Dedication

The Seventh Volume of the Saddleback Journal of Biology
is dedicated to
Dr. Steven Wickler

“Wick” was one of Professor Steve Teh’s Masters professors at California State Polytechnic University, Pomona. Dr. Wickler passed away on May 26, 2007. He was a graduate of the University of California, Riverside and held a Ph.D. from the University of Michigan and a DVM from the University of California, Davis. He was the University Veterinarian and Director of Lab Animal Facilities and Equine Research at the California State Polytechnic University, Pomona. He was the recipient of numerous awards and honoraria, including the 1999-2001 Teacher in the College of Agriculture. He authored 58 peer-reviewed papers, 32 symposia articles and 124 professional presentations. During his tenure he generated $3.4 million in external funding to support research by graduate and undergraduate students. His passion for teaching was reflected by his stewardship of nearly 70 graduate students working on their Masters degree in science. He also served a large number of students working toward their doctorates in veterinary medicine.

Dr. Wickler is survived by his wife Nicole, daughter Kaitlyn and son Eric. He is remembered and missed by his countless graduate and undergraduate students whose lives he touched in so many ways. To further the success of students in the sciences, the Steven J. Wickler Scholarship was set up in his name at California State Polytechnic University, Pomona.
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The Effect of Proanthocyanidins on Bacterial Growth

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Proanthocyanidins are flavonoid molecules found in many plants. They are believed to have antibacterial properties and increase longevity. This study tested the hypothesis that grape seed proanthocyanidins inhibit bacterial growth. Using the Kirby-Bauer disk-diffusion method, 24 nutrient agar plates were used to test the effects on gram-positive and gram-negative bacteria, *Escherichia coli* and *Streptococcus mutans*. The proanthocyanidins were placed on the agar in 0.1 g/200 mL, 0.2 g/200 mL, 0.3 g/200 mL, and 1.8 g/200 mL concentrations, as well as in powder form. The plates were then incubated for 48 hours at 37°C. No inhibition was observed for any of the concentrations with either bacterium.

Introduction

Many commercial sources claim grape seed extract supplements that contain oligomeric proanthocyanidins may be responsible for fighting bacteria and increasing longevity. Proanthocyanidins are molecules of the flavonoid family known mostly for their antioxidant properties and potential health-promoting effects (Wu, et al., 2004). Proanthocyanidins can be found in the skin and seeds of red grapes, cranberries, tea, tree bark, peanuts and a variety of plants where it is believed that they serve to protect against microbial pathogens (Corder, et al., 2006). Each source contains proanthocyanidins of different molecular structures with different biological effects (Dixon, et al., 2005). Previous studies have shown proanthocyanidins from green tea inhibit the growth of both gram-positive and gram-negative bacteria (Ikigai, et al., 1992). Other studies have shown that proanthocyanidins from cranberries alter the lipid bilayer of the bacteria, which eliminates the bacteria’s adhesive properties (Howell, et al., 2005; Foo, et al., 2000).

Because of the untested claims that grape seed extract kills bacteria, we will be testing the antibacterial effects of grape seed proanthocyanidins on gram-positive and gram-negative bacteria. One study suggests that proanthocyanidins have different effects on different bacteria types. Gram-positive cell walls have only one membrane, and gram-negative cell walls have two (Ikigai, et al., 1992). This double membrane gives the gram-negative bacteria better protection, making them more resistant to antibiotics and other compounds with antibacterial properties than gram-positive bacteria (Avison, 2005). If the proanthocyanidins from grape seeds work on the membrane walls of the bacteria in a similar way, gram-negative bacteria would be more resilient to them. In response to these claims of grape seed extract killing bacteria, this experiment will test the hypothesis that grape seed extract will inhibit the growth of both bacteria types, with a larger effect on gram-positive bacteria.

Materials and Methods

The antibacterial activity of the proanthocyanidins was determined using the Kirby-Bauer disk-diffusion method (Bauer, et al., 1966). This experiment required a total of 24 nutrient agar plates. Twelve plates were used to test the effect of the proanthocyanidins on gram-negative *Escherichia coli* and the remaining 12 were used to test its effects on gram-positive *Streptococcus mutans*. The diluted bacteria were evenly spread on the agar in a sterile fashion. The powder oligomeric proanthocyanidins were obtained from Natural Factors (Coquitlam, British Columbia, Canada) and claimed at least 95% purity. They were dissolved in sterile deionized water to prepare four solutions at different concentrations. These concentrations were 0.1 g/200 mL, 0.2 g/200 mL, and 0.3 g/200 mL. Sterile deionized water was used for the control. For each plate of agar, four sterile 6.0 mm diameter filter paper disks were dipped in one of the solutions and evenly placed on the agar. For each solution and the control, three plates were made for each bacteria type. The plates were all incubated at 37°C for 48 hours. After being incubated, the diameter of the zone of inhibition around each disk was measured in millimeters.

Proanthocyanidins in a concentration of 1.8 g/200 mL and 2.0 g in powder form were also used. A sterile filter paper disk was dipped in the 1.8 g/200 mL solution and placed in the center of six *E. coli* plates and six *S. mutans* plates. On six additional plates of each type of bacteria, 1.0 g of
proanthocyanidin powder was placed directly on the agar. These plates were incubated at 37°C for 48 hours and the zone of inhibition was measured.

Results
At the end of the two days of incubation, no inhibition was detected. With both bacteria types, the bacteria grew contiguous to the filter paper discs. All concentrations, as well as the powder form, of proanthocyanidins displayed the same results of no inhibition.

Discussion
This experiment indicates that the oligomeric proanthocyanidins found in grape seeds do not inhibit bacteria growth. With the vast variations within this family of flavonoids, it is hardly surprising that one of these forms lacks the antibacterial properties seen in many other proanthocyanidins. This common claim of antibacterial effects in grape seed proanthocyanidins may likely be due to a misunderstanding of proanthocyanidins’ variations in physical properties.

Typically, suppliers of these supplements will cite the potency of the proanthocyanidins from different sources to claim superiority while drawing no attention to the individual properties of the molecules. It cannot be assumed that all proanthocyanidins share the same properties. Compounding this issue further, proanthocyanidins may be classed as either oligomeric or polymeric; the oligomeric form has the most beneficial properties (Dixon, et al., 2005). Oligomeric proanthocyanidins are clusters of less than six identical molecules bonded to one another, while polymeric proanthocyanidins are six or more and are usually referred to as condensed tannins (Wu, et al., 2004). The proanthocyanidins obtained for this experiment claim to contain at least 95% oligomeric proanthocyanidins; however this cannot be a guaranteed analysis.

Although these results suggest that grape seed oligomeric proanthocyanidins do not inhibit the growth of bacteria, it is possible that it has other antibacterial properties beyond the scope of this experiment. Instead of killing the bacteria, it may alter the lipid bilayer and eliminate the adhesive properties of the bacteria, similar to the effects of the cranberry proanthocyanidin (Avison, 2005).

To ensure purity of the proanthocyanidins in future experiments, researchers should personally extract the oligomeric proanthocyanidin in the laboratory. It would be beneficial to also test the molecule’s effect on humans. This may be done by monitoring the output of bacteria in the urine after ingesting the proanthocyanidins. More research needs to be done to investigate the potentials of the various types of oligomeric proanthocyanidins.

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Lactate Threshold and Correlation to Recovery Rate in Male Marines

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As a result of lack of oxygen during strenuous activity, the body converts the pyruvate from glycolysis into lactic acid. The bloodstream picks up the converted form of lactic acid, lactate, and delivers it to the liver where it is converted back to pyruvate in order to continue with ATP production by cellular respiration. Lactate threshold is the point in anaerobic activity when lactate begins to accumulate in the blood faster than it is being metabolized. After intense activity, the body is able to convert the lactate back to pyruvate and remove it from the bloodstream over a period of time, known as the recovery process. It was predicted that there would be a significant correlation between the lactate threshold and rate of recovery. To determine this, blood lactate samples were collected from male marines. Mean value for the lactate threshold was \(0.0222 \pm 0.00229\) mM/W (±SEM, n=6). Mean recovery rate was \(0.024 \pm 0.00214\) mM/min. (±SEM, n=6). No correlation was found between the lactate threshold and recovery rate (\(R^2=0.007\)).

Introduction

When oxygen is not present immediately after glycolysis, the end result is lactate (the ionized form of lactic acid). Lactate is formed by fermentation, a process of removing hydrogen ions from pyruvate, resulting in the formation of \(\text{NAD}^+\) (nicotinamide adenine dinucleotide) (Brooks et al., 1999). During intense activity when not enough oxygen is being taken in, the body begins to accumulate lactate through lactic fermentation. Although the blood always has some amount of lactate in it, levels do not build up in great quantities unless the lactate threshold is reached. In anaerobic activity, the lactate threshold is the point at which the lack of oxygen begins to cause a more intense accumulation of lactate in the cells and blood (Davis et al., 2007). This buildup can be detected in the bloodstream. Once in the blood, 65% of the lactate is converted back to pyruvate by lactate dehydrogenase (LDH) to be used to synthesize ATP in the rest of the respiration process (Tomlin and Wenger, 2001).

Once the body has stopped intense exercise, a recovery period begins where the lactate accumulated is converted back to pyruvate. Full recovery is noted when blood lactate levels have returned to what they were at rest; this process varies in length between a few minutes to a few hours (Gaesser and Brooks, 1984). It has been shown that lactate recovery rate is an indicator of endurance training (Pelayo et al., 1996). It has also been established that endurance training can affect lactate threshold (Sjodin and Karlsson, 1981). Since endurance training is a factor in both studies, it was thought that a correlation between the two values might be possible.

In order to strengthen the evidence for any correlation in lactate threshold (LT) and recovery rate (RR), the subjects in this study were chosen for their similarity in cardiovascular training. If a correlation was found within a group of individuals training at the same standard, then evidence would indicate a correlation should exist between less fit individuals and those who are at a higher state of aerobic fitness. This experiment was designed to determine whether or not a significant correlation exists between the LT and the RR within a group of physically fit individuals.

Materials and Methods

Six male marines from Marine Corps Base, Camp Pendleton, volunteered to participate in an experiment to determine if a correlation between lactate threshold and lactate recovery rate exists. The subjects ranged in age from 22 to 32 years of age and were all training at the same level of cardiovascular fitness.

All six subjects were tested on the morning of March 12, 2009, between 7:30 a.m. and 10:30 a.m. The testing was conducted at the Camp Taleza Fitness Center at Camp Pendleton. Located on-site were two Nautilus NR 3000 Recumbent Bicycle Ergometers, both of which were calibrated according to the manufacturer’s specifications prior to the testing date. Testing supplies were provided by Saddleback College Department of Biology, and
included Polar T31 heart rate monitors and Lactate Scout portable testers. Both Lactate Scouts were calibrated before the commencement of the experiment to ensure accuracy.

At the start of the experiment, the subjects were fitted with the heart rate monitors and observed for two minutes to determine the at-rest heart rate. Then the level of increasing heart rate was calculated by the formula:

\[
\frac{[220 - \text{age}] \times 0.9}{\text{at-rest heart rate}}
\]

The direct method of measuring lactate threshold can only be obtained when incremental testing of blood lactate is done (Candotti et al., 2008). The inclusion of this formula ensured that the subject would reach anaerobic exercise by the end of the timed portion, allowing for the correct determination of the lactate threshold. At the start of the exercise, the subject’s left ring finger was cleaned with an alcohol swab, air-dried, and pricked to obtain a drop of blood to determine the baseline lactate level. The hand being used was then placed in a heating pad in order to increase the circulation of blood to the fingers. The individuals then began to exercise for two minutes at their predetermined at-rest heart rate, plus the increment of increase indicated by the previous calculation. This addition was made every two minutes, incrementally for 14 minutes. Thirty seconds before the end of each two-minute interval, the lactate level was again obtained while the subjects continued to exercise. In addition, the power output in the form of watts was noted for each increase in rate. Final readings were taken at 90% of the subject’s maximum heart rate.

Once the lactate threshold data had been collected, the individuals were instructed to remain inactive. Lactate readings were obtained every 15 minutes for a total of 60 minutes. This method was adapted from a previous experiment performed by Bauer, et al. (2004), at the University Hospital of Munich.

In the analysis of the data, Microsoft Excel was used to calculate all values that would be significant. The accumulation of lactate is essentially a linear relationship when plotted against work rate (Beaver et al., 1985). The results for each individual were calculated as log functions and placed in a scatter plot to determine the LT. The values are calculated as common logarithm functions in order to obtain a best fit line that is not as curvilinear (Davis et al., 2007). The linear best-fit line was established using the least square method and extrapolated to intersect and determine the lactate threshold. Also, the LT was determined by dividing the lactate concentration by the work rate of the individual and plotted against the time increased during the exercise portion of the project. This method was not discussed within any of the literature found but yielded the same value for the LT. Lactate recovery rate was then calculated using the exponential decay formula and the points plotted in Microsoft Excel. Finally, the lactate threshold and the recovery rate were plotted to determine if there was a linear correlation between the two values. In order to affirm a correlation, the R² value should be equal to one.

**Results**

Analysis was first performed on the lactate thresholds using log of lactate to log of watts inspection method (Figure 1). Mean log of lactate was 0.5352 ± 0.033 mM (±SEM, n=6), while the mean log of watts was 2.193 ± 0.0178 W (±SEM, n=6). These numbers were then used to obtain the mean lactate threshold. Mean value for the lactate thresholds was 0.0222 ± 0.00229 mM ⋅ W⁻¹ (±SEM, n=6). For further analysis purposes, the lactate threshold was graphed versus weight of each individual and found not to have a significant correlation (R²=0.4023) (Figure 2). Next, the recovery rates were calculated using exponential decay graphs (Figure 3). Mean recovery rate was found to be 0.024 ± 0.00214 mM⋅min⁻¹ (±SE, n=6). A one-way ANOVA for uncorrelated samples was run on the recovery rates, producing a significant difference (p=0.0001). Completion of the Bonferroni Dunn post hoc test showed significant differences between start and end values of the data.

The LT was then plotted versus the RR and there was found to be no significant correlation between the two values (R²=0.0075) (Figure 4).

**Figure 1.** Scatter plot with trend line of log blood lactate concentration versus log work rate for subject AL. Lactate threshold for AL was reported as log of watts equal to 2.147, and the log of lactate concentration was equal to 0.577.
Discussion

Our results indicate that no significant correlation exists between lactate threshold and recovery rate. A possible explanation and alternate ideas can be addressed using the experimental data.

Population Variation

Within the experiment, the subjects were chosen from an unrepresentative small population. All the participants had the same physical training regimen and workout intensity. When deciding the subjects to be used in the experiment, the conformity within the group was selected to strengthen the evidence that the correlation between RR and LT did exist. With the population sample being reduced to individuals that were all at the same level of physical fitness, the correlation among the individual RR and LT could have been hindered. Since LT is affected by the amount and type of exercise (Plowman and Smith, 2007), the experiment could have shown a correlation between groups that had greater differences in daily physical activity.

Lactate as Fuel

It is expected that during intense exercise, blood lactate concentration accumulates with the increase of power output (Candotti et al., 2008), wherein blood lactate levels can be collected and analyzed to find the LT. In the results from this study, specifically Figure 5, the idea of lactate as a fuel can be sufficiently analyzed.

Lactate as a fuel is by no means a new idea. Cell-to-cell lactate shuttles that transport lactate are found in skeletal muscle, where the lactate is said to be mostly consumed as an oxidative fuel (Brooks et al., 1999; Gladden, 2008; Van Hall, 2000). Brooks’ studies also touch on the ability of the human body to reuse lactate specifically during intense exercise (1999). This information, coupled with the idea that
the lactate threshold is the point at which the lack of oxygen begins to cause a more intense accumulation of lactate in the cells and blood (Davis et al., 2007), was effectively traced when the lactate concentration per watt produced (mM-W⁻¹) was plotted against time (min).

In Figure 5, as the work rate increases over time, the amount of lactate per watt being produced decreases, indicating that the body is using the lactate as fuel reserves to continue production of ATP as oxygen is depleted. At a certain point in the graph, the blood lactate per watt begins to increase as time increases. The point where this trend begins is effectively the LT. The points thereafter indicate that the body is accumulating lactate faster than it can clear or use it. The LT point is affirmed by taking the log of lactate and the log of watts for the LT and converting them back to rational numbers. Then the two numbers were divided resulting in the same value as on the graph in Figure 5.

This idea continues to be supported by the data when examining Figure 4, the exponential decay of lactate (mM) against time (min). The more aerobically fit are seen to have a superior lactate-recovery ability to those who are not aerobically fit (Tomlin and Wenger, 2001). As the graph effectively indicates, lactate did not remain at high levels in the bloodstream, but was rather recovered by the body over a period of time by LDH. Figure 5 graphically supports the idea of the role of lactate being used as a fuel during intense exercise.

Lactate Threshold Versus Body Mass

When LT was graphed versus body mass, there was no significant correlation. However, the R² value of 0.4023 did indicate that there could be a correlation found with further testing. When these values were graphed, it seemed to indicate that there was an increase in LT as body mass decreases. The LT is affected by the rate of gas exchange in individuals (Davis, et al., 1997). It could be possible that smaller subjects are able to transport the oxygen needed for ATP production faster than larger subjects can.

Lactate production due to the absence of oxygen is affected by physiological and physical differences in males and females, such as a larger lung capacity, also affecting the lactate threshold in individuals (Schwartz et al., 1988). In the experiment, the lung capacity was not measured and therefore it cannot be used as a prediction of the LT. If it could be shown that the larger subjects also had larger lung capacity, then a correlation to the LT could be ascertained. However, this would seemingly lead to a different conclusion than what was shown by the graph in Figure 2. Further testing between the LT and body mass is needed to determine if a correlation is present.

Literature Cited


The Effect of Concentration of $\textit{Rhizobium}$ Bacteria on the Growth of Alfalfa Sprouts

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$\textit{Rhizobium}$ is a soil bacterium that functions as the dominant symbiotic fixer of nitrogen. It can infect the roots of legumes, resulting in the development of nodules where nitrogen fixation occurs. The effectiveness of $\textit{Rhizobium}$ bacteria on plant growth was compared at low and high inoculations. It was expected that alfalfa seeds grown at a high inoculation would yield a greater amount of plant growth than seeds grown at a low inoculation with $\textit{Rhizobium}$ bacteria. The samples in the control group were found to weigh an average of 0.0164 ± 0.00132 grams (±S.E.M.), n=10. The samples in the low inoculation group weighed an average of 0.0147 ± 0.00122 grams (±S.E.M.), n=10. The samples in the high inoculation group weighed an average of 0.0256 ± 0.00323 grams (±S.E.M.), n=10. Significant differences were found between the control and high inoculation groups and between the low and high inoculation groups (ANOVA, p=0.002, with Bonferroni correction post hoc). Nodules were found on each condition: control had a mean value of 2.90 ± 0.623 nodules (±S.E.M.), low inoculation 1.20 ± 0.337 nodules (±S.E.M.), and high inoculation 1.20 ± 0.573 nodules (±S.E.M.), n=10. The experiment supported the hypothesis that high inoculation of alfalfa seeds would produce a larger amount of plant growth than alfalfa seeds grown at a low inoculation.

Introduction

Nitrogen is an important compound used for plant growth. Atmospheric nitrogen, $N_2$, is the most abundant gaseous compound. Nitrogen must be reduced to $NH_3$ by prokaryotic organisms before plants can use it. $\textit{Rhizobium}$ forms an association with legume plants and changes the root structure by adding nodules containing the soil bacteria that fix nitrogen. This symbiotic relationship allows the nodules to obtain carbohydrates from the vascular tissue of the roots. Legumes, like alfalfa, can produce enough ammonia with nodules to fulfill all the nitrogen needs of the plant (Erker and Brick, 2006). Inoculating legumes with $\textit{Rhizobium}$ bacteria has been found to increase plant growth. Rupela and Sudarshana (1990) found that $\textit{Rhizobium}$ inoculation of chickpea increased plant growth and dry matter after 20 and 51 days of growing. Some $\textit{Rhizobium}$ strains, even of the same species, can be more effective in yielding a greater amount of plant...
growth. Species differentiation in the genus *Rhizobium* are classified by the particular legume(s) they infect (Bohlool and Schmidt, 1974). The *Rhizobium* bacteria used were intended for alfalfa plants. Guar, Sen, and Subba Rao (1979) found that *Rhizobium* synthesis was better obtained by double inoculation. It was hypothesized that alfalfa seeds grown at high inoculation would yield a greater amount of plant growth than seeds grown at low inoculation with *Rhizobium* bacteria.

**Materials and Methods**

Alfalfa seedlings were grown in sterilized planting soil in one of three conditions: without inoculation, after low inoculation, and after high inoculation of *Rhizobium* bacteria. Two-thirds of a cup of seeds and water were mixed and left to sit for a little over seven hours. The soil was sterilized in a drying oven at 149°C for 20 minutes. It remained in the oven until it was used for planting to insure sterilization. After seven hours, the two-thirds cup of seeds was divided into three groups, each with two-ninths of a cup. The sterilized soil was removed from the oven and put into three aluminum-baking pans. Three tablespoons of alfalfa seeds were planted into three rows in the control pan. Another two-ninths of seeds and five tablespoons of *Rhizobium* were mixed together for the low group; three tablespoons of this were planted in three rows. The last two-ninths of seeds were mixed with 15 tablespoons of *Rhizobium* for the high group; three tablespoons of this were planted in three rows. The plants were kept in the greenhouse at Saddleback College in Mission Viejo, California. The seeds were watered equally every four to five days as needed. According to Braun (2002), good nodule formation takes roughly four weeks. At the end of eight weeks, the ten largest plants from each group were selected. The number of nodules on each plant was recorded and weight of the plants determined plant growth. The plants were weighed and logged before and after drying. To ensure water weight was not a factor in the results, the plants were dried in an oven at 79°C for 15 minutes till constant weight.

**Results**

An ANOVA (p=0.04) on the number of nodules on the samples indicated significance. However, no significance was found using a Bonferroni correction post hoc (Figure 1). Though there did appear to be large differences between the samples, the control had a mean value of 2.90 ± 0.623 nodules (±S.E.M.), low inoculation 1.20 ± 0.337 nodules (±S.E.M.), and high inoculation 1.20 ± 0.573 nodules (±S.E.M.), n=10. The mean value for the number of nodules and the dry weight in grams of the plants were recorded (Table 1). An ANOVA (p=0.002) indicated significance and a Bonferroni correction post hoc showed significance between the dry weight of the low and high inoculation groups and between the dry weight of the control and high experimental groups. Control had an average of 0.0164 ± 0.00132 grams (±S.E.M.), low inoculation had an average of 0.0147 ± 0.00122 grams (±S.E.M.), and high inoculation weighed an average of 0.0256 ± 0.00323 grams (±S.E.M.), n=10 (Figure 2).

![Figure One](image)

**Figure One.** Average quantity of nodules per sample. Error bars indicate standard error of the mean. Control was found to contain an average of 2.90 ± 0.623 nodules (±S.E.M.), low inoculation 1.20 ± 0.337 nodules (±S.E.M.), and high inoculation 1.20 ± 0.573 nodules (±S.E.M.), n=10.

<table>
<thead>
<tr>
<th>Mean value for:</th>
<th>Control</th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
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<tr>
<td>Number of Nodules</td>
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<td>1.20</td>
<td>1.20</td>
</tr>
<tr>
<td>Dry Weight (g)</td>
<td>0.0164</td>
<td>0.0147</td>
<td>0.0256</td>
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</table>

**Table One.** Mean values for the number of nodules and the dried constant weight in grams for the control, low inoculation, and high inoculation groups.

**Discussion**

The experiment would appear to be successful since a significant difference between the low and high inoculation experimental groups was found. However, there were some unaccounted for variables that arose during the course of the experiment. Mold was found two and a half weeks into the experiment. Although most of the mold was removed, it was nearly impossible to remove it all. Also, the control group had more nodules than both the low and high inoculation group, which should not have occurred. If *Rhizobium* inoculation is responsible for the development of nodules, which in turn yields greater
plant growth, it would be expected that the high and low groups would have a significant difference between the numbers of nodules. A possible explanation for the control group having nodules is that some small-seeded legumes, like alfalfa, are often preinoculated upon purchase (Erker and Brick, 2006). It cannot be ruled out that the alfalfa seedlings purchased weren’t already inoculated, though the package did not suggest this. Some past researchers have found that rhizobial inoculant was ineffective in consistently improving seedling herbage accumulation when compared to no inoculant (Seguin et al., 2001). Due to a few unexpected results, further experiments should be conducted on the effect of different concentrations of *Rhizobium* bacteria on the growth of legumes, such as alfalfa seeds.

**Figure Two.** Dry weight means for control, low inoculation, and high inoculation experimental groups. Error bars indicate standard error of the mean. Control had an average of 0.0164 ± 0.00132 grams (±S.E.M.), low inoculation had an average of 0.0147 ± 0.00122 grams (±S.E.M.), and high inoculation weighed an average of 0.0256 ± 0.00323 grams (±S.E.M.), n=10.

**Acknowledgements**

We would like to thank Dr. Huntley for his insightful suggestions and support in our experiment.

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The Relationship of Bilateral Facial Symmetry and Desirability Based on Body Odor in Male Humans (*Homo sapiens*)

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Bilateral facial symmetry in humans has been associated with desirability. The same is true for body odor, which plays a role in mate selection. This study tests the correlation between facial symmetry and body odor on desirability and thus selection of potential mates. In the study, facial symmetry of male subjects (n=7) was measured and these subjects were requested to wear a t-shirt for a period of twenty-four hours without taking a shower or using external fragrances. The female testers (n=38) smelled each of the t-shirts and chose the one they found most desirable based strictly on body odor. Based on the results, one subject stood out with having the greatest facial symmetry as well as the highest selection count. These results showed a significant difference between the male subjects. Thus a positive relationship was found between greater bilateral facial symmetry, body odor, and desirability.

**Introduction**

Selection of suitable mates by either males or females is the driving force in sexual evolution due to natural selection. Studies conducted on humans (*Homo sapiens*) on the relationship between bilateral facial symmetry and mate selection indicate that men with greater symmetry are involved in more sexual activity than men with less symmetry (Penton-Voak, et al., 2001). Greater bilateral facial symmetry is also correlated with the production of pleasurable pheromones and mate selection as women in the most fertile phase of the menstrual cycle choose the odor of more physically symmetrical men (Rikowski and Grammer, 1999). The main objective of this study is to find a correlation between facial symmetry and the production of favorable body odor. Based on this relationship, males with greater bilateral facial symmetry should produce more desirable body odor, and thus increasing their chance to be chosen as a potential mate.

**Materials and Methods**

This experiment was conducted in the Biological Sciences building at Saddleback College throughout March and April, 2009. On March 23, 2009, female Saddleback College students (n=38, age range 18-40 years) signed up for the olfactory response test. Thirty white t-shirts were washed in odorless detergent and then sealed in zip-lock bags while wearing clean latex gloves to prevent contamination from external odors.

On March 31, 2009, male Saddleback College students (n=7, age range age range 18-25) signed up for the pheromone production test. On the same day, facial pictures of each subject were taken and the pre-washed t-shirts were distributed to each of them. Subjects were instructed to wear the shirts over a twenty-four hour period starting the following morning to ensure sufficient absorption of the subject’s own natural body scent. Over the twenty-four hour period subjects were requested not to use any deodorant, cologne, body wash, aftershave or any other scented product. This ensured the t-shirts did not absorb scent from an external influence.

On the morning of April 2, 2009, the subjects were to take off the t-shirts and seal them in the provided zip-lock bags, which were collected the same afternoon. Upon collection, the t-shirts were sealed once more in Tupperware to be used later for the olfactory response test. On the same day, measurements were conducted on the headshots taken two days prior. Three different measurements were collected using the program ImageJ (1997-2009 National Institute of Health, USA). The first measurement originated from between the eyebrows and ended at the outer tip of the left and right eye (measurement S1), the second measurement was taken from the tip of the nose and extended horizontally to the bottom of the left and right earlobe (measurement S2), and the final measurement was taken from the cleft of the upper lip to the left and right tip of the mouth (measurement S3), as shown in Figure 1.
On April 13, 2009, the olfactory test was set up in SM 246 of Saddleback College and 15 female testers, from the total 38, were asked to individually examine the scent of each shirt. The females were notified that each shirt was worn by a mating male and they were to select a shirt based upon scent for the male they would most likely find pleasurable to mate with. The mate choice of the testers was recorded individually and throughout the scent test the t-shirts were randomized to insure the female testers did not influence each other’s decisions. An equivalent procedure was conducted two days following the initial data collection with the remaining 23 female testers.

The left and right side measurements taken from the headshots were recorded into MS Excel (1985-2009 Microsoft Corporation). Using the respective left and right side measurements, a ratio was calculated to find the subject with the greatest bilateral facial symmetry. This was done for all three measurements and then an average of the three ratios was calculated for each subject. In the olfactory data, the number of t-shirts chosen for each subject was counted and totaled. Based upon the total, a percentage was calculated by dividing the count of each subject by 38, thus giving the probability in selection of each subject. These percentages were compared to a calculated theoretical probability each subject had in being selected. Using the experimental and theoretical percentages, a chi-squared contingency table was constructed.

The experimental percentages of each of the male subjects were compared to the theoretical percentage (14%) that each subject had in being chosen as a potential mate. The results of the Chi-squared show significance in the selection of male subject with the highest count among other male subjects (p=0.001, Chi-squared contingency table).

**Results**

Ratio averages and selection count for the three facial symmetry measurements of each subject are shown in Table 1. In the data collected from the male subjects, one subject in particular has the highest selection count by the female testers and in addition, the male subject also has the greatest overall ratio average among the measurements of all male subjects as shown in Figure 2.

**Discussion**

In a limited sample size of male subjects, there was one subject who exhibited the greatest bilateral facial symmetry and also yielded the highest selection count among the female olfactory testers. Based on the results of this test, there is a significant difference between the subjects. In accordance with these results, a correlation can be deduced between greater bilateral facial symmetry and body odor. The choice of male subjects based solely on body odor may be related to the pheromones the males produced. The transfer of body odor in the form of pheromones is indeed relevant in mate choice even if the odor is dependent on a variety of factors (Rikowski and Grammer, 1999). In order to understand this in greater detail, further experiments must be conducted to find a correlation between pheromones and mate selection based on facial symmetry. Specific preferences for mate selection could imply that genetic traits, favorable hormone production, and external environmental cues influence the selection (Cornwell, et al., 2004).

Potential errors that could have occurred were from the female olfactory testers. Females respond differently to external olfactory cues during different periods of their menstrual cycle. In our experiment we do not have the females’ phase of menstrual cycle as a variable, thus our results do not account for this.
Table 1. Average ratio percentage and selection count of male subjects.

<table>
<thead>
<tr>
<th>Subject #</th>
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<th>Subject #</th>
<th>Subject #</th>
<th>Subject #</th>
<th>Subject #</th>
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<tr>
<td>Average Ratio (%)</td>
<td>95.02</td>
<td>92.12</td>
<td>94.97</td>
<td>92.36</td>
<td>95.87</td>
<td>90.98</td>
</tr>
<tr>
<td>Selection Count</td>
<td>2</td>
<td>4</td>
<td>0</td>
<td>5</td>
<td>17</td>
<td>4</td>
</tr>
</tbody>
</table>

In a study conducted previously, it has been found that females in their least fertile phase find intense smells to be unattractive, while contrarily, those same intense smells are deemed desirable to females in their most fertile phase of their menstrual cycle (Rikowski and Grammer, 1999). Therefore, by not recording the phase of the female’s menstrual cycle a variable might not have been counted for in the results.

As a modification to the experiment, one could separate the experiment into two trials between healthy and unhealthy subjects. It is probable that the unhealthy subjects would produce less desirable pheromones since their body would be more focused on fighting off infections in contrast to the healthy subjects. Another modification could be the use of female subjects instead of male subjects. This would test to see whether or not the results would be similar to the findings found in the male subjects. A last alteration could be to increase the sample size for both male subjects and female testers. In having larger samples of subjects and testers, error greatly decreases, as does the chance of coincidence, which would entail more accurate results.

In implementing various techniques of research to see the effect of symmetry on body odor, a broader understanding can be applied to supplement research conducted by deodorant and cologne companies. Even though symmetry alone does not determine mate selection for females, since other factors also influence their decisions, future research would be necessary to get a broader understanding in mate choice. (Scheib, Gangestad and Thornhill, 1999). With greater research in symmetry and its relationship with body odor and possible pheromone production, companies can enhance their products in ways that help ordinary individuals achieve greater sexual success with the opposite sex.

**Literature Cited**


Comparisons of Bacterial Growth in Desktop Keyboards and Toilet Seat Handles

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Recent research has revealed that computer keyboards can harbor more bacteria than a toilet seat which poses a health hazard and increases the chances of transmitting disease between computer users. In available studies testing the amount of bacteria in keyboards the last cleaning of the computer keyboards was unknown. Therefore, reasons behind the high amount of bacteria could be due to personal negligence instead of a keyboard’s enhanced ability to sustain bacteria. In this experiment the desktop computer keyboards (n=11) and toilet seat handles (n=11) that were found were only used by one person for the length of the experiment. They were cleaned at the same time with Clorox disinfectant wipes; samples were taken exactly 12 days later, where, after incubation, bacteria colonies were counted. Computer keyboards accumulated bacteria significantly faster, as shown by a mean bacteria colony count of 37.09±7.48 (± S.E.M.) and a mean bacteria colony count for toilet seat handles of 12.45±4.26 (± S.E.M.), rejecting the null hypothesis. There was a positive correlation between bacteria colony count on computer keyboards and toilet seat handles. On average, keyboards had an average of 4.05±0.57 (± S.E.M.) times more bacteria colonies than toilet seat handles shared by the same person. The higher the bacteria colony count on each person’s keyboard, the higher the count on their toilet seat handles. To find the reason behind the correlation, more research would need to be done.

Introduction

Recent census studies have revealed that more than half of households in the U.S. have computers due to lowering computer prices and a rising need for constant communication (Newburger, 2001). Along with the increasing proliferation of computers in our technologically emphasized society also comes a new health concern over whether computer keyboards are harboring harmful bacteria. U.S. scientists have actually linked the increase of computer technology in hospitals to the antibiotic-resistant methicillin-resistant Staphylococcus aureus (MRSA) (Fellowes and Kerstein, 2006) and research conducted at Northwestern Memorial Hospital has found that certain bacteria, such as MRSA and vancomycin-resistant Enterococcus faecium (VRE) are capable of prolonged survival and growth on computer keyboards, and can even spread to bare or gloved hands (Bates, 2005). The study by Fellowes and Kerstein (2006) actually found MRSA on ICU computer keyboards. Although these bacteria usually do not cause many problems in healthy people, except in hospitalized patients whose immune systems are compromised by other disease or illness, recent outbreaks of MRSA skin infections in otherwise healthy persons have raised concern among infectious disease experts. These findings might be due to the fact that bacteria thrive exceptionally on keyboards due to personal negligence, keyboard design, or both. One microbiologist has found keyboards in an office building with 150 times the recommended limit of bacteria (Goldsborough, 2008), which is considered a health hazard. It was also five times as much bacteria as a toilet seat swabbed at the same time (Goldsborough, 2008). Another study that tested the bacteria from many surfaces found that computer keyboards had the highest proportion of plates positive for bacteria at 63% on mannitol salt agar (Brooke et al., 2009). This research suggests that computer users in a community setting, such as at the school computer labs, where many people use the computer a day, might be more at risk of acquiring and transmitting harmful bacteria. The majority of studies done so far regarding the bacterial content of keyboards test those that have not been cleaned on a regular basis. They might have five times the bacteria as a toilet seat, but the toilet seats tested could be cleaned regularly. The main aim of this experiment was to find if keyboards accumulate bacteria faster than toilet seat handles if both are
cleaned thoroughly at the same time. It was predicted that the keyboards tested will have a significantly greater amount of bacteria than toilet seat handles.

