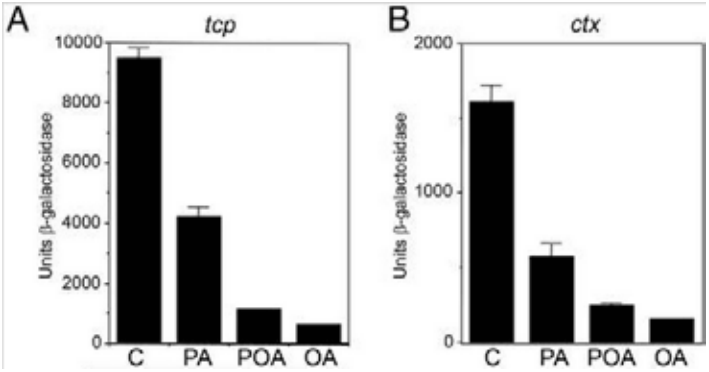


**Figure 3. Effects of fatty acids on tcp and ctx expression measured with units of beta-galactosidase (measure of expression). Individual graphs depict varying activities for respective fatty acids. (C - control with methanol; PA - sodium palmitate; POA - palmitoleic acid; OA - oleic acid) (1).**

Once the *V. cholerae* bacterium penetrate the intestine, charge-charge repulsion between K31 and K230 destabilize the closed conformation and an opening occurs in the N-terminal and C-terminal domains. In this “open” conformation, K230, helix

a7, and helix a6 are no longer restrained; thus, ToxT is able to bind to DNA. When a fatty acid binds, ToxT is inhibited. The negatively charged carboxylate head groups of the fatty acid form salt bridges with K31 from the N-terminal domain and K230 from the C-terminal domain. With the N and C terminal domains in a "closed" conformation, the overall structure of ToxT becomes non-parallel, preventing ToxT from binding to DNA and inducing expression of TCP and CT.

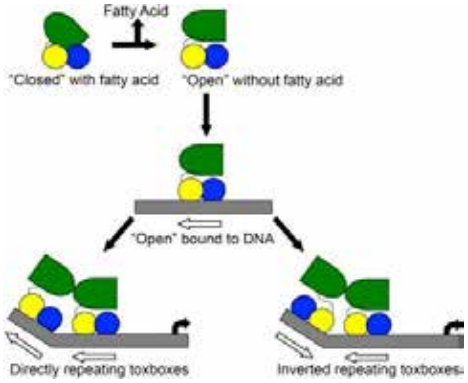


**Figure 4. Model for the regulation of ToxT by monounsaturated fatty acid. When fatty acid is bound to ToxT, it undertakes a "closed" conformation, which cannot bind to DNA (3).**

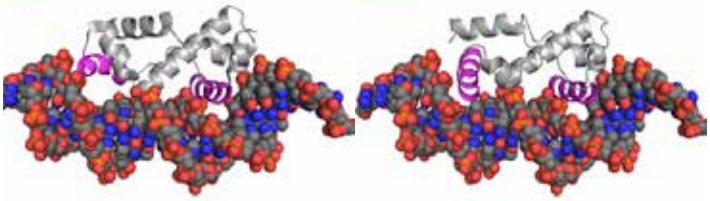

**Figure 5. Model for the regulation of ToxT by monounsaturated fatty acid. When fatty acid is bound to ToxT, it undertakes a "closed" conformation, which cannot bind to DNA (3).**

The left figure in Figure 5 illustrates MarA, a protein in the AraC family. Its two DNA binding helices are parallel to each other, as shown. These two helices are the a6 and a9 helices. Because they are parallel, MarA is able to fit into the major grooves of DNA. ToxT operates similarly to MarA. When ToxT does not have a lipid bound, its a6 and a9 helices are aligned in a parallel formation and allow the cholera toxin to eventually be expressed. However, when ToxT has a lipid bound in the ligand-binding domain, ToxT's structure changes. Once the fatty acid is bound, the N and C-terminal domains close together. One of the residues that interacts with the fatty acid, Lys230, moves helix a7 into a different position. This in turn causes helix a6 to become distorted. Therefore, when ToxT has a lipid bound in its ligand-binding domain, helices a6 and a9 become divergent and restrict ToxT from binding to DNA.

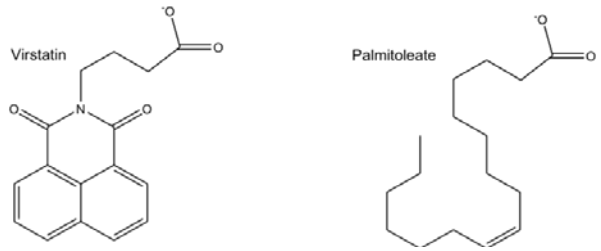


**Figure 6. Virstatin and Palmitoleate.**

Virstatin is a small molecule that may provide an alternate way to inhibit virulence regulation in V. cholerae. With a similar structure to the typical environmental inhibitor, cis-palmitoleic acid, virstatin may be able to disrupt the same conformational pathway. As a molecule that can be chemically synthesized, virstatin presents opportunities for future studies as a treatment or preventative for cholera.