**Materials and Methods**

All keyboards (n=11) and toilet seat handles (n=11) were cleaned thoroughly with Clorox disinfectant wipes twelve days prior to when samples were taken. Eleven subjects participated in the experiment, each person using one keyboard and one toilet each. During the twelve days neither keyboards nor toilets were used by more than one person, and none were cleaned. Five of the participants stated that they ate at their computer daily. Participants used their toilet an average of two times a day and their computers an average of three hours per day. Standard nutrient agar plates were made using sterile technique, and sterile swabs were used to take samples. Samples on each keyboard were taken in between the J and K keys. The average length of a key from the keyboard is 2cm, so a total of 4cm were swabbed on the sterile swab in between the keys, and therefore 4cm were swabbed on each toilet seat handle, 2cm on each side of the swab. After a sample was taken it was quickly smeared onto a standard nutrient agar plate in a zigzag motion. The twenty-two plates were labeled A-K and then incubated at 37 degrees Celsius for forty-eight hours. After incubation period ended the bacteria colonies were counted on each plate and then the data were analyzed. Results were compared using a one-tailed, unpaired t-test assuming unequal variances. Differences were considered significant at P<0.05; all data were expressed as a mean ± S.E.M.

**Results**

All plates were found positive for bacteria. The average bacteria colony number from the keyboards was significantly higher than the average bacteria colony number from the toilet seats, p=0.0057. The mean colony number from the keyboards was 37.1 ± 7.5 (± S.E.M.) and the mean from the toilet seats was 12.5 ± 4.3 (± S.E.M.) (Figure 1). There was also a significant difference of bacteria colonies between the keyboards where subjects reported eating food at (mean = 58.2) and keyboards where no food was eaten at (mean = 19.5), p = 0.0011, which could account for a much higher range of results (range = 70) compared to the range of bacteria colony count of the toilet seats (range = 49).

Figure 2 reveals a positive correlation (although not perfect correlation) from bacteria colony count on keyboards and toilet seat handles between subjects. On average, keyboards had an average of 4.05 ± 0.57 times more bacteria colonies than toilet seat handles shared by the same person. The trend shows that the higher the bacteria count on the toilet seat handles, the higher the bacteria count on the keyboard from one person congruent to other persons.

**Discussion**

Results were congruent with previous research testing bacteria on keyboards. After being disinfected the keyboards accumulated bacteria significantly faster than the toilet seat handles, which supported the hypothesis. Similar to research done by Brooke et al. (2009), there was more bacteria on the computer keyboard than other surfaces. Although there was a difference, the keyboards did not show 5 times the bacteria colony count of toilet seat handles as stated by Goldsborough (2008). The evidence supports
statements by Bates (2005) that bacteria can sustain survival and growth on keyboards easily.

The positive correlation between bacteria colony count on the computer keyboards and toilet seat handles could be due to multiple reasons, such as cause and effect, where toilet seat bacteria is transmitted to keyboards, where it grows more easily, or a third variable, such as the person using the toilet and keyboard is just dirtier in general. Consequently, the importance can not truly be measured by only counting the bacteria colonies. It might be beneficial to elaborate upon this research and test for the types of bacteria on the surfaces. Also, results would have been more accurate if bacteria colonies were counted from surfaces directly after being cleaned, so the percent increase could be found.

The main causes to the huge amounts of bacteria are unclear. This could be due to personal negligence, such as forgetting to clean the keyboard, or the keyboard design, or both. Eating lunch at the desk and filling the keyboard with crumbs encourages the growth of bacteria, as well as not washing hands after using the toilet. The design of keyboards creates small warm spaces safe from easy cleaning and disturbance. Computers quickly become magnets for airborne dust and bacteria-harboring dirt. Dust also settles in between the keys, which creates a moist environment for bacteria to thrive.

This experiment is significant since cleaning keyboards may not be a priority for most people. Research suggests that bacteria thrive on keyboards, and if the bacteria that survive are actually harmful (such as MRSA), it could pose huge problem in hospitals as computer technology is increased in close proximity to patients, such as stated by research done by Fellowes and Kerstein (2006). This experiment tested keyboards that had been recently cleaned and used by one person; therefore keyboards in a community setting, as in a computer lab or hospital, could be subject to much greater bacteria accumulation and consequently, transmittal.

**Literature Cited**


Effect of Water Temperature on Dive Response in Humans

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During this experiment the human dive response was tested to see if there was a significant difference between the heart rates of resting and being immersed in water. Secondly, the effect of water temperature was tested to see if there was any significant difference between submerged in warm and cold water. Only the first hypothesis was supported by this study. In eleven young healthy saddleback students, the mean heart rate (bpm) was found to be significantly different between resting and submerged in water, p = 4.83x10^-7. This illustrates that humans do in fact have a dive response. The percent drop in heart rate from resting to either cold or hot water did not provide any significant differences, p = 0.13. A two-tailed paired t-test was used for both of these calculations.

Introduction

The ability of prolonged dives without surfacing in marine animals has interested scientists for many years. In order to remain functional for extended periods without needing to obtain more oxygen requires a number of adaptations. These adaptations are collectively referred to as “dive response”, named by Paul Bert (Huntley, 2009, pers. Comm. 29 March). The dive response is characterized by respiratory, vascular, and cardiac responses prompted by breath-hold diving (Gooden, 1994). Tests of most other taxa have revealed that these responses are initiated (to dissimilar degrees) in a wide range of vertebrate types, including almost all birds and mammals. This will even occur in fish that are out of water, thus unable to obtain oxygen (Scholander, 1963; Hainsworth, 1981).

Of these responses, diving bradycardia is usually the first response to be given attention (Bron et al, 1966). Bradycardia is the slowing of heart rate, usually to about 60 bpm or lower, reduction of limb blood flow, and a gradual rise in the mean arterial blood pressure (Gooden, 1994). The bradycardia effect results from the increase in parasympathetic activity via the vagus nerves that inhibit the cardiac pacemaker. Limb vasoconstriction is due to acetylcholine being released by the sympathetic nerves, supplying the arteries in the calf and forearm (Finely et al, 1979; Fagius 1985). These responses are necessary to maintain and preserve the functions of the brain and the rest of the central nervous system. The peripheral vasoconstriction minimizes delivery of oxygen reserves to all organs except the brain and heart. Reduction of work by the heart due to bradycardia preserves most of the oxygen for the brain to use (Barman & Belzer, 1988).

Although there is clearly a dive response in humans, because we are not required to dive in everyday life there is a less pronounced quantitative response than natural marine divers such as seals (Gooden, 1994). It is known that direct contact of water on the face while diving is the greatest stimulus, this initiates a dive response. This could be due to heat lost from the face, therefore stimulating the cold receptors of the face (Parfrey & Sheehan, 1975). According to Gooden (1994), subjects who were immersed in warm water, 35°C, had an average heart rate deduction of only 11% compared to 41% in those immersed in cold water, 5°C. Therefore this study seeks to first determine if humans do have a dive response and determine if there is a significant difference between young fit individuals submerged in water opposed to resting. It is anticipated that not only will heart rates drop when submerged in water, but also that the percent dropped will be significantly different when submerged in cold water opposed to warm water.

Materials and Methods

Subjects

Electrocardiographs were taken of eleven healthy volunteers (n= 9), seven males and four females aged 17 – 24 (mean 22) years. All subjects gave their informal consent.

Electrocardiography

All eleven subjects were seated and connected to a Burdick EKG machine with three leads. The first
two leads were connected to the left and right upper chest, equal distances from center on the individuals body. The third lead was connected to the upper mid-chest. The skin was first cleaned with alcohol wipes to assist adhesion of leads; tape was also used. Subjects were then given a thirty second period to rest before the dive. This helped to calm them and was used to record resting heart rate. Subjects were then asked to take a breath and slowly submerge their face in a bucket of water. During the dive they were asked to remain as steady as possible so they would not disturb the recordings. Once subjects reached thirty seconds underwater (not all reached thirty seconds) they were asked to slowly move their heads out of the bucket. They were then permitted to dry their faces. All warm water temperatures were recorded from the subjects, and then all the cold water temperatures were recorded.

**Controlling water temperature**

To obtain warm water, hot water was first poured into the bucket straight from the sink. The temperature was then recorded with a thermometer. Cool water was then added until the desired temperature was reached, between 35°C and 42°C (mean 39°C). Between each subject, warm water was added as needed to maintain the desired temperature. To obtain cold water, cold water was poured into a bucket straight from the sink. Ice was then added to the bucket to bring the temperature down until the preferred temperature was reached, between 7°C and 17°C (mean 12°C). Between each subject, ice was added as needed to maintain the preferred temperature.

**Reading recordings from EKG**

Readings were taken of the thirty seconds of resting time and for as long as the subject could hold their breath underwater. Subjects who were able to reach 30 seconds had their number of beats multiplied by 2 to get bpm. Those that were unable to reach thirty seconds had their time counted then divided by 30 then multiplied by how many beats they had in that time. That number was then multiplied by 2 to get bpm.

**Results**

The mean resting heart rate of all eleven subjects was 85.63 ± 1.97 bpm (±S.E.M); this includes two reading from each individual. The mean dive response heart rate of all eleven subjects was 70.91 ± 2.12 bpm (± S.E.M) including both warm and cold water. A two – tailed paired t-test of two samples for means indicates that there is a significant difference between these two values, p = 4.83x10⁻⁷, (Figure 1).

The mean percent drop in heart rate from resting to submerged in cold water was 19.73 ± 3.01 bpm (± S.E.M), (N= 9). The mean percent drop from resting to hot water was 14.18 ± 3.13 bpm (± S.E.M), (n = 9). A two – tailed paired t-test of two samples for means indicates that there is not a significant difference between these two values, P = 0.13, (Figure 4).

**Figure 1.** Average heart rate of resting and submerged individuals. The average resting heart rate was 85.63 ± 1.97 bpm (±S.E.M), (n = 11). The average submerged heart rate was 70.91 ± 2.12 bpm (± S.E.M), (n = 11). Error bars indicate standard error. A two – tailed paired t-test of two samples for means indicates that there is a significant difference between these two values p = 4.83x10⁻⁷.

**Figure 2.** Mean drop in heart rate while submerged in cold and hot water. The mean percent drop from resting to cold water was 19.73 ± 3.01 bpm (± S.E.M), (N= 9). The mean percent drop from resting to warm water was 14.18 ± 3.13 bpm (± S.E.M), (n = 9). A two – tailed paired t-test of two samples for means indicates that there is not a significant difference between these two values, P = 0.13.
Discussion

The overall difference between resting and being immersed in water did produce a significant difference. This shows that there is a dive response that humans exhibit while holding their breath under water. The heart rates dramatically fell when under water. Although only four subjects out of the eleven actually showed a true bradycardia response, a fall in heart rate to about 60 bpm. This is actually a much greater percentage (36%) of individuals exhibiting bradycardia than other studies which observed about 18% (Arnold, 1985). This could have to do with the relative age of the subjects; it is believed that the older humans get, the more likely they are to not have a response (Gooden, 1994).

There was no significant difference found between the percent drop in heart rate from resting to submerged in either cold or warm water. This is interesting because it would appear that hitting cold water would shock the heat sensors of face more than warm water. Studies done on humans have shown that when completely immersed in very cold water, there is less damage done to the brain than when immersed in warm water (Barman and Belzer, 1988). This means that there was more oxygen available to the brain, therefore there would appear to be a greater dive response. The fact that ten of eleven subjects had a different resting heart rate before the dives into hot and cold water could possibly have caused this insensitivity. If it is possible to keep their resting heart rates uniform between each dive, perhaps a significant difference can be found.

It is expected that animals that dive regularly are going to have a more superior response than humans. Although most humans do not spend a majority of the time underwater, they do have a dive response. The magnitude of that response varies greatly and can be affected by a number of variables including; age, fear of water, time spent around water and individual health. Dive response also may not be an instinctual trait, but instead learned. Further studies on human dive response can shed light on how human dive response varies from individual to individual and other species.

Literature Cited


Escherichia coli is not Transported Through Roots to Leaves in Cabbage, Brassica oleracea

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Escherichia coli has become responsible for the sickness and death of many people around the world and dangerous strains of E. coli have been traced to spinach and lettuce plants. Much controversy surrounds exactly how contamination of the plants occur and how it leads to ingestion by humans causing sickness. The question is: do cabbage plants have a transport system to carry bacteria from their roots to their leaves? This is a concern because crops are being grown in potentially E. coli infected fertilizer, therefore making it possible for contamination to occur in the plant if there is indeed a transport system. In this experiment, cabbage plants were grown in water containing an E. coli bacterial concentration of 3.34x10^6 CFU/L. The E. coli used was transformed to express a green fluorescent protein and ampicillin resistance. After the plant’s roots were exposed to the contaminated water for forty-eight hours, there was no evidence of transport to the leaves. None of the plates from the experimental cabbage plant’s leaves showed any E. coli growth supporting the hypothesis being tested that cabbage plants do not contain a translocation system for E. coli bacteria.

Introduction
Animal and human manure have been used for many years to fertilize crops around the world. In the United States, an estimated 10.4 million dairy and feedlot cattle produce anywhere between 23.6 and 35.5 kg of manure daily (Andrews, et. al, 2004). It is not the manure used that causes concern but the E. coli concentrations found in the manure which are known to cause sickness. Previously conducted studies are often contradictory providing altering possibilities to how and if bacteria can be transported by plant systems. E. coli, specifically, is under question due to its ability to withstand cold temperatures and live longer on cover crops (Johannessen, 2005). One study was done on lettuce plants that were grown in E. coli contaminated fertilizer in which the plant was able to translocate the bacteria from its roots into the leaf tissue (Matthews, et. al, 2002). This is serious because the bacteria that enter the tissue of the leaves could not be killed before raw consumption. In contrast, another similarly conducted study was done in which no bacterial content was found in the leaves of lettuce plants after having been grown in contaminated fertilizer (Johannessen, 2004). We hypothesized that healthy cabbage plants would not be able to translocate the E. coli bacteria from the roots into the leaves.

Materials and Methods
Ten Brassica oleracea plants were purchased from the Home Depot Store in Laguna Niguel, California. These plants were stored in the greenhouse at Saddleback Community College, Mission Viejo, California, until all materials for the experiment were prepared. Thirty-two LB Miller Agar plates were created with ampicillin and arabinose added. The E. coli bacteria were then placed in the LB broth, and incubated at 36.6°C to promote growth.

The incubated LB broth was diluted with 1800mL of water, and in order to determine the concentration of bacteria, three different concentrations were spread on the plates. One mL of the broth was placed in a mason jar, filled with water, topped, and inverted to ensure mixing of the bacteria with the water. This was poured into a beaker and three plates of the three various bacterial concentrations were spread onto a total of nine plates and incubated for five days. The diluted concentrations were as follows: 1/100, 1/10000, 1/1000000. The concentration of bacteria used for the experiment was calculated to be 3.34x10^6 CFU/L.

A leaf was clipped from each plant and refrigerated in a plastic zip lock bag as the control group. The ten plants, in individual containers, were then set in a metal pan that was filled with the diluted bacterial broth solution. After five days, the plants
had soaked up all of the contaminated water and the leaves were cut off and individually bagged. Each plant’s leaves were blended with 50mL of water in sterile mason jars and 1mL of this solution was placed in an individual test tube containing LB broth. Each leaf homogeny was tested twice in two separate test tubes, yielding 22 total test tubes. After incubating for 48 hours, 50μL were extracted from each test tube and spread on the LB Agar with ampicillin and arabinose added. Each leaf homogeny was tested twice, totaling 22 plates spread including the control. These plates were incubated for three days and analyzed for bacteria growth using a black light.

**Results**

There was no sign of *E. coli* bacteria growth on any of the 22 plates when examined under black light after three days of incubation.

**Discussion**

Our results supported our original hypothesis which predicted that cabbage plants would not transport *Escherichia coli* bacteria from the roots to the leaves. This hypothesis was constructed based upon the knowledge of the regulative membrane, the endodermis and casparian strip, in the root system which limits what is allowed, through apoplastic passage, to enter the xylem and phloem. Our findings are important because they contradict some results found in previous experiments. One particular experiment showed that bacteria from fertilizers were found in the leaves of lettuce plants which supported the idea that plants possess a transportation system that enables them to transport bacteria (Matthews, et. al, 2002). In contrast, our results were supported by a similar experiment in which no bacteria were found to have been transported from the fertilizer to the leaf tissue (Johannessen, 2004). These developments are important in consideration of food safety because there is wide spread concern over the raw consumption of contaminated leaf tissue. More experimentation would have to be performed to completely eliminate the idea of internal contamination from popular belief. Based on our results, it appears that using fertilizers that contain animal feces and potentially harmful bacteria is not dangerous in terms of contaminating the actual cells and tissues of our food sources.

**Literature Cited**


Nitric acid has an impact on the growth of wheatgrass (Triticum aestivum). The experiment was conducted at Saddleback College Biological Sciences greenhouse. Due to the low environmental impact: temperature, pressure and humidity were controlled in the greenhouse which, limited external influences on the results. Sixteen plots of wheatgrass (n=4/tray) were evenly distributed in four trays in a 2x2 fashion. Each tray was separated based on a volumetric concentration of 1M nitric acid of 1.0 mL, 10.0 mL, and 30.0 mL respectively and the last tray served as a control. Based on the results obtained, 1M HNO₃ did affect the growth of wheatgrass in the three experimental groups in comparison to the control group. In the control group, the mean length of growth from 80 blades of grass extracted was 6.35 cm, and in comparison to the experimental groups, 7.27 cm mean growth in the 30.0 mL group, 8.80 cm mean growth in the 10.0 mL group and 7.00 cm mean growth in the 1.0 mL group. These results suggest that the nitric acid component in acid rain affects the growth of wheatgrass.

Introduction

Acid rain is produced mostly as a result from human emissions such as sulfur dioxide and nitrogen oxides being released into and reacting with the atmosphere. Acid rain is harmful to many things, plants being an example. When acid rain falls onto soil, it adds a surplus of hydrogen ions which displace nutrients already in the soil by washing these nutrients deep into the soil or washing them off the top off the soil, therefore disabling roots from up taking these nutrients. If plants continue to be exposed to acid rain, subtle changes that lead to declining production may occur (Stroo and Alexander, 1984).

The main objective of this experiment was to study the effect of various volumetric concentrations of 1M HNO₃ on the growth of wheatgrass, Triticum aestivum. The expectation of this study is that groups exposed to higher concentrations of nitric acid will have inhibited growth compared to the control group.

Materials and Methods

The experiment on growth of wheatgrass (Triticum aestivum) was conducted in the Biological Sciences greenhouse at Saddleback College. Four plastic trays (measuring 54 cm x 26 cm x 5 cm) were labeled: Control, 1.0 mL, 10.0 mL, and 30.0 mL. Four water cans were labeled respective to the trays. 16 plots of wheatgrass were then evenly distributed (four plots per tray) in a square fashion, as seen in Figure 1.

All the plots were then shaved down to 2.54 cm using a garden sheer and scissors. Using a 100 mL graduated cylinder, 1000 mL of deionized water was poured into the control water container. This procedure was repeated for each of the remaining three water containers. Using a 10 mL graduated cylinder 30.0 mL of 1M HNO₃ was measured and poured into its respective container. Using the same graduated cylinder 10.0 mL of 1M HNO₃ was poured into the container labeled 10.0 mL. Using a 1 mL graduated cylinder, 1.0 mL of 1M HNO₃ was measured and poured into its respective container.

The trays were then watered with their respectively labeled containers. In all, 1000 mL of water was poured into the control tray, 1030 mL of water and acid solution was poured into the tray labeled 30 mL, 1010 mL of water and acid solution was poured into tray labeled 10 mL, and 1001 mL of water and acid solution was poured into tray labeled 1mL. 143mL of solution would be poured to their respective plots for the next seven days. The water was poured evenly across all 16 plots. Remaining solution more than 142mL on the last day was emptied out to their respective plots of grass.

No changes of any sort were made to the wheatgrass over the seven days given for growth. After seven days, the 16 plots of grass were removed from the trays. 20 blades of grass from each plot were selectively plucked and measured from their trays. The blades of grass were reduced by 2.54 cm to account for only the length of growth that occurred.
during the experiment. The resulting data was inputted into MS Excel (1985-2009 Microsoft Corporation) and statistical computations were applied using analysis of variance (ANOVA single factor).

**Results**

The mean wheatgrass length of the control group was 6.35 cm, the 1.0 mL group was 7.00 cm, the 10.0 mL group was 8.80 cm, and the 30.0 mL group was 7.27 cm (Figure 2). Measuring the grass length (cm) of 80 blades from each tray (80 blades X 4 Trays = 320 blades), the mean length of wheatgrass from each experimental group (1.0 mL, 10.0 mL, and 30.0 mL) was overall greater than the control group (Figure 2). There is a significant difference in average length of wheatgrass between the four groups (p=4.24x10^{-5}, ANOVA single factor). The data suggests statistical significance at P <0.05 within the four groups.

**Discussion**

The experimental groups mean length of growth turned out larger than the control group’s mean length of growth; these results do not confirm our initial hypothesis that nitric acid inhibits growth of wheatgrass. This suggests that the nitric acid has little to no effect inhibiting growth of wheatgrass. The 1M HNO₃ applied in the experiment has a pH of zero, lower than simulated acid rain that has a pH of 3 which also resulted with no effect on seed yield of soybeans (Kohno and Kobayashi, 1989).

In measuring the length of wheatgrass from the root, and after subtracting 2.54 cm to each blade after, the results provided interesting insight into the effect nitric acid has on the growth rate of wheatgrass. Instead of inhibiting growth, the nitric acid improved the growth of wheatgrass. In the four groups the control has the shortest mean growth length in comparison to the three experimental groups. With the addition of nitric acid in low volumetric concentrations, the grass length progressively increased. The increased grass length with greater volumes of nitric acid indicates that a low pH may be beneficial for stimulating grass growth. However, further studies on nitric acid and wheatgrass growth rate would be needed to provide evidence that low pH does increase growth rate in wheatgrass.

There could have been potential errors in the experiment that could have led to inconsistent results. One of these errors is that during the course of our experiment, there was heavy rainfall that occurred during that week. The greenhouse was protected, so barely any rain got through into our experiment, yet the weather throughout our experiment was considerably lower than normal room temperature. Another factor that could cause potential errors in the experiment was the chemistry of the soil, cation losses played a role in growth of white pine, *Pinus strobus* (Wood and Bormann, 1973) and could had played a role in our wheatgrass soil. The wheatgrass plots were purchased from Crown Valley market and it wasn't known if they put fertilizer within the soil when we purchased the plots. If fertilizer was mixed in with the soil, then the fertilizer could have also played a role in the growth of the wheatgrass.

Alterations to this experiment could have also caused different results. One example is to have done the experiment in multiple temperature conditions to have seen if temperature played a role in the growth. Another experiment that can be done is with the use of sulfuric acid, the other component of acid rain. By conducting an experiment using 1M of sulfuric acid instead of nitric acid, the results of wheatgrass growth could change. Sulfuric acid could potentially...
cause a significant decrease in growth as volumetric and molar concentration increases.

Implementing various techniques of research on wheatgrass is important to understanding the most efficient and useful method of producing this grass. Wheatgrass is used in juice bars as a nutrient supplement. Even though nitric acid improved the growth rate of wheatgrass, it probably isn’t safe to consume it because of the pH (Stroo, et al., 1984). With more field research it can be possible to better understand how acid rain and its components impact wheatgrass and other agricultural sources that are used daily.

Literature Cited

Fasting has been found to reduce the oxygen consumption of white feeder mice (Mus musculus). The results suggest that the metabolisms of the mice are reduced during periods of fasting; suggesting a lowered oxygen intake. A group of ten feeder mice were fed equal amounts of food and water for a period of 24-hours. The rates of their oxygen consumption were measured early the previous morning. The mice were fasted for a period of 48 hours, in which they were only given water. Their rates of oxygen consumption were then measured at the end of the 48-hour period, for a second time. All ten mice were housed separately and within the same environmental conditions; including temperature, lighting, insulation, and cage sizes. Within the 48-hour duration of fasting, the mice had lost between 0.5 to 1.2 grams of bodyweight. Their rates of oxygen consumption had also dropped (6.79 mean ± 0.16 mL/g/hr ± S.E.M.) compared to (5.51 mean ± 0.12 mL/g/hr ± S.E.M.) post-fasting. Due to the results, we were able to support our hypothesis that the oxygen consumption did lower. Our thesis is that fasting white mice will have a lower metabolic rate to conserve energy versus white mice that are on a regular diet.

Oxygen Consumption of Fasting White Mice (Mus musculus)

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Introduction

It is possible to alter an animal’s rate of oxygen consumption based on the amount of food intake received. From the point of consumption, the rate of oxygen in the animal’s body increases, allowing the animal to process the food and metabolize properly. Some possible variables such as; sex, body condition (i.e. body fat storage) and/or inactivity might have on the relationship between heart rate and rate of oxygen consumption (Butler, Froget, Woakes, and Handrich, 2001).

By using measurements of VO$_2$ it is possible to receive the volume of airflow. VO$_2$ is the volume of oxygen that can be consumed during intense, whole-body exercise, while breathing air at sea level. This permitted not only the direct measurement of the oxygen consumed but also the elimination of periods during which the animal was active (Davis, Van Dyke, 1933).

Measures of minimal, or resting, metabolic rates (also termed basal, BMR, or standard, SMR, depending on details of measurement conditions) are often used to infer the minimum maintenance requirements of an organism (Dohm, Hayes, Garland Jr., 2000). The objective of our experiment is to show that fasting white mice will have a lower metabolic rate to conserve energy versus white mice that are on a regular diet.

Materials and Methods

Studies were performed on feeder mice (*Mus Musculus*). Ten feeder mice were purchased at Sand Bar pet shop in Mission Viejo, California. The group of ten mice received the same amount of care and was exposed to the same environmental conditions. The mice were well maintained and kept healthy throughout the duration of the experiment.

Each mouse was placed into a labeled transparent plastic cage with a removable top and ample ventilation. The cages were 17.8 cm long by 11.4 cm wide, and 15.2 cm tall. This allowed the mice to be isolated and prevented one mouse from eating more than another. The cages were insulated with crumpled paper towels as bedding, and each mouse was given a Dixie cup filled with 10 mL of water twice a day. Before the initial fast the mice had a diet of oatmeal and cheerios. Each cage was cleaned and re-bedded on a daily basis, keeping the mice dry, warm, and healthy. The cages were placed indoors, at an average temperature of 22.5 °C and checked on every three hours. Each mouse was also let out on a clean dry surface to run around and exercise for 10 minutes daily.

Two sets of measurements were collected using the same methods and conditions on all 10 mice for both the 48-hour fasting and 24-hour non-fasting periods. The process was identical, including the weighing process and the measurement of O$_2$ consumption using the FoxBox Oxygen Analyzer. The apparatus was setup as in Figure 1. This setup included one end of the cylinder to be attached to the FoxBox Oxygen Analyzer and the other end to an air pump. The FoxBox Oxygen Analyzer was then powered on to allow for the normalization with the air inside the room. Each mouse was placed in the cylinder (3.6 cm in diameter and a length of 11.5 cm). The air inside the cylinder was neutralized for five minutes, before and after the initial recording of the O$_2$ rates for 10 minutes. Moisture and water vapor were ignored due to the use of dry right, which absorbs the moisture within the FoxBox Oxygen Analyzer.

The data collected using the FoxBox was then run though the Withers equation solving for VO$_2$. By using a paired t-test and allowing us to calculate the CO$_2$ using the Vo$_2$ calculation (Withers, 1977). The equations; VO$_2$ = ((Flow Rate)*(O$_2$ Entering – O$_2$ Exiting)) / (1 – O$_2$ Entering).

After all the data was run through the equation, an average of the rate of O$_2$ consumption was taken and adjusted to STP (Standard Temperature Pressure) using the combined gas law. The values obtained were then divided by the mass of each mouse to establish the amount of O$_2$ consumed per gram of mouse. (Each mouse was weighed on a balance before and after the fasting process). The final results were compared from the fasting and non-fasting mice using a one tailed t-test (p<0.05).

![Figure 1. FoxBox apparatus set up for normalization, with FoxBox on the left and Air pump on the right.](image)

Results

The oxygen consumption of mice can be manipulated with different environments, or amounts of food. Mice have higher oxygen consumption rates when they are active or have consumed their daily intake of food. The oxygen consumption difference between fasting and non-fasting mice was determined by measuring the oxygen consumption of normally fed mice, and then after a 48-hour fasting period. A one-tailed t-test was conducted to find the resulting
p-value ($p = 1.40 \times 10^{-1}$). With fasting, there was a significant decrease in the $O_2$ consumption rates compared to that of the non-fasting mice (Figure 2). The percentage of body weight did also reduce by an average of 0.95% after the 48-hour fasting period.

Non-fasting and fasting mice mean $O_2$ consumptions were compared. The mean $O_2$ consumption rate of the non-fasted mice ($6.79 \pm 0.16$ mL/g/hr ($\pm$ S.E.M.), N=10) was compared to the mean $O_2$ consumption rate of the fasted mice, ($5.51 \pm 0.12$ mL/g/hr ($\pm$ S.E.M.), N=10). This suggests that there is a significant loss of $O_2$ consumption in the fasting mice (Figure 2). This shows that there is little need for high oxygen content, which results in a minimal amount oxygen to sustain life and internal functions.

**Figure 2.** Mean $O_2$ consumption of pre-fasting ($6.79 \pm 0.16$ mL/g/hr ($\pm$ S.E.M.), N=10) and post-fasting ($5.51 \pm 0.12$ mL/g/hr ($\pm$ S.E.M.), N=10) white mice (*Mus musculus*). ($p = 1.40 \times 10^{-8}$).

**Discussion**

The results show that there was a significant difference between the non-fasting and fasting mice oxygen consumption rates. The non-fasting mice had a much higher rate of $O_2$ consumption than that of the fasting mice. Although we did not find any studies based on pre and post fasting mouse $O_2$ consumption rates, a study conducted by Davis and Van Dyke showed that the rate of oxygen of fasting mice fluctuates based on behavior (1933). It is possible that other types of mice would have varying rates that differ from that of the results obtained from these experiments.

During the initial testing of the mice many of them were in a state of panic in the beginning of the test and relaxed for the remainder of the testing. When an animal is under stress they begin to take in more oxygen than when in a calm state. Some of the mice on the other hand were calm and fell asleep when placed in the cylinder, altering the results because in Davis and Van Dyke’s study the mice that were sleeping had lower $O_2$ consumption rates than that of the calm nonmoving mice (1933). During the 48-hour fasting period many of the mice became fatigued and were unable to move around as easily as before the fasting period. The temperature could have had an affect on how the mice acted during the testing. The lower the temperature of the environment, the slower the metabolism will become, whereas in our study the mice were kept in a standard room temperature (Geizer, 2003).

This experiment could be expanded upon by studying different ages, genders, and species of mice to compare the different rates of $O_2$ consumption between these categories of mice. Another opportunity to further explore these findings would be to compare the mean $O_2$ consumptions of fasting and non-fasting mice along with mice that have been exercised a set distance and time.

**Literature Cited**


The Effect of *Laminaria porra* on the Growth of Common Black Mold, *Rhizopus stolonifer*

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Mold is a major cause of losses due to food spoilage and property damage in buildings. In this experiment, different concentrations of algae were mixed with potato dextrose agar plates and swabbed with mold to determine if these concentrations of algae would inhibit mold growth. Mold was allowed to grow for 7 days. One group mixed with a 10% algae solution, and another with a 12% algae solution. Our hypothesis was rejected, and no significant difference was seen between the two groups of potato dextrose agar plates. Regardless of algae concentration mold covered 100% of all the agar plates.

**Introduction**

Many marine algae species contain secondary compounds that have been studied to show antiviral and antifungal properties (Nezha et al. 2004). With increasing environmental temperatures and a suspected increase in both algae blooms and fungi growth, we have conducted an experiment to see if algae, *Laminaria porra*, has an inhibiting affect on common black mold, *Rhizopus stolonifer*.

According to Nezha et al (2004), anti-microbial activities on bacteria and fungi subjected to algae extracts were reported. Different extracts from marine algae (*Cystoseira tamaricifolia*) were tested. The extracts were examined for their inhibitory effect on growth of molds, yeast, and mycotoxines. Fungal species were held at 10% concentration and were mixed with algae extracts. The minimal inhibitory concentration was determined in every case of dilutions and a total of 100% inhibition was obtained from all fungal species (Nezha et al., 2004).

Several works have been carried out on extracts from marine algae. Antifungal activities on fungi were also reported by Hellio (2000). According to her, marine algae have evolved sufficient survival skills by producing chemical and defensive compounds (2000). One of these compounds is CYN, sesquiterpene caulerpenyne (Hellio, 2004). An earlier experiment conducted by Di Guardia, found that CYN exhibited antibiotic activities (Guardia et al. 2000). Advancing on this knowledge, Hellio collected specimens of algae, *Caulerpa racemosa*, to conduct a series of tests on algal metabolites. Her results revealed that algae could be used as an antifouling agent (Hellio, 2001).

However there is great debate over whether brown algae inhibit mycotoxigenic fungi. Some studies have shown, in opposition, that extracts of brown algal, *Laminaria japonica*, can actually be used as a fungus stimulator (Hartmut and Laatsch, 2008). Conversely, Hartmut Laatsch reared insignificant results (2008).

In our current experiment, we hypothesize crude extracts of brown algae *Laminaria porra* will exhibit inhibiting effects on mold, *Rhizopus stolonifer*. If tests suggest this to be correct, antimicrobial activities of marine algae could be applied to food as a natural preservative. Or even be used in home industries as a method of preventing mold from ruining home structures.

**Methods and Materials**

The seaweed (*Laminaria porra*) was collected from Laguna Beach’s main beach. The seaweed was then osterized and made into a puree. Utilizing 40 agar plates, the seaweed puree was mixed with potato dextrose agar and placed into the agar plates. The puree was mixed with the potato dextrose in two different percentages in order to determine if differences in concentration of seaweed would result in increased mold inhibition.

Twenty agar plates were filled with a 10% solution of seaweed and the other twenty were filled with a 12% solution of seaweed. The agar plates were left to solidify for a few days prior to further experimentation. Common black mold (*Rhizopus stolonifer*) was then applied to each agar plate, and the plates were left to culture at room temperature for seven days. Five test tubes were also used to provide a control for the growth of the common black mold.