The next step is to conduct animal model studies. Sea Buckthorn, a plant found in Eurasia, Australia, and North America, may be a possible agent for these studies (4). Sea Buckthorn has natural berries that contain 26% palmitoleic acid and 17% oleic acid. Further clinical trials must be conducted to examine the effects of both palmitoleic and oleic acid on the *V. cholerae* transcriptional cascade. In these trials, scientists must also test other members of the AraC family for UFA inhibition. Future studies may show that unsaturated fatty acids, such as virstatin, palmitoleic acid, and oleic acid, may have an effect in preventing other diseases regulated by AraC proteins.

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# Effect of Methane Digesters on Global Greenhouse Gases

By Pradyuth Maganti'15 and Matthew Rice'15


## ABSTRACT

The increased use of home methane digesters has the potential to limit the exposure of greenhouse gases directly into the atmosphere. We made home-scaled methane digesters with mostly recycled parts, easily accessible for people constructing them with a budget under $100. They also featured the innovative use of two holding chambers and an s-trap.


## INTRODUCTION

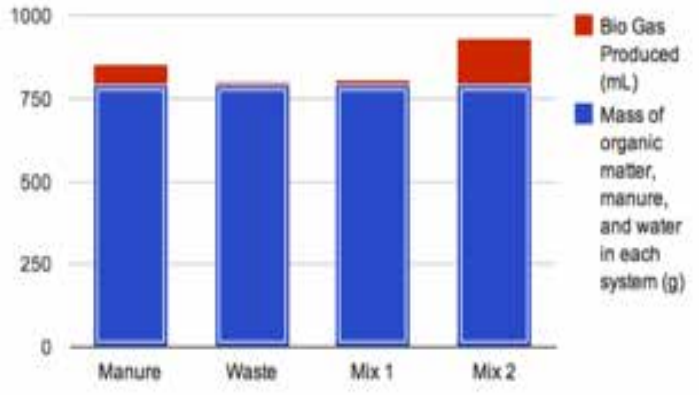
Methane digesters address three different environmental issues: non-renewable energy consumption, land management, and greenhouse gas emissions. We were inspired to do this project after learning that throwing away organic waste is extremely harmful to the environment (7). Every day millions of people in the U.S. and other countries put their garbage outside to be taken away to landfills. In landfills, organic waste is buried under tons of other waste, thus creating an anaerobic environment. In anaerobic environments, organic waste, and generates the extremely harmful greenhouse gas, methane. "The US EPA has identified landfills as the single largest source of methane (CH4), a potent greenhouse gas that is 23 times more efficient at trapping heat than carbon dioxide (CO2)" (7). On an annual basis, approximately 600 million metric tons of methane are emitted in the United States alone. Many of these emissions are derived from agriculture and wastes from homes and businesses (5). In addition, landfills in general are harmful to the natural landscape and destroy natural habitats.

Fossil fuels and petroleum are not renewable after the initial consumption and are being exhausted quickly. The world consumes over 11 billion tons of oil annually. Organic waste has traditionally been disposed of because people assume that there is no practical use for it. Instead of simply ignoring this potentially beneficial resource, the possibility exists to utilize it in the mission to slow the growth of non-renewable energy consumption. (1, 2, 4) While researching the effects of methane it was discovered that it can be useful in partially solving the energy crisis. Methane has a high content of hydrogen and is very combustible (6). Methane is widely used for heating, cooking, lighting, and a source of hydrogen. In fact, many people have noticed this and tried to capture the methane produced from landfills but their efforts have not proven to be efficient because the organic material in landfills decomposes and emits methane before the landfills can be capped and controlled (7). We decided we liked the idea of using the methane generated by the organic waste and looked more into the generation of methane. The controlled generation of methane is one of our main goals.

Methane digesters provide the perfect oxygen free environment to produce biogas, which is predominantly composed of methane. Surprisingly, there are very few methane digester resources and projects. There are only "6 federally recognized 'bioreactor' projects underway" (7). The further development of methane digesters would be extremely beneficial.

Organic wastes not only have the ability to help in the crisis in energy production, but also with the greenhouse gas concern. After installing a methane digester, some farms have reported a 99% reduction in "greenhouse potential" (1). Greenhouse gas emissions have long been documented as a factor in the heating of the earth. (3) This global warming crisis will not be tremendously affected by these methane mechanisms on a small scale, but with a global effort they can make an impact. Greenhouse gases are in the atmosphere around the world and not centralized in one specific area. Creating awareness of the issue and a possible solution is vital for methane digesters to make an impact.