**Results**

After allowing the common black mold to culture in the 10% and 12% solution of seaweed potato dextrose agar plates for seven days, the plates were removed from storage for observation. Both the 10% and 12% seaweed concentration groups...
exhibited 100% growth of common black mold. Control tubes also facilitated mold growth as expected. There was no visible inhibiting effect of the algae on the mold in any of the sample groups since the mold filled the agar plates, and caused the lids to tend to stick to the plate. Results did not support our initial hypothesis.

**Discussion**

Mold placed on agar plates mixed with brown algae were not inhibited. All plates regardless of concentrations had 100% growth of mold. These results did not provide support for our hypothesis. The end result of this experiment could be an outcome of low concentration amounts of algae in the agar plates. The inhibition which occurred on previously conducted experiments could have been the result of higher concentrations of algae, or possibly a particular type of algae different than the algae used in this experiment. This implicates that if algae were to have inhibiting effects on common black mold, it would possibly have to be at a much grander scale. However, further research would have to be conducted to test that analysis.

In the future, another experiment could be conducted using higher concentrations or different species of algae to try and support our initial hypothesis. This is important because if results prove positive, algae could be a used as an effective natural preservative for foods. As health concerns focusing around chemical preservatives are growing, the market for a natural, more effective preservative is greatly needed. This could also be applied to the field of construction, as mold is a major problem in homes and business buildings.

**Acknowledgements**

A special thanks to Saddleback College for providing the materials needed to conduct this experiment including potato dextrose agar plates and mold. The assistance of Dr. Huntley to help aid specific processes in this experiment was crucial to this experiment's success, and we would like to thank him as well.

**Literature Cited**


The Effect of Salt Stress on the Inhibition of *Staphylococcus aureus* by Ampicillin

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The continual evolution of bacteria to survive in their environments is well known and has caused a major concern in the health industry. The objective of this experiment is to test whether salt stress affects the zone of inhibition of the gram-positive bacterium, *Staphylococcus aureus* when exposed to ampicillin. Inhibition was tested on nutrient agar of varying salinities: 0%, 5%, 7.5%, 10%, 12.5% and 20%. A two-tailed t-test with unequal variances showed a significant difference between the inhibition at 0% and 10% in the first experiment (p = 2.2x10^{-33}). An ANOVA with Bonferroni corrections in the second experiment indicated a significant difference within the 5%, 7.5%, and 10% plates (p < 0.05). (As salt level increased, the bacteria performed well in the presence of ampicillin until the salinity, 12.5% and above, reached a critical point where the bacterium was unable to grow.)

**Introduction**

*Staphylococcus aureus* is a spherical gram positive bacterium that is often found in epithelium of the nose and skin of a person. *S. aureus* is the most common *Staphylococcus* infection causing a range of illnesses, including toxic-shock syndrome. About 20% of the human population is long-term carriers of these bacteria. *S. aureus* seems to have very high binding capacity for epithelial cells adhering to nasal epithelium and to labium major cells exceptionally well. *S. aureus* concentrations in neonatal nasal epithelial reach adult levels by the fifth day (Aly, R. and Levit, S. 1987).

Beta-lactam antibiotics like ampicillin combat bacteria in the body by inhibiting bacterial division and the enzyme transpeptidase. The disruption of the peptidoglycan walls is caused by beta-lactam. The peptidoglycan wall is important to cell structure and integrity. Beta-lactam antibiotics inhibit cross linking of peptidoglycan by mimicking the binding site and competitively inhibiting transpeptidases. Ampicillin has an attached amino group that helps it penetrate the cell wall in gram negative bacteria.

*S. aureus* has also been known to have strains resistant to methicillin and vancomycin through various changes in the cell’s wall and or the creation of enzyme destroying chemicals. Strains of *S. aureus* that are resistant can cause because they can be difficult to treat. The ability to produce beta-lactamase plays an important role in the resistance of bacteria to beta-lactam antibiotics like ampicillin. Research shows that it is probable that all bacteria create a minimum of one chromosomally mediated beta-lactamase (Mathew and Harris, 1976). Bacteria also have plasmids that can specify beta-lactamases. The enzymes are inducible and occur most rapidly when a beta-lactam antibiotic is present (Hennessey 1967). Due to this resistance in bacteria and its large presence in our environment, it is necessary to understand the processes of bacteria and their reaction when exposed to various environments and antibiotics.

The objective of this experiment is to see if salt stress would have any effect on *S. aureus* when combating the antibiotic ampicillin. This experiment will help better understand the function of bacteria and its resistance to antibiotics while dealing with the outside environment.

**Material and Methods**

During the first trial, one flask was set aside to be used as the control containing no salt. Salt was added to the other flasks until the second one reached 10% salinity and the third flask obtained 20% salinity.

For the second trial, the first flask contained 5% salt, the second flask contained 7.5% salt, the third flask contained 10% salt, and the last flask contained 12.5% salt.

Each plate in both trials was spread with 50 microliters of *S. aureus*. Four sterile paper disks were coated with ampicillin and were evenly spaced to allow for zones of inhibition in each plate. In the first trial, the plates were incubated with the ampicillin disks at 37°C for a 24-hour period. In the second trial, the plates were incubated with the
ampicillin disks at 37°C for a 72-hour period. Zones of inhibition were measured and recorded after the plate’s incubation.

**Results**

A significant difference between nutrient agar containing salt and the control was observed in this experiment. The first trial showed an increase in bacterial growth in nutrient agar enriched with salt up to a critical point. After the critical point, bacteria were unable to grow at all. Figure 1 shows that the control plates which contained 0% salt in the nutrient agar showed a mean inhibition of 2.6 cm ± S.E.M. The mean inhibition of the plates that had 10% salt in the nutrient agar was 1.4 cm ± S.E.M. The significance between the 0% and 10% salt plates were found using a two-tailed unpaired t-test (p = 2.2x10^{-33}). All of the plates containing 20% salt contained no bacterial growth at all.

The results of trial two resulted in a similar trend to trial one. The salt decreased the inhibition up to a critical point. In Figure 2, the mean inhibition for the plates containing 5% salt was 3.8 cm ± S.E. The mean inhibition for the plates containing 7.5% salt was 3.5 cm ± S.E. The mean of the inhibition for the 10% salt nutrient agar plates was 2.6 cm ± S.E. An ANOVA single factor analysis yielded 81 degrees of freedom and a means squared of 0.0669. Bonferroni corrections explained that each salt concentration variable showed a significant difference from the others (p<0.05). The range in size of zones of inhibition for both trials is shown in Table 1. The second trial also showed a limit to the benefit of salt in the nutrient agar like in trial one. All of the plates containing 12.5% salt in the nutrient agar had no *S. aureus* growth.

**Table 1.** Diameters of the zone of inhibition of *S. aureus* by ampicillin on varying salt nutrient agar concentrations.

<table>
<thead>
<tr>
<th></th>
<th>0% salt zone range</th>
<th>10% salt zone range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First Trial</strong></td>
<td>2.0cm - 3.4cm</td>
<td>1.0cm - 1.8cm</td>
</tr>
<tr>
<td><strong>Second Trial</strong></td>
<td>3.4cm - 4.0cm</td>
<td>2.8cm - 4.1cm</td>
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</table>

**Discussion**

The experiment resulted in a significant difference in the means of inhibition of *S. aureus* by ampicillin in a salt stress environment. The difference in the inhibition between the first and second trial was most likely due to the differences in incubation periods. The exact point at which salt levels stop the growth of bacteria is unknown and should be discovered through further studies. Researchers hypothesize that the bacteria ceases to grow due to cell lysis. Once the bacterium absorbs too much salt and the concentration gradient reaches a certain point the cell would most likely absorb too much water and rupture. The direct cause of why the presence of salt in the nutrient agar causes smaller zones of inhibition is still questionable and may be caused by the salt hindering the ampicillin and or aiding the *S. aureus*. Bacteria can become resistant to antibiotics through environmental pressure. Early research shows that antibiotics cause the selection of variants that already exist, not the creation of new mutants (Demerec, 1948).

When bacteria are exposed to antibiotics, the bacteria with a mutant gene suitable for survival will be able to reproduce and transfer its genes to the next generation. It could be possible that bacteria with genes better suited for a high salt environment also carry more defenses against beta-lactum antibiotics like ampicillin. Bacteria that already contain mutant genes are usually the ones that survive and pass their...
genes. There are also bacteria that can become a hyper-mutable bacterium by inactivating the proofreading system that usually corrects errors in DNA copying.

The researchers hypothesize that the salt concentration in the nutrient agar caused the bacteria to absorb less of the ampicillin into their cell walls which allowed them to travel closer to the ampicillin disks where the ampicillin solution is more concentrated and the solute gradient changes. A similar study suggested the salt effect is exerted on the same features of the surface layers that impede the entry of some penicillins (notably ampicillin) to their targets in the E. coli inner membrane (Bongaerts and Bruggeman-ogle, 1980). Another hypothesis is that the bacteria more suited for high salt environments are also better adapted to defend against beta-lactam antibiotics by speeding up the production of beta-lactamases or hindering the affects of beta-lactam.

The ability to produce beta-lactamase has become widespread amongst the pathogenic bacteria, primarily through the mechanism of plasmid exchange. As a result, the usefulness of first generation beta-lactam antibiotics has been considerably reduced (Herzberg, O. and Moult, J., 1987). If there are other environmental presences that also aid bacteria like salt, the danger and the growing number of drug resistant bacteria calls for information on the bacteria due to the dramatic impact they can have on human health.

### Literature Cited


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**Figure 2.** Mean inhibition of *S. aureus* in nutrient agar containing 5%, 7.5%, and 10% salinity. 5% had mean inhibition of was 3.8 cm ± S.E.M., 7.5% had mean inhibition of 3.5 cm ± S.E.M., and 10% had mean inhibition of 2.6 cm ± S.E.M. Error bars indicate 95% confidence interval.
Allometric Relationship Between the Mass and Metabolic Rate of Giant Madagascar
Cockroaches *Gromphadorhina portentosa*

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We studied the effect of mass on the metabolic rate of Giant Madagascar Cockroaches, *Gromphadorhina portentosa*. *G. portentosa* range in mass from 0.1g to 12.0g. This unusually high intra-species mass range allows a study of metabolic rate allometry. Our hypothesis was that there is an allometric relationship between oxygen consumption and the mass of the cockroaches. We predicted this relationship to be linear. We also predicted that metabolic rate will scale with a value of 0.75 in accordance with Kleiber’s scaling laws. Thirty individuals ranging in mass from 0.36g to 11.29g were used in this study. Our results indicated that metabolic rate does not scale to 0.75; it scales to 0.58.

Introduction

The idea that bigger animals use more energy was first presented by German chemist Max Rubner in 1908, when he compared the metabolism and life spans of five different animals. He noted that larger animals live longer. While this idea was subsequently thwarted by the work of other scientists, it still appeared that evolution seemed to have found way to partially overcome the limitations posed by pure geometric scaling. This is evident by research that shows that the surface area of an organism grows more slowly than its size. Before we conducted our own experiment, we noted that in previous studies, when the basal metabolic rate of an organism has been tested, the exponent b in the allometric equation $VO_2=aM^b$, varies taxonomically in the order and family levels of organisms (Speakman, 2005). This equation describes the relationship between basal metabolic rate and body mass, where $VO_2$ is the resting oxygen consumption for the cockroaches *Nauphoeta cinerea* (Coelho & Moore, 1989). In other studies involving mice, elephants and humans, the equation $M=a.x^k$ was applied to calculate the allometric relationship number, where $M$ is the metabolic rate, $x$ is the size of the organism, and $a$ and $k$ are constants (Kleiber, 1932). Kleiber also showed that taking the logarithm of both sides of the equation would yield $\log (M) = \log (a) + k*\log (x)$, which plots as a linear line. The general correlation between the body mass and the metabolic rate was thus shown to be scaled to the three-quarters power (Hopley & Schalkwyk, 2009).

Kleiber’s findings are now recognized as the one-quarter power scaling law used to successfully find a relationship between metabolic rate and body mass of an organism as well as other allometric relationships (Johnson, 1999). Although this allometric relationship seems to be constant between species not much is known regarding a relationship within a species. Hence, we conducted this experiment on an organism such as *G. portentosa*, which has significant variance in size within the species itself. Our hypothesis was that just as Kleiber’s scaling law applies to a vast variety of species, it will also apply to organisms within a species and show an allometric relationship between metabolic rate and body mass. We also predicted that the metabolic rate of *G. portentosa* will scale with its body mass to the three-quarters of power.

![Figure 1. Log of mass (g) of *G. portentosa* against the log of its metabolic rate (W). Trendline through the data points is linear with a slope of 0.58](image-url)
**Results**

We found that a linear relationship does exist when the data points are plotted on a scatter point graph. In addition, when a trendline is added to the graph, its slope is 0.58. This means that as the mass of *G. portentosa*, increases, metabolic rate increases by a factor of 0.58, which is significantly lower than our predicted value of 0.75. (See Figure 1)

**Discussion**

Most of our research on this topic indicates that as you increase the mass of the cockroach, the metabolic rate increases by a factor of 0.75. However, our figure was 0.58. This finding is closer to the findings of several researchers such as Coelho & Moore (2006) and Spaargen (1994), who claim that the "true" value of the exponential component is 0.66 because Kleiber did not take into account the surface-to-volume ratio of the organism. Heusner (1982) reported that the exponent is approximately 0.67 for any single mammalian species and suggested that the interspecies value of 0.75 is a statistical artifact. Agutter & Wheatley (2004) pointed out that metabolic rate data for small and large mammals lie on parallel regression lines, each with a gradient of approximately 0.67 but with different intercepts. Heusner (1982) found the value to be 0.65 for small mammals and 0.86 for large ones, again suggesting that 0.75 is a cross-species "average" with no biological significance. Hochachka et al. (2003) reported value of 0.60 for small mammals. Another reason why the Kleiber’s exponent does not match our exponent may be that Kleiber’s law has limitations. According to Hochachka et al. (2003), Kleiber’s original treatment is limited to a narrow definition of metabolism, which only incorporates oxygen consumption and synthesis of ATP as its ultimate factors. However, we took the recharge rate into account when we converted our data into Watts.

**Literature Cited**


The Effect of Atmospheric Pressure on Wheatgrass, *Triticum aestivum*, Growth

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A large number of experiments conducted in mountain areas reveal the effects of altitude on plant growth. However, these experiments include several factors such as temperature, solar radiation, and availability of nutrients. None of the studies assessed the effect of atmospheric pressure on plant growth rate. In this experiment, we manipulated levels of pressure while keeping other variables such as temperature and light exposure constant. Our hypothesis was that the higher pressure would result in better growth than lower pressure. This hypothesis was investigated with wheatgrass, *Triticum aestivum*, and the growth rates of *T. aestivum* under three different atmospheric pressures, 760 mmHg, 497 mmHg, and 235 mmHg. The Analysis of Variance and Bonferroni post hoc test showed that there are significant differences between each group (p<0.05). Thus our results support the hypothesis that the higher pressures result in better growth.

**Introduction**

Plant species distribution exists along altitude differences. Trees in high altitudes have larger spacing and decreased heights. Treeless space exists in areas of high altitude creating a boundary called the forest line. These treeless spaces occur because of unfavorable climates. The forest line is affected by several factors. Previous studies focus on the longitudinal differences and have found that the height of mountains influences the altitudinal distribution of many plants (Odland, 2008). However, the effects of altitudinal differences are greater than those of longitudinal differences because altitudinal differences affect temperature, solar radiation, and water vaporization (Korner, 2002). For instance, Morecroft and Woodward (1996) state that plant growth differs at different altitudes. The total basal area of all trees including larger trunk-resprouted trees declines linearly in response to altitude (Pyrke and Kirkpatrick, 1994).

According to Woodward, et al. (1986) there is a difference in leaf extension at lower and higher altitudes. Research done by Odland (2008) listed factors that are affected by altitudinal change: temperature, solar radiation, and availability of nutrients. In Odland’s research, temperature is the most important factor in altitudinal change and plant growth. On the altitude-pressure-temperature table, temperature at sea level is 15°C, while the temperature decreases to 0°C at 2250m above sea level (Brombacher, 1936). Based on the table, every 300m increase in altitude there is a 1°C decrease in temperature. Plants have an optimal temperature between 0°C to 40°C and extreme temperatures may destroy the plant’s structure and halt growth (Went, 1953). The higher altitude decreases evapotranspiration, and therefore, increases solar radiation (Korner, 1999; Odland, 2008). Water and nutrients are less available in high altitudes.

Since most of the previous research was conducted in mountain areas, none of the studies showed the simple effect of atmospheric pressure on plant growth rate. In this experiment we manipulated levels of pressure while keeping other variables such as temperature and light exposure constant. This study will allow other researchers to have a better understanding of pressure and its affect on plant growth. In the experiment, our hypothesis is that at higher pressures plant growth improves. This hypothesis was investigated by observing the growth rate of *T. aestivum* at three different atmospheric pressures.

**Materials and Methods**

Six 9cm by 9cm containers of wheatgrass were purchased at Mother’s Market & Kitchen in Laguna Hills. The experiment spanned three weeks. Before the experiment began, wheatgrass was cut to four centimeters from the top edge of the container. Each container was watered with 200mL of de-ionized water before being put in their respective pressure environments. Then, they were placed under timed lighting of 12 hours of exposure to light and no light. Observation of *T. aestivum* growth in differing pressure levels were measured. Three groups were tested at different levels of pressure, group 1 at 760
mmHg, group 2 at 497 mmHg, and group 3 at 235 mmHg. Groups 2 and 3 were placed in a pressure chamber, and the pressure was decreased by the vacuum system. Group 1 was placed in a transparent cage to control the intensity of light. On the eighth day, all wheatgrass were again cut to four centimeters from the container’s top edge. Then 150 blades from each package of wheatgrass were measured.

A single factor analysis of variance (ANOVA) was used to compare groups, accompanied by Bonferroni post-hoc test. Significance was observed at P<0.05. All data were reported as mean±SEM.

**Results**

In the experiment, the mean growth lengths of wheatgrass under 760 mmHg, 497 mmHg, and 235 mmHg were 6.2 ± 0.2 cm, 2.3 ± 0.1 cm, and 1.5 ± 0.04 cm (± S.E.M.) respectively. The mean temperature for 760 mmHg and 235 mmHg was 21.8 ± 0.3 °C (n=4). The mean temperature of 497 mmHg was 21.9 ± 0.3 °C (n=4). Analysis of Variance (ANOVA) and the post-hoc test showed significant differences between 760 mmHg and 496 mmHg, 760 mmHg and 235 mmHg, and 496 mmHg and 235 mmHg (p<0.05 for each).

![Figure 1](image_url)

**Figure 1.** The mean growth length under each pressure 760mmHg, 497mmHg, and 235mmHg. The mean growth lengths under 760, 497, and 235 mmHg were 6.2 ± 0.2 cm, 2.3 ± 0.1 cm, and 1.5 ± 0.04 cm (± S.E.M.) respectively. Analysis of Variance (ANOVA) and post-hoc test indicated that there were significant differences between each group (p< 0.05).

**Discussion**

In this experiment, three different pressures were used: 760mmHg, 497mmHg, and 235mmHg. Previous research showed the relationships between altitude and pressure (Brombacher, 1936). A pressure of 760mmHg is equal to zero meters above sea level. Atmospheric pressure of 497mmHg is equal to 3440.0m above sea level. The height of Mount Fuji is 3776m and the data showed growth at lower pressures is possible. Atmospheric pressure of 235mmHg is equal to 8868.5m above sea level. This altitude is equivalent to Mount Everest’s height of 8840m resulting in decreased growth. A previous study done by Brombacher (1936) also shows the basic relationship between altitude and temperature. The temperatures at 760 mmHg, 497 mmHg, and 235 mmHg are about 15.0°C, -6.8°C, and -42.5°C (Brombacher, 1936).

Our analysis showed the effect of pressure on wheatgrass growth. According to the data, wheatgrass can in fact grow in higher pressures. Lower pressures showed a decrease in growth and leads to the conclusion that lower pressures are not ideal environments for plant growth. Based on the data one can assess that growth does occur above 235mmHg. The findings suggest a linear pattern in growth. As pressure increases growth rate increases and as pressure decreases growth rate decreases. Research that expands on our experiment can help discover new ways to sustain life and grow plants in areas where plant life may not be possible such as low-pressure environments.

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The Relationships among Altitude, Chlorophyll Concentration and Stomatal Density in Toyon (Heteromeles arbutifolia)

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The objective of this inquiry was to determine whether chlorophyll concentration and stomatal density would increase in parallel to altitude. Ten samples of toyon (Heteromeles arbutifolia) were taken from twelve different altitudes, cored, and incubated in a liquid medium. Chlorophyll concentrations were obtained using a Beckman DU 750, calibrated according to the Beer-Lambert Law. Mean stomata counts were obtained via “fossil imprints” from ten leaves from each altitude. Analysis of variance for chlorophyll concentrations indicated significance among the data set ($p = 9.99 \times 10^{-18}$). Post-hoc comparisons did not support an increase and/or decrease in chlorophyll concentrations with altitude. Analysis of variance for mean stomatal counts indicated significance among the data set ($p = 5.14 \times 10^{-6}$). Post-hoc comparisons supported significant increases in stomatal density with increases in altitude. Chlorophyll concentrations were determined to be constant, with no apparent trend between points. Stomatal density increases in parallel with altitude. We suggest this effect to be primarily influenced by the inverse relationship between CO$_2$ partial pressures and altitude.

Introduction
An ecosystem is often characterized and diversified by the organisms that make it up and contribute to its stability. Plants play a major role in this cycle of life and offer a great example of one of the more complex components to nature’s order: photosynthesis. This process can be loosely described as a balanced intermingling of light and respiratory energy (Warburg, 1958), and is synthesized by the basis of this inquiry: chlorophyll.

Investigators were interested in the effects of elevation on chlorophyll concentrations, not only to understand the adaptive qualities of Earth’s organisms, but to better understand why there may exist a high and low critical point for life as it correlates to elevation. In a similar study performed by Covington (1975), bark samples of Populus tremuloides taken at lower elevation were found to contain higher chlorophyll concentrations than those taken at the higher elevations. Although trees were greener and had a larger canopy spread at higher elevations, the outer mesophyll and external tissues of these samples were found to be more or less dead. Current investigators expected similar results, potentially attributed from the increased UV intensity at higher elevations and the variance in stomata diffusion (Ellsworth et al, 2001). Chlorophyll concentrations were expected to be lower closer to sea level primarily due to an adaptive adjustment to a smaller, less invasive proportion of sunlight and higher concentration of carbon dioxide.

The diffusion of carbon dioxide and water through the stomata of a plant is an essential aspect to the plant’s survival and success of its complex internal system (Loomis and Ting, 1963). Carbon Dioxide acts as a trigger for the opening and closing of the stomatal pores (guard cells) when concentrations are parallel to that of ATP and...
variations in CO₂ concentration could account for concentration/gradient diffusion rates. These et al., 1980), thus altering the decrease with elevation and temperature (Bischof 1980), at an elevation of 583.08 m; the base and point at vertical meters apart. The first samples were taken meters and were spaced an average of 67.95 vertical range of approximately 762.00 m. Altitudes ranged between 457.20 and 1219.20

Early stomatal development and density are thought to be under direct influence from atmospheric water vapor and CO₂ concentrations (Martin and Warner, 1984), both of which make up and contribute to a fluctuation in the pressure of Earth’s lesser and greater atmospheres (Fussler and Gassmann, 2000). Although partial pressures stay fairly constant throughout the various atmospheric layers (Humphreys, 1913), CO₂ mixing ratios
decrease with elevation and temperature (Bischof et al., 1980), thus altering the concentration/gradient diffusion rates. These variations in CO₂ concentration could account for an unbalanced relation between the reaction of light and water, and the incorporation of CO₂ into the production of inorganic energy, i.e. Calvin cycle. Due to these environmental constraints, stomatal counts were expected to be higher, and thus, closer to each other at greater elevations.

Material and Methods

Toyon (Heteromeles arbutifolia) samples were collected from a total of eleven different altitudes along the Santiago Peak Trail, Trabuco Canyon, CA, on the ninth day of April, 2009. Ten samples were collected from each altitude for a total of 110. Altitudes ranged between 457.20 and 1219.20 meters and were spaced an average of 67.95 vertical meters apart. The first samples were taken at an elevation of 583.08 m; the base and point at which Santiago Peak Trail branches from the Holy Jim Falls Trail. The last samples were taken at an elevation of 1041.81 m for a (final minus initial) vertical range of approximately 762.00 m.

Samples were taken back to the Saddleback College Biology Lab, Mission Viejo, CA and prepared for analysis of chlorophyll concentration and stomata count as it correlates to altitude. The stomatal count technique was introduced to the current investigators by Dr. Tony Huntley, Saddleback College. The process of chlorophyll quantification comes from the concepts described in the Beer-Lambert Law.

Results

Chlorophyll. Mean chlorophyll concentrations ([C]) were sporadic (R² = 0.00370); all data points fell between a range of 0.200 and 0.438 μg/mm². Analysis of variance indicated significance among the data set (p = 9.992x10⁻¹⁸), but post-hoc comparisons between the Saddleback College (SBC; Alt35.66), and first (Alt583.08), middle (Alt810.16), and last (Alt1041.81) Santiago Peak Trail (SPT) points indicated an insignificant increase and/or decrease in parallel to altitude (significance was assessed purely on increase or decrease in concentration with altitude and not on absolute differences). Significance among the data set is likely influenced by the Alt583.08 point, as it is much lower than all other points. A complete summary of these data are represented in Figure 1.

Stomata. A mean value of three separate FOV counts per leaf was taken to control against variables. These data were then evaluated in a similar fashion to that of [C], (nleaves = 10) x (naltitude = 12). Stomatal density fell between a range of
171.861 and 254.299 units/FOV. Analysis of variance indicated significance among the data set, \( p = 5.142 \times 10^{-05} \). Post hoc comparisons between SBC Alt 35.66, Alt 583.08, Alt 810.16, and Alt 1041.81 support significant increases in stomatal density with increases in elevation. A complete summary of these data are represented in Figure 1.

**Figure 1.** Mean chlorophyll concentration ([C]) and stomatal density (nS) in toyon (Heteromeles arbutifolia) growing at different altitudes (Altreadings; naltitudes = 12). FOV = 0.159 mm². Alt 35.66 locale – SBC; Alt 583.08 – 810.16 locales – SPT. Analysis of variance for [C], Alt 35.66 – 1041.81 (\( p = 9.992 \times 10^{-18} \)); post-hoc comparisons between Alt 35.66 / 583.08 / 810.16 / 1041.81 indicated no significance. Analysis of variance for nS, Alt 35.66 – 1041.81 (\( p = 5.142 \times 10^{-05} \)); post-hoc comparisons between Alt 35.66 / 583.08 / 810.16 / 1041.81 indicated significant increases in stomatal density with increases in altitude.

**Discussion**

Among the altitudinal variables that influence the physiology of a plant, CO₂ concentrations are determined to have the greatest change with increases in altitude. This being the case, it was expected that a decrease in CO₂ would ultimately have the greatest effect on chlorophyll concentration ([C]) in toyon. However, within the confines of this study it is clear that [C] does not significantly change with altitude. It is shown in a similar study that the underlying cause of change in [C] was related to the duration and intensity of sunlight (Martin, 1984). Our samples were collected a mean 67.95 m apart in elevation. We did not account for sunlight variations, slope face, or brush cover, which may account for some of the variation seen in [C] in Figure 1.

Due to the inverse relationship that exists between CO₂ partial pressures and altitudinal gradient, plants have developed several mechanisms by which they can extend their range. By increasing not only the number of stomata per leaf, but also the diameter of its stomatal apertures, a plant could adapt to decreased CO₂ concentrations and exist at higher altitudes (Beerling et al, 2001). Kelly and Woodward (1995) conducted a study of the influence of CO₂ concentration on stomatal density in several tree and herb species. Eighty percent of their Hamamelidae and 87% of their Rosidae species were found to have a significant increase in stomatal counts with decreased CO₂ concentrations. As shown in Figure 1, it is apparent that a trend exists between altitude and stomatal density; as altitude increased so did the number of units/FOV.

In this study we found no significant relationship between altitude and chlorophyll concentration in *H. arbutifolia*. Stomata increase in density with an increase in altitude, in what we believe to be an adaptation to decreasing CO₂ partial pressures. In future studies it would be interesting to isolate and analyze the effect of specific variables not tested for in this inquiry and possibly extend the range at which samples are taken.

**Acknowledgments**

Special Thanks to Dr. Tony Huntley and Professor Steve Teh for their devotion to each of us individually. Their time and knowledge is much appreciated and we will miss being a part of their classes. Thanks to Kyle Meade and Amir Zand for their assistance in sample collection and preparation.

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**The Metabolic Rate of White Mice (Mus musculus) Throughout Pregnancy**

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This study examined the effects of pregnancy on the metabolic rate of *Mus musculus* at different pregnancy stages. It was predicted that the overall metabolic rate would rise as the pregnancy progressed in the mice tested. As their pregnancy progressed the mice’s weight and metabolic rate increased. However, there was no significant difference between a pregnant and non-pregnant mouse. The average metabolic rate of the pregnant mice was 4.82 ppm/g (± S.E.M.), while the average of the non-pregnant mice was 4.31 ppm/g (± S.E.M.). The mice’s average weight before pregnancy was 24.70g (± S.E.M.), during pregnancy, it was 25.46g (± S.E.M.), and after pregnancy, 30.39g (± S.E.M.). This study showed that the pregnancy factors, such as developing more body fat and the rapidly growing fetuses, raises the metabolic rate, but not significantly.

**Introduction**

The white mouse, also commonly known as *Mus musculus*, is generally utilized for laboratory experiments due to their high degree of homology with humans, (Martinez et al., 2008) their short life span and their ability to quickly reproduce. They make good test subjects because they can go through similar gestation as other mammals, in less time. An average gestation period for the domestic mouse is about 22 days. A female mouse after giving birth is able to give birth to another litter of babies in...
about 3 weeks after the birth of her first litter. Out of the energy the mice use, respiration uses the largest proportion (87%) of assimilated energy and the remainder was allocated to production (Polley et al. 1977). The oxygen consumption that can be calculated is part of the 87%. It is important to know the respiration rate in order for one to figure out the total amount of energy the mouse uses. The energy not used for respiration is used for the development of fetal mice.

In this experiment ten female mice will be impregnated to show the effects of gestation on metabolic rate using the Fox Box. The average metabolic rate of the mice’s pregnancy and lactation data will be taken and compared to the average metabolic rate data of non-pregnant mice. The magnitude of the change (of displacement in the metabolic rate from the minimal resting rate) is related to body weight and ambient temperature and its duration is related to the activity pattern of the species (Kenagy, 1973). This means that the weight and temperature must also be considered when taking the metabolic rate. The hypothesis being tested here is that the metabolic rate will be higher in the pregnant mice due to the fact that she is supporting developing fetal mice inside her body. The pregnant mice are also developing maternal body fat, which is also a factor of a higher metabolic rate.

**Materials & Methods**

Ten female laboratory white mice (*Mus musculus*) were purchased at the Petco in Rancho Santa Margarita in the month of February 2009. Two cages housed three mice and one housed four mice in order to prevent over crowding. Different colored mice were purchased in order to identify them and prevent any mistake and mix up of data. The mice were each individually weighed on an electric balance and were placed in specific plastic cylindrical tubing that was calculated to have a volume of 144.51 cm³. Each mouse was placed in this plastic tube with corked ends. Connected to the corked ends were rubber hoses that connected one side to an air pump and the fox box on the other side. The mice were left alone for an average of about ten minutes, in order for the specimen to adapt to her environment. Oxygen was applied and no readings were attempted; only when the mouse became quiet (Davis & Dyke, 1933) the recording began. The temperature was acquired inside the tubing by placing a special thermometer, attached to a GLX probe. The average for the temperature throughout all the testing was 24 degrees Celsius. The Fox Box, provided by Saddleback Colleges department of biological sciences, requires the tube to be left alone in order for carbon dioxide to build up. Once carbon dioxide has built up it was then connected to the Fox Box to record the amount of carbon dioxide produced in five minutes. Some of the mice data came out unusable and was thrown out completely. After retrieving the data for pre-pregnancy, male mice were placed in the cages and left there for about 6 days to assure conception of all mice. After the 6 days, the mice were weighed again and tested again using the Fox Box for the oxygen consumption. As the pregnancy progressed, a total of three samples of data were obtained for each mouse. The female mice were then left alone until they each gave birth to a litter. The average of the litters was about 6 pinkies per litter. The litters were placed carefully in a tube and the oxygen consumption was recorded, then they were removed. The mother was then put into the tube and was recorded alone. After obtaining all the data, the metabolic rate was obtained, in accordance with the Withers (1977) equation:

\[
V_{O2} = \frac{Ve*(F_iO_2 - F'eO_2)}{1 - F_iO_2 + RQ*(F_iO_2 - F'eO_2)}
\]  

**Results**

The metabolic rate of the female mice rose slightly between “before pregnancy” and “during pregnancy” but there was no significant difference (p-value= 0.224) as shown in Figure 1. The average before pregnancy was 4.31 ppm/g, during pregnancy it was 4.82 ppm/g and after pregnancy it was 4.60 ppm/g (± S.E.M.). There was a mouse whose metabolic rate was not similar to the rest when going through the calculations and never became pregnant so this data was not used, but the other mice showed a small trend to increase with pregnancy. The greatest increase with most the mice were between the pre-pregnancy and the first test during pregnancy; which was six days after introducing the male. Two of the mice tested, had their greatest growth in metabolic rate between the second and third tests, day nine and day fourteen of pregnancy, going against the average pregnant mouse trend shown in Figure 2. The average at day six was 4.57 ppm/g, at day nine was 4.86 ppm/g and at day fourteen was 5.25 ppm/g (± S.E.M.). The mice babies’ metabolic rate was greater than the difference of
metabolic rate of the female mice before and during pregnancy as shown in Figure 3. The metabolic rate of the female mice in the final term of pregnancy was 4.22ppm/g (± S.E.M.), while the total metabolic rate of its offspring was at 1.78ppm/g (± S.E.M.). The metabolic rate decreased after pregnancy by very little (P value at 0.760), though all, except one, mice’s weight increased, this trend in weight is shown in Figure 4.

**Discussion**

There was not a large difference between the metabolic rates before and during pregnancy, so our hypothesis wasn’t supported on the basis that there wasn’t a significant difference. The p-value would have decreased if there were a greater amount of samples collected and if the sample group used was larger. A smaller p-value would have coincided with the given hypothesis and it would have proved a significant difference. A reason there wasn’t a significant difference may have been because the differences in resting metabolic rates between the rodents in different reproductive states probably was masked by variation in activity during measurements (Oswald & Polley 1990).
left alone to calm down for a few minutes. They were all tested in the same area, so temperature stayed fairly constant, meaning this was not left as a completely inconsistent variable that would alter the data. This was all a requirement because body temperature is regulated by physical and chemical factors; metabolic rates fluctuate to accommodate for environmental surrounding. Testing the mice in the same place, on the same days of pregnancy kept variables to a minimum but the activity of the individual mouse is not easily controlled and could’ve resulted in the main reason for any errors in the recording. If more samples were recorded and more mice were tested then there would be more data from calmer mice available, leading to more accurate readings of oxygen consumption. Considering there was a slight increase, in order to conclude a significant increase, it would mean more mice would have to show an increase in metabolic rate between before and during pregnancy. If this is done and there is still no significant increase it would mean the female mouse’s metabolic rate does not increase with pregnancy and that it just incorporates the fetus’s metabolism into her own.