## RESULTS



Of our four small-scale tests, all produced some gas. Now we understand that the gases collected from our test digesters do not contain strictly methane. The gas we collected is biogas and an unverified amount of oxygen. As the EPA points out "BMP"'s typically overpredict the amount of biogas and/or methane produced" (10). We estimate that half of the gas collected is actually biogas. Our first digester filled with only cow manure produced 60 mL of biogas, which is what we expected. The cow manure is rich with methanogens and half digested materials. Our second digester, filled with food waste, produced 9.8 mL of biogas. This was also expected since there was a lack of methanogens to produce methane. Our last two digesters, with mixed proportions of manure and food waste, produced about 15 mL and 140 mL of biogas. We were extremely surprised at these results.

After extensive research and observing the systems we concluded that a single stage digester would be very prone to failing. One of our mini test digesters did not generate much biogas, while the other did, but both digesters were constructed at the same time with the same materials and very similar proportions. We believe that the organic waste we put in the one digester decomposed too quickly and lowered the pH of the slurry, killing off the methanogens (9). Thus only a limited amount of biogas was produced. We quickly concluded that a single stage digester would not be efficient and reliable.

We developed a 2-stage system that has a 3 gallon influent input per day. The first stage holds 9 gallons of slurry, giving it a HRT (hydraulic retention time) of 3 days. The second stage holds 27 gallons of slurry, giving it a HRT of 9 days. This will effectively separate the acidogenesis stage and the methanogenesis stage of the anaerobic digestion. This makes it much more likely for the pH of the digester to remain stable between the operating level of 6.5-8.5 (9).

## DISCUSSION

Methane digesters have the potential to make a tremendous impact on the environment with the increased use of them in individual homes. Methane digesters can limit the household emissions of greenhouse gases (predominantly methane) by approximately 60%. This device also has the ability to use the greenhouse gases as alternative energy sources. Some appliances such as refrigerators, stoves, and lights are already being sold that run solely on biogas produced from machines such as methane digesters. This field of appliances continues to grow rapidly.

Methane digesters themselves are a fairly new technology, developing in the last few decades. The digester is cost-effective ($100 budget) and entirely eco-friendly. Our digester is innovative for two reasons. First, the multi-chambered approach consisting of a 30 gallon drum and a 9 gallon PVC tank effectively separates the acidogenesis stage and the methanogenesis stage of the anaerobic digestion. This makes it much more likely for the pH of the digester to remain within the optimum range. Second, the utilization of the s-trap prevents any backflow of the gases into the chambers holding the organic matter. These devices are commonly used in plumbing to prevent the backflow of odorous gases, but we thought it would be useful to use in the construction of a methane digester, and it was. In all of the tests, the gases flowed smoothly into the collection duct.

Our data are evidence of the viability of our solution. When organic matter is discarded into a landfill, methane collects and is emitted into the atmosphere. Our data show that when organic matter is put into a methane digester, the gas that is formed as a result of anaerobic digestion does not emit into the atmosphere. It simply remains in the completely sealed collection duct. Since methane generally stays in the atmosphere for a total of 12 years (11), this greatly reduces the concerns with greenhouse gases.

Our plan was made possible by the great deal of research that was conducted on both the issue of greenhouse gases and possible solutions. This helped us formulate our own ideas and potential solution to the problem. Research on previously built methane digesters also enabled us to improve upon them and make them more efficient as previously mentioned.

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# Effect of Coffee Grounds on Lettuce Growth

By Rebecca Muller'14 and Lauren Ru'14

## ABSTRACT

For our biology research project, we mixed varying concentrations of coffee grounds into the soil of multiple lettuce plants and measured leaf growth over the course of several months. We measured not only the total amount of growth, but also the rate of growth to determine the efficiency and overall production. We were attempting to determine if coffee grounds could be used as an effective organic fertilizer for lettuce plants to speed growth and increase the size of the lettuce. Here we show that coffee grounds can be utilized as a natural fertilizer in the farming industry to increase production and efficiency.

## INTRODUCTION

Lettuce was chosen as the plant to test because it is easy to visually observe growth and measure without having to uproot the entire plant. Coffee grounds were chosen because they are a common waste product in households and businesses with no conventional use to date. Coffee grounds also contain a variety of vitamins and minerals that are known to aid plant growth by increasing root and leaf growth and speeding flowering. Phosphorus in coffee has been proven to stimulate root growth because phosphorus is a component in the process of photosynthesis, as well as nutrient transport and energy transport. A heavy amount of phosphorus is beneficial to the plant's root development and flowering. Coffee also contains potassium, which aids flower and fruit development as well, because potassium controls the opening and closing of the stomata. Without enough potassium, the stomata do not efficiently use water, causing the plants to become vulnerable. Potassium aids enzymes that produce proteins and sugar, which are essential to growth. Lastly, nitrogen promotes leaf growth because nitrogen is part of the chlorophyll molecule, which gives plants their green color and is involved in creating food for the plant through photosynthesis. Small amounts of the other nutrients found in coffee grounds are also known to facilitate growth by conditioning and replenishing the soil.