**Literature Cited**


The Effect of Light Intensity on the Nitrogen Fixing Cyanobacteria, *Anabaena*

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*Anabaena* is a species of cyanobacteria that undergoes photosynthesis and nitrogen fixation. To undergo nitrogen fixation, heterocyst cells form from vegetative cells when there is a lack in fixed nitrogen. Under increased light concentration, cyanobacteria undergoes nitrogen fixation more readily than lower light exposures. Three groups of *Anabaena* were grown under three different light intensities, 375 Lux, 300 Lux, and 225 Lux. The ability to undergo nitrogen fixation was determined by the amount of heterocyst cells present in each filament. The average percentage of heterocyst cells in each filament at 375 Lux was 5.32 % (± S.E.M.), N = 10. The average percentage of heterocyst cells in each filament at 300 Lux was 5.28% (± S.E.M.), N =10. The average percentage of heterocyst cells in each filament at 225 Lux was 4.15% (± S.E.M.), N = 10. An ANOVA indicated no significant difference weekly (p = 0.77, p = 0.65, and p = 0.24).

**Introduction**

*Anabaena* are filamentous, colonial cyanobacteria known as blue-green algae that contain two types of cells: vegetative cells and heterocyst cells. They are the only known prokaryote with plantlike, oxygen-generating photosynthesis. When water is plentiful the cyanobacteria can provide large amounts of food for freshwater and marine ecosystems. *Anabaena* is found in many freshwater lakes. Nitrogen fixing cyanobacteria is thought to be one of the first organisms to colonize the earth. Cyanobacteria are not harmful to plants or animals in small amounts, however in large quantities may increase the biological oxygen demand which then depletes fish and other aquatic residents of their required oxygen (Johnson and Golden, 1999). The simple structure of *Anabaena* is a filament of vegetative cells, a small amount of randomly placed heterocyst along the strand visual through a microscope.

This study focused on heterocyst cell formation. Heterocysts are nitrogen fixing cells formed during nitrogen starvation. Heterocysts fix nitrogen from the air to provide nitrogen throughout the organism to undergo biosynthesis, which is a formation of chemical compounds by a living organism. When heterocyst cells are no longer needed to fix nitrogen they cannot revert into a vegetative cell, therefore it differentiates into akinetes which are dormant cells that serves as a survival structure for the organism. *Anabaena* is known for its nitrogen fixing abilities, and the formation of symbiotic relationships with certain plants. (Akinyanju and Smith, 1986). The *Anabaena* exposed to high light intensity is expected to form more heterocyst cells compare to low light.

**Materials and Methods**

Thirty large test tubes of *Anabaena*, 10 at 375 Lux, 10 at 300 Lux and 10 at 225 Lux were used in this study. At 25°C, heterocyst cell formation was monitored for twenty-one days. Each group place at light intensities of: 375 Lux, 300 Lux, and 225 Lux (measured with a Lutron light meter) for eighteen hours a day and left in the dark for the last six hours of the day. To determine the heterocyst cell formation percentage, a specific *Anabaena* filament was viewed under a microscope and followed from end to end. Heterocyst cells and vegetative cells were counted throughout the filament and the heterocyst cell formation percentages were attained as follows:

\[
\%\text{heterocyst} = \frac{\#\text{heterocyst}}{\#\text{vegetative} + \#\text{heterocyst}} \times 100
\]

**Results**

An ANOVA showed no significant difference between weekly means (p = 0.77, p = 0.65, and p = 0.24). The mean combined heterocyst cell formation percentage in each *Anabaena* filament at 375 Lux, 300 Lux, and 225 Lux were 5.32%, 5.28%, and 4.15%. Heterocyst cells were significantly greater in Group A (375 Lux) than Group C (275 Lux).
Figure 1. Weekly mean percentages of heterocyst cells in each Anabaena filament at 375 Lux, 300 Lux, and 225 Lux. Mean percentage of heterocyst cells in Group A at 375 Lux is greater than Group B at 300 Lux and Group C at 225 Lux. Error bars indicate 95% confidence interval.

Discussion

These differences from 375 Lux, 300 Lux, and 275 Lux displayed the amount of light available to the Anabaena corresponding to the production of heterocyst cells. The mean combined percentage of heterocyst cell formation in each Anabaena filament at 375 Lux, 300 Lux, and 225 Lux was 5.32%, 5.28%, and 4.15% (Figure 1). Anabaena begins as a filament of vegetative cells interspersed with heterocyst cells throughout the chain (De Nobel et al., 1998). Anabaena is considered a “sun” species because the nitrogen fixing heterocyst cells count is higher with an increased amount of light, indicating that the organism is actively fixing nitrogen (De Nobel et al., 1998).

Anabaena includes both photosystem I and photosystem II. The vegetative cells carry out photosynthesis and release oxygen into the atmosphere (Hoeger et al., 2002). When nitrogen sources, such as NH$_4^+$ and NO$_3^-$, are limited, vegetative cells differentiate into heterocyst cells (Vagnoli et al., 1992). Heterocyst cells are terminally specialized for nitrogen fixation. Anaerobic conditions are necessary for the nitrogen fixation to take place in the heterocyst cells because oxygen is deadly to nitrogenase, so they form a thick protective cell wall. This process takes up to 24 hours as the surrounding vegetative cells continue to multiply by binary fission (Gallon et al., 1993). An increase in heterocyst cell formation indicated that the sample has actively undergone nitrogen fixation.

In conclusion, the results indicated that Anabaena heterocyst cell formation was greatest at the high light intensity of 375 Lux followed by 300 Lux and 225 Lux.

Acknowledgements

We would like to thank Dr. Huntley and Saddleback College for the experience of presenting our proposal, conducting the actual experiment, and supplying the necessary equipment.

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The Effect of Salinity on Dark Respiration Rates in Pickleweed (*Salicornia virginica*)

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Marsh plants are known to be extremely tolerant of different salinities and they provide much of the methane that contributes to the greenhouse gases. However, these plants are not very well known for contributing to a decrease in carbon dioxide or an increase in oxygen. The current body of knowledge suggests that higher salinities will inhibit these photosynthetic processes of marsh plants, including pickleweed, and it was predicted that there would be significant differences in dark respiration rates between three areas of varying salinities located in Newport Back Bay. Pickleweed samples taken from the Newport Back Bay were tested for carbon dioxide rates while in the dark in order to assess this. The pickleweed samples did not have significant differences between salinities (ANOVA, p = 0.87). Due to this result, it is suspected that if the marsh areas continue to decrease in salinity due to the melting ice caps, the plants will not have a significant change in photosynthetic processes.

**Introduction**

Marsh plants are highly adapted to life in changing salinities. These plants use salinity tolerance mechanisms to gain the necessary amount of freshwater from their naturally saline environment. These salinity tolerance mechanisms were defined as salt metabolism by Cheeseman (1988). Cheeseman (1988) goes on to explain the different aspects of salt metabolism including the cellular compartmentalization of salt and the salt’s initial movement into the cell across the “root cell plasmalemma” and through multiple transport systems found within the plant. Due to this excess work necessary to deal with the salt in the water, the plant loses energy by performing the various mechanisms. Seemann (1985) defined this excess work as salt stress. He also went on to describe a decrease in stomatal conductance when there is salt stress on the plant, *Phaseolus vulgaris* (cv. Hawkesbury Wonder). In his experiment, Seemann also saw a decrease in carbon dioxide uptake and photosynthetic performance. In an experiment, conducted by Alexander and Dunton (2002), it was discovered that flora germination, growth and productivity of salt marsh vegetation increased with fresher waters, due to the decrease in salt stress. Williams et al. (1980) found that by blocking ocean connection to a marsh and having fresh water runoff accumulate, the plants did have a higher productivity, reinforcing the higher productivity of marsh land plants when inundated with fresh water. Another study, by Robert Pearcy and Susan Ustin (1984), on the effects of salinity on C4 and C3 plants of California marshes showed that the C3 plants fluctuated in their growth and photosynthesis in low salinity where as the C4 plants did significantly better in the low salinity.

The major greenhouse gases include carbon dioxide and methane. Wetlands are a natural contributor to the methane released into the atmosphere. Whalen (2005) states that the wetlands account for as much as one fourth of all emissions from all anthropogenic and natural sources. Fredrick and Major (1997) describe an inundation of salt water over marshland areas due to global climate change. While most papers discuss the effects the methane from the marshland have on global climate change, or the ways the marshland will be affected by global climate change, there has not been a significant amount of data collected on photosynthetic properties of the marshland plants which can contribute to the conversion of the green house gas, carbon dioxide into oxygen.

This experiment was performed in order to determine the effects of varying salinity on dark respiration rates in pickleweed (*Salicornia virginica*). It is predicted that the pickleweed from the less saline areas will have greater respiration rates than that from the pickleweed in the higher saline areas.

**Materials and Methods**

The samples of pickleweed were collected from the Newport Back Bay at three different locations along the pathway, one near the Newport Back Bay
Science Center, one just before the dredging areas, and one midway between the other two areas. Salinities were measured at time of extraction using a refractometer. The Back Bay Science Center area was considered as the highest saline area, labeled area one, the midway area corresponded to a mild saline area, labeled area two, and the third, northern area near the dredging was regarded as the low saline area, labeled area three. The collection of the pickleweed occurred at approximately the same time on all days and was taken from consistent areas.

After extraction from their respective areas, the pickleweed groups were measured out to between eight to ten grams per sample for ten samples per area, they were next placed into plastic containers, which were placed into drawers, and then tested for fifteen minutes. Two PASCO GLxplorers were used to measure carbon dioxide rates in the pickleweed over the fifteen minutes. After the fifteen minutes of measurement, average respiration rate was recorded. All measurements for the salinities were taken at approximately the same time, with no more than a thirty minute gap between areas. Including collection, transportation, and equipment set-up time, the samples were kept in the dark for approximately two hours before their measurement.

An ANOVA was then performed on the resulting data from the thirty samples to determine if the differences in respiration rates were significant between varying salinities.

Results

Salinities for the three areas were at 34% for area one, 22% for area two and 20% for area three. After recording the average values of respiration rates per sample, the average respiration rate per group was taken. The average rates per sample were as follows: (area one) between 2.73 and 5.95 cc/g/s, (area two) between 2.29 and 5.16 cc/g/s, and (area three) between 2.80 and 5.07. The average respiration rates per group were as follows: (area one) 3.821, (area two) 3.802, (area three) 4.003 (Table 1).

An ANOVA run between all three areas resulted in a p-value of 0.87, therefore the differences between the three area dark respiration rates are insignificant.

Respiration rates were recorded and showed small differences between the mean values, which corresponds to their large p-value (Figure 1). The salinities were plotted against the mean respiration rates showing a weak correlation between the two variables (Figure 2).

<table>
<thead>
<tr>
<th>Area</th>
<th>Avg Dark Resp. (cc/g/s)</th>
<th>Salinity (%)</th>
<th>Std. Err.</th>
</tr>
</thead>
<tbody>
<tr>
<td>One</td>
<td>3.821</td>
<td>34</td>
<td>0.347</td>
</tr>
<tr>
<td>Two</td>
<td>3.802</td>
<td>22</td>
<td>0.310</td>
</tr>
<tr>
<td>Three</td>
<td>4.003</td>
<td>20</td>
<td>0.220</td>
</tr>
</tbody>
</table>

Table 1. Average dark respiration rate and standard error for the various areas with corresponding varying salinities.
**Discussion**

The results of the experiment show no significant differences between the dark respiration rates of the three areas with varying salinities. It can be concluded from this experiment that the carbon dioxide rates were not influenced by outside air because the measurement system, involving the PASCO GLXplorer, is a closed system. These results support the null hypothesis that pickleweed has no change in dark respiration rates in varying salinities.

The data does not correspond to the body of scientific work already presented. Both Cheeseman and Seemann found that with the salinity tolerance mechanisms, the plants would need to set aside energy, which would otherwise be used in photosynthetic processes (1988 and 1985). It is possible however that the pickleweed is so highly adapted to the varying salinities that the change is not enough to deter normal photosynthetic processes. This would suggest that as the ice caps melt due to global warming created by green house gases, the process of changing carbon dioxide into oxygen by marshland plants will not increase or decrease. Therefore the marshland areas, while important to methane and oxygen production, are not a type of global negative-feedback system to excess green house gases, and offer a only a fixed output of oxygen and decrease in carbon dioxide.

There were several possible sources for error. The distance between extraction sites, which was limited due to dredging in the far inland area of the bay, could have been too small. The extent of stress due to the dredging in the bay is unknown and it may have affected the plants’ metabolic properties. The area where the majority of the pickleweed grows is off limits due to native birds that nest in the marsh area. As a result, the collection of the pickleweed occurred along the bike pathway that circles the bay. This would have affected the study because the bike trail areas are closer to sources of freshwater from homes on the cliffs around the bike trail. The plants from this area might also be undergoing stress from anthropogenic contact.

There are possibilities for furthering this experiment. It is possible to perform the same basic experiment with pickleweed when the marshland area is not being dredged. It is also possible to look at greater varying salinities not found in this population of pickleweed.

**Literature Cited**


The Effect of Mycorrhizal Symbiosis on the Growth and Development of the California Buckwheat plant, *Eriogonum fasciculatum*

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Mycorrhizae are an endosymbiotic relationship between a fungus and a plant’s roots. In exchange for sugar provided by the plant, the fungi increase surface area to improve water uptake and provide plants with greater access to important minerals essential for optimal plant growth. The short-term benefits of mycorrhizal inoculation on *Eriogonum fasciculatum* (California buckwheat) was studied over a four week period. Two experimental groups of *E. fasciculatum* seeds were inoculated with a low concentration and high concentration of mycorrhizal fungi, respectively, and were compared with a group grown without any mycorrhizae. The dry weights of the plants were calculated and an ANOVA indicated no significant differences between the three groups (p=0.247).

**Introduction**

The mutualistic relationship between plants and fungi may have been one of the evolutionary adaptations contributing to the colonization of land by plants. Mycorrhizae or “fungus roots,” is the symbiotic association between fungi and the root systems of plants. The benefits of mycorrhizae include: increase in water uptake, access to important minerals, secretion of growth factors, and protection from infectious pathogens. In exchange, the plant provides the fungi with a portion of the carbohydrates that it produces via photosynthesis. Although not all plants utilize this endosymbiotic association, research has shown that 92% of plant families are mycorrhizal (Wang et al., 2006). The various species of fungi that form mycorrhizal relationships belong to the phyla Basidiomycota and Ascomycota. The most prevalent mutualistic symbiosis is the arbuscular mycorrhizae or endomycorrhizae, which is found in 85% of all plant families (Wang et al., 2006). In arbuscular mycorrhizae (AM), the hyphae of the fungi penetrate the cortical cell walls forming arbuscules, which act as nutrient transfer sites. In contrast, ectomycorrhizae form a sheath in the apoplast of the roots to facilitate nutrient exchange and never penetrate the cell walls. Mutualistic associations involving ectomycorrhizae are formed in ten percent of all plant families.

Research regarding the benefits of mycorrhizae varies depending on type of inoculation, soil environment, and plants used. Abbot and Robson (1979) determined that levels of soil phosphorus greater than what was required for plant growth inhibited arbuscule growth and limited the effectiveness of mycorrhizal fungi. Experimental research of several tropical plants inoculated with vesicular-arbuscular mycorrhizae (VA) in lowland tropical soils resulted in a significant increase in seedling growth and bacterial nodule size (Janos, 1980). Furthermore, the seedlings of some plant species were completely dependent on mycorrhizae and failed to grow in its absence. Baylis (1967) determined that plants lacking mycorrhizae did not survive in some New Zealand forest soils. Experiments conducted by Hayman et al. (1970) on *Allium* and *Coprosma* plants supported the notion that mycorrhizal inoculation had significantly increased shoot dry weight in comparison to plants grown without mycorrhizae. The main focus of this study is to determine the potential short-term benefits of growing California buckwheat plants, *E. fasciculatum*, inoculated with different concentrations of mycorrhizal fungi. It is expected that significant differences in growth rates will exist between the groups inoculated with mycorrhizae and those grown without it.

**Materials and Methods**

A four ounce bag of *E. fasciculatum* seeds (Handy Pantry Sprouting Inc, Springville, UT) were purchased online through Amazon.com (Amazon.com Inc, Seattle, WA). A one ounce package of Myco Grow™ Soluble Mycorrhizae was purchased from Fungi.com (Fungi Perfect® LLC, Olympia, WA) containing spore masses of various species of both ectomycorrhizal and endomycorrhizal fungi. Miracle-Gro® Potting Mix (The Scotts Company LLC, Marysville, OH), a potting mix...
containing sphagnum peat moss, wetting agent, coconut fibers, and other organic material, was purchased from Green Thumb International Nursery in El Toro, California. In order to standardize the growing media, the potting mix was spread one inch deep into three baking sheets, covered with aluminum foil, and heated in an oven at 90°C for two hours. Care was taken to not exceed 90°C as higher temperatures may produce plant toxins. The heated potting mix was placed aside to cool while any microorganisms initially present was assumed to have been destroyed.

After cooling at room temperature for four hours, the potting mix from each of the three baking sheets was carefully transferred over to their respective clean potting containers and labeled as low concentration, high concentration, and control. Next, 1.5039g ± 0.0001g of the mycorrhizae spore mass was added to the potting mix in the low concentration group. The potting soil in the high concentration container was treated with 3.5283g ± 0.0001g of mycorrhizae spore mass. The values used for the mass of the added mycorrhizae correlated with the manufacturer’s recommended suggestions.

Deionized water was added to the potting soil for each group until the soil was damp. *E. fasciculatum* seeds (n=20) were placed on top of the soil one inch apart in each container. The seeds were covered with a damp paper bag as per manufacturer’s instructions and were allowed to germinate for three days. Daily misting with deionized water was done twice a day to avoid desiccation, with special care taken to avoid excessive saturation of the delicate seedlings. Root and shoot formation was observed in at least 70% of the seeds. The three containers were placed outside in close proximity to ensure equal exposure to sunlight and similar environmental conditions.

The plants were carefully maintained and watered approximately twice a week with deionized water for the entire four week duration. Upon completion of the four week growth period, all plants were carefully uprooted and separated. Roots were rinsed with lukewarm water to wash away any soil mass adhering to it. In order to obtain dry weight, all the plants were placed back onto baking sheets and heated at 90°C until a constant weight measurement was found. Results of the dry weight of all the plants were recorded in grams and analyzed using an analysis of variance (ANOVA).

**Results**

A comparison between the mean dry weights of the plants showed no significant differences in growth rates among the three groups tested in this experiment (ANOVA, p=0.247). The amount of growth (mean dry weight) was 0.9952g ± 0.03165 (±S.E.M.), 1.0896g ± 0.0445, and 1.0423g ± 0.0389 (±S.E.M.), for the low concentration, high concentration, and control group, respectively.

**Discussion**

This experiment indicates that the California buckwheat plants grown in soil containing mycorrhizae did not grow significantly greater than those grown in standardized soil containing no mycorrhizae. It cannot be assumed that California buckwheat does not form any symbiotic relationship with fungus based solely off this experiment. Other variables such as soil pH, sun exposure, temperature, and most importantly, soil quality, were possible variables that influenced growth rates. Research conducted by Virant (1998) concluded that the existence of VA mycorrhizal fungi was in fact present in buckwheat plant roots, however, its presence was rare. The benefits of mycorrhizal inoculation can be best distinguished when the amount of available phosphate in the soil is a limiting factor. The fungi help acquire phosphorous from areas of the soil that the plant’s roots do not have access to, increasing uptake.

![Figure 1. Mean dry weight in grams of California buckwheat plants grown with various concentrations of mycorrhizal inoculation. Error bars indicate 95% confidence intervals.](image-url)

To expand on this experiment, it would be interesting to observe the difference in growth rates among California buckwheat grown in various soil environments with differing concentrations of mycorrhizae. Applying these procedures to other species and families of plants could provide different results as well, as other plants form different mycorrhizal associations. Further, contrasting growth rates at various time durations may provide information regarding the mechanisms of mycorrhizal associations.
Acknowledgements

I would like to thank the wonderful staff at Green Thumb International Nursery in El Toro, California, for being so helpful and providing so much useful information regarding plant growth. Special thanks go out to my mother for allowing me to use the oven to heat soil and dry plant material.

Literature Cited


The Effects of Antibiotic Sensitivity in *Staphylococcus aureus* Selected for Increased Halotolerance

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*Staphylococcus aureus* is capable of growing in environments of high NaCl concentrations. This heightened tolerance for salt has been associated with an increase in resistance to β-lactam antibiotics and methicillin-resistant *Staphylococcus aureus* (MRSA). *S. aureus* was grown in a medium of increasingly higher concentrations of NaCl, allowing selection for the most halotolerant strain. A broth was prepared using *S. aureus* grown in 12.5% Mannitol Salt Agar and then spread onto six plates of 7.5% NaCl agar. For comparison, the wildtype strain was spread onto six additional plates of 7.5% NaCl agar. Both strains were allowed to grow in the presence of ampicillin. After incubation at 37°C for 48 hours, diameters of zones of inhibition were measured. The mean diameter of the zones of inhibition for the halotolerant strain was significantly smaller than the mean diameter of the zones of inhibition for the wildtype strain (n = 6, one-tailed, unpaired, t-test, p = 1.68 x 10⁻¹⁰) implying an increase in resistance in the halotolerant strain. Current research has shown that in the presence of NaCl there is an increased production of altered penicillin-binding proteins (PBP 2a) that is directly linked to an increase in antibiotic resistance.

**Introduction**

*Staphylococcus aureus* is unique among members of the Domain Bacteria in that it has the capacity to grow in environments of high-salt concentration. A key factor contributing to the halotolerance of *S. aureus* is the ability to accumulate what are called compatible solutes (Stapleton et al., 2006). Under osmotic stress, bacteria respond with an efflux of water, resulting in a decrease in cell volume and increasing intercellular concentrations. In lieu of a strictly passive adjustment, which could bring intercellular concentrations of certain molecules to damaging levels, the cell responds to the initial decrease in volume by actively transporting solutes into the cell that are considered “compatible” with cellular processes. The primary compatible substances consist of K⁺ and a group of amino acids called osmoprotectants, primarily proline and glycine betaine, which when transported into the cell, serve to adjust the intercellular osmolarity without greatly inhibiting cellular processes (Csonka, 1989).

The ability of *S. aureus* to survive in a high salt environment has endowed it with a unique pathogenicity. *S. aureus* is responsible for one-third of all food-borne illnesses in the United States and can survive in food of high osmolarity better than any other pathogenic bacteria (Wengender and Miller, 1995). Growth of *S. aureus* in food is favored under the high-salt, low-moisture conditions which are commonly utilized in food preservation (Graham and Wilkinson, 1992). In addition, the halotolerant characteristics of *S. aureus* have also been linked to methicillin resistance through an increased production of a cell wall enzyme capable of thwarting the antibiotic mechanism (Madiraju et al., 1987). MRSA infections often occur as a result of an initial colonization of a high-salt environment such as the skin (Stapleton et al., 2006).

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a strain of *S. aureus* that is resistant to a group of antibiotics called beta-lactams (Russell et al., 1990). Beta-lactam antibiotics work by binding to and inhibiting the enzymes (commonly called penicillin-binding proteins) responsible for cross-linking of the peptidoglycan layer in bacterial cell walls, thus compromising cell wall integrity and resulting in autolysis (Lewis et al., 2002). MRSA is closely associated with an altered penicillin-binding protein (PBP 2a) that exhibits a low affinity for beta-lactam antibiotics (Madiraju et al., 1987). Studies have indicated that the expression of PBP 2a is increased in the presence of NaCl (Madiraju et al., 1987).
It was hypothesized that the halophilic strain of bacteria, grown in the presence of ampicillin would exhibit a higher degree of antibiotic resistance than that of the wildtype (initial) bacteria. The effect of beta-lactam sensitivity on saline tolerant bacteria was examined by observing growth of a strain of *S. aureus*, selected for extreme halotolerance in the presence of ampicillin.

**Materials and Methods**

On 7 April 2009, Mannitol Salt Agar (Difco) was prepared at concentrations of 7.5%, 10%, 12.5% and 15% NaCl. Ingredients were obtained from the Saddleback College Biology stockroom. All agars were poured into sterile Petri dishes and allowed to solidify. Plate counts were as follows: 21 plates of 7.5% NaCl agar, 19 plates of 10% NaCl agar, 20 plates of 12.5% agar and 23 plates of 15% agar. All 21 of the 7.5% NaCl agar surfaces were plated with *S. aureus* and incubated for 48 hours at 37°C, after which time, any surviving organisms were selected and re-plated onto 19 sterile Petri dishes containing 10% Mannitol Salt Agar. Plates were allowed to incubate for 48 hours at 37°C. Any surviving organisms were selected and re-plated onto 20 sterile Petri dishes containing 12.5% Mannitol Salt Agar. All plates were allowed to incubate for 48 hours at 37°C, after which time, plates were reexamined for growth and re-plated onto 23 sterile Petri dishes containing 15% Mannitol Salt Agar and incubated for 48 hours at 37°C.

On 28 April 2009, a broth was prepared using a colony of bacteria grown on the 12.5% salt agar plates. Fifty microliters of broth containing the wildtype strain of *S. aureus* (attained from Saddleback College Biology stockroom) was pipetted onto the surface of six agar plates containing 7.5% NaCl and spread using aseptic technique. Fifty microliters of broth containing the halotolerant strain of *S. aureus* was pipetted onto another six agar plates containing 7.5% NaCl and also spread using aseptic technique. Sterile paper discs were dipped in 6 µg/mL ampicillin solution before being placed onto each of the 12 plates. Four discs were placed onto each plate. All plates were incubated at 37°C for 48 hours. On 30 April 2009, diameters of the zones of inhibition were measured enabling comparisons to be made regarding antibiotic resistance. A one-tailed, unpaired t-test was run to compare the mean diameters of the zones of inhibition exhibited by both the original and the halotolerant strains of *S. aureus*. All data analysis was performed using Microsoft Excel 2007.

**Results**

Maximum salt concentration permitting survival of bacteria was 12.5% NaCl. This strain was therefore used in subsequent testing with ampicillin. All plates exhibited distinguishable zones of inhibition. Descriptive statistics were used to ascertain the mean diameter of zones of inhibition for plates containing both halotolerant and wildtype strains of *S. aureus*. The mean diameter for the zones on the halotolerant plates was 1.66 ± 0.05 cm (n = 6, mean ± S.E.M.). The mean diameter for the zones on the wildtype plates was 2.22 ± 0.04 cm (n = 6, mean ± S.E.M.). A one-tailed unpaired t-test revealed the mean diameter of the halotolerant plates to be significantly smaller than that of the wildtype plates (p = 1.68 x 10⁻¹⁰). It was concluded that the halotolerant strain of *S. aureus* displayed an increased resistance when grown in the presence of ampicillin.

**Discussion**

*Staphylococcus aureus* was successfully grown on plates of Mannitol Salt Agar with NaCl concentrations of 7.5%, 10%, 12.5% and 15%. Though literature cited successful growth on media containing 15% NaCl, results were only witnessed as high as 12.5% NaCl (Ochiai, 1998). One possible explanation for this was a shortage of amino acids in the medium. Growth of the pathogen at high salt levels is directly dependent on the presence of exogenous proline and glycine betaine in the agar which the cell utilizes for osmoregulation in high salt environments (Bae and Miller, 1992). Therefore it can be proposed that growth was inhibited at 15% NaCl due to an insufficient concentration of amino acids in the agar.

Although the growth of *S. aureus* was only witnessed up to 12.5% NaCl, this concentration
would be lethal for most other pathogens. This begs the question as to why \textit{S. aureus} is capable of surviving in conditions considered fatal to other bacteria. Most bacteria are able to cope with an increase in the osmolarity of their external environment, however not to the same extent as \textit{S. aureus} (Bae and Miller, 1992). Despite this discrepancy, most bacteria use the same osmoregulatory strategies as \textit{S. aureus}. Like \textit{S. aureus}, most bacterial pathogens react to high osmotic environments through a passive efflux of water and an active influx of potassium, proline, glycine betaine and other such osmoprotectants (Csonka, 1989). In addition, both \textit{S. aureus} and \textit{Escherichia coli} utilize a similar transport system for the active uptake of proline. Proline uptake in \textit{S. aureus} and \textit{E. coli} is mediated by two different transport systems, a low-affinity transport system and a high-affinity transport system, however it is the low-affinity system in both pathogens that is primarily responsible for the accumulation and uptake of proline (Bae and Miller, 1992). Therefore, given these similarities, what is it that allows \textit{S. aureus} to grow in environments of higher osmolarity? This ability stems from two distinctions between proline transport systems. First, the low-affinity proline transport system of \textit{S. aureus} has a greater tolerance for NaCl than does that of \textit{E. coli}. In fact, the NaCl concentrations needed for optimal activation of the proline transport system in \textit{S. aureus} are so high that they would inhibit the parallel system in the \textit{E. coli} (Wengender and Miller, 1994). Second, the low-affinity proline transport system of \textit{E. coli} has the ability to transport glycine betaine in addition to proline. The effect of this is a reduction in proline uptake by the cell as glycine betaine competes for access to the active site of the transporter. \textit{S. aureus} utilizes an additional transport system for uptake of glycine betaine thus preventing inhibition of proline uptake.

The \textit{S. aureus} strain grown in a 12.5% NaCl Mannitol Agar displayed smaller zones of inhibition than those of the wildtype strain when plated and allowed to grow in the presence of ampicillin. These results suggested an increase in the antibiotic resistance of the halotolerant strain thus providing support for our hypothesis. Sabath & Wallace (1977) attained similar results by observing a 1600-2500-fold increase in the proportion of highly resistant cells when cultivated on a growth medium containing 5% NaCl in the presence of methicillin. One probable mechanism for the increased resistance is an increase in the production of an altered penicillin binding protein that exhibits a low affinity for β-lactam antibiotics. The target for β-lactam antibiotics are a group of enzymes localized on the outer leaflet of the bacterial cytoplasmic membrane that are responsible for catalyzing the formation of cross-linking in the peptidoglycan layer of bacterial cell walls. These enzymes have a high affinity for β-lactam antibiotics and are therefore referred to as penicillin-binding proteins (PBP’s) (Wilke et al., 2005). Binding of the antibiotic with the enzyme interferes with the cross-linking reaction and results in weakening of the cell wall and eventual cell lysis (Stapleton and Taylor, 2002). Studies have revealed the existence of an additional altered penicillin binding protein (PBP 2a) that is intimately involved with increased antibiotic resistance. PBP 2a exhibits a decreased affinity for β-lactam antibiotics and is capable of taking over the functions of the other four PBPs. There are two schools of thought in explanation of the increased resistance. Studies have shown that in the presence of NaCl there is an increased expression of PBP 2a which is directly linked to an increase in resistance (Madiraju et al., 1987). An alternative hypothesis was proposed by Chambers and Hackbarth (1987), who asserted that increased NaCl enhances resistance through inhibition of antibiotic-triggered autolysis when in the presence of PBP 2a.

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Effects Of Simulated Acid Rain On Earthworms (*Lumbricus terrestris*)

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Earthworms (*Lumbricus terrestris*) play an essential role in the biodiversity of forested areas of the world, such as decomposing organic matter into necessary minerals for plants and providing a source of food for many organisms. In this experiment, earthworms were placed in a controlled environment with two pH solutions to mimic areas affected by acid rain and areas that are not. Earthworms (n=50) were allowed to migrate to their desired pH environment for 24 hours. There was a significant difference in preference for earthworms between the two-pH soils (p=1.19x10^-10). 84.42% Earthworms preferred the 6.0-pH compared to the 3.5-pH soil.

Introduction

Earthworms (*Lumbricus terrestris*) play a significant role in many of the world’s soil biomass. Earthworms are essential to maintaining and aerating soils to help promote root elongation, reduce runoff, and decompose organic material to provide nutrients for plants. Earthworms influence soil microbial communities and hence, have effects on microbial processes related to soil organic matter status and nutrient dynamics. They also affect the activities of other soil-inhabiting invertebrates, either by modifying their environment or through competition for feeding resources (Edwards, 2004). However, increasing pollution in the environment has led to acid rain and the development of a higher acidic concentration in soils. Earthworms are generally absent from very acidic soils (pH less than 3.5) and are scarce in soils with pH less than 4.5. Although there are considerable differences among species in their pH preference, the majority of temperate climate species are found in the pH range 5.0 to 7.4 (Satchel 1967, Bouche 1972). As a result, increases in acid rain in parts of the United States have the potential to decrease earthworm populations in areas with high acid rain levels. In a controlled experiment done by Malmer (1976) a supply of artificial acid rain with a pH of 3.0 was added to a podsol soil along with normal precipitation, which showed a decrease in earthworm activity.


in the soil’s pH. Acid rain can drop the soil pH to as low as 3.5 in some areas of the United States which will diminish nutrients in the soil. This can lead to reduced plant growth and fewer numbers of earthworms in the soil. In our experiment, earthworm preference on soil pH was tested using two solutions; it is predicted that earthworms will migrate to the less acidic side.

Materials and Methods
Fifty earthworms (Lumbricus terrestris, n = 50) were purchased from the Bait and Tackle shop in Dana Point, CA. One plastic gutter three meters in length and topsoil were purchased from Home Depot in Mission Viejo CA. The gutter was cut into three pieces, each piece being one meter in length. Fifty, one millimeter diameter holes were drilled into the bottom of the gutters so the water could percolate through the soil but not pool up and drown the worms. An acid rain solution consisting of 1M HNO₃, 1M H₂SO₄ and tap water, which was obtained from the Saddleback College biology lab was made. Ten milliliters of HNO₃ and ten milliliters of H₂SO₄ were added to eighteen liters of water to make the 3.5-pH acid rain solution. 0.5 milliliters of HNO₃ and 0.5 milliliters of H₂SO₄ were added to eighteen liters of water in a separate container to make the 6.0-pH acid rain solution. Soil was placed in the gutters at a depth of fifteen centimeters. The 3.5-pH acid rain solution was added to the right side of the three gutters and the 6.0-pH acid rain solution was added to the left side of the three gutters. Once the soil had sufficiently drained, ten earthworms were added to each of the gutters, all of which were placed in the center of the two soil pH solutions. The environments were watered with their respective pH solutions two times a day to keep the soil saturated at their respected pH levels. The saturated soil will allow the earthworms to move through the soil and keep them from dehydrating. The worms were allowed to move freely to their desired pH soil solutions for twenty-four hours after which each side of soil was completely removed and each worm was located and counted in their respected locations. Four separate trials were conducted using a total of thirty worms per trial. The removed soil was thrown out and new soil was used for each subsequent trial to prevent mixing of the two different soil pH levels.

Results
A small amount of worms died in each trial, the earthworms that died were removed from the study. Earthworm deaths had no correlation to acidity as an equivalent amount of worms died on both sides. Deaths were most likely due to the manner in which they were stored prior to their insertion in the study. The living earthworms on the 6.0 pH side were counted after the each trial and divided by the total number of living earthworms. The same calculation was made for the 3.5 pH side. As shown in figure 1, 82.42% of the earthworms migrated to the higher pH (6.0). 16.75% of the earthworms migrated to the lower pH (3.5). A significant difference was found between the earthworms preference of the two respected pH’s (p=1.19x10⁻¹⁰ one tailed unpaired T-test assuming unequal variances).

Discussion
Based on our results, earthworms prefer low acidic to non-acidic soil environments. Earthworms are the backbone of many environments, providing a nutrient rich setting to promote plant growth. Earthworms are a beautiful example of the natural cultivation of the soil; for year after year the thrown-up castings cover the dead leaves; the result being rich humus of great thickness (Darwin, 1881). As the world becomes more populated, issues such as carbon emissions, vehicle emissions, and deforestation are increasingly becoming an issue with the environment. As a result, we are seeing phenomena such as acid rain which can drop soil pH’s to as low as 3.5 in some areas of the United States. As a result, environments that are exceedingly affected by acid rain can start to see a decline in the earthworm population.

As Darwin (1881) first noted, earthworms move large amounts of soil from the deeper strata to the surface. The amounts moved in this way range from
2 to 250 tons per hectare per annum, equivalent to bringing a layer of soil between 1 mm and 5 cm thick to the surface every ear, creating a stone free layer on the soil surface (Edwards, 2004). A decrease in earthworm population will lead to a decrease in plant and animal biodiversity due to lack of essential resources that earthworms provide and degradation in essential nutrients found in the soil. Not only do earthworms provide the soil with nutrients but earthworms are also an essential part of the food chain. Animals such as birds, lizards, frogs, toads, turtles, gophers and moles include earthworms in their daily diet.

As stated by Edwards (2004) a major difference between short-scale experiments and the real world is that, in confined small experiments, earthworms have limited opportunities to choose their environments and move away. This probably explains why they often lose weight or die in laboratory experiments. In our short-scale, confined experiment we had very conclusive results with the trials that were performed. As a result, areas affected by acid rain could potentially see a deterioration of earthworm populations in greater numbers than we saw in our experiment. Other than soil moisture, the soil properties that appear to be most important include texture, depth, pH and organic matter content (Edwards 2004).

P. Borella (pers. comm.) has suggested that earthworms may alter the pH environment of the soil by ingestion and excretion. In our study we demonstrated that earthworms preferred less acidic soil, an interesting area of further research could involve the change in low pH environments over time in the presence of earthworms.

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Metabolic Rate and development stages in White Mice (Mus musculus)

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This study examined the effect of ontogeny would have an effect on the metabolic rate of mice (Mus musculus) at ambient temperature. It was expected that metabolic rate of adult mice would be the highest. Six to thirteen day old, two to three week old, and four to six week old laboratory white mice were used. The test showed a significant difference among the metabolic rates (ANOVA, p=1.49 x 10^{-11}). A post hoc test showed the hopper mice (two and three weeks old had the highest metabolic rates, thus refuting our original hypothesis.

Introduction
Metabolism sustains life. It is the process by which energy and materials are transformed within an organism and exchanged between the organism and its environment (Gillooly, et al., 2001). Ontogeny is the study of the development of an organism from fertilized egg to maturation. Mice (Mus musculus) were used to test if metabolic rate would be higher during developmental stages or during adulthood. Metabolic rate is influenced by an organism’s size, activity, insulation and temperature of its surroundings. Measurements of metabolic rate in newborns are interesting not only in relation to thermoregulation; the way in which basal metabolic rate varies with age and with weight poses fundamental questions about the physiological changes that occur during the period of transition from being part of the adult to being a free living organism (Hill and Rahimtulla, 1964). Gillooly (2001) observed that body mass has a significant effect on the metabolic rate of an organism; as body mass increases, the daily energy required for an organism also increases. Variables that have an influence on metabolic rate are insulation, which helps maintain homeostasis, and efficiency of energy production (Mount, 1971). The environment affects how much energy the organism exerts in attempting to maintain stable homeostasis (Eldgar and Harvey, 1987). Two factors, among others, that affect metabolism are the organism’s type and temperature. A homeotherm is an organism that can maintain a constant body temperature across a range of environmental temperatures. An endotherm is an organism in which the majority of its body heat is derived from its own metabolism. Mice are homeothermic endotherms, an animal that generates majority of body heat by metabolism and maintains consistent internal temperature. When determining the resting metabolic rate, the organism is left undisturbed, and the organism will use minimum required energy. If the organism is stimulated and exerts energy, more energy is required to perform actions, therefore increasing metabolic rate (Degen, et al., 1998). Given that adult mice utilize more energy due to larger body mass and activity, metabolic rate should increase throughout development of the mice.

Materials and Methods
Fuzzies - six to thirteen day old mice, hoppers- two to three week old mice, and adults- mice greater than three weeks of age, were tested to see if metabolic rate increased or decreased during developmental period. On March 10, 2009, ten female fuzzies were purchased from Sandbar Pet Shop, Mission Viejo. Mice were separated at time of purchase to minimize stimulation. The study began on March 10, 2009. The mice were first marked along the tail with different patterns for identification, and then weighed on an electronic balance. After recording the weights, the mice were left undisturbed in a partially lit room at 23.5°C in containers with a volume of 50.51 mL for a period of ten minutes in order to reduce activity and obtain resting metabolic rate. A FoxBox Sable System, Las Vegas, Nevada was used to record atmospheric oxygen levels; it was set at a standard of 20.95%. Each trial was given a total time of twenty minutes. The first and last five minutes were recorded in order to determine any changes within atmospheric oxygen levels. The fuzzies were placed in the 50.51 mL tube and was given oxygen from pumps while the FoxBox recorded oxygen levels. After the first five minutes, the oxygen pump was taken out of the tube and the FoxBox was then connected to the tube, recording oxygen levels of each fuzzies for a period of ten
minutes. After the ten minutes, the FoxBox tube was taken out and left undisturbed in order to retain original atmospheric oxygen level. Same procedures were used for determining metabolic rate of adolescent (hoppers) placed in a 78.95 mL container and adult mice place in a 162.01 mL container. The data was corrected for the difference in starting and ending O₂ levels. The metabolic rate was determined by using Wither’s (1977) 4a. equation standardized to STPD.

Results
The amount of O₂ consumed by the hoppers is significantly greater than the amount of O₂ consumed by the fuzzies and the adults. \( (p = 1.49 \times 10^{-11}, \text{ANOVA Single Factor}) \) Figure 1. The mean O₂ produced by the fuzzies was \( 4.56 \pm 0.0533 \text{ cc O}_2 \text{ g}^{-1} \text{ hr}^{-1} (\pm \text{S.E.M}) \). The mean O₂ produced by the hoppers was \( 6.78 \pm 0.118 \text{ O}_2 \text{ g}^{-1} \text{ hr}^{-1} (\pm \text{S.E.M}) \). The mean O₂ produced by the adults was \( 5.21 \pm 0.0729 \text{ O}_2 \text{ g}^{-1} \text{ hr}^{-1} (\pm \text{S.E.M}) \). Due to the significance of the ANOVA, post hoc was run and showed significance among all three groups.

Discussion
Metabolic rate of animals is influenced by developmental stage, body mass, insulation, and temperature. Metabolic rate correlates with body mass due to the increase of energy needed in accordance to the increase in cell counts and cellular activity. As body mass decreases, an organism loses heat more readily due to the surface area to volume ratio (Dilla, et al., 1949; Burton & Edholm, 1955), therefore, increasing metabolism to bring body temperature back to standard conditions. Temperature changes metabolism by affecting the metabolic rates. The effect of temperature on a biological process is traditionally expressed as a Q₁₀, which quantifies temperature

Dependence across a limited temperature range (Gillooly, et al., 2001). Temperature affects it in such a way that when temperature decreases, homeotherms increase metabolic rates in order for them to maintain their constant body temperature. The ontogeny of metabolic rate in white mice was investigated at three developmental stages in regulated environment.

The study showed that fuzzies had the lowest metabolic rate out of the three groups that were tested. This could be due to the fuzzies lacking a layer of insulation, causing them to increase metabolism to compensate for the heat loss. Muscles and sense of sight are in the process of full development, causing weakness in movement and lack of stimulation from environment. As a result of deficient muscle movement, not as much cellular activity is needed, causing metabolic rate to be low. The fuzzies have not yet opened their eyes, therefore, is not as stimulated in comparison to organisms that have fully developed senses. Through instinct, the infant mice are able to exert minimal energy required to find warmth and food.

In the next stage of development, hoppers, muscles have fully developed to the point of free movement and eyes have fully opened; hoppers attained the highest metabolic rate. If the organism is stimulated and exerts energy, more energy is required to perform actions allowing increase in metabolic rate (Degen et al., 1998). The fully developed muscles and senses allowed hoppers to become more stimulated to their surroundings.

The adult mice decreased in metabolic rate as a result of growing accustomed to their surrounding environment. Based on our previous study on the effect of temperature and insulation on metabolic rate of white mouse, it was proven that insulation reduces cost of homeostasis. Lack of insulation will make the white mice more susceptible and sensitive to the outside temperature, causing them to expend more energy to keep its body temperature constant. The adult mice have grown into full maturation and their muscles and joints are not as newly developed as hoppers.

Body size, developmental stages, insulation, and temperature have a substantial effect on metabolic rate of homeothermic endotherms. Fuzzies, hoppers, and adult mice were used to look at changes in metabolic rate during developmental stage. Fuzzies attained the lowest metabolic rate. Although fur was starting to develop, fuzzies used minimal energy to maintain body temperature. Organisms with insulation were able to conserve body heat and maintain a constant body temperature (Kenagy & Pearson, 2000). Hoppers had highest metabolic rate
due to their newly developed muscles which allowed them to exert more energy. Although adult mice have reached full maturation, their metabolic rate decreased due to less activity and stimulation in comparison to hoppers. In conclusion, metabolic rate fluctuates through stages of development, depending on how much energy is being used, body mass of the organism, and surrounding environments that can affect it.

Acknowledgments

The researchers would like to thank Tony Huntley and Steve Teh for their contributions to the experiment.

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Differences in Respiration and Heart Rate Between Male Track Runners and Male Football Players

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Football players and track runners tend to be, overall, physically fit, but each has its own strengths and weakness. With the differing in strengthening routines and main focuses put on different muscle groups, yet still aimed at a common goal, these two sports make a good study for testing differences between heart rates and respiration rates. Saddleback College football players and track runners had their heart rates and respiration rates taken prior to running and directly after the run. Track runners should show lower respiration rates and lower heart rates, before and after physical exertion, than football players. The initial hypothesis was supported by the collected data. The track runners had significantly lower heart rates (\( p = 2.0 \times 10^{-8} \)) and significantly lower respirations (\( p = 9.8 \times 10^{-4} \)) after physical exertion.


**Introduction**

Every athletic sport has its own training and exercise routines that are geared at making the participant physically fit according to the standards of that sport. Football and track are two sports that seem to have the same exercise goals; speed, strength, and endurance. The ability to exert oneself for a long period of time is an essential part of sports and competition. Aerobic and anaerobic exercises are used to facilitate endurance training. Aerobic, for with oxygen, is also known as cardio and tends to have a heart rate within the range of 40% to 80% of maximum heart rate. Anaerobic, for without oxygen, is training that is done at a heart rate above 80% of maximum heart rate. For instance, sprinting would be an example of anaerobic exercise, because the runner will become out of breath during the run. Jogging would be an example of aerobic exercise, because the runner would only be slightly out of breath during the run. Due to the amount of time spent running, track runners should have better endurance levels than football players, as well as better control over their respiration rates. High endurance running increases the strength of heart muscles. A strong heart tends to have a high “stroke volume,” which means the heart can pump more blood with each beat than a weaker, untrained heart (Thompson, 2002). The training and exercises football and track athletes perform strengthen and prepare their bodies for the strain and fatigue the sport puts on the player. The practice regiment of football and track athletes affects the regular respiration and heart rate during the physical activity and affects the overall threshold level of the athletes (Fukouka, 1997). It is believed that the track players’ practice routine would cause their hearts to have a higher variability and to be more adaptable to the stresses of physical exertion (Tsai, 2006). In turn, the track runners should exhibit lower heart rate and lower respiration rates after physical exertion. The lack of studies on differences between these two athletes, led to this current study.

**Materials and Methods**

Sixteen male Saddleback College athletes, eight football players and eight track runners, were used in this study. No specificity on position for the football players or event for the track runners was used in selection of subjects. The data collecting occurred in the late afternoon at the Saddleback College track and football field. For both groups, data was taken before practices, so extraneous exercise prior to the data collecting would not occur. For both groups, resting heart rate and respiration rates were taken as the subjects arrived at the track or football field. The resting heart rate and respiration rate were taken at one-minute intervals by counting heart beats and counting breaths (one inhale and one exhale= one breath) respectively. Once resting data was taken, each subject ran two laps around track. The first lap was a paced run and the second lap was sprinted. After the two laps, the heart rates and respiration rates were again taken in the manner previously explained. Statistical analysis was run on all four data pairings by using unpaired, one-tailed t-tests.

**Results**

A one-tailed unpaired t-test was performed to determine significant differences between the heart rates and respiration rates of football players and track runners. The mean resting heart rate and respiration rate of football players was 83.875 ± 3.686 (± S.E.M., n=8) beats per minute and 22.125 ± 0.789 (± S.E.M., n=8) breaths per minute respectively. The mean resting heart rate and respiration rate of track runners was 52.25 ± 0.940 (± S.E.M., n=8) beats per minute and 24.5 ± 0.982 (± S.E.M., n=8) breaths per minute respectively. The track runners had a significantly lower resting heart rate, (p= 1.7 x 10⁻⁵) (figure 1), and a significantly lower resting respiration, (p= 4.1 x 10⁻²) (figure 2), than football players. The mean heart rate and respiration rate after exercise for the football players was 155.75 ± 3.039 (± S.E.M., n=8) beats per minute and 44.625 ± 2.521 (± S.E.M., n=8) breaths per minute. The mean heart rate and respiration rate after exercise for the track runners was 91.375 ± 0.844 (± S.E.M., n=8) beats per minute and 32.875 ± 0.639 (± S.E.M., n=8) breaths per minute. The track runners also had significantly lower heart rates, (p= 2.0 x 10⁻⁸) (figure 3), and respiration rates, (p= 9.8 x 10⁻⁴) (figure 4), after exercise than football players.

![Figure 1](image_url)

**Figure 1.** Mean resting heart rate of football players and track runners. The football mean resting heart rate was 83.875 ± 3.686 beats per minute (± S.E.M., n=8). The track mean resting heart rate was 52.25 ± 0.940 beats per minute (± S.E.M., n=8). The track runners’ resting heart rates were significantly lower than football players’ (p= 1.7 x 10⁻⁵ one-tailed unpaired t-test). Error bars represent S.E.M..
Figure 2. Mean resting respiration of football players and track runners. The football mean resting respiration rate was 22.125 ± 0.789 breaths per minute (± S.E.M., n=8). The track mean resting respiration rate was 24.5 ± 0.982 breaths per minute (±S.E.M., n=8). The track runners’ resting respiration rates were significantly lower than football players’ (p=4.1 x 10⁻² one-tailed unpaired t-test). Error bars indicate S.E.M.

Figure 3. Mean heart rate of football players and track runners after exercise. The football mean heart rate was 154.75 ± 3.039 beats per minute (±S.E.M., n=8). The track mean heart rate was 91.375 ± 0.844 beats per minute (±S.E.M., n=8). Track runners’ heart rates were significantly lower than football players’ (p=2.0 x 10⁻⁸ one-tailed unpaired t-test). Error bars indicate S.E.M.

Discussion

Both football and track have rigorous endurance training, but in football the endurance only has to last a short amount of time, less than a minute on some plays, compared to track. Track has events that require both long term endurance, such as any event over 3000 meters, and short term endurance, such as sprints or relays. Track runners have higher endurance levels than football players, because they train closer to their maximum heart rates. By exercising at the anaerobic level, the body is increasing the exertion thresholds as well as increasing the amount of time the body can stay at the aerobic level during exertion. With the data supporting our hypothesis that track runners statistically have lower heart rates and respiration rates before and after physical exertion, this suggests that track runners have the means to better control their heart rate and the amount of respiration during exercise and physical activity. Respiration rate is the number of breaths a living being takes in a given period of time and heart rate is the number of beats the heart of a living being makes in a given period.

Figure 4. Mean respiration after exercise of football players and track runners. The football mean respiration rate after exercise was 44.625 ± 2.521 breaths per minute (±S.E.M., n=8). The track mean respiration rate was 32.875 ± 0.639 breaths per minute (±S.E.M., n=8). Track runners’ respiration rates after exercise were significantly lower than football players’ (p=9.8 x 10⁻⁴ one-tailed unpaired t-test). Error bars indicate S.E.M.

The both rates were used in determining the endurance levels of the athletes. The track runner’s heart and lungs are better adapted to stay at the aerobic level for a longer period of time, because the endurance training trained the heart to keep the heart rate below the 80% of maximum heart rate during exertion. A runner’s heart can pump the same amount of blood in a minute using fewer beats than a football player’s, so even though both did the same level of activity, the track runners exhibited lower more steady heart beats and less labored breathing than the football players. The average resting heart rate of endurance athletes is around 50 to 60 beats per minute (Thompson, 2002). All of the tested track runners’ resting heart rates fit into this range and all of the football players’ resting heart rates were above this range. With these results, the suggestion can also be made that football training and practice regiments do not significantly affect how fast the heart beats or how much oxygen intake is needed during physical activity. Also, the training for football players is considered not significantly affective on the kinetic development of oxygen intake and heart rate when the exercise is submaximal, but it does increases oxygen intake maximum levels (Fukuoka, 1997). Our study provides evidence that endurance exercise
programs can increase heart rate variability, or the beat-to-beat alterations in the heart. A heart rate with high variability is an indication of good heart adaptability, implying that the individual has good autonomic control mechanisms (Tsai, 2006). The autonomic system controls the visceral functions of the body, including actions of the heart. With this statement it can be concluded that track runners have higher heart rate variability than football players since the track runners heart rates were more controlled and adapted better to the strains physical activity than the football players’. It should also be kept in mind that individuals with cardiac abnormalities could have distorted heart rate variability. In such cases the heart rate variability could be lowered as a whole.

The study was restricted to only male subjects due to hormones and the level differences between males and females. Males have a higher level of testosterone in the body, this causes the bone and muscle growth to be greater than in females. Also, males tend to have larger hearts and lungs, which allows for a higher lung capacity and heart rate than females. Males can also transport more oxygen to the body more efficiently due to increased surface area of the red blood cells, which allows the blood cells to have a higher hemoglobin capacity. The football data would not be affected by these hormone differences because there are not normally females on football teams. With there being many female track athletes, the combined female and male data could read as a false “no significant difference” between track and football heart rates and respiration rates. It might be beneficial to have this study done on both male and female track runners, and compare theses two heart rates and respiration rates to football players’ heart rates and respiration rates to see if, even with the lower testosterone levels, the female track runners will have lower heart rates and respiration rates.

Acknowledgments
We would like to thank the Saddleback College football coaches Mark McElroy (head coach) and Steve Crapo (assistant coach) and the Saddleback College track coaches Mark Blethen (head coach) and Matt Sherman (assistant coach). And a special thank you to all of the athletes who were willing to participate in this study.

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Effects of Salinity on Wheatgrass (*Triticum aestivum*) Growth

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Wheatgrass grows in highly variable soil compositions, which can affect the overall growth of the plant. Variation in soil salinity (salt content upon irrigation) can affect the overall hydration of the plant tissues, causing the shoot growth to either vary or diminish. This experiment tests shoot growth among four groups of wheatgrass cultivated in saline soils. Solutions with concentrations of 150mM, 175mM and 200mM NaCl were prepared as an irrigation source for three of the groups. A fourth solution of deionized water was set up as a control group. The predicted outcome was that an increase in salinity in the soil would cause a decrease in growth of the wheatgrass. The wheatgrass samples were placed in a shaded climate at temperatures 24°C for seven days; the samples were trimmed to be 7.0 cm before the salinity treatment. After the seven days, the 10 longest blades for each sample were measured and averaged. The average differences in heights (Hf - Hi) were taken for each group and an analysis of variance (ANOVA) was conducted between all four groups, each with 10 samples of wheatgrass. There were no significant differences found between the four groups of wheatgrass treated with varying salt concentrations.

Introduction

Wheatgrass is the common term used for the immature grass of our everyday wheat plant, *Triticum aestivum*. The contribution of *T. aestivum* to agricultural expansion has increased at the expense of the soil surface occupied in the world, giving the species a higher chance of existing among varying compositions of soil (Bazzigalupi et al., 2008). Agricultural environments all over the world have land and soil upon which the plant must adapt to in order to have a proper harvest each year. For example, saline soils represent eight percent of the total agricultural soil surface in Argentina (Bazzigalupi et al., 2008). Salt tolerance in tall wheat grass, *Thinopyrum ponticum*, was tested and it was found that this species of wheat grass thrived in breeding and germination in some of these specific areas in Argentina that have natural sodic characteristics. Salinization of ions existing in soil is seen primarily among the ions Na⁺, K⁺, Ca²⁺, Mg²⁺, and Cl⁻; Na⁺ being the predominate ion causing soil to become sodic.

Vegetation fertility is dependent upon many factors, one being the composition of soil. Therefore successful growth occurs when the precise concentration of ions in the soil reaches maximum growth using minimum energy is attained. The objective of this project was to evaluate the variation of growth among different groups of wheatgrass grown under different salinity treatments.

The absorption of water into the roots of plants from the surrounding soil occurs by osmosis, meaning water movement is dictated by the concentration gradients of ions generating an osmotic pressure inside the soil and in the roots. The combined pressures and solute concentrations form what is known as water potential, causing there to be two different water potentials for both the soil and roots. Therefore, absorption of water among roots will change depending on water potentials in the soil and roots themselves. Water bound to solutes or surfaces moves from regions of higher water potential to lower water potential. Therefore, as the solute concentration increases within the soil, the water potential will become more negative in the soil. Theoretically, an increased sodium chloride concentration within the soil can inhibit the overall “hydration” or uptake of water that moves through the xylem tissue of this monocot by generating a more negative water potential within the soil thereby causing less absorption into the roots.

It has been observed in previous studies that an increase in the salt content within a medium growing wheatgrass can cause the osmotic potential (water potential) in the soil to prevent water absorption necessary for mobilization of particles and nutrients within the plant tissues. (Johnson, 1991) However,
other conflicting research shows that a greater intensity of saline stress is beneficial at certain stages of growth and has no effect or a negative effect at other stages of growth (Rahman et al., 2008). It was observed that wheatgrass in higher saline soil responded poorly during germination but grew well during the seedling stage of growth. (Rahman et al., 2008)

Based on this research, our goal was to observe change in growth of wheat grass in soils of varying salinity (NaCl) content. Therefore, we predicted that wheatgrass exposed to high concentrations of salt (NaCl) will have less growth than those with lesser concentrations of salt in the soil.

**Materials and Methods**

Forty samples of *T. aestivum* were bought from Sprouts in Tustin Ranch, CA. Ten samples were placed into four groups. Each sample was contained in a plastic container with 10 holes at the bottom to allow for water absorption from the outside of the container. The samples were pruned to have an initial height of approximately 7.0 cm. Before the testing commenced, the exact initial height was measured. Each group was treated with salt solutions prepared using a 1L volumetric flask. The salt water solutions were prepared by weighing the specific amount of Morton™ table salt needed to make a 300 mOsM (8.77g NaCl), 350 mOsM (10.23g NaCl), and 400 mOsM (11.87g NaCl) concentration by using an analytical balance. The amount of salt needed for each group of 10 samples was prepared in 1 liter of deionized water.

Each of the liter salt solutions was poured into a Pyrex™ baking dish, and 10 samples were placed into the four different solutions for a period of 30 minutes. After all 40 samples of wheatgrass were soaked in the concentrated solutions, they were placed into a 24°C room with 24 hours of fluorescent lighting exposure. The samples were watered daily at 16:00PST with 50mL of deionized water. After a seven day period of treatment, the samples were measured by finding the 10 longest blades of each sample and the average final height was obtained. The difference (Hf – Hi) for each sample was calculated, and the 10 samples for each of the four groups were averaged (Figure 1).

**Results**

Each sample of wheatgrass was measured individually after the soil was soaked in different solutions; the tallest 10 blades were measured on each sample. The difference between the initial height and final average height for each sample was found to be: Group A) 2.4 cm (300 mOsM), Group B) 1.4 cm (350 mOsM), Group C) 1.1 cm (400 mOsM), and Group D) 2.0 cm (DI water = 0 mOsM). An analysis of variance (ANOVA) on the averages showed there was no significant difference between any of the groups (A-D).

**Discussion**

The results from this experiment, after running the appropriate statistical analysis, display a lack of any significant difference. As seen in Figure 1 above, there is a clear pattern that is beginning to form based on the relationship between growth and salinity content within the soil. As the osmolarity content within the soil increased, the average difference in growth before the saline treatment and after the saline treatment decreased. Although the analysis of variance showed no difference, if further testing was done for a longer period of time, or the number of groups was increased from four to five, it would seem that the pattern would continue to occur making it more likely for there to be a significant difference between all of the average growths, which would then support the findings of Johnson.

In our day and age, our surrounding bodies of water that make up 75% of the Earth’s surface have much more of an affect on our land than we think. As we use energy from fossil fuels, such as coal and oil which release gases such as carbon dioxide, we increase the partial pressure of carbon dioxide within our atmosphere. This eventually leads to the global warming effect where the water level of the ocean rises and infiltrates our soil. The approximate osmolarity of seawater is 1,300 mOsM, meaning that our current agricultural irrigation practices would be inefficient in the future due to the dramatic variation in water potential caused by the salinity in seawater. This small but continuous change of our Earth’s composition is another reason for our population to continually seek alternative sources of efficient energy that allows maximum production but uses minimum energy. Vegetation will continue to evolve as we do, therefore our ability to adapt to changing composition will result from further research on growing plants in different environments.
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*Grand fourmilier (Myrmecophaga jubata).*
The Effect of Day Length on the Metabolic Rate of *Sceloporus occidentalis*
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Thermoregulation and metabolism are essential components in reptilian survival, with each being heavily influenced by ambient temperature. Studies on heliotherms have shown that both temperature and body size influence metabolic rate in lizards. The purpose of this study was to see the effect of day length on western fence lizards’ (*Sceloporus occidentalis*) metabolic rates. It was predicted that ten wild western fence lizards exposed to both 12 hour and 8 hour day lengths (two weeks for each period) would have a higher metabolic rate in the 12 hour day lengths. Our study did not result in a significant difference between the two in a one tailed t-test (*p* = 0.0863), with the average cc/CO₂/ per minute being .0189 (S.E.M. ± 0.00285) in 12 hour day lengths and.0151 cc/CO₂/produced per minute (S.E.M. ± 0.00337) in 8 hour day lengths. An unforeseen event was a 4.1% net gain in weight in the lizards from when they were initially tested for the 12 hour days to when they were tested for the 8 hour days (no significant difference; *p* = 0.0779). Although there was not a significant difference in the sample size used, a larger sample size may result in a significant difference.

Introduction
Thermoregulation and metabolism are both essential components in reptilian survival. Thermoregulation is an organism’s regulation of internal body temperature, allowing cells to function while in fluctuating external temperatures. Metabolism is the required chemical processes that occur within a living organism that allow for life to exist. In thermoregulation, animals are classified as endotherms or ectotherms. Endotherms use internal mechanisms for body temperature control. Ectotherms, such as reptiles, perform thermoregulation primarily through external heat and non-heat sources. In ectotherms, heat gain and loss are heavily influenced by the environment. Radiation is one type of heat gaining process. Radiation transfers heat through electromagnetic waves between objects that are not in direct contact. The sun emits electromagnetic waves as sunlight. Sunlight used by heliotherms, such as reptiles, absorb these electromagnetic waves as heat, in turn, thermoregulating internal body temperature. Lizards can increase their internal body temperature by absorbing sunlight directly or indirectly (Benton, 1978). Lizards also increase body temperatures through convection and conduction, depending on habitat and time of day. Animals have either a varied or somewhat constant body temperature. Constant body temperature animals are called homeotherms. Animals that exhibit in their metabolism and activity some degree of independence of their temperature are called poikilotherms (Bullock 2008). Hence lizards are classified as poikilotherms.

There has been a lack of well-documented studies conducted on the relationships between day length and metabolism in lizards, such as the western fence lizard (*Sceloporus occidentalis*) a Californian native species. Previous studies have shown that as body temperature increases so does oxygen consumption, and vice versa (Bennett and Gleeson, 1975). The purpose for this study was to see if there was a correlation between day length and metabolic rate in western fence lizards. It was predicted that longer day lengths would correlate with higher CO₂ productions and hence higher metabolic rates.

Material and Methods
Ten western fence lizards, (*Sceloporus occidentalis*) were captured using a slipknot end fishing pole apparatus at Saddleback College, Mission Viejo, California, in mid October 2008. Four male lizards were identified by having blue chins and their territorial aggression, while six female lizards were identified. Mean lizard weight was 4.91 (g) (± 0.789 S.E.M) during 12 hour day length runs and 5.11 (g) (± 0.7047 S.E.M) during the 8 hour day length runs. All lizards were assigned a number. Six lizards were housed in a large glass aquarium and four lizards were housed in a small glass aquarium. Temperature of aquariums were maintain between
20-22°C at night and 24-26°C during the day. Lizards were given two weeks to adjust to new day lengths. Lizards were placed in 12 hour and 8 hour day lengths. Each lizard was fed 7 crickets every three days and each aquarium was supplied with two water holders. Lizards were fed at the same time (4pm) every three days.

After two weeks of being exposed to either 8 hour or 12 hour day lengths, CO₂ production was measured for each lizard using a carbon dioxide gas sensor (Pasco Xplorer GLX and Pasport Carbon Dioxide Gas Sensor). Lizards were measured two days after being fed between 3pm and 4pm. Before each trial, lizards were allowed to rest for ten minutes inside the container being used to measure CO₂ in order to reach resting metabolic rate. Three trials of 600 seconds were run on each lizard. Trials were run in complete darkness to prevent interference from outside stimulus.

**Results**

The average cc CO₂/minute was 0.0189 (±0.00285 S.E.M.) when the lizards were exposed to 14 days of 12 hour environments, compared to the average of 0.0151 cc CO₂ per minute (S.E ±0.00337) (Figure 1) when exposed to 14 days of 8 hour environments. There was an overall net gain in weight of 4.1% in the lizards (Figure 2), from when they were initially tested in the 12 hour days to when they were tested for the 8 hour days. There was no significant difference in either weight gained (p=0.077) or amount of cc CO₂ produced (p=0.086) under a one-tailed paired t-test.

![Figure 1.](image.png)

**12 Hour Days**

The lizards were visibly hyperactive and easily agitated by any movements. When placed in containers, they would move their bodies up and down, showing both signs of aggression and territorial claim.

**Figure 2.** The bar graph displays the mean weight of ten western fence lizards (Sceloporus occidentalis) in 12 hour and 8 hour environments. There was a 4.1% net gain in weight in 8 hour environments vs. 12 hour environments, there was no statistical difference between the two (n=10).

**8 Hour Days**

Three lizards were found burrowed underneath dirt. The lizards appeared less agitated, and when placed in containers did not show any signs of aggression. There was an overall net gain in weight by the lizards, but those lizards found burrowed had lost weight (Figure 3; Lizards #1, 3, & 5). One lizard, that had not burrowed, was found to have lost weight as well (Figure 3; Lizard #4).

**Discussion**

The ambient temperature of a heliothermic lizard greatly affects its ecology and well being. Studies on mud turtles also showed that higher temperatures resulted in increased activity and metabolic rates, as well as an increase in oxygen consumption (Litzgus and Hopkins 2003). This study though, which looked at the effect of day length on metabolism, through CO₂ production, in western fence lizards (Sceloporus occidentalis), did not result in a significant difference between 12 hour and 8 hour day lengths (p=.086; Figure 1). A larger sample size though, may result in a significant difference though.

There were several factors that may have influenced the CO₂ production of the lizards. One of which, is that the lizards appeared to have become more dormant towards the end of the study. Whether the lizards’ dormancies were due to shorter day lengths or if they had become more tamed overtime is unclear. A possible method to make this clear would be to give each lizard greater space, and to better mimic their outside environment, in order to have them in a constant state of alertness.

![Figure 2.](image.png)
During the study, the lizards’ CO$_2$ productions were measured at the same time of the day, and each lizard had its “daytime” start at the same time.

Figure 3. Bar graph: The weight of ten western fence lizards (Sceloporus occidentalis) before having their CO2 production rate measured. There wasn’t a significant difference between the two. Line graph: Mean cc/CO2/per minute produced by the ten western fence lizards over a 10 minute period (n=3).

Studies at La Trobe University have shown that temperature has a positive effect on oxygen consumption in lizards (Clark, Butler, and Frappell, 2006). Since temperature appears to influence a lizard’s metabolic rate, it’s unclear whether the lizard’s CO$_2$ production was influenced more by the temperature within their cages or whether it was more influenced by the day length. A possible method to fix this is unclear, but possibly measuring the lizards’ metabolic rate halfway through the daylight session may resolve the problem.

Several of the lizards’ burrowed underneath the soil, for extended periods time (several days). This fact may have influenced our study, since “small ectotherms undergo rapid heat exchange with their environment, so they can act as thermal opportunists and regulate their body temperature almost exclusively by behavioral means” (Herczeg, Herrero and Saarikivi, 2007); which would have lowered their metabolic rate. To prevent this, harder soil should have been used, and is recommended in further studies.

Although the study did not result in a significant difference between the two day lengths; greater day length differences, a larger sample size, and better control of the lizards’ environment may result in a significant difference in a later study.

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Comparison of the Resting Metabolic Rate among Aerobic Exercisers and Anaerobic Exercisers

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Metabolism is the sum of all chemical and physical processes occurring within a cell or an organism. Resting metabolism is the amount of energy needed to maintain vital physiological functions when a person is at complete rest. In order to measure a person’s basal metabolism resting VO$_2$ is measured. Resting VO$_2$ is the volume of oxygen that the body consumes at rest, during daily life activities, or while exercising. Twenty-three (n=23) women between the ages of 18 and 30 were chosen and broken down into three (3) groups. The first group consisted of 8 women that engaged in exercise that was more anaerobic, the second group consisted of 6 women that engaged in exercise that was more aerobic, and the final group of 9 was the control group of women that did not exercise regularly, or at all. It was hypothesized that aerobic exercisers have a higher RMR than anaerobic exercisers. The mean VO$_2$ for the aerobic group was 4.62 ± 0.41 mL•min$^{-1}$•kg$^{-1}$, the mean for the anaerobic group was 3.87 ± 0.27 mL•min$^{-1}$•kg$^{-1}$, and the mean for the control group was 3.68 ± 0.30 mL•min$^{-1}$•kg$^{-1}$. Among the three groups tested there was no significant difference in the RMR of the women (p=0.134, ANOVA single factor).