Coffee grounds are a common waste product in households and businesses, but there are not many well-known and productive ways to use them. If used coffee grounds could be successfully recycled as a fertilizer, this would eliminate a major source of waste and improve the efficiency of crop growth without in-

creasing the cost. There is an urgent need for organic fertilizers because traditional intensive agriculture uses large amounts of inorganic fertilizers and pesticides for maximum productivity, which can lead to declining soil fertility and harmful environmental effects in the long run (3). The best fertilizers should sustain soil management as well as yield successful results. Coffee grounds could be a step in the right direction, because they are easily accessible, inexpensive, cost less than store bought fertilizers, and are significantly less harmful to the environment (2).

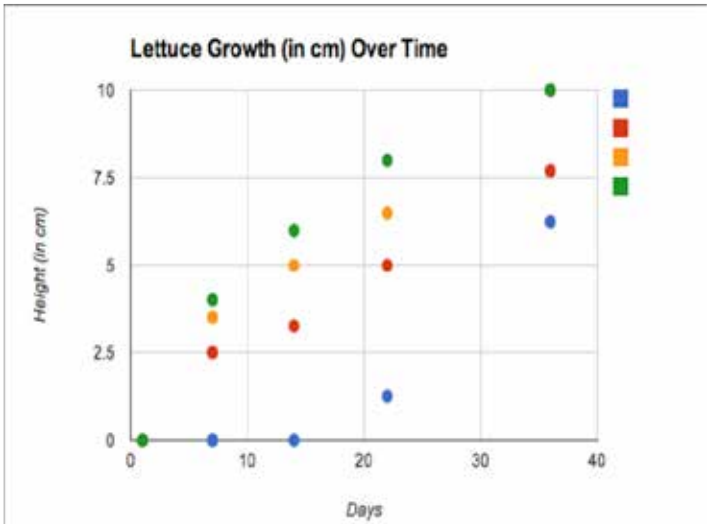
We mixed varying concentrations of coffee grounds into the soil of various lettuce plants and measured leaf growth over the course of several months. We measured not only the total amount of growth, but also the growth rate to determine the efficiency and overall production. Our hypothesis is that coffee grounds will allow the plant to grow faster and larger than the plants without coffee grounds.

## RESULTS

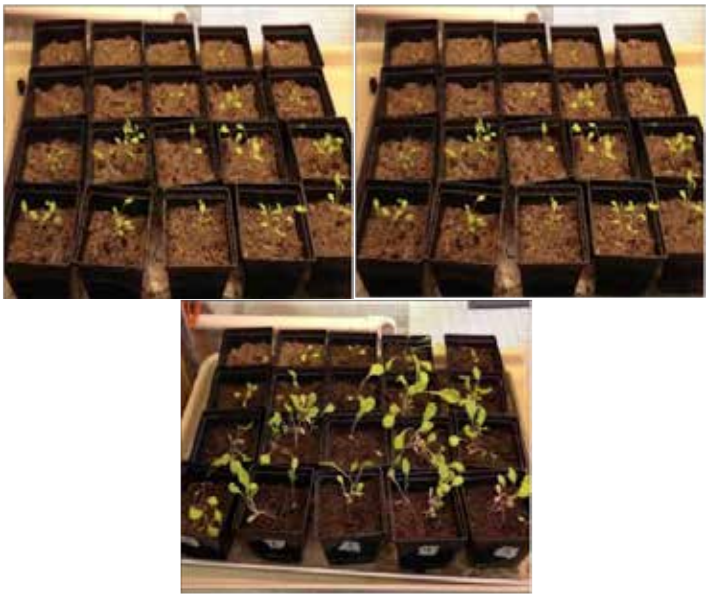
| Averages | | | | |
|---|---|---|---|---|
| | Measure 1 (cm) | Measure 2 (cm) | Measure 3 (cm) | Measure 4 (cm) |
| Control | 0 | 0 | 1.25 | 6.25 |
| 1 Tbsp | 2.5 | 3.25 | 5 | 7.7 |
| 2 Tbsp | 3.5 | 5 | 6.5 | 10 |
| 3 Tbsp | 4 | 6 | 8 | 10 |

This chart shows the average recorded heights of the five samples from each concentration in centimeters. The different numbered measures are different times that we recorded the data, and they are approximately equally spaced out from each other.

This data are graphed in the following scatter plot to show the rates of growth among all the plants over the course of the two months that we measured them.




The pictures below the graph are examples of our setup detailing growth on day 7, 14, and 36. The plants in the row closest to the front in all of the pictures have the most coffee grounds (3 Tablespoons) and the row farthest to the back has no coffee grounds. There is no difference between each of the columns, other then the fact that they were numbered to increase our sample size and minimize the possibility of error. One can see that the row closest to the front consistently has the tallest leaves.



## DISCUSSION

We found that the addition of coffee grounds had a beneficial effect on the growth of lettuce. Physical and quantifiable evidence shows that the lettuce plants with more coffee grounds had more growth than those that didn't. We, therefore, accept the hypothesis that coffee grounds aids lettuce growth. We believe that this is a crucial step in branding and using coffee grounds as an organic fertilizer, and we would propose further experiments with other plants; for example, tomatoes are very acidic and the acidity could affect the results.