Introduction

Metabolism is the sum of all chemical and physical processes occurring within a cell or an organism. It is the catabolic process that takes oxygen and biochemical energy from nutrients and forms adenosine triphosphate (ATP). This study examined the resting metabolism of *Homo sapiens*. Resting metabolism is the amount of energy needed to maintain vital physiological functions when a person is at complete rest. The resting metabolic rate (RMR) is measured after a minimum 12 hour fast to ensure that the subject is in the post-absorptive stage of digestion. Foley (1928) states that scientists have “proven” that the volume of oxygen a patient inhales is a direct index of metabolism. Therefore, when a patient is at rest and the volume of oxygen (VO$_2$) inhaled is collected, the RMR can be calculated.

*Homo sapiens* make ATP through specific cellular pathways. Carbohydrates, fats, and proteins are processed and used as fuel for all of life’s functions. Cellular respiration in general is the term used to describe both aerobic and anaerobic pathways which break down organic molecules and produce ATP. The equation for cellular respiration is:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{Energy (ATP)}$$

*C$_6$H$_{12}$O$_6$* (glucose) in the presence of oxygen is broken down into carbon dioxide, water and ATP. In order to evaluate a person’s metabolic rate, the VO$_2$ utilized during cellular respiration is analyzed. VO$_2$ can be the volume of oxygen that the body consumes at rest, during daily life activities or while exercising. The VO$_2$ is expressed in units of mL•min$^{-1}$•kg$^{-1}$. Each and every cell in the body uses oxygen in order to do its cellular work and there is a direct, linear relationship between VO$_2$ and metabolic rate. When the body is exercising, it will have higher oxygen consumption (VO$_2$ max) which is the maximum amount of oxygen the body can consume during very intense exercise. Conversely, when the body is at rest, it will have lower oxygen consumption (VO$_2$) per minute composing the RMR which was tested in this study.

The RMR varies largely among individuals. This variation is due to numerous factors such as age, sex, height, weight etc. Individual metabolic differences are also partially due to variations in skeletal muscle because diverse muscle types use different metabolic pathways. The muscle fibers that primarily make ATP using oxygen, and are therefore using aerobic metabolic pathways, are called oxidative fibers. The muscle fibers that make ATP without oxygen (anaerobic metabolic pathways) are called glycolytic.
fibers. These fibers use glycolysis to make ATP. Muscle fibers are more specifically described as either (1) slow-twitch oxidative fibers (type I) which are rich in oxidative enzymes and lack the enzymes used in glycolysis, (2) intermediate fast-twitch (type IIa), containing both oxidative and glycolytic enzymes, or (3) fast-twitch glycolytic (type IIb) which predominantly contain glycolytic enzymes and fewer oxidative enzymes. Type I and type IIa produce smaller contractions but are capable of working for long periods of time due to their capability of continually regenerating ATP. Fast-twitch glycolytic fibers provide much larger contractions due to a surge of ATP generation, but are unable to maintain the high ATP levels and therefore fatigue quickly.

It is characteristic of the muscles in trained athletes such as distance runners and cyclists to contain large amounts of type I muscle fibers. Anaerobic exercisers like weightlifters and volleyball players develop many more type IIb muscle fibers. It is also important to note that most of the muscles in the human body contain a mixture of all three fiber types whether trained or not. Genetics is thought to contribute partly to the amount of each muscle fiber type a person’s body contains (Park et al., 1988). However, routine exercise habits can cause one muscle fiber type to dominate in one specific muscle, limb, or person (Vandenborne et al., 1991). For example, one study found that individual performances in a wrist flexion exercise routine differed immensely among endurance athletes and non-athletes. The athlete muscles were able to perform wrist flexion with increasing amounts of resistance for an extended period of time due to the recruitment of type I fibers. In contrast, all of the non-athletes grew weak and fatigued quickly, and some were not able to finish the exercise routine at all. A magnetic resonance spectroscopy (MRS) was used to find the composition of each subject’s muscle fibers. As expected, the athlete’s muscles did indeed contain more type I fibers and the non-athlete’s muscles recruited more type IIb fibers during exercise. Additionally, in three out of four subjects the more dominant arm (trained like athletes) of subjects were also shown to contain more type I fibers when compared to the less dominant arm (untrained like non-athletes) which recruited more type IIb fibers for exercise (Park et al., 1987).

In this study 3 different groups of women participated in a RMR test. These women represented either aerobic exercise utilizing type I fibers, anaerobic exercise utilizing type IIb fibers or a control group of non-exercisers. It was hypothesized that aerobic exercisers would have a higher RMR than anaerobic exercisers.

**Materials and Methods**

Twenty-three (n=23) women between the ages of 18 and 30 were chosen and broken down into 3 groups. The first group consisted of 8 women that engaged in exercise that was more anaerobic, the second group consisted of 6 women that engaged in exercise that was more aerobic, and the final group of 9 was the control group of women that did not exercise regularly, or at all. The parameters for the aerobic and anaerobic exercisers were that they exercised for at least 30 minutes, at least three times per week, and had been doing so for the last 3 months. The parameters for the inactive women were that they had not exercised consistently for at least 30 minutes, at least 3 times per week in the last 3 months.

The Cardio Coach VO2 Fitness Assessment System Model 9001-RMR by Korr Industries was used to test the RMR of the subjects. The subjects did not workout the day before the testing and had fasted for 12 hours. Initial information was collected and entered into the system. This information included age, height, and gender. The subjects were also weighed barefoot and their current weight and goal weight were then entered into the system. Once the information had been entered in, the Cardio Coach began calibration by drawing room air across its oxygen sensors and establishing a baseline.

The barometric pressure is required to calibrate blood gas analysis equipment. Normal barometric pressure is 760 mm of Hg at sea level minus the water vapor pressure. The Cardio Coach converts gas volumes to standard pressure and temperature, for dry air (STPD: 0 °C, 760 mm of Hg) using Boyle's law to adjust the gas volumes to STP:

\[
P_1 V_1 = P_2 V_2 \frac{T_1}{T_2}
\]

Where \(P_B\) is the room barometric pressure (in mm of Hg), \(V_1\) is the gas volume (in Liters), \(T_1\) is the room temperature in Kelvin (K), \(P_2\) is standard pressure (760 mm of Hg), \(T_2\) is 273K, and \(V_2\) is the adjusted volume (at STP).

Once calibration was complete, the hose was placed on the Cardio Coach and the test subject was given the other end with a one way valve. The purpose of the one way valve was to ensure that all of
the exhaled gas was picked up and that there was no cross contamination with inhaled air. The test subject placed a soft nostril pinching device on their nose to ensure air was entering only through the mouth. At this point the subject began breathing into the one way valve. The Cardio Coach waited for the subject’s breathing to stabilize and then began a 90 second countdown, after which the actual testing began. At the conclusion of the test the Cardio Coach saved the data for later analysis.

The Cardio Coach measures the amount of oxygen (VO₂) inhaled at a lower limit (resting). Based on this information it was possible to determine how much oxygen the body was consuming and thus established RMR. The data from all 23 subjects was entered into Microsoft Excel and a statistical analysis was run.

**Results**

The mean VO₂ for each individual was determined and, in turn, the mean for each of the 3 groups was computed. The results show that the mean VO₂ for the anaerobic group was 4.62 ± 0.41 mL•min⁻¹•kg⁻¹, the mean for the aerobic group was 3.87 ± 0.27 mL•min⁻¹•kg⁻¹, and the mean for the control group was 3.68 ± 0.30 mL•min⁻¹•kg⁻¹ (Figure 1). There was no significant difference between the three groups (p=0.135, ANOVA single factor).

![Mean VO₂ mL/min/kg](image)

**Figure 1. Comparison of the mean VO₂ measurements between anaerobic exercisers, aerobic exercisers and non-exercisers. There is no significant difference in the mean VO₂ between the groups (p=0.134, ANOVA single factor). Error bars indicate S.E.M.**

**Discussion**

Among the three groups tested there was no significant difference in the RMRs and the results did not support the hypothesis. The conclusion that there is no difference between RMR in anaerobic and aerobic exercisers is consistent with a previous 12 week study. This study found no significant change in RMR between high-intensity resistance training (anaerobic exercise) and endurance training (aerobic exercise) (Broeder et al., 1992). Broeder’s results suggest that both high-intensity resistance training and endurance training may help prevent a reduction in RMR by either preserving or increasing an individual’s fat free mass. Broeder et al (1992) also cited for those individuals seeking to reduce body fat without reducing RMR, the best exercise program includes both endurance and/or resistance training. Additional studies have been performed comparing the RMR of individuals after a 6 month controlled exercise program (Sweeney et al., 1993). The program which included strength and endurance training along with moderate dieting showed no significant difference in RMR between aerobic and anaerobic exercise while dieting.

While it is consistent among the research to conclude that RMR does not differ between anaerobic and aerobic exercisers, it is not consistent to say that RMR is similar for exercisers and non-exercisers like the results in this study showed. It has long been known that total metabolism per kilogram of body weight is much larger in athletes versus non-athletes (Benedict, 1915). Because metabolism is affected by many factors, it has been concluded that a variety of uncontrolled factors could be better managed in future studies. This study provided a narrow snapshot of individual RMRs. In future studies a more complete picture of RMR is desirable. This would be accomplished by testing individuals throughout the day and while the individuals are in both a pre-absorptive and post-absorptive state. Benedict (1915) reports the findings of differences in RMR throughout the day. This is due partly to the intensity of daily activity, rate of digestion, and the activity of the sympathetic and parasympathetic nervous system. It is also recommended by Foley (1928) that some particular preparations for RMR testing be followed by each test subject. These include (1) retiring by 10 o’clock the night before testing, (2) eating the same dinner the night before of 1 pint of milk and two slices of bread (no butter), and (3) no drugs may be taken before testing. In future studies more of the metabolic variables should be controlled.

**Literature Cited**


The Acquisition of Spatial Learning in *Mus musculus* as Compared between Levels of Difficulty

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In this study, two identical groups of *Mus musculus* were assigned a maze to learn. One group was given a rudimentary maze, while the other was given a complex maze. The mice completed their mazes once a day for two weeks, during which the time taken to complete the mazes was recorded. It was expected that the mice memorizing a more complex maze would learn the route as effectively as those given an easy maze. This seems to suggest that the rate of spatial learning was due more to the amount of repetition than the difficulty of the task. The mean time for group A, the easy maze, was 126.3 seconds ± 16.36 S.E.M. (N = 10), while the mean for the complex maze, group B, was 145.2 seconds ± 18.66 S.E.M. (N = 10). The difference between these two times is not statistically significant (p = 0.46).

**Introduction**

Many scientific experiments have used the ability of mice to learn, or memorize, a maze. Many variations on this idea have resulted in different behavioral or genetic aspects of the mice being observed in order to determine how variances in these traits might affect the ability of the mice to complete the task. An example would be the study which examined the relationship of differences in the structure of the hippocampus of mice to spatial learning abilities (Schwegler 1988). Other studies have suggested that differences in learning and memory between the strains are most likely not due to non-cognitive factors, such as anxiety or activity (Podhorna, 2002). In the course of this experiment, the investigator hopes to observe the rate at which two identical groups of mice learn two mazes of disparate difficulty. It is expected that with identical...


time spent learning each maze, there will be no difference in how effectively each group is able to spatially negotiate their maze.

Materials & Methods
At the start of the experiment, sixteen male mice were split into two groups containing 8 mice each. These mice were housed in separate aquariums during the entirety of the experiment, with each group assigned to complete one of two mazes daily. Mice were given food and water ad libitum. Both of the mazes were 18 inches by 20 inches and were of a simple block design. The maze assigned to group A was designed to have a very simple solution, with far less possible faulty choices than maze B. Peanut butter was used as the reward at the end of the maze, with the purpose being that the subjects would learn to navigate the mazes with the smell of peanut butter to provide sensory clues. The mice were made to run through the maze once per day and the time taken to reach the correct chamber of the maze, which was kept constant throughout the experiment, was recorded. Mice which became distracted and stopped moving through the maze were encouraged with nudging. Once data was recorded, analysis was done by an unpaired t-test to determine the statistical significance.

Results
Comparison of the times recorded between the subjects solving a simple maze and those solving the more complex maze showed that the difference in mean times was not significant. The mean for group A, the mice assigned to the easy maze, was 126.3 sec ± 16.36 SE (N=10). The mean of group B was 145.2 sec ± 18.66 SE. An unpaired t test showed that the difference between these two means was not significant (p= 0.46). A line graph of the daily mean of each group’s time shows that any apparent difference in the time between group A and B at the start of the experiment seems to even out in a plateau of similar times for both groups.

Discussion
In a previous study comparing the rate and methods between mice and humans completing mazes, data exhibited a trend in which humans completed mazes more slowly than mice but with one third of the errors (Stanford et al, 2001). It could have been assumed that for mice, which do not rely on visual information as much as humans, a complicated maze with a greater opportunity for error would have increased the time taken to solve the maze. However, other experiments have noted identical levels of learning between mice running different types of mazes (Stanford et al, 2001). It cannot be determined from this study how the mice seemed to recall the maze they were assigned to, however the investigator did note that mice running the complex maze began to avoid portions of the maze which lead further away from the end chamber. Mice running the simple maze seemed to be deterred by the greater amount of open spaces, which may have resulted in decreased motivation to solve the puzzle. Such behavior is similar to the observations in a prior experiment which indicated that groups of mice performing the best in maze-running trials seemed to do so due to an ability to build up maze-running skills based upon the demand of the situation (Ammassari-Teule 1992).
Whatever the cause, the ability of the group B mice, running a more demanding maze, to reach a mean time so similar to that of the mice performing the easy maze is clear. As the investigator had hypothesized, the same amount of repetition was effective for both groups of mice to begin memorizing their respective maze.

**Literature Cited**


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**Ambient Temperature and Honeybee (Apis mellifera) Metabolic Rates**

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Six honey bee metabolic rates were taken at varying ambient temperatures (10, 23, 40°C) to see if ambient temperature would affect honey bee metabolic rate. The stated hypothesis was that honeybees, like many organisms, would show a decrease in metabolic rate in cold ambient temperature and an increase in metabolic rate in hot ambient temperature. Metabolic rates were taken in a closed system as a function of CO₂ consumption normalized by mass over time in minutes. Metabolic rates were averaged for each honey bee at each minute. Results show that although metabolic rates for each bee varied for each ambient temperature, they were inconsistent and did not support the initial hypothesis. There was no significant difference in bee metabolic rates for the three different ambient temperatures (p=0.298 ± SE, single factor ANOVA Post Hoc). Varying degrees in honeybee behavior and physiological adaptations coupled with the agitation of the closed system can be attributed to inconsistent and insignificant CO₂ consumption readings.

**Introduction**

Bees are endothermic, meaning that they are warmed mainly through heat generated by metabolic processes. However, due to their small size, bees are subject to rapid changes in temperature and heat exchange and thus are poikilothermic endotherms (Morgan, K.R. & Heinrich, B. 1987; Stone, G. N., and Willmer, P.G. 1989.). Bees, like many flying insects, can regulate their temperature through their behavior.

In cold weather, honeybees huddle together to generate and retain heat. Bees in a cluster will actively produce heat by shivering and thus have been shown to maintain a core temperature of 20-35°C during winter (Stabentheiner et al. 2003). Flying insects can increase their body temperature by vibrating their wings which are powered by strong flight muscles (Heinrich 1975; Kammer, A.E. and Heinrich, B. 1974.). This preflight warm up enables flying insects to reach a high thoracic temperature.
needed for flight. Honeybees can maintain continuous flight at extremely high temperatures and can reach thoracic temperatures greater than 40° C (Heinrich 1979).

Flying insects often run into the risk of overheating during periods of extended flight. Studies have shown that bees use countercurrent heat exchangers to allow heat loss from the thorax to escape to the abdomen. (Heinrich 1979). Honey bees also have been shown to regurgitate water and honey droplets to reduce body temperatures through evaporative cooling (Cooper et al. 1985).

Honeybees have various behavioral and physiological adaptations for thermoregulation. Thus, Honeybees are fascinating subjects for metabolic experiments. In our experiment the CO2 production of the bees will be measured at three different ambient temperatures: at room temperature, a reduced temperature of 10°C, and an increased temperature of 40°C. Our hypothesis is that the honeybees will show a decreased metabolism at a reduced temperature and will have a greater metabolism at an increased temperature.

**Materials and Methods**

Honeybees were obtained at the horticulture grounds on Saddleback College. The foraging bees were captured as they landed on flower by using a large 12.5cm fish net. The captured bees were then transferred in a Herphaven® round container. A total of thirteen bees were captured for the experiment. The bees were given lavender flowers and honey by dipping wooden coffee stirrers in honey and placing it in their container.

The following day, CO2 production of the bees was measured at three different temperatures: room temperature, a reduced temperature of 10°C, and an increased temperature of 40°C. The mass of the bee was recorded using an Ohaus Scout Pro lab balance scale. The mass of the bee was obtained by first recording the mass of the container plus the bee and then subtracting that number by the mass of the container alone. The bee was then placed in the respirometer and CO2 production was measured for five minutes using the Xplorer Glx CO2 Sensor. Afterwards, the mass of the bee was recorded again. The temperature session was repeated for each of the six bees.

The bees were then tested at a reduced ambient temperature. A VWR incubator was set at 10°C. The containers were flushed with air in between temperature sessions and before a trial run. Using the same bees from the first temperature sessions, the bees were acclimated to the cold for 2-3 minutes before being placed in the respirometer. In order to allow airflow, and prevent escape, cheesecloth was placed and taped over the opening of the bottle container. After acclimating, the mass of the bees were recorded. The respirometer was then placed in the incubator and CO2 production was recorded for five minutes. Again, the mass of the bees were recorded after the temperature session. This was repeated for each of the bees from the room temperature session.

Lastly, CO2 production was measured at an increased ambient temperature. The VWR incubator was set to a temperature of 40°C. Then using the same methods described above the mass and CO2 production was measured for each of the ten bees.

**Results**

![Figure 1. Honey Bee Metabolic Rates and Varying Ambient Temperature. Ambient temperatures at 10, 23, and 40 degrees Celsius. Marker represents metabolic rate at a specific temperature. N=6 for each ambient temperature.](image1)

![Figure 2. Average Relationship between Varying Ambient Temperatures and Honey Bee Metabolic Rates. Ambient Temperatures at 10, 23, and 40 degrees Celsius. Averages at 8.73, 11.8, and 9.4 ml CO2/g per minute. Statistical analysis shows no significant amount of variance between metabolic rates as a result of varying ambient temperature, p=0.298 ± SE ANOVA, Post Hoc. Error bars set for standard error.](image2)
Honeybee metabolic rates were calculated as a function of CO₂ consumption normalized by mass over time in minutes. Results show that metabolic rates between bees do vary in different ambient temperatures. However, the results lack consistency throughout the test group. An overview of metabolic trends for the six bees is shown in Figure 1.

Average bee metabolic rate data analysis showed no significance as a result of ambient temperature changes (p=0.298 ± SE, single factor ANOVA). A multiple comparison test (post hoc) confirmed the analysis by comparing data between groups (Figure 2). ANOVA statistical analysis was accomplished using Microsoft Excel (Microsoft Corporation, Redmond, WA). Post hoc confirmation was done through an online post test calculator (http://graphpad.com).

**Discussion**

The metabolism at increased and reduced temperatures were shown to be insignificant. Our study showed that metabolism was reduced when temperature was increased. Other studies have shown that there is an inverse relationship between MR and T₀ (Woods, W.A., Heinrich, B., and Stevenson, R.D. 2005.) However, this relationship was not seen under decreased ambient temperatures. Other research experiments have shown that bee behavior varies and metabolic differences could be due to agitation. Furthermore, these metabolic differences could be due to differences among, colonies, genotypes, seasons, age, and foraging tasks (Woods, W.A., Heinrich, B., and Stevenson, R.D. 2005.). For example, attacking or agitated bees have been shown to have increased thoracic temperature and metabolism. Moreover, African bees have a higher metabolic rate than their European relatives (Heinrich, B. 1979).

Metabolic rates are also closely related to body mass. Since surface area to volume ratios decrease with increasing size, the rate of heat loss decreases with increasing mass (Stone, G. N., and Willmer, P.G. 1989.). This relationship has been well studied with mammals and other endothermic animals.

Honeybees display various behavioral and physiological adaptations for thermoregulation. While, honeybees pose fascinating subjects for metabolic experiments, they are also very difficult subjects to study due to the many variables involved.

Since many of these factors were not controlled, further studies are needed to determine the relationship between ambient temperature and metabolism.

**Literature Cited**


Competition Effects on Growth Rates of Various Mixtures of Ryegrass and Fescue Grasses

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Competition among plant communities is mainly for the availability of nutrients. In areas of abundant nutrient availability, intermixed plants will compete for light as well. Research is unclear as to whether competition among plant species causes interference, therefore inhibiting growth and/or causing death, or if it causes an increase in nutrient uptake and an abundance of growth. In this experiment, five groups of Ryegrass and Fescue mixed in different amounts were nurtured and observed over a five-week period. Four groups of each seed mixture were evaluated: 10 mL Fescue, 10 mL Ryegrass, 2.5 mL Fescue with 7.5 mL Ryegrass, 5.0 mL Fescue with 5.0 mL Ryegrass, 7.5 mL Fescue with 2.5 mL Ryegrass. With all mixtures of Ryegrass and Fescue being planted in equal amounts of soil and watered equally, it was certain that only the different mixtures and how they compete could be factors as far as growth rate. The results showed that our hypothesis was accepted; indicating that competition in high concentrations causes increased growth rates among Ryegrass and Fescue. The statistically significant difference was between the 10 mL Ryegrass group and the 5.0 mL Ryegrass with 5.0 mL Fescue group.

Introduction

Competition among plant species is a relatively new area of science currently being researched. Past research suggests that competition can sometimes help facilitate faster plant growth (Holmgren, 1997). Experimental research indicates that there are some plants that are complimentary, with each plant being helped in some way, and some that are detrimental to the survival of one or both species (Callaway, 2008). Some research has indicated that competition is harmful to the plants. This harmful type of competition is often referred to as interference (Weiner, 1988). Competition for sunlight in which one plant has a large size advantage over another plant has proven to be a major factor on why competition is sometimes detrimental to plant growth (Weiner, 1986). This could occur when a tree and a bush are competing for the same sunlight. The tree being taller would shield the bush from sunlight and thus the growth effects of the bush would be negative. There also appears to be certain species that are very invasive and detrimental to other species (Callaway, 2000). The purpose of our experiment was to determine if our hypothesis, that our two chosen grass species would compete with each other and increase growth rates, was true.

Materials and Methods

Twenty small four inch pots were filled with 400 mL each of potting soil and placed in the greenhouse at Saddleback College in Mission Viejo, CA. We obtained two bags of grass seed: Annual Ryegrass and Rebel Exeda Tall Fescue. We mixed the seeds into different groups with 10 mL of each seed mixture being placed in each pot on top of the soil. 100 mL of soil was then poured on top of the seeds. These pots of soil were equally watered twice a week with 200 mL of water in each pot. The ratios of grass seed mixtures were as follows: 10 mL of Ryegrass seed was put into four pots of soil. 10 mL of Fescue grass seed was put into four other pots of soil. These eight pots were the control groups. Another group of four pots of soil contained 7.5 mL of Ryegrass seed and 2.5 mL of Fescue grass seed. Another group of four pots of soil contained 5.0 mL of Ryegrass seed and 5.0 mL of Fescue grass seed. The final four pots of soil contained 2.5 mL of Ryegrass seed and 7.5 mL of Fescue grass seed. The purpose of this project was to determine that a grass species will grow at a faster rate when in the presence of another grass species. This is known as positive competition or facilitation. Documentation of results was taken weekly, every Monday throughout the five weeks of the experiment. Grass blades were measured from the soil level to their peak. The tallest blades of each group were recorded, along with the shortest and an average height. From the very first week, the pots containing equal mixtures of Ryegrass (5.0 mL) and Fescue (5.0 mL) grew at a noticeably faster rate. Our data collected confirmed this observation.
Consistently each week, this trend continued, however the gap between this mixture and the others became less visibly noticeable over time. At the end of the five weeks, the blades of each pot were cut off at the surface of the soil for a total of 20 groups. Each group of grass blades from each pot was then weighed on an analytical balance in the Biology Lab at Saddleback College. The data collected (mass in g) was then entered into a spreadsheet and a one-tailed Analysis of Variance (ANOVA) was performed. Since we wanted to observe the differences between groups, it was necessary to perform a post hoc multiple comparison test using a post test calculator: an online calculator for scientists (Graphpad software, http://graphpad.com/quickcalcs/posttest1.cfm).

Differences were considered significant at $P<0.05$.

**Results**

Each week throughout the five week experiment, data was consistent in showing that the greater the mixture (5.0 mL Ryegrass with 5.0 mL Fescue) the greater the growth, confirming our hypothesis that grass will grow at a faster rate in the presence of another grass type. After running the ANOVA, a statistically significant difference was found between groups. In order to see specifically which groups the differences were between, the post hoc multiple comparison was run. Results showed that the only significant difference was between the 10 mL of Ryegrass group and the group mixed with 5.0 mL of Ryegrass and 5.0 mL of Fescue.

**Competitive Increases Growth Rate**

![Graph showing competitive increases growth rate](image)

**Figure 1.** Mean weight ± S.E.M in grams of the five groups. Results show that the equally mixed (50%/50%) group grew significantly more biomass than the other groups.

**Discussion**

The idea that increased cross species competition can have positive effects on plant growth is counterintuitive to many of us, as competition is often viewed to have negative effects on plant growth. While researching this idea we found that there have been relatively few research projects studying the causes of this positive competition phenomenon. As a result of our project we have found that even among two closely related grass species there can be some beneficial effects of competition. Since the statistically significant difference was between 100% Ryegrass and a group with equally mixed (50%/50%) Ryegrass and Fescue, this indicates that positive competition did take place. The growth rate does increase when two grass types are in competition with one another for nutrients and/or light. Our group of equal parts Ryegrass and Fescue had the most direct competition of any group and it is intuitive that this group would show the largest effects positive or negative from competition. Because this mixture was the only mixture that was significantly different from the others, it is apparent that for competition to have its full positive effects, the mixture must be equal or close to it. One theory that is supported by the work of Rien Aerts is the idea that in nutrient rich environments competition is generally thought to be mainly for light (Aerts, 1999). This competition for sun can be negative to growth in situations where one competitor has a general height advantage over another, but we believe that this competition for light was positive in our case because our grass species are very close in size. Our environment was nutrient rich and the different grass species grew more quickly when they were combined in a 50%/50% ratio because they were continually competing to grow taller and thus have more of the available sunlight. Our observed phenomenon that plants can grow more quickly when competing with other plants is consistent with past projects (Holmgren, 1997, Aerts, 1999). It is also important to note that in more harsh environments in terms of soil moisture or nutrients the competition between species may have been negative. In fact there may have been a negative competition effect...
occurring in our pots but it was not noticed because the positive competition for sunlight was superior.

**Literature Cited**


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![Fig. 13. Preparing hydrogen.](image-url)
The Effect of Green Tea on the Growth and Metabolic Rate of the Common Laboratory Mouse (Mus musculus)

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Metabolism is the chemical process that is necessary for the maintenance of life. In metabolism, some substances are broken down to yield energy for vital processes, while other substances that are also necessary for life are synthesized. Temperature, organism type and eating behaviors affect this vital process. To find out if the consumption of green tea has an effect on the metabolic rate and growth of the common laboratory mouse (Mus musculus), ten female, non-pregnant mice were placed in a respirometer to measure oxygen consumption at room temperature. Prior to measuring, five of them were placed into Group A which was used as a control group and fed only mouse food and water, while the other five were placed into Group B which was fed mouse food, water, and 4 mL of Lipton Green Tea. The results acquired show that the control Group A had a significant increase in weight with a mean of 4.33 g ± 0.30 (±se) compared to the increase in weight in Group B that had a mean of 3.23 g ± 0.45 (±se), (p=0.04, one-tailed t-test). In addition, Group A showed a significantly lower metabolic rate with a mean of 5.12 mL/O2/gm/hr, STPD compared to Group B, which were given green tea, with a mean metabolic rate of 9.02 mL/O2/gm/hr, STPD (p = 0.01; one tailed t-test). It can be affirmed that the use of green tea has substantial effects on the metabolism, as well as growth rate of the common laboratory mouse (Mus musculus).

Introduction

Metabolism sustains life. It is the process by which energy and materials are transformed within an organism and exchanged between the organism and its environment. (Gilooly, Brown, West, Savage & Charnov, 2001). As mammals, mice are an imperfect surrogate for humans that can be used to analyze certain human conditions such as metabolism, thermoregulation processes, hunger, as well as weight gain management. Their body plan, physiology and genome share many features with that of humans. The mouse brain for example, although laid out in a more linear pattern, contains the same array of basic components, which is generally a great tool for comparison to human brain architecture. The hypothalamus in the brain of mice, which is responsible for certain metabolic processes and other activities of the autonomic nervous system, serves the same function in humans. Other parts of a mouse brain also function in the same way as a human brain; therefore, when mouse brain function is examined, it can provide a general view to human brain architecture and function.

With a current high prevalence of chemical weight-loss pills in the market that have harsh side effects, it becomes important to test for alternatives in which people have a different option to reduce their weight without drastic measures. Since ancient times green tea has been considered a health-promoting beverage. Green tea can be regarded as food components useful for the maintenance of cardiovascular and metabolic health (Wolfram, 2007). When incorporated with a balanced diet, green tea can provide a healthy additional alternative for weight reduction. Green tea contains high quantities of several polyphenolic components, such as epicatechin, epicatechin gallate, epigallocatechin, and, the most abundant and probably the most pharmacologically active, epigallocatechin gallate (EGCG). The catechins in green tea may stimulate thermogenesis and fat oxidation through inhibition of catechol O-methyl-transferase (COMT), an enzyme that degrades norepinephrine (Diepvens, et al. 2007). During the last couple of years, green tea consumption has become reportedly associated with various health-promoting properties. It has also been shown to promote fat oxidation in humans at rest and to prevent obesity and improve insulin sensitivity in mice (Venables et al., 2008). Other research shows that Green Tea Extract (GTE) inhibits intestinal lipid
absorption and may regulate hepatic lipid accumulation, which could lead to hepatic steatosis, or fatty liver, that is influenced by obesity and high alcohol consumption (Bruno et al., 2008). In addition to that, previous experiments’ results indicate that oral consumption of green tea can lower rat and human serum cholesterol levels and lower rat blood glucose and triglyceride (Hsi-Kao, et al., 2000).

In recent studies, results show that daily cups of the tea or an extract can lead to significant weight loss. Because of this, it was hypothesized that the metabolic rates of mice that are given green tea are expected to be significantly higher than that of those that are only consuming plain water. Moreover, increase in weight of mice is expected to directly correlate with the increase in their metabolic rates. A relatively higher weight gain is expected to occur in mice that are not given green tea, as opposed to those that are given the concoction. This can be done in two fold: First, by having a group of mice that are fed only mouse food and water, and another group that is fed mouse food, water and green tea; Secondly, by comparing their weights and metabolic rates subsequent to the experiment.

Materials and Methods

This study intends to provide a better understanding of varying metabolic rates for average weight humans who are living a sedentary lifestyle. For this experiment, ten female, non-pregnant mice Mus musculus were purchased from Sand Bar Pet Shop (Mission Viejo, CA). In order to prevent cross over data, the mice were each housed separately. Five small cages were obtained from Pet Co. in Rancho Santa Margarita, CA. Each cage was then divided into two, using a solid plastic sheet. This was done to prevent the mice from eating through the divider. The cages were then labeled “A” on one side and “B” on the other side. Each mouse was weighed prior to the experiment. The mice were placed inside a tared box in order to determine its initial weight. The mice were then subjected to a standardization period in order to determine their metabolic rates. After a week, similar procedures were followed in administering the determination of oxygen consumption for both groups. The data collected for the control group of mice were utilized to calculate the oxygen consumption of the mice that were given green tea.

Weight specific metabolic rate measured in mLO_{2}/gm/hr was calculated using:

\[ MR = \frac{10cc}{t} \times \frac{60sec}{min} \times \frac{60min}{hr} \times \frac{1}{wt} \]

where 10 cc is the volume injected into the respirometer, t is the time in seconds to return to initial manometer reading after 10 cc injection and wt is the weight of the mouse in grams.

Gas volumes were then converted to standard temperature and pressure, for dry air (0°C, 760 mmHg). Water vapor pressure was disregarded due to the presence of the soda lime in the jar, which was assumed to have dried all the air in the respirometer.
The volumes were then adjusted to STP by applying Boyle’s Law:

\[ \frac{P_1 V_1}{T_1} = \frac{P_2 V_2}{T_2} \]

where \( P_1 \) is the barometric pressure of the room (in mmHg), \( V_1 \) is the gas volume (MR), \( T_1 \) is the temperature of the respirometer in K, \( P_2 \) is standard pressure (760 mmHg), \( T_2 \) is 273 K, and \( V_2 \) is the adjusted volume (STP).

**Results**

![Figure 1](image1.png)

**Figure 1.** Average weight gain of female, non-pregnant mice before and after being given just water and water and Green Tea. Average weight gain for Group A is 4.33 g ± 0.30 (± se), N=5. Average weight gain for Group B is 3.23 g ± 0.45 (± se), N=5. Significant difference exists between the two groups (\( p=0.04 \), one-tailed t-test). Error bars indicate standard errors (±se).

![Figure 2](image2.png)

**Figure 2.** Weight Specific Mean Metabolic Rate (mL/O2/gm/hr, STPD) of control Group A and experimental Group B before being given green tea. N=5 per group. No significant difference exist between the two groups (\( p=0.14 \), one tailed t-test).

**Figure 3.** Weight Specific Mean Metabolic Rate (mL/O2/gm/hr, STPD) of control Group A and experimental Group B after being given green tea. N=5 per group. Significant difference exists between the two groups (\( p=0.01 \), one tailed t-test).

**Discussion**

A correlation was made between the metabolic rates of two *Mus musculus* groups: one that was not given green tea (Group A), and the other that was given the herbal tea (Group B). Following to the standardization period, the mice showed no significant increase in weight when they were only fed regular mouse food and water (Figure 1). Moreover, Figure 2 shows that the weight specific metabolic rates remained at a fairly similar range for all mice. In contrast, even though all mice showed an increase in weight after a week, a relatively higher increase in weight became more evident with control Group A, than experimental Group B. Figure 1 explains that an increase in weight was clearly visible for the mice that were not given the green tea concoction. Conversely, the metabolic rates of Group B increased significantly than that of Group A. The results shown in Figure 3 show that with the help of green tea, the oxygen consumption of Group B mice increased significantly. Consequently, it was made apparent that green tea, when consumed along with a balanced diet, can aid in weight loss, and also can provide a higher metabolic rate.

The results of the experiment supported the hypothesis that metabolic rates of mice that are given green tea will be higher than those that are not. Furthermore, since the body mass of the mice who were not fed green tea showed a relatively higher increase than those who were, the results provided an insight that green tea can in fact be used as a tool for weight loss or possible aid in weight management. As previous experiments found out, green tea or its catechins decreases intestinal lipid absorption, lipogenesis, and fat storage while promoting fat.
utilization for energy (Bruno et al., 2008). Thus, these potential mechanisms may provide a possible explanation the results acquired for this experiment.

Future experiments might include the study of the variation between the oxygen consumption by running the mice on a treadmill and comparing an increase in metabolic rates for active mice that are given green tea and active mice that are not. Different concentrations and amounts of green tea given and its effects can also be tested.

Literature Cited


The Effects of Salinity on Photosynthetic Rates of Red Algae, *Porphyra perforata*

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*Porphyra perforata* is a red algae that is tolerant to salinity changes due to its ability to develop methods of surviving in a marine environment. *P. perforata* lives on the inter-tidal zones where there is a higher probability of salinity fluctuations that could affect the oxygen production of these species. The hypothesis being tested is that a greater salt concentration will cause red algae to produce less oxygen. In this experiment the oxygen production rate (mg/L) was measured after incubating the algae in three different salt solutions: 0 %, 3.5% and a 6.0%. The average oxygen production of the 0% solution was 11.8 ± 0.49 mg/L. For the 3.5% solution it was 20.0 ± 0.0 mg/L. The 6.0% solution was 6.05 ± 0.21 mg/L. Results concluded that the red algae in the 3.5% solution produced the most oxygen, while the algae in the 6% solution had the least amount of oxygen. According to the ANOVA test and Bonferroni Correction there was a significant difference between the 3.5% solution and the 6% solution. (p=0.0001)

Introduction

*Porphyra perforata* is a type of red algae that lives in marine environments throughout the coast of the United States. Some of these algae are found living in the upper tidal splash zones. In these zones, the amount of salt in the ocean fluctuates drastically due to environmental changes. Two factors that could change the amount of salinity would be hot weather and precipitation. In a warmer climate, the ocean water will evaporate, leaving more salt in the ocean,
which causes the concentration of salt to be higher. In the presence of rain, the amount of salt in the ocean would be diluted causing the concentration of salt to be lower. According to a study made by Cyrus Charles et. al (1958), in southern California, P. perforata disappears in winter from the ocean due to precipitation, which is a strong factor that contributes to the mortality of the species. P. perforata is able to have a great tolerance for salinity due to its transport mechanisms. Red algae rely on active mechanisms and require energy derived from cellular metabolism. Previous studies have indicated that red algae take ions from seawater actively but sodium is pumped out of the cell actively against an electrochemical gradient. (Eppley, 1959). The active mechanisms of ionic exchange between algae and the sea water has been obtained through studies of kinetics where the rate of the uptake of ions increases in direct ratio to the exterior concentration of that ion at a low concentration but steadily becomes saturated at a higher concentration. (Dring, 1982).

P. perforata therefore has a great tolerance to low and high levels of salt. P. perforata is a marine plant that uses the Calvin cycle for initial steps of incorporating CO₂ from the outside air into organic materials present in the chloroplast. This is known as carbon fixation. ATP is generated by the light reactions that convert solar energy to chemical energy. This chemical energy is used to activate proton pumps and produce a hydrogen gradient. Water is then separated and O₂ is given off (Campbell, et. al 2008). Salinity can be a major factor on the oxygen production of marine plants; however exposure to intense sunlight, rain, or freezing can also have an impact on the salinity acceptance. The purpose of this experiment is to measure the different photosynthetic rates by calculating the rate of oxygen produced in P. perforata. In this experiment the red algae will be put into three different NaCl solutions: 0%, 3.5%, and 6%. These percentages show the concentrations of salt such, as 3.5% is equivalent to thirty-five pounds of salt for every 1,000 pounds of seawater, which corresponds to the amount of salt in regular ocean water.

The hypothesis presumes that a greater concentration of salt in the solution will cause the red algae to produce less oxygen. Since the photosynthetic process uses water and releases oxygen, too much salt may inhibit photosynthesis. Fork et. al (1983) tested the change in photosynthetic rate in various salt-water solutions. They concluded that photosynthesis was weak in higher concentrations of salinity. This research agrees with the hypothesis being tested in this experiment.

**Materials and Methods**

**Design of Study**

Porphyra perforata were collected on the rocky shores of Laguna Beach on November 8, 2008 and November 15, 2008. They were collected and placed in its natural seawater. Within forty-eight hours, the algae were brought to the Science building at Saddleback College to begin testing for dissolved oxygen consumption. Three different salinity solutions were used to determine any difference in the readings of the algae placed in them.

**Measurements of the Salinity Solution**

Calculations determinations for sodium chloride concentration were made: 0%, 3.5%, and 6%.

Using the calculation:

\[
\frac{500 \text{ mL of } H_2O \times N \text{ grams of } NaCl}{100 \text{ mL of } H_2O} = X \text{ g NaCl}
\]

No NaCl was needed for the 0% solution because it contained no salts.

**P. perforata conditions and measurements**

The red algae were incubated for forty-eight hours in its natural seawater in the refrigerator (10°C) in order to preserve them. The red algae were kept alive inside a four by eight inches plastic container. Approximately ten grams of red algae were measured using the Scout Pro scale to the nearest 0.01 grams. Each piece was then placed into their respective solutions for fifteen minutes for stabilization. Each solution contained 0%, 3.5% and 6% of NaCl in 600 (mL) of water.

**Testing procedure**

The three 600 mL beakers were placed on the tables of the laboratory at Saddleback College. Three 100-watt lamps were set up on each table. A light meter (model: LX-101A) was used for the same amount of light penetrating the algae. It was measured at 260 nanometers. Three PASPort Dissolved Oxygen machines were used and their probes were placed in each beaker to record the dissolved oxygen consumption levels. Each solution was tested for one hour. Two trials were completed for each solution. The second trial was tested the following week after collecting new algae.

**Statistical Analysis**

Data were analyzed, using Microsoft Excel 2003 (Microsoft, Redmond, WA) with Analysis of Variance (ANOVA test) using the means of dissolved
oxygen consumption in each solution from each trial to determine if there were significant differences between them. Differences were considered significant at P ≤ 0.05. The data are shown in mean ± standard error. Because there was a significant difference, the Bonferroni Correction was scaled between the intervals of: (0% vs. 3.5%), (0% vs. 6%), and (3.5% vs. 6%).

Results

Figure 1 shows that the 0% solution had the second highest rate of oxygen consumption levels and photosynthetic rate. The 3.5% solution had the highest rate and the 6% solution had the lowest. Oxygen production significantly differs between the three salinity solutions (p=0.0001, ANOVA). The oxygen productions in the salinity solutions were 11.8 ± 0.49 mg/L in the 0%, 20.0 ± 0.0 mg/L in 3.5%, and 6.05 ± 0.21 mg/L in 6%. The ANOVA test also resulted in a Mean square of 12.45 and a deference value of 248. Running the Bonferroni Correction resulted in a significant difference between 3.5% and the 6% solutions. The 3.5% solution was forty-seven percent greater than the 6% solution. There were no significant differences between (0% vs. 3.5%) and the (0% vs. 6%) solutions.

Discussion

The photosynthetic rate of the P. perforata was significantly different in the salinity solutions containing 3.5% vs. 6% NaCl. The average 0% solution was 11.8 ± 0.49 mg/L (±se). The 3.5% solution produced the average of 20.0 ± 0.0 mg/L (±se) of oxygen while the 6% solution produced 6.05 ± 0.21 mg/L (±se). The oxygen given off by the red algae is what was being measured in this experiment.

To measure the photosynthetic rate it was imperative to use a light source. Light is a form of energy used to generate the light reactions and the Calvin cycle (Campbell, et. al 2008). The light source that was used in this investigation was three 100-watt lamps. A light meter was used to have the most optimal wavelength in driving photosynthesis.

P. perforata was subjected to salinity stress. Most marine plants have adapted to the salt in the ocean, however it is difficult for a plant to revert back to fresh water. In the 0% solution, it contained no salts, thus putting the algae under pressure. The algae would have a harder time to produce oxygen when they have already developed mechanisms to cope with salt water. Even towards the end of each trial, oxygen production was at a level 1.1 mg/L. Given a longer time to test the algae would have had lethal results according to Coudret et al (1983). He demonstrated that a decrease in salinity causes a decrease in photosynthesis and metabolism. Rain is a big factor by filling up the tide pools diluting its salt content. In another previous study, Casey et. al (1979), stated that their marine algae in fresh water had a limited survival time of 1.5 to 3.5 days. That is why in the 3.5% solution the algae had no trouble producing oxygen. Lüning et.al (1990) attested that fluctuating salinities could cause a hyperosmotic stress or a hyposmotic stress depending on the increasing or decreasing amount of salt. These rapid environmental changes can cause the turgor pressure to become irregular. This is dangerous for the P. perforata because its stability, shape and growth depend on the turgor pressure. Marine plants have developed mechanisms to regulate the changes in salinity by osmotic regulation, however the 3.5% solution is the natural and optimal salinity rate of the ocean, therefore providing a stable turgor pressure to the species.

All living things need salt, but too much can also be sickening and fatal. In the 6% solution, the production of oxygen was the least amount. The concentration of salt in the water was too high for the red algae to handle. It inhibited photosynthesis by decreasing carbon fixation and reducing respiration (Macler, 1988). This can also happen at its natural course if there was no rain and the water was evaporating, leaving the ocean more concentrated with salts. The high salinity can destroy the cell walls of some marine plants causing them to become dehydrated. There needs to be a balance with the NaCl in the water for marine life to survive.

Some variables could have changed this experiment. Taking the red algae out from their natural environment could have upset the balance of life between them and other sea life. Incubating the red algae in the plastic container could have had
some effect to the readings because it was not their natural habitat. Different weight measurements of the algae could be another factor in the readings of the oxygen levels because they already had water mass in them.

Previous studies have determined that stress responses to altered salinities directly affected not only photosynthesis and respiration, but also nitrogen assimilation and photosynthetic flow. (Macler, 1988.) Algae are incapable of nitrogen fixation so nitrogen is needed mostly for the synthesis of amino acids and nucleotides. Nitrogen needs to be moved into the cell and converted into ammonia and then changed into amino acids. The transport mechanisms of ions are thus slowed down unable to perform the work to survive. Changes in the environment affecting the salinity of the ocean, can ultimately devastate many marine species including the Porphyra perforata.

Acknowledgments
The authors acknowledge Professor Steve Teh for his guidance and the Saddleback College Biology lab for providing the necessary measuring devices and other equipment used for this experiment.

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Salicornia virginica is a halophytic shrub plant species which has adapted to environments with high salinity. *S. virginica* uses storage vacuoles within the cells to hold excess salt and also filters salt using sodium pumps within the root system. Cellular respiration was determined by measuring oxygen consumption at various saline solutions: 0%, 3.5%, 7%, 9%, and 10%. The oxygen consumed 5.03 ± 0.43 mg/L at 0%, 4.23 ± 0.23 mg/L at 3.5%, 3.8 ± 0.058 mg/L at 7%, and 2.7 ± 0.058 mg/L at 9%. At salinity levels at and above 10%, no oxygen was consumed due to cell death. It was hypothesized that the increase in saline concentration would correlate to a decrease in respiration rate. The results indicated a decrease in cellular respiration as salinity increased. There was a significant difference between the oxygen consumption at the various saline levels (*p* = 0.001 ANOVA single factor).

Introduction

Pickleweed (*Salicornia virginica*) is a halophytic species of plants which inhabits coastal and marshland areas throughout the United States. It has flourished in areas of high saline which would otherwise be toxic to most other plant species. Pickleweed and other halophytes must tolerate high salt concentrations while also absorbing water which is essential for cellular respiration and photosynthesis (Khan and Ungar, 2000). Therefore, these halophytic plant species have developed mechanisms to filter, excrete, and store excess salt.

In California, *S. virginica* regularly encounters changes in salinity due to incoming and outgoing salt water flow from the oceans and lack of summer precipitation. These plants also generally inhabit the high tide marsh areas where the water levels fluctuate throughout the day (Pearcy and Ustin, 1984) where salt is deposited into the soil after the waters recede. Due to these environmental pressures, this plant species has developed mechanisms to tolerate salt concentrations greater than that of normal sea water.

*S. virginica* can tolerate a wide range of salt concentrations by utilizing ion pumps and specified storage vacuoles. In the root system, salt is filtered using small sodium-potassium pumps. The rest of the cells use ion pumps to move salt into storage vacuoles at the tips of the plant segments. When the vacuoles are unable to store additional excess salt, cell death occurs and the plant segment falls off. Younger cells are then responsible for the storage and removal of excess salt. This cellular compartmentalization has been shown as one of many methods for salinity toleration (Cheeseman, 1988).

The objective of this study was to observe salinity effects on the cellular respiration of *S. virginica* and determine how increased concentrations affect the oxygen consumption. And, also determine the threshold at which cellular respiration ceases. It is hypothesized that the cellular respiration rate will decrease as salinity levels increase.

Materials and Methods

*S. virginica* was collected from a marsh in Newport, California. The samples were cut at the stems, leaving the lateral branches intact. Each plant was weighed out to 30.0 grams for uniform comparison.

The following saline solutions were prepared using distilled water and table salt: 0.0%, 3.5%, 7.0%, 9.0% and 10%. Each plant sample was allowed a thirty minute acclimation period in the particular saline solution prior to experimentation. The plants were then placed in a completely dark environment for a one hour testing period. All plants were in similar ambient conditions prior to and during testing.

The oxygen levels in mg/L were measured using a Pasco Xplorer GLX dissolved oxygen probe (Pasco Scientific, Roseville, CA). The probes were placed into the beakers containing the plant and saline solution after the acclimation period and were left in for the duration of the trial period. At the conclusion of each testing period, the initial and final oxygen...
levels were recorded. Three trials were conducted for each saline solution.

A threshold for salt tolerance was determined through trial and error using various saline solutions between 8-14%.

**Results**

Oxygen consumption rates decreased as salinity concentrations increased, with the highest oxygen consumption occurring in solutions without any salt (Table 1). The average oxygen consumption was 5.03 ± 0.43 mg/L at 0% concentration, 4.23 ± 0.23 mg/L at 3.5%, 3.8 ± 0.058 mg/L at 7%, and 2.7 ± 0.058 mg/L at 9% (Figure 1). Saline concentrations at and above 10% yielded no oxygen consumption. The plants were unable to withstand salinity levels at such a high concentration.

**Discussion**

The results indicate that salinity increases led to decreases in the respiration rate of *S. virginica*. At salinity levels up to 10%, the plant was able to function and perform cellular respiration. These results reflect regular encounters with fluctuating saline concentrations that occur in the natural habitat. However, salinity is still toxic and high levels of salinity were determined to be a factor in mortality of *S. virginica* (Ungar, 1987). This is demonstrated by the plant’s inability to function at concentrations at or above 10%. A significant difference (p=0.001, ANOVA single factor) was determined between all of the groups, which suggest that salinity has a large effect on respiration rate.

**Figure 1.** The average oxygen consumption of *Salicornia virginica* at various saline solutions. The oxygen consumption at 0%, 3.5%, 7%, and 9% saline solutions were 5.03 ± 0.43 mg/L, 4.23 ± 0.23 mg/L, 3.8 ± 0.058 mg/L, and 2.7 ± 0.058 mg/L, respectively. Error bars indicate standard error of the mean.

<table>
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<th>Trial 2</th>
<th>Trial 3</th>
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<td>4.2</td>
<td>5.6</td>
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<td>3.8</td>
<td>4.3</td>
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<td>3.9</td>
<td>3.8</td>
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<td>0</td>
</tr>
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</table>

The plants placed in the 0% solution demonstrated the highest oxygen consumption rates. Additionally, plants placed in 3.5% solution and 7% solution also showed similar oxygen consumption rates. This correlates to natural exposure to sea water which is approximately 3.5% saline on average as well as exposure to salt pans which are areas of high salt concentrations resulting from deposited sodium chloride after the tides recede.

Previous studies have shown that respiration rates were determined to increase at lower salinity and decrease at higher salinities in red alga (Macler, 1988). Additionally, coral (*Siderastrea sideria*) showed similar responses when exposed to increasing levels of salinity (Muthiga & Szmant, 1987). This corresponds to the similar trend observed in *S. virginica* because red alga and *S. sideria* encounter regular gradations and changes in salinity.

Halophytes have demonstrated adaptations to salt solutions that would be unsuitable for non-halophytes. Halophytes show a stimulation of growth at sodium chloride concentrations that are inhibitory to the growth of non-halophytes (Khan and Ungar, 2000). *S. virginica* has also shown to inhabit areas that experience low precipitation during the year. Their adaptation to this can be seen in their ability to tolerate salt concentrations up to 10%.

Further research can be conducted on other *Salicornia* species and other halophytic species to determine similarities in salt tolerance on respiration rate. Comparative studies between photosynthetic rate and respiration rate as well as impact of salinity on growth rate can be conducted.

**Literature Cited**


The Effects of Sugar Water on Potato Starch Concentration

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If plants can be bred to grow shorter or taller, produce more flowers or fruit, could vegetables be bred to taste differently than normal; make a sour apple more sour, a grapefruit less tart, and a potato sweeter? This experiment attempts to determine if a potato (Solanum tuberosum) watered with sugar water will become sweeter, in other words store more starch, therefore this experiment's hypothesis is that these potatoes will in fact store more starch and become “sweeter.” The results yielded by this experiment unfortunately were not representative of the potato population however, due to the fact that half of the potatoes died while being grown, reducing the viable potatoes to be tested to a number lower than required. The results recorded however do suggest that the hypothesis was correct. The mean starch concentration of the experimental potatoes was 0.85 ± 0.098 mg/100 mL (N=6), while the control group concentration was 0.58 ± 0.0046 mg/100 mL (N=4). The P-value determined by an unpaired t-test was 0.046.

Introduction

Imagine really sweet potatoes, or grapefruits. Should sour apples be less tart, or made more sour? Plants absorb nutrients to nourish themselves, grow and procreate; oftentimes these nutrients are stored within the plant to the point that they may change a particularly interesting characteristic, their taste. For humans, this particular ability of plants can be very beneficial, allowing a person to pick and consume foods according to their personal preferences. Imagine making a sweet grapefruit just a bit tarter, or a sour apple just a little sweeter. What if those plants and the food they produced could be modified on the stalk, not through genetic manipulation, but simply by what was fed to them?

Potatoes, Solanum tuberosum, generally come in two varieties, “sweet” and “regular” (non-sweet). In the case of this experiment, Russet Potatoes will be tested to determine if they can be “made sweeter.” This result will attempted by watering potato tubers with slightly sugared water. These experimental potatoes will then have their starch levels compared to a control group of potatoes that have received nothing except plain water. It is hypothesized that the experimental potatoes will have a higher sugar concentration than the control group potatoes, stored in the form of starch.

Materials and Methods

Plant Preparation

A total of 10 Russet Potatoes were used for this experiment, purchased from Ralph’s Grocery Store (San Clemente, CA). The 10 potatoes were picked due to their approximate equality in size and numerous “eyes,” a potato’s growth spot where the plant stalks grow from. In an attempt to eliminate the possibility of one group being “more starchy” than another before the experiment began, each of the 10 potatoes were cut in half. Each half of a potato was then put into the control and experimental groups in the hopes that if the potato naturally had, or was naturally prone to absorb more starch, that this possible variable would be eliminated.

The potato halves were then put, cut side down, into plastic planting pots supplied by the Saddleback Biology Department and covered with potting soil, also supplied by the biology department. After all the potatoes were planted, each of the control plants...
was watered with two-hundred mL of tap water. Experimental plants were watered also with two-hundred mL of tap water, but mix with a 1% concentration of C & H Pure Cane Sugar (C&H Sugar Company Crockett, CA). Each plant was watered in this same manner for 43 days. The plants were watered as needed, which was usually once a week.

**Testing Procedures**

On the 43rd day after being planted, all plants viable for testing were removed from their pots and soil, and washed. A 10 gram sample of each plant was then ground up in a blender (supplied by the Saddleback Biology Department) with 100 mL deionized water. The potato juice was then tested for starch concentration in a spectrophotometer (supplied by the Saddleback Biology Department).

The spectrophotometer was set to a wavelength of 620nm, and the starch in the potato juice to be read was colored by iodine, the starch indicator. In addition to the unknown concentration of potato juice starch being recorded, three readings were made from potato juice including one of three standards: 0.5%, 2.5%, and 5.0% starch concentrations. The standard solutions of potato juice were made up of nine mL potato juice and one mL standard, as well as the two to three drops iodine indicator. The unknown solution was simply ten mL of potato juice and indicator.

The calculation used to determine the unknown concentration of starch was

\[ X_1 Y_1 = X_2 Y_2 \]

where \( X_1 \) = standard concentration solution (any of the three could be used), \( Y_1 \) = absorbance reading of standard solution (again any of the three), \( X_2 \) = unknown concentration, and \( Y_2 \) = unknown absorbance. Since the unknown concentration was being calculated for, the above calculation was changed to solve for \( X_2 \).

**Results**

The standard solutions all had an absorbance reading of 0.61 despite their different concentrations of starch. This was true for both the control and experimental standard solutions. The control’s four unknown absorbance readings ranged from 0.52 to 0.60 with an average of 0.55, while the experimental group’s six absorbance readings ranged from 0.21 to 0.52 with an average of 0.39.

The average unknown concentrations from the control group standards, 0.5%, 2.5%, and 5.0%, were calculated to be 0.55, 2.76, and 5.52 respectively, while the average experimental group concentrations were 0.78, 3.79, and 7.79 respectively. An unpaired t-test assuming unequal variances (Microsoft Excel, Redmond, WA) was taken using the data mentioned above, it determined that the starch concentration mean of the control group (\( N=4 \)) was 0.58 ±0.0046, while the concentration mean of the experimental group (\( N=6 \)) was 0.85 ±0.098 (fig. 1). The one tailed t-test also determined that the P-value to be 0.046 (\( N=10 \)).

**Discussion**

This experiment’s results suggest that the hypothesis was in fact correct. The concentration of the experimental group does appear to have a higher concentration of starch. This in turn is very likely a result of the experimental potatoes being watered with a slightly elevated amount of sugar water.

Unfortunately at the time of harvest, only ten, four plants from the control group and six plants from the experimental group, of the twenty plants had survived in a state that would allow them to be utilized in the testing phase of the experiment. Therefore, while results were obtained from the plants and data was then analyzed, these results should not be considered by any means conclusive as the sample population was not large enough to be considered representative. The data that was collected however was interesting nonetheless.

**Areas of Improvement**

While watering the plants, it was noticed that for the experimental group a white crust was forming on top of the soil, and it is likely that this crust was dried sugar collected from the waterings. While this top dry top layer appears to have little effect on the plants themselves it may have had an unexpected effect on the control group.
Each week the plants were inspected to determine when they should be watered. To avoid watering one plant more then another, or one group more then the other, all plants were watered at the same time. The crust layer on top of the experimental groups appears to have acted as a moisture trap, drying out the top layer, but keeping the soil beneath it rather damp. Therefore, to avoid over watering the experimental group the top layer had to be broken up, exposing the damp soil beneath. Then a few days later the soil would be dry enough to consider watering. All the while however, the control group soil would be in a much drier state.

Since the potatoes were cut in half, it is my belief that as the soil became drier and drier, the potato cutting would also begin to dry out. This would likely have occurred especially quickly since the skin was no longer in place to prevent water loss. This dehydration likely contributed to the death of many of the control potatoes, but not why some of the experimental potatoes died.

The experimental potatoes that died may have occurred due to the exact opposite reason that the control potatoes died; excess moisture keeping the soil moist to the point that fungus or other parasitic organisms were able to grow on the plant. It is likely that the sugar present, aided in such organism growth as well.

It is also likely that in the case of both groups, due to the lack of protective skin over the cut portions of the potato, that the potatoes were more prone to disease and parasites. Therefore the lack of protective skin around the potatoes combined with the possible lack of or excess of water likely contributed to the demise of many of the potatoes.

It should therefore be considered in the future that the potatoes should not have their skin removed. Also to be considered for future experiments, is how to control soil moisture. Instead of attempting to give the plants equal amounts of water, each plant’s individual soil should be monitored and maintained at a particular level of moisture, ideally a level that stimulates potato plant growth.

Relevance to other Studies

This experiment attempts to determine if by simply feeding a plant a particular nutrient, one could change the characteristics of the plant, in this case, the taste of a potato. While no studies were found replicating this type of experiment, attempting to change the plant’s taste, there were a few studies that did include the effects of starch and sucrose on plants.

Most similar to the above experiment was one done in 1993, testing the effects of $CO_2$ and temperature on potatoes, and attempting to determine if they had any effect upon the plants starch concentrations (Cao and Tibbitts 1993). This experiment was obviously attempting to determine the ideal concentration of $CO_2$ and temperature growing conditions, to grow potatoes. While this experiment was attempting to manipulate starch concentration, it was not necessarily doing so to modify the taste of the plant, but rather give farmers better information as to how to best raise their crops.

Five years later scientists tested to see how plants, in this particular case Kentucky bluegrass, dealt with having a large portion of their nitrogen (N) metabolism removed, i.e. mowing (Jiang and Hull 1998). Several groups were grown and fertilized using different nutrients including one group that received increased sucrose levels. The results showed that grass grown with sucrose nutrient tended to rebound faster then most other types fed differently after losing most of their N metabolisms. Of course while this experiment does not deal directly with potatoes, it does suggest that plants treated with sucrose do differ in growth and metabolism then those that do not receive such nutrient.

In 2000 scientists attempted to see what effects sucrose would have upon three strains of potato plant growth, i.e. shoot length, leaf length, etc (Barbosa de Franca 2000). The results varied from plant to plant and ranged from some growing more buds, others showing longer shoot growth, and such results. Again, an experiment similar to this one; attempting to determine how sugar may affect the plant, but not its taste.

That same year, sweet potatoes were tested in much the same manner as the Barbosa de Franca experiment; however, this experiment was created to determine the regeneration of plant growth through the effects of sucrose nutrient given during callus development (Chen 2000). This experiment showed that modifying the sucrose levels of the sweet potato plants did in fact effect the regeneration of the buds, shoots, and leafs of the plant.

While none of these experiments attempted to determine how to manipulate the taste of potatoes, they nonetheless showed that sucrose and starch levels do have a very significant effect upon plants. These effects may vary from storing starch to regenerating lost parts. Possibly in the future, with some modified procedures, this experiment may be attempted again and more reliable data could be collected to determine if we may be able to change just how sweet our potatoes are, how sour our apples have to be, or be able to change a variety of other food flavors.
The Efficiency of Common Hygiene Measures by Salivary Acid Neutralization

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For many years dental professionals have suggested that the general public brush after meals to prevent dental cavities. This is not always easy for busy people. It would be nice for them to know a more practical technique that may have a significant effect at decreasing the risk of dental cavities. This study has served to shed light on the issue of oral hygiene by quantifying the pH of saliva after different hygiene measures. The experiment consisted of 50 adults. The participants were randomly split into 5 groups of 10 each. They all discharged 1 mL of saliva into plastic cups after eating a Snickers® (58.7g). Next the pH of their saliva was measured with pH strips. The first group was given a piece (3 g) of Koolerz® sugarless gum, the second group, a dry toothbrush, the third group, Listerine® (20 mL), the fourth group, a cup of water, and the fifth group was given nothing. The participants in the study brushed, chewed gum, and gargled with water or Listerine for 1.5 minutes. Afterwards, they waited 3.5 minutes and once more discharged 1 mL of saliva into a different cup. They continued to do so every 5 minutes for a total of 30 minutes. It was expected that chewing the Koolerz® sugarless gum would be the most efficient and effective way of balancing the pH of saliva after meals. The mean pH result after the consumption of the Snickers® was 7.40 ± 0.0534 se (N=50). After 30 minutes the mean pH of saliva of the subjects who did no hygiene measure was 6.70 ± 0.0828 se (N=10), the subjects treated with the Listerine after thirty minutes was 7.22 ± 0.0448 se (N=10), gum was 7.03 ± 0.0333 se (N=10), brushing was 7.02 ± 0.0326 se (N=10), and water was 6.54 ± 0.1355 se (N=10). In conclusion, Listerine® showed to be the most efficient and effective way of balancing the pH of saliva after meals.
Materials & Methods

The measure of pH was performed in 50 healthy adults between 18-30 years currently without braces who did not smoke. The participants were randomly split into 5 groups of 10 each. Participants were asked to withhold from brushing their teeth or doing any dental hygiene measures for twelve hours prior to the experiment; furthermore, helpers were asked to fast two hours from 6–8 PM. Then at 8 PM, each participant discharged 1 mL of saliva into plastic cups (Carthage® 5 fl oz) and the pH of their saliva was measured with pH strips (Alkalive pH Stix™ range: 4.5 - 9.0). They all discharged 1 mL of saliva into a different cup and the pH of all the subjects’ saliva was measured. They continued to do so every 5 minutes for a total of 30 minutes.

ANOVA single factor tests were done to interpret the results using Microsoft Excel (Microsoft Corporation, Redmond, Washington). These tests were followed by Multi-comparison post-hoc tests between groups. Differences were considered significant at P < 0.05. All data was expressed as a mean ± standard error.

Results

The acidification of saliva inside of the mouth occurred during the process of consuming food. The mean pH of the saliva of the subjects treated with nothing, right after consuming Snickers®, was 7.555 ± 0.1691 se (N=10), and after 30 minutes, the mean pH of saliva of these subjects was 6.70 ± 0.0828 se (N=10). The pH of the saliva of the subjects treated with Listerine® after thirty minutes was 7.22 ± 0.0448 se (N=10), gum was 7.03 ± 0.0333 se (N=10), brushing was 7.02 ± 0.0326 se (N=10), and water was 6.54 ± 0.1355 se (N=10). After running an ANOVA single factor test, a significant difference was seen throughout the groups (p= 1.30938 x 10⁻⁰⁸). After the ANOVA test was run, a multiple comparison test was done in which there was no significant difference between the baseline data and the individual five groups. In addition to an ANOVA test on the baseline pH versus the results from the five categories, an ANOVA test was run on the average pH results without the baseline. The ANOVA also showed that there was a significant difference between the five groups (p= 4.93 x 10⁻⁰⁷). The multiple comparison test showed no significant difference when comparing the groups individually. A significant difference was seen in all time periods 0 min. to every 5 min. until 30 min. (P=7, 0–30min, ANOVA single factor, Table 1)

![Table displaying the ANOVA test value between all treatment groups at each time period (the total period was seven, 0 min to 30 min). Each group had a statistical difference.](image)

Discussion

In the study of the pH neutralization, through the use of sugarless gum, brushing one’s teeth, rinsing with Listerine®, or rinsing with water, the results showed Listerine® was the most efficient at maintaining a balanced pH at the end of the 30 minutes, followed by chewing Koolerz® sugarless gum, brushing one’s teeth, rinsing with water, and doing nothing.
The group, whose subjects were treated with Listerine®, Koolerz® sugarless gum, and brushing, demonstrated more rapid neutralization of the pH of their saliva (to about 7). The group whose subjects received water and the group who did not receive any hygiene measure showed a lower pH than the optimal pH of 7 and slower speed of reaching this optimal pH point (Figure 1). The results summarized in (Figure 2) reject the hypothesis by statistically showing that Listerine maintained the pH, the most high, during the specified time period of 5 to 30 minutes. This study demonstrates how an increase in the salivary flow, stimulated by chewing gum, results in a significant difference in the buffering of the saliva’s pH. Cavities form when plaque, made of many oral bacteria, build up on a tooth and demineralize the outer enamel. Studies have shown a direct relationship between the increase of a buffer quality in saliva and chewing sugarless gum. A notable incident in our results was that our pH data did not drop as low as the pH results in similar experiments (Dodds, Hsieh, and Johnson, 1991).

**Literature Cited**


Effect of a Change in Length of Whiskers on Maze-Behavior in *Mus musculus*

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Many humans think that the most important sensory organ is the eye. However, past experiments have shown evidence that in many animals, like the house mouse *Mus musculus*, have evolved a more useful feature in whiskers. It was predicted that when the mice’s whiskers are trimmed, their ability to navigate through a maze would be significantly decreased. First, the mice were trained to seek food at the end of a maze. Then, the mice were run through a maze in light. Next, the mice were run through the maze in dark. After that, the whiskers were trimmed the half. Then two trials were held each in light and dark. The mean times required to exit the maze were 68.36 ± 4.49 s (±s.e.) for the first trial, 64.08 ± 3.82 s (±s.e.) for the second trial, 185.76 ± 12.51 (±s.e.) for the third trial, and 178.09 ± 7.70 s (±s.e.) for the forth trial. The results were analyzed with ANOVA along with Post Hoc test. There was a significant difference between the trials that the mice had full whiskers and the trials that the mice had half-length whiskers (p=4.62 X 10^-15). The difference reflects that a reduction in length of whiskers but the absence of light decreased the ability of mice to run through a maze.

**Introduction**

Most humans think eyes are the most important sensory organ, but this is not true to all animals. Early in their evolution, when mammals were derived from cynodonts, they became nocturnal in order to avoid competition with other animals such as reptiles (Menaker et al, 1997). Many of these mammals are still nocturnal including the house mouse, *Mus musculus*. Since mice are not only nocturnal but also tunnel living, they improved their abilities such as hearing, smelling, and the sense of touch (Heffner and Masterton, 1980).

Because there is a limited amount of light at night and the mouse has developed other, more dominant, sensory organs, the vision of the mouse was thought to be weak. However, while the physiology and genetics of the mouse eye were popular study subjects, only a few studies were done on the actual function of the eye for the mouse. Augustsson and Meyerson found that the mouse has depth perception and uses the visual information in making decisions (2004). Yet, which sense is dominant in closed spaces, such as tunnels or mazes, was not studied. This study was aimed at determining which sense *M. musculus* relies in a maze. It was hypothesized that mice would be affected by a change in the length of their whiskers but not by the presence of light.

**Materials and Methods**

Eleven female mice, 1-2 weeks old, were obtained from Reptropolis, San Clemente, CA, 92672. Before the maze-behavior test, all the mice were trained to run a maze by the method used in a previous study (Shirai et al, 2006). We starved mice for 12 hours and placed them in a maze. Food was located at the exit, while mice searched for the food. In the maze-behavior test, a maze with eight blind alleys was used (Figure 1).

Mice were starved 12 hours prior to the trial so that they sought for the food at the exit of the maze. The time required to escape the maze was recorded. The escape was considered to be when the mouse touches the hand holding a cookie. The first trial was in light, and the second trial was in dark. After the second trial, whiskers of the mice were trimmed to half. They were run through the same maze in light and then in dark.