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# Comparing the Effects of Simple and Complex Fish Diets on Lettuce (*Lactuca sativa*) Growth

By Christina Ou'15


## ABSTRACT

Aquaponics is a sustainable plant and fish farming method designed to utilize the nutrients from fish waste to promote growth in plants. The plants at the same time purify the water for fish by absorbing the nutrients from fish feces. I tested the effects of a complex, varying high-quality diet and a simple, high-quality diet on fish feces as fertilizers for plants. I hypothesized that a complex high-quality fish diet would promote higher plant growth than a simple fish diet. The difference of plant mortality and the average plant height in the two systems was not statistically significant, showing that a simple diet can produce the same yields as a complex diet.


## INTRODUCTION

Waste products from fish farmeries are serious environmental pollutants. In 2000, the nutrient pollution from Scottish fish farmeries was more that of its people. Concentrated pollutants from fish farms can travel significant distances to reach coastlines (2). The fish-plant farming technique called aquaponics can be used as a solution to reduce pollutants from fish farming.

Aquaponics is the integration of hydroponics with aquaculture in one system (2, 4, 6, 7). Hydroponics is a method of cultivating plants in nutrient-rich water. Aquaculture is the farming of aquatic organisms such as fish (2, 6, 7). Aquaponics combines the two by having the two methods share the same water. The fish produce waste in water which double as plant fertilizers, and the plants use the waste and nutrient-rich water to grow. Because the plants absorb the fish waste and nutrients, they purify the water and reduce fish pollutants by acting as a filter. Through this linkage of plant and fish culture, fish pollutants are reduced and the economical advantages include shared operational costs and a profit potential because two cash crops are produced in one closed system (7). Figure 1A shows a commercial aquaponics setup.

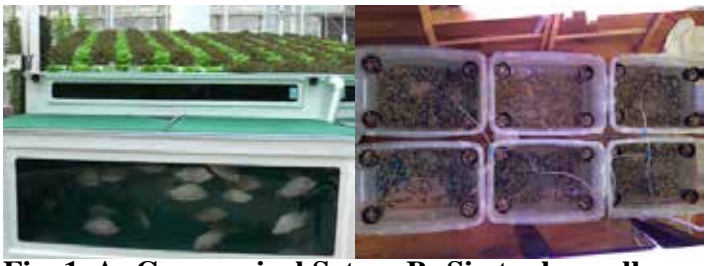

**Fig. 1. A: Commerical Setup. B: Six tank small-scale aquaponics setup**

For my experiment, I built a small-scale aquaponic system using white cloud fish and lettuce plants (Fig. 1B) to test how fish food affects the quality of fish feces, and measured the difference by comparing plant height. The simple diet consisted of a single fish food made by Hikari (fish meal, wheat flour, and multiple vitamins and minerals). The complex diet consisted of the aforementioned Hikari food, Omega One's kelp flakes (whole kelp, spirulina, whole salmon, vitamins and minerals, etc), and frozen brine shrimp. Plant height was measured over a 3-week period.

## RESULTS

There were 12 plants in both the simple and complex systems so 24 data points were taken daily. The average plant height of both systems is graphed in Figure 2. At the end of the 3-week period, 7 plants in the simple system had lived and 10 plants in the complex system had lived. Both systems started out with 12 plants.

Nitrate levels were relatively consistent throughout the trial. I took nitrate readings weekly for 3 weeks for 6 tanks , and out of 18 total readings, 16 read 40 ppm, and 2 read 20 ppm, 1 from the simple tank and 1 from the complex tank.

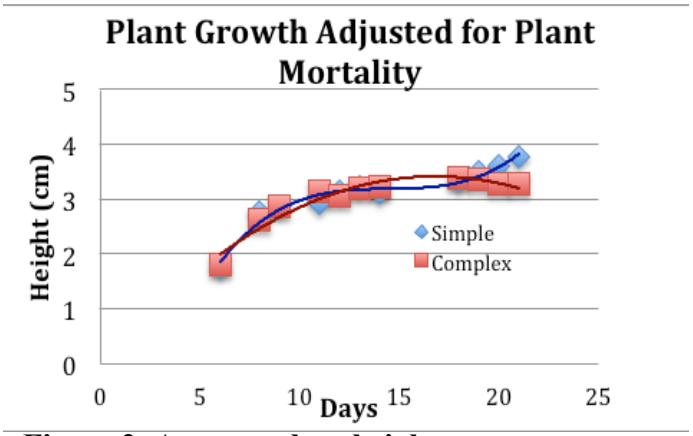


**Figure 2: Average plant height**

## DISCUSSION

Using my data from plant height readings and mortality numbers, I carried out two data analyses. For the plant height readings, I used a T-Test for each day's plant heights, and for mortality numbers, I used a chi-square analysis with one degree of freedom. Both of the statistical values showed that the results were not statistically different, and so we accept the alternate hypothesis that a complex fish diet compared to a simple fish diet has no effect on plant height growth.