**Results**

The mean times required to reach the exit were 68.36 ± 4.49 seconds for the first trial, mice with full whiskers in light, 64.08 ± 3.82 seconds for the second trial, full whiskers in dark, 185.76 ± 12.51 seconds for the third trial, half whiskers in light, and 178.09 ± 7.70 seconds for the forth trial, half whiskers in dark. There was a significant difference between the trials (N=10, p=4.62 X 10^-15, ANOVA). With Post Hoc test, the places of significant differences were identified; there was a significant difference between trials with full-length whiskers and trials with half-length whiskers. However, there were no significant differences within the trials with the same whisker lengths. After the whiskers were reduced, mice more often showed washing motions, touching their nose area with their forepaws. They also touched the walls with whiskers, smelled, and hesitated to move forward more frequently. Because one mouse had not opened its eyes yet, the records from this mouse were excluded.
resulting in a decrease in the sample size from eleven to ten.

**Discussion**

The ability to run the maze was greatly influenced by the change in the length of the whiskers (Figure 2). However, the presence of light did not have significant effect on the ability to run the maze. In addition, it was observed that mice touched the walls with their whiskers before they moved. These results support the hypothesis that mice rely on their whiskers to run through a maze rather than their eyes.

It was found that mice learn mazes and reduce the time trial (Shirai et al, 2006). Mice significantly reduced time trials at each trial in the previous study. Because one maze was used for behavior test, in order to prevent the effect of learning the maze, mice were run the maze once in each condition. There was no significant difference between trials with the same length of whisker. Thus, changing the condition prevented mice from learning the maze.

It was observed that they more often stood up and sniffed as they approached the exit. As doing that, they oriented the exit with the smell of the cookie. No research was done on the olfactory capability of *M. musculus*. However, a previous study found that other rodents have good olfactory capabilities (Wall, Vander S. B. 1995). With these, it is reasonable to say *M. musculus* also has a good olfactory capability.

A previous study showed that mice utilize visual information from their eyes (Augustsson and Meyerson, 2004). However, in a closed space, like a maze, mice cannot see far away. Moreover, in a tunnel, due to lack of light, the visual information is limited. Although mice have a good olfactory capability, it still cannot help mice to move fast in a limited space. Regarding mice are active in dark and narrow places, it can be concluded that the whisker is the most important sensory organ for mice.

**Literature Cited**


The Effect of Different Sugar Environments on the Growth of *Streptococcus lactis*

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In this experiment, the effect of different types of sugar environments on the growth of *Streptococcus lactis*, a common oral bacterium, was measured to determine if there are any sugars or substitutes that promote oral health. It was hypothesized that there would be a statistically significant difference between the growth of *Streptococcus lactis* at each experimental treatment (honey, Splenda®, raw cane sugar, control). Utilizing an ANOVA multiple comparison test, it was determined that there was indeed a significant difference between the growth of *S. lactis* in each experimental environment (*p*=1.74x10⁻¹³, ANOVA). A Bonferroni Correction was used to determine where the significant difference lies and it was determined that there is a statistically significant difference between every experimental environment. Ultimately, since the hypothesis was validated by the data collected, it was determined that honey is the sugar substance that promotes oral health the most, by inhibiting the growth of oral bacteria whereas raw cane sugar is the substance that does not promote oral health by increasing the growth of *S. lactis* drastically.

**Introduction**

The objective of this experiment is to determine if different sugars affect the growth rate of oral bacteria. Dental caries are bacteria that damage tooth structures, resulting in what is commonly called tooth decay or cavities. This damage first affects the hard tissues of the teeth, such as the enamel. Caries prevalence is related to the increasing consumption of fermentable carbohydrates, such as sugars and starches (Marsh, 1997). These fermentable carbohydrates are digested by the oral bacteria to produce acids, decreasing the pH of the oral environment (Kandelman, 1997). The composition of oral micro flora is closely related to the pH of the oral environment. Dental caries thrive in acidic conditions and increase the adhesion of dental plaque on the tooth surface (Kandelman, 1997).

Sugar consumption has been considered to be the main causative dietary factor in caries development (Miller, 1890). Once consumed, the teeth are exposed to an increase in oral acidity and possible demineralization could be initiated at the outer enamel surface (Kandelman, 1997). But alternative sweeteners and honey are not readily fermented by the oral bacteria (Vadeboncoeur, 1983). Artificial sweeteners possess non-carcinogenic and even anti-carcinogenic properties. Some studies have shown an 85% reduction in dental caries with xylitol consumption than in comparison to sucrose consumption (Vadeboncoeur, 1983). Honey also has been found to have an inhibitory effect on bacterial growth in vitro. The antibacterial effect of honey may be attributed to its osmolarity effect (Steinberg, 1996).

In this study, the researchers’ hypothesis stated that artificial sweetener (Splenda®; McNeil Nutritional, Fort Washington, PA) and honey will inhibit oral bacteria growth significantly while sugar will stimulate oral bacteria growth. With the gained knowledge of this experiment, advances could be made in sweetener development to improve oral health.

**Materials and Methods**

Before the experiment was run, forty agar plates were prepared. There were ten agar plates for each of the following samples: control, raw cane sugar (C&H, Yonkers NY), Splenda® and honey (Sioux Honey Association, Sioux City, IA). 250mL of Criterion Nutrient Agar (Hardy Diagnostics, Santa Maria, CA) was used per 10 plates. To each 250mL agar solution was added 12.5g of the respective experimental treatment, making it a 5% experimental agar solution; the control agar plates were plain nutrient agar with no additives. The solutions were then autoclaved and after cooling each solution was poured evenly into 10 sterile agar plates. The *Streptococcus lactis* bacteria (Saddleback College, Mission Viejo) was looped into a 5mL nutrient broth and incubated at 36°C for a 24 hour period (Saddleback College).
After sufficient incubation of the bacteria, each agar plate was streaked with *S. lactis* utilizing aseptic technique. After all of the plates were streaked, they were incubated at 36°C for 24 hours. One-fourth of each of the plates was counted to get an accurate estimate of the number of colonies of *S. lactis* per agar plate. These numbers were then multiplied by four to get the number of total colonies per plate.

**Results**

The results of this experiment were obtained by performing the aforementioned techniques for a total period of 48 hours. The average number of colonies per plate per experimental treatment is represented in Figure 1. The average number of colonies per plate for the control sample was 727.6 ±67.2 (±S.E.). The average number of colonies per plate for the honey sample was 40.4±7.9 (±S.E.). The average number of colonies per plate for the Splenda® sample was 413.2±40.2 (±S.E.). The average number of colonies per plate for the raw cane sugar sample was 1212.8±108.8 (±S.E.).

![Figure 1. Average number of colonies of *S. lactis* per each experimental environment. The average number of colonies per plate for the control sample was 727.6 ±67.2 (±S.E.), for the honey sample 40.4±7.9 (±S.E.), for the Splenda® sample 413.2±40.2 (±S.E.), for the raw cane sugar sample 1212.8±108.8 (±S.E.). There was a statistically significant difference between the growth of *S. lactis* at each experimental environment (p=1.74x10^{-13}).](image)

The principle findings of these studies led the experimenters to propose that as hypothesized there will be a significant difference between the growth of *S. lactis* in each environment. More specifically, sugar will speed up the growth of the oral bacteria *Streptococcus lactis*. Also as theorized the presence of an artificial sweetener, such as Splenda®, will delay rates of bacterial growth. This illustrates that Splenda® possesses a non-carcinogenic trait. The most surprising finding of this study was the result with the honey sample. In concurrence with Steinberg’s findings, honey inhibited bacterial growth. The surprising result was that honey seemed to have almost an antibacterial quality in that it almost completely restrained bacterial growth, in comparison to the control, which had a reasonable number of colonies formed. These findings confirm Vadeboncoeur’s suggestion that alternative sweeteners and honey are not readily fermented by the oral bacteria. Since they are not fermented by bacteria, the oral environment stays at a relatively neutral pH. Sugar on the other hand is quickly fermented, and in doing so decreases pH levels to an acidic state, which is the preferred environment for micro flora to thrive. The acid conditions created by cane sugar increase the adhesion of dental plaque on the tooth surface, causing increases in enamel demineralization.

This study could be useful in further studies related to oral bacterial health. These finding suggest a beneficial swap in sweetening methods of materials that have direct oral contact. The researchers also feel that this study could be continued to find honey’s inhibitory effect on bacteria species other than oral *Streptococcus lactis*. The researchers would also like to make a note that if proper oral health maintenance methods (i.e. brushing with fluoride toothpaste) are conducted after sugar consumption, fermentation is significantly decreased and enamel deterioration is less likely to occur.

**Discussion**


Comparison of Lactate Dehydrogenase Activity Between Shrimp (*Litopenaeus vannamei*), Crab (*Callinectes sapidus*), and Prawn (*Penaeus monodon*)

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**Abstract**

During lactic acid fermentation, the enzyme lactate dehydrogenase (LDH) catalyzes the reduction of pyruvate to lactate as NADH is oxidized to NAD⁺. Muscle samples were taken from *L. vannamei* (shrimp), *C. sapidus* (crab), and *P. monodon* (prawn) to compare LDH activity between the three marine crustaceans. These species vary significantly in size: prawn being the largest and shrimp being the smallest. Because LDH activity has been found to increase as the mass of an organism increases, it was expected that the LDH activity would be highest in prawn, followed by crab, and then lowest in shrimp. A UV light spectrophotometric assay was applied to measure Δ Absorbance/time as the substrate NADH was oxidized. *L. vannamei* had a mean LDH activity of 3.95 μmol/min/g ±0.2206 (±SEM), N=10. *C. sapidus* had a mean LDH activity of 0.862 μmol/min/g ±0.8617 (±SEM), N=10. *P. monodon* had a mean LDH activity of 0.896 μmol/min/g ±0.1787 (±SEM), N=10. The data shows that shrimp had a significantly higher enzyme activity than both the crab and the prawn; the crab and prawn were found to not be significantly different. The data was analyzed using an ANOVA with a Post-Hoc test. It certainly seems that the original hypothesis has been invalidated. Because of this, it can be inferred that size is not the only factor that needs to be taken into account for varying LDH activity among different species, but a species’ unique habits and skills that nature has selected for it.

**Introduction**

Lactic acid fermentation occurs at the beginning of strenuous activities (i.e. hunting as a predator, fleeing as a prey, human exercise, etc.) as the demand for energy in the form of ATP is higher than the supply of oxygen in muscular cells. In all animals and some plants, this process of fermentation can be chemically characterized as the reduction of pyruvate to lactate as NADH is oxidized to NAD⁺. During this process, a net of 2 ATP molecules are produced by substrate level phosphorylation, the NAD⁺ molecule is produced in order to start at the glycolysis pathway once more, and lactate, or ionized lactic acid is formed. This spontaneous process is catalyzed by the enzyme Lactate Dehydrogenase. The higher density of enzyme in an animal’s muscle tissue means that more substrate can react at a given time, and the reaction rate of this chemical process increases as well (Campbell et al, 2008).

In the early twentieth century, Archibald Vivian Hill had suggested that increased lactic acid concentration in muscular tissue contributes to muscular fatigue and soreness (Hill et al, 1923). This had been the thought of scientists and athletes alike until the end of the 20th century and early 21st century. Newer studies propose that muscle fatigue and lower muscle excitability is the result of increased concentrations of K⁺ ions in muscle cells and that a lower potassium ion concentration causes muscle fatigue when muscle acidosis is halted in humans. This acidosis is an integral part of not becoming fatigued as quickly (Mohr et al, 2004). Furthermore, the acidosis of muscle cells by lactic acid has been shown to actually increase muscle excitability and contraction endurance in animals (Karelis et al, 2004). Because of these studies one can conclude that the importance of fermentation during the early part of strenuous exercise in an animals muscle cells lies not only in energy production but more so in the production of lactic acid. These studies are also used to validate the correlation that can be made between lactic acid production and overall muscular contraction effectiveness in strenuous exercise. An animal’s
ability to produce higher amounts of lactic acid in a shorter amount of time increases that animal’s muscular endurance and effectiveness more quickly. Childress and Somero (1990) found that in some pelagic fish, there is a correlation between glycolytic enzyme activity and mass of the animal. As the mass of the animal increases, so does the enzyme activity in muscle cells. They also found that nature seemed to select for higher enzyme concentration per muscle mass in fish that demonstrated exceptional sprint swimming abilities and related habits. The shrimp, crab, and prawn being studied are known as scavengers of the ocean bottom and are not known for their exceptional sprint swimming abilities. However the three different species do have significant differences in size, which is the basis for the hypothesis.

The Penaeus monodon species of prawn is significantly larger than the Callinectes sapidus species of crab. Both the crab and prawn are significantly larger than the Litopenaeus vannamei species of shrimp. It is expected that the LDH activity would be lowest in the shrimp, higher in the crab, and highest in the prawn. To compare LDH activity among these crustaceans, enzymes were extracted from equal muscle masses taken from each animal. Reaction rates were measured using a UV light spectrophotometric assay, and LDH activity was calculated as light spectrophotometric assay, and LDH activity was calculated as μmol of substrate NADH converted per minute per gram of muscle.

Materials and Methods

Animal and Solution Preparation

Ten shrimp (19.075 g ±0.691), 10 crabs (119.973 g ± 11.808), and 10 prawns (245.67 g ±8.356) were purchased fresh and uncooked from 99 Ranch Market (Irvine, CA). These fish were held in a freezer until actual muscle samples were taken to keep the proteins from denaturing. The Worthington Biochemical Corporation Lactate Dehydrogenase Assay was used as a guide to perform the preparation and procedure for this experiment.

A stock solution of 0.2M Tris Buffer (BDH Electran Molecular Biology Grade) was dissolved in 1M HCl to obtain a pH of 7.3. Because LDH is a particularly stable enzyme at a relatively wide pH range, using a pH meter to get an exact pH of 7.3 was superfluous. Getting the pH between 7-7.5 using pHydron paper was a satisfactory measurement. Stock solutions of 5.6mM NADH salt (Sigma β-NADH disodium hydrate) and 30mM Sodium pyruvate (Sigma Reagent Plus) were then dissolved in Tris buffer. All stock solutions were kept in a refrigerator. Because of the substrate specificity of enzymes, meaning there are no other enzymes in the crude muscle extract that could have any catalyzing effect on the reaction, attaining a purified LDH solution was unnecessary. Therefore, the enzyme solutions used in the experiment were solely crude muscle extracts.

Using razor blades, approximately 0.50g of muscle tissue was cut out of each sample and weighed using a laboratory top-scale. This tissue was then minced as finely as possible on a glass plate and then homogenized in 4mL of Tris buffer using a glass mortar and pestle that had been sitting on ice. This homogenized solution was then centrifuged for 2 minutes and 1mL of the supernatant was put into 4mL of Tris buffer and centrifuged again for 4 minutes. The liquid was then decanted into a small beaker and placed on ice to be used as one working enzyme sample. The dilution of this crude extract was essential to getting a constant Δ Absorbance/time over a 3 minute period; otherwise the reaction would proceed too quickly, converting all of the substrate providing no useful data. The same extraction process was repeated for all shrimp, crab, and prawn samples used in the experiment.

Absorbance Analysis

The Tris HCl buffer, Sodium pyruvate, NADH, and LDH solutions were all kept on ice throughout the experiment as the integrity of these reagents deteriorates if kept at room temperature for extended periods of time. However, the actual spectrophotometric analysis is performed at room temperature (20-21°C). The spectrophotometer (Beckman Coulter DU730 Life Science UV/Vis) was set at a wavelength of 340nm. The blank solution used for every run was a solution containing 2.7mL Tris buffer and 0.1mL Sodium pyruvate. The solutions were put into the cuvette in the following order: 2.7mL Tris buffer, 0.1mL Sodium pyruvate, 0.1mL NADH, and 0.1mL LDH. The cuvette was placed inside the spectrophotometer prior to adding the LDH so that absorbance readings could be taken promptly after adding the LDH solution. The control run for the experiment was taken by adding 2.7mL Tris buffer, 0.1mL Sodium pyruvate, and 0.1 mL NADH, leaving out the LDH. Absorbance readings were taken at 12 second intervals over a 3 minute period. After three minutes, the cuvette was discarded and the spectrophotometer was blanked again before the next run. For each species, this procedure was repeated for 3 separate runs per muscle sample from the 10 organisms, totaling 90 runs overall.

Calculations

Lactate Dehydrogenase activity is defined as μmol of NADH converted per minute per gram of muscle tissue.
Equation 1 represents the reaction rate of substrate used in the experiment, where S is change in Absorbance per time (ΔA/Δt/min) and R is substrate converted per time (μmol/min). Absorbance per time was obtained by scatter plotting all absorbance readings vs. time for all 3 runs per each sample and adding a trend line. The slope of this trend line represents the average ΔA/min for each sample.

\[
R = \frac{0.0056 \text{ mol NADH}}{1000 \text{ mL}} \times 0.1 \text{ mL} \times S
\]  

Equation 2 represents the extraction and dilution of the muscle sample, where \( m_m \) is initial mass of muscle tissue, and \( m_e \) represents the working sample mass after extraction and dilution used in the actual procedure.

\[
m_e = \frac{m_m}{4 \text{ mL}} \times \frac{1 \text{ mL}}{4 \text{ mL}} \times 0.1 \text{ mL}
\]  

Equation 3 represents enzyme activity of LDH where A is in units of μmol/min/g, R is the result of equation 1 and \( m_e \) is the result of equation 2.

\[
A = \frac{R}{m_e}
\]

**Results**

The samples for the shrimp, crab, and prawn (Figure 1) show a decrease in absorbance observed from each trend line slope. Table 1 shows experimental and calculated values used in equations 1-3 to obtain LDH activity (A) for shrimp, crab, and prawn samples, \( N=10 \). Slope of the trend line (S), Reaction rate (R), initial mass of muscle tissue (\( m_m \)), and working enzyme sample mass after extraction and dilution (\( m_e \)).

<table>
<thead>
<tr>
<th>Species</th>
<th>S</th>
<th>R</th>
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As shown in Figure 2, LDH activity of shrimp was significantly higher than that of both crab and prawn (p < 0.05, ANOVA with a Post-hoc test). However, there was no statistical difference between LDH activity for crab and prawn (p > 0.05, ANOVA with a Post-hoc test). Mean LDH activity for shrimp was 3.9544 μmol/min/g ±0.2206 (±SEM). Mean LDH activity for crab was 0.8617 μmol/min/g ±0.1787 (±SEM). Mean LDH activity for prawn was 0.8962 μmol/min/g ±0.1791 (±SEM).
Figure 1. Absorbance readings at 340nm vs. time for shrimp samples, crab samples, and prawn samples. Trend lines show average slope ($R=ΔA/time$) from 3 runs per sample, from the trend line equations for samples 1-10 and the control respectively.

Discussion

From the laboratory experiment performed, for each individual species, LDH activity showed a decrease in absorbance observed from each trend line slope. As NADH is oxidized to NAD$^+$, absorbance decreases over time because NAD$^+$ does not absorb UV light ($λ=340$nm), whereas the initial substrate, NADH, does. The LDH activity was higher in muscle samples taken from shrimp than in crab and prawns. Based on the experiment’s analysis and observations, the original hypothesis has been disproved. The mean LDH activity was not higher in muscle samples taken from the larger animals, prawn and crabs; in fact, the highest enzyme activity was found in the smallest animal being analyzed, the shrimp.

It is difficult to say why the shrimp has higher enzyme activity than both the crab and the prawn. Behaviorally they are all very similar, so a relevant conclusion cannot be drawn from that stance. However, a conclusion can be drawn that although in other LDH activity comparison experiments, the larger animal has the higher enzyme activity, that hypothesis has been refuted for these three crustaceans.

For further research, it would be interesting to build upon this experiment to draw some valid conclusions about the effects of natural and artificial selection on LDH activities between species, by using animals that have a great deal of information about behaviors and traits in each. For example, comparing two different species of canines, both wild and domestic species, and drawing legitimate conclusions about the effects of natural and artificial selection on LDH activity. Another possible experiment would be to compare LDH activity between a plant that uses lactic acid fermentation (the fungus that the dairy industry uses to make cheeses and yogurts), and any animal. The great thing about this experiment is that it is so flexible that there are countless interesting undertakings while still keeping the same experimental framework intact. For future biology students at Saddleback, this experiment can be carried out relatively quickly and efficiently with the tools that students at Saddleback have at their disposal.
Acknowledgements
Very special thanks to the Saddleback College Department of Biological Sciences and Professor S. Teh for facilitating such a smooth experiment.

Literature Cited


The Effect of Mild Escherichia Coli Resistance to Cefaclor on Resistance to Cefixime
Tracy L. Kubas and Tommy L. Roberts
Department of Biological Sciences
Saddleback College
Mission Viejo, Ca 92653

Natural selection is a major source of increased antibiotic resistance in bacteria. In this experiment, antibiotic resistance in Escherichia coli was increased against one type of Cefaclor then exposed to Cefixime to find any differences in the zones of inhibition when compared to an E. coli sample exposed only to Cefixime. Zones of inhibitions were measured after agar plates were exposed with an E. coli and an antibiotic disk was incubated. The most resistant colonies of bacteria were used to grow the next generation of bacteria. Differences in zones of inhibition were measured from bacteria exposed to only Cefaclor, only Cefixime, and Cefaclor followed be Cefixime. An ANOVA was used to compare all the generations exposed only to Cefaclor and a statistical difference was found between the groups (p-value = 3.98 x 10^-5). A Bonferroni correction found that there was a statistical difference between generation one and generations two, three, four and five. A statistical difference was also found between generation five and generations two and three. In comparing the Cefaclor generations to those exposed to Cefaclor and Cefixime a statistical differences was observed between the two groups (p-value = 8.39 x 10^-21). A Bonferroni correction, however, indicated that there was no difference between the fist generation Cefaclor and the first generation exposed to both antibiotics. A difference was observed between the first generation Cefaclor and the second generation exposed to both.

Introduction
Escherichia coli is a gram- negative bacterium commonly found in the lower intestine of warm-blooded animals. The majority of E. coli strains are harmless; however, some can cause serious food poisoning in humans. Harmless strains are part of the normal flora of the intestines, and they benefit their hosts by producing vitamin K2 and by preventing the establishment of harmful bacteria within the intestine (Reid, 2001).

Bacteria, such as E. coli can be hindered or killed through the use of antibiotics. This study utilizes a
grouped of antibiotics called Cephalosporins. Cephalosporins are a family of broad-spectrum β-lactam antibiotics. They were first introduced in the 1960’s, and have become very widely used in the treatment of infections such as gonorrhea, pneumonia, and meningitis (Marshall, 1999). New types of cephalosporins are produced frequently and offer new advantages over their predecessors. The many cephalosporins are grouped into generations based upon their spectrum activity. First generation cephalosporins, such as cefaclor are most active in fighting aerobic gram-positive cocci. Second generation agents are active against gram-negative organisms. Third generation agents, such as cefixime, are the most active against gram-negative bacteria. Lastly, fourth generation cephalosporins are designed to work against gram-positive and gram-negative bacteria (Marshall, 1999).

Cephalosporins work because they disrupt the synthesis of the peptidoglycan layer of bacterial cell walls, which is important to cell structure and integrity. In the final step in the synthesis of peptidoglycan, transpeptidases bind to the end of the peptidoglycan precursor to create the peptidoglycan layer. β-lactam antibiotics inhibit the cross linking of peptidoglycan by mimicking the binding site and competitively inhibiting transpeptidases.

Bacteria, such as E. coli can counter the effects of β-lactam antibiotics through the use of bacterial enzymes called β-lactamases. These enzymes, which are produced by bacteria, can render antibiotics inactive by hydrolyzing the amide bond in the β-lactam ring of cephalosporins. The ability to produce β-lactamases plays an important role in the resistance of bacteria to β-lactam antibiotics. Research shows that it is probable that all bacteria create at minimum one chromosomally mediated β-lactamase (Matthew and Harris, 1976). Bacteria also have R plasmids that also specify β-lactamases. Additionally, the enzymes are inducible, with the most enzyme production occurring when the β-lactam antibiotic is present (Hennessey, 1967).

In addition to the production of β-lactamases, the permeability barrier of gram-negative bacteria also helps to resist β-lactam antibiotics. This barrier restricts the rate at which antibiotics can reach the sites which they target on the bacteria. This barrier is a result of gram-negative bacteria’s unique cellular envelope, which consists of 3 parts; a bilayered inner cytoplasmic membrane, a peptidoglycan band, and a bilayered out membrane. The outer membrane functions as a permeability barrier, however studies demonstrate that the barrier may be species specific (Sykes, 1972).

Bacteria exhibit antibiotic resistance when an antibiotic is no longer effective in controlling growth. In such a case, bacteria will continue to grow and exhibit resistance despite the presence of antibiotic. When bacteria are exposed to antibiotics, any resistant bacteria will have the greatest chance of survival. As a result, the antibiotic kills the susceptible bacteria, causing a selective pressure for the survival of resistant types of bacteria. Bacteria can acquire bacterial resistance through genetic mutation or by acquiring resistance from other bacterium. Furthermore, antibiotic resistance spreads through bacteria populations “horizontally”, when genetic material is shared with other bacteria, “vertically,” when subsequent generations inherit resistance.

A great body of research has been conducted to examine the mechanism of bacterial resistance and ways to combat bacteria through the use of antibiotics. The ability of bacteria to become resistant to multiple antibiotics has posed a serious health threat due to increase use of broad-spectrum antibiotics. Research is commonly concerned with bacterial resistance to antibiotics that the bacterium has been exposed to. The researchers aim to study the resistance of bacteria to antibiotics similar to those it already exhibits a resistance to. The objective of this work was to investigate whether previous exposure to a first generation cephalosporin, cefaclor, would influence the reaction E. coli would have to the third generation cephalosporin, cefixime. Since all cephalosporins function in a similar matter the researchers hypothesized that E. coli exposed to cefaclor and then exposed to cefixime would exhibit more resistance than E. coli only exposed to cefixime.

Materials and Methods

The original Escherichia coli bacteria sample was obtained September 29, 2008 from Saddleback’s Department of Biological Sciences. The two original test tubes of E. coli were grown in nutrient broth incubated at 37.0º C for 48 hours.

Nutrient broths were made by mixing 8 grams of nutrient broth powder made by Citeron into 1 liter of deionized water in a 1 liter glass flask. The mixture was heated and stirred until thoroughly mixed. The prepared nutrient broth was then placed into glass test tubes. Each test tube was filled with 10 mL of nutrient broth from the 1 liter glass flask using micropipettes. The test tubes were loosely capped and placed into an autoclave, made by Consolidated, to be sterilized.

Nutrient agar plates were created by mixing 20 grams of agar nutrient from Citeron with 1 liter of deionized water in a 1 liter glass flask. The nutrient...
Three generations of *E. coli* on agar plates were grown. All the zones of inhibitions were recorded. The third generation of bacterial colonies agar plates was used to grow two sets of ten labeled nutrient broth test tubes. Using the same methods outlined above, two sets of ten agar plates were covered with *E. coli*. One set of ten was given continued exposure to the Cefalcor antibiotic disks while the other ten were exposed to 5 mcg of Cefixime antibiotic disks. These plates were grown into a second generation of agar plates.

As a control, two additional samples of *E. coli* were grown. One was the sample of base *E. coli*, used in the first sample, exposed to cefaclor. And the other was the base *E. coli* exposed to cefixime. Two sets of nine agar plates were given a lawn of *E. coli*. The first set of these agar plates was exposed to the Cefalcor antibiotic disks and the other nine were exposed to Cefixime disks. All zones of inhibition on each agar plate was measured and recorded into the laboratory notebook. Data was used in an Anova test with Bonferroni corrections. The difference in zones of inhibition between bacteria with both antibiotics and bacteria with just one antibiotic was not significant.

**Results**

The zone of inhibition of the *E. coli* exposed to cefaclor was measured once a week for five weeks. From week one to week two the zone of inhibition decreased from 1.04 cm to 0.82 cm. The zone of inhibition remained at 0.82 cm on week three, on week four the zone of inhibition decreased to 0.8 cm, and on week five the observed zone of inhibition had decreased to 0.69 cm. The remaining samples were initiated on week three and first measurements were taken on week four. The second *E. coli* sample exposed to Cefaclor demonstrated a decrease in the zone of inhibition from 0.99 cm to 0.84 cm. The *E. coli* exposed to cefixime remained relatively constant from week four to week five, with observed zones of inhibition of 0.67 cm and 0.68 cm respectively. The *E. coli* sample exposed to cefaclor and then to cefixime had an average zone of inhibition of 0.98 cm on week four and exhibited two zones of inhibition on week five with the larger zone at 0.62 cm and the smaller at 0.312 cm (Table 1, Figure 1).

The measurements from week five exhibited a final average zone of inhibition of 0.69 cm and a standard error of 0.020 for the *E. coli* exposed to cefaclor for the full test period. *E. coli* exposed to cefaclor at week three and for only for two generations exhibited a final average zone of inhibition of 0.84 cm with a standard error of 0.024. *E. coli* grown for two generations in the presence of...
Cefixime exhibited a final average zone of inhibition of 0.68 cm with a standard error of 0.022.

**Table 1.** The average zone of inhibition (cm) observed in E. coli samples exposed to cefaclor (n=10), cefaclor (n=9), cefixime (n=9), and a combination of the two antibiotics (n=10).

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**Figure 1.** The average zone of inhibition (cm) exhibited by E. coli samples exposed to cefaclor (n=10) over ten days. The average zone of inhibition (cm) exhibited by E. coli samples exposed to cefaclor (n=9), cefixime (n=9), and cefaclor and cefixime (n=10) from day eight to day ten. Measurements were obtained every two days for a total of ten days.

E. coli grown for three generations in the presence of cefaclor and then grown for two generations in the presence of cefixime exhibited two distinct zones around the antibiotic disk in the final generations. The average larger zone of inhibition was 0.62 cm with a standard error of 0.039 and the smaller zone of inhibition had an average of 0.31 with a standard error of 0.023 (Figure 2).

An ANOVA single factor analysis of the measurements from all five generations of E. coli exposed to cefaclor showed a degrees of freedom of 44 and a mean squared of 0.0083. A Bonferroni correction found that there was a statistical difference between generation one and generations two, three, four and five. A statistical difference was also found between generation five and generations two and three.

A t-test comparing the samples of E. coli exposed to cefixime showed no statistical difference between the zone of inhibition of the first and second generations (p = 0.38, one tail). Additionally, a t-test comparing the first and second generations of E. coli exposed to cefaclor and then cefixime showed that there was a statistical difference between the zones of inhibition (p = 8.09 x 10^{-8}, one tail). T-tests comparing the first generation of bacteria exposed to cefaclor and the first generation exposed to cefixime showed a statistical difference between the zones of inhibition (p = 1.74 x 10^{-7}, one tail). Additionally, a t-test comparing the second generation of bacteria exposed to cefaclor and the second generation exposed to cefixime also showed a statistical difference between the zones of inhibition (p = 5.67 x 10^{-5}, one tailed).
An ANOVA: single factor analysis of the first three generations exposed to cefaclor and the two generations of the bacteria previously exposed to cefaclor an then exposed to cefixime yielded a degrees of freedom of 52 and a means squared of 0.0099. The Bonferroni correction indicated that there was a statistical difference between the average zone of inhibition of the first generation bacteria grown in the presence of cefaclor and the second generation of bacteria that was exposed to both antibiotics (larger and smaller zones). There was also a statistical difference between the second generation grown only in the presence of cefaclor and the first and second generation of bacteria that was exposed to both antibiotics (larger and smaller zones). Additionally, there was a statistical difference between the third generation grown only in the presence of cefaclor and the second generation of bacteria that was exposed to both antibiotics (larger and smaller zones). No statistical difference was observed between the first generation of the cefaclor bacteria and the first generation of the bacteria exposed to cefaclor and then cefixime.

An ANOVA: single factor analysis comparing the first and second generations (larger and smaller) of the *E. coli* exposed to cefaclor and then cefixime yielded a degrees of freedom of 25 and a means squared of 0.00803. A Bonferroni correction test showed a significant difference between all three groups.

**Discussion**

Both samples of *E. coli* exposed only to cefaclor exhibited increased resistance as the average zone of inhibition decreased overall from the first to the last generations. This is consistent with current research, as bacteria have been shown to become resistant to an antibiotic through environmental pressures. The strain of *E. coli* exposed only to cefixime showed no significant decline in the zone of inhibition from the first to the second generation. Although this sample did not behave in a similar fashion to the cefaclor samples, it does not mean that the bacteria will not exhibit increased resistance. Cefixime is a 3rd generation Cephalosporin and is designed to be an improvement upon Cefaclor; thus it may take more generations for the bacteria to begin expressing significant resistance.

The sample exposed to cefaclor and then exposed to cefixime did not show any signs of increased resistance in the first generation exposed to cefixime. The researchers hypothesized that because the bacteria were allowed to build up a resistance to cefaclor that when exposed to cefixime it would have had a smaller zone of inhibition than bacteria exposed to cefixime alone. However, the sample exposed to both antibiotics had an average zone of inhibition of 0.98cm, which was similar to zones of inhibition from the first generations grown in the presence of cefaclor. Interestingly, when a second generation of the bacteria exposed to both antibiotics was grown the average zone of inhibition was drastically smaller, with a larger zone of 0.62cm and a smaller zone of 0.31cm.

Bacteria can become resistant to an antibiotic through environmental pressures. When a drug is introduced into a population those bacteria that posses a mutation that enables them to survive will be able to reproduce. The surviving bacteria then pass the resistance on to future bacteria, which creates a resistant strain. Antibacterial resistance, however, is not a straightforward process. Early research shows that antibiotics cause the selection of variants that already exist, not the creation of new mutants (Demerec 1948). However, it has also been observed that bacteria can become hypermutable through inactivation of the proofreading systems that normally correct DNA copying errors. Hypermutators have up to 200 times greater mutation rates than normal cells and are thus more likely to become resistant to a first antibiotic by mutation rather than selection. Once selected by the original antibiotic, they are then “primed” to develop resistance to future agents. Thus antibiotics may cause the creation of variants with a greater aptitude to develop further resistance (Bridges 2001). Current research has also shown that resistance to one antibiotic can affect a bacteria’s resistance to another. Mutants grown through selection by low concentrations of tetracycline or chloramphenicol were resistant not only to the agent of selection, but also to a number of structurally unrelated antibiotics (Cohen 1989).

Measurements and procedures were also subject to possible errors. Although sterile technique was observed during all of the procedures it is possible that some samples could have been compromised. Additionally, the times that each generation was allowed to stay in the incubator did vary. Lastly, all measurements of the zone of inhibition were done by the same experimenter to ensure consistency. However, the measurements were still subject to limitations in accuracy due to imprecise measurements.

Although our data did not demonstrate increased resistance in the first generation of the test bacteria, the second generation did show a smaller zone of inhibition than any of the control samples. The researchers believe that a disruption in the bacteria caused by the change of the antibiotic may be at fault for the poor resistance of the first generation after the new antibiotic had been administered. The bacteria
seem to be able to overcome this problem as the second generation improved greatly. Further research would need to be done to investigate the mechanism of the possible disruption caused during the change in antibiotics. Given the growing number of drug resistant antibiotics, information