Although we were not able to conclude the benefits of either a simple or complex diet, my aquaponics setup was shown to be viable for fish and reduce fish pollutants. The nitrate reading stayed at a constant 40 ppm showing that plants are indeed able to remove excess fish waste and pollutants from water.

Since the plants were getting a constant supply of water and nutrients, and 9 hours of light, the causes of plant mortality could have included bacterial/fungal infections, too much water and not enough aeration, or too little water due to evaporation. Other projects with aquaponics include testing quality of food given, different flow systems, and comparisons with conventional and hydroponic systems. With further research, aquaponics can become an even more efficient and sustainable farming technique for the future.

## ACKNOWLEDGEMENTS

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# Using Olive Oil to Improve the Effectiveness of Nepetalactone as an Insect Repellant

By Adriano Taglietti'14 and Charlie Wollmuth'14

## ABSTRACT

Nepetalactone is a compound present in the oil of *Nepeta cataria* (catnip) and has been previously proven to have an insect repellant effect. However, previous trials have shown that the effectiveness of the nepetalactone solution lasts no more than 5 minutes against fruit flies as a result of the compound's volatility. In this project, we combined steam distilled and purified oils from catnip with olive oil. Then, the solution was tested on fruit flies to see its relative effectiveness to a common synthetic repellant, DEET. Although the solution does not compare with DEET with regards to longevity yet, distilled nepetalactone mixed with olive oil has strengthened and extended the repellant effect of nepetalactone in these trials. With further research, nepetalactone can become a viable natural alternative to synthetic insect repellants in the future.

## INTRODUCTION

Nepetalactone is an organic compound that can be steam distilled from *Nepeta cataria*. It is a chemical of interest due to its ability to repel some insects. Nepetalactone is an environmentally friendly compound that has the potential to be used in place of

synthetic chemicals, such as DEET, which is known to irritate the skin and contaminate plants and soil (5). Repellents such as DEET and its predecessor TCEP have been found to be absorbed into the seeds, roots, and leaves of various crops (6). If nepetalactone is found to be an effective insect repellent, it could replace widely used harmful chemicals, such as DEET.

The major concern with nepetalactone's effectiveness as an insect repellent is its extremely high volatility. In the experiments on fruits, the nepetalactone evaporated and was no longer effective after five minutes. Before conducting the experience, we planned to mix the nepetalactone with olive oil, which is very rich in oleic acid. Oleic acid has been identified as a mosquito repellant; we plan to test the effectiveness of a combination of two insect repellants in one compound (2). We proposed that olive oil would lengthen and improve the repelling effects of nepetalactone on flies by reducing nepetalactone's rate of evaporation based on the previous group's research.

## RESULTS

The conclusion is that surfaces covered in nepetalactone repel fruit flies most effectively in comparison to those covered in oil, nepetalactone and oil, DEET, or water. In the seven fifteen minute tests on apple slices, water received 124 touches. Nepetalactone had an average touch-per-test score of two, and a mixture of nepetalactone and oil sustained an average of eight. DEET was even less effective, receiving an average of 116 fruit fly touches. Each experiment was run with 30-50 flies, and the touches of the flies per test for each substance was averaged to create our graph. While the concentrated, nepetalactone worked best; flies ventured near the slice after about 12 minutes, unlike the oil based version. Thus, our solution suggests an extended efficacy in need of further exploration in tests over longer periods of time.

| Solution | # of Tests | # of Touches | Average Touches |
|---|---|---|---|
| Nepetalactone | 4 | 8 | 2 |
| Oil | 1 | 4 | 4 |
| Nepetalactone & Oil | 4 | 32 | 8 |
| DEET | 3 | 248 | 116 |
| Water | 3 | 372 | 124 |

## DISCUSSION

The hypothesis we proposed is confirmed by our results. The Nepetalactone-oil solution had increased efficacy with respect to a pure Nepetalactone solution over a longer period of time, but the pure solution had a greater repellant effect in the short term (<10 minutes). The strength of these results was bolstered by the fact that we distilled our Nepetalactone in a fractional distillation chamber rather than a teakettle, and introduced oil, which was high in oleic acid, to the solution. Our results have been more consistent and distinctive than last year's (7), due to our solution's increased potency and stability. Regardless, Nepetalactone has been shown to be an effective bug repellent in a more convincing manner. In comparison to DEET, both the pure nepetalactone solution and the nepetalactone-olive oil solution were clearly more effective at repelling fruit flies within 15-minute periods. The high toxicity levels of synthetic insect repellants display a need for an organic repellent. Our oil-Nepetalactone solution results provide evidence that further experimentation with Nepetalactone-based solutions is required to explore the full potential of this chemical. Additionally, tests on human skin with the oil stabilizer should be performed to investigate the effectiveness of nepetalactone as a skin-based repellant rather than crop-based. If nepetalactone and stabilizing oil is shown to be effective on the skin as well, nepetalactone may become a viable alternative to DEET for repelling mosquitoes. Further refining the purity of the catnip oil solution in the distillation process and trying new stabilizing agents and repellent additives with the nepetalactone will continue to increase the effectiveness of the solution in the future.

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# Protein Tyrosine Phosphatase S (PTPRS) Is a Growth Suppressor in Lung Adenocarcinoma Cell Lines

By L. George Zachary'14, Alexandra Snyder, Logan Walsh, Timothy Chan

## ABSTRACT

Protein tyrosine phosphatase receptor S (PTPRS) is a tyrosine phosphatase receptor with a role in insulin regulation, and neuronal and urothelial system development that has hitherto been little studied in malignancy. Data from groups at Memorial Sloan-Kettering in head and neck squamous cell carcinoma found this gene to be frequently deleted, with preliminary data in lung adenocarcinoma cell lines to suggest that PTPRS might be a tumor suppressor gene. We created stable knock-down of PTPRS in 5 cell lines using 2 independent lentiviral shRNAs as compared to Scramble control. We also used a PTPRS-specific small molecule inhibitor to further dissect pathway alterations resulting from decreased PTPRS activity. Our study shows that inhibition of PTPRS increases growth and colony formation in a context-dependent manner. Furthermore, PTPRS knock-down (KD) desensitizes EGFR-mutant cell lines to erlotinib. In cells affected most dramatically by PTPRS inhibition, the mTOR pathway was implicated.

## INTRODUCTION

Growth suppressor receptors on cells are critical to natural and stable growth within our body. These suppressors regulate essential signaling pathways in the cell. These pathways have direct access to the nucleus and the DNA of each cell and are important in expressing and inhibiting the expression of certain genes on the chromosomes. Such receptors are involved in lung adenocarcinoma. PTPRS, protein tyrosine phosphatase receptor S, is predicted to be a growth suppressor receptor. It was determined through experiments at Memorial Sloan-Kettering that PTPRS is mutated or deleted in the minority of lung adenocarcinomas; however, its expression is decreased in the majority of cases. This was the incentive to study PTPRS. My mentor and I wanted to determine, through cell culture experiments, Western Blots, and phosphokinase arrays, if PTPRS is a growth suppressor in lung adenocarcinoma and, if so, which specific pathways it activates. These findings would also give researchers access to important data, allowing them to manufacture a drug to reactivate PTPRS in lung cancer patients and potentially save lives afflicted with this specific cancer.

## RESULTS

| Cell Line | Growth | Colony Formation |
|---|---|---|
| 11-18 | ↔ | ↓ |
| 827 | ↑ | ↑ |
| PC9 | ↔ | ↔ |
| 460 | ↑ | ↑ ↔ |
| 1975 | ↑ | ↑ |

**Figure A: Growth and Colony Formation of Cell Lines using shRNA inhibitors vs. control**

In Figure A, we concluded that PTPRS knock-down has cell line-dependent effects on growth velocity and colony formation. The chart shows changes in both parameters using 2 shRNAs to PTPRS (shPTPRS4 and shPTPRS5) as compared with control in cell culture plates.

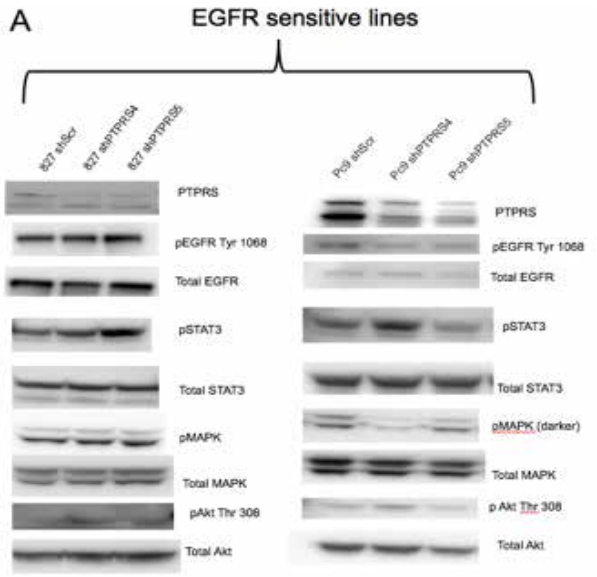



**Figure B: Western Blot of signaling pathways of 827 and PC9 cell lines**

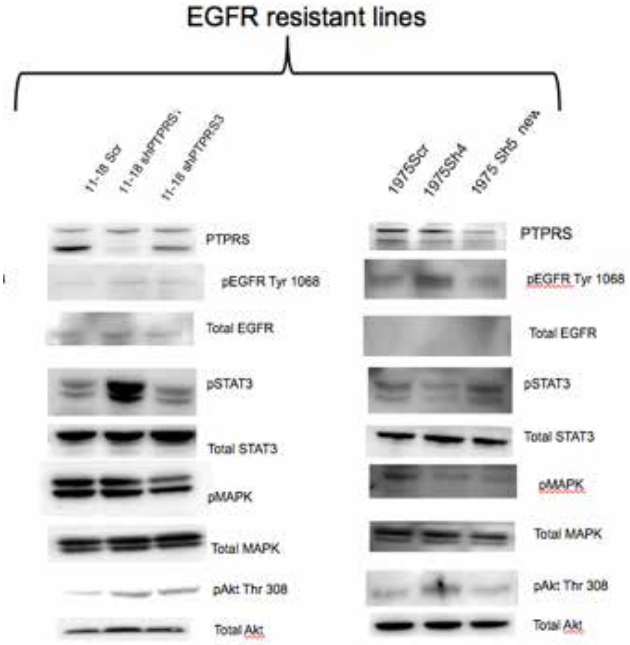



**Figure C: Western Blot of signaling pathways of 11-18 and 1975 cell lines**

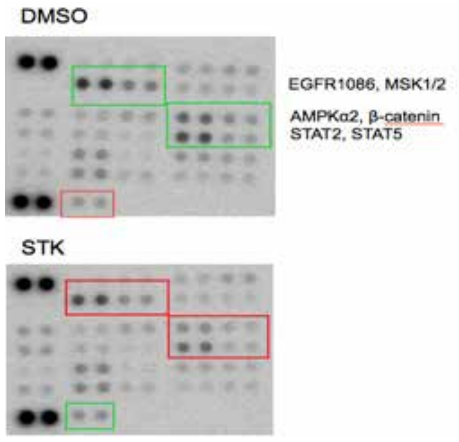



**Figure D: 1975 phospho-kinase array treated with STK and DMSO**

The 1975 cells exhibited the most pronounced effects in both parameters, having consistent increase in growth and colony formation. Figure B and C display the results of the western blots of the 827, 11-18, 1975, and PC9 cell lines. We determined that PTPRS knock down has cell line-dependent effects on signaling pathways. An increase in phospho-Akt is the most consistent change across all cell lines.

Figure D displays a phospho-kinase array, which shows a decreased expression of MSK 1/ 2, AMPK2, STAT2, and STAT5 when PTPRS was inhibited by an STK and an increase in PRAS40.

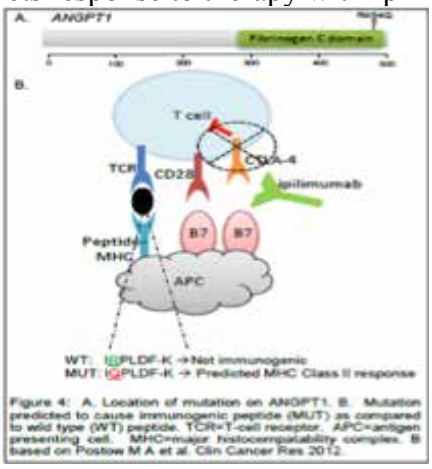
## DISCUSSION

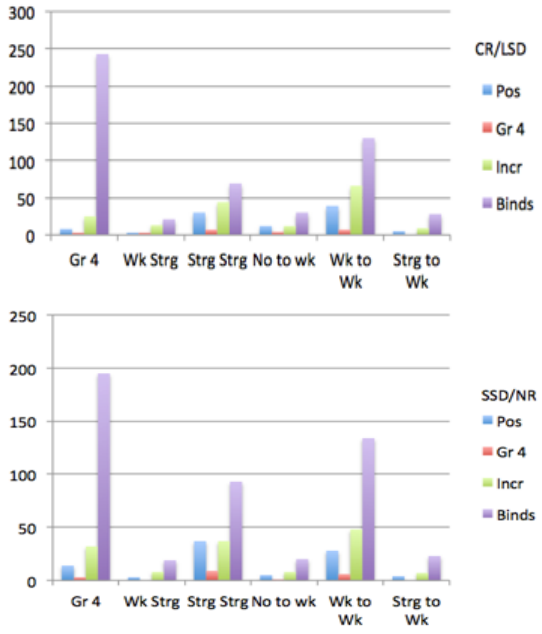
Our data highlights that PTPRS has growth-suppressive effects in a context-dependent manner. These effects display increases in growth and/or colony formation in cell lines. Furthermore, PTPRS knock down has context-dependent effects on cell signaling, with increased phospho-Akt seen in all EGFR-mutant lines. In the 11-18 and 827 cell lines, signaling appears to involve the AKT pathway, with putative binding partners for PTPRS that require validation.

The increased expression of AKT in these lines indicates the activation of AKT, a regulator of cell survival and growth. This evidence is well supported and demonstrates that PTPRS is, in fact, a growth suppressor receptor in lung adenocarcinoma. Future studies will include in vivo models of PTPRS effects on tumor growth and the development of targeted drug therapy.

## ADDITIONAL PROJECT

Melanoma is the most dangerous form of skin cancer. Immune blockade with drugs such as Ipilimumab has had significant effects on treatment. By comparing tumor DNA mutations vs. normal DNA we are investigating whether the tumor's mutational landscape predicts response to therapy with Ipilimumab.



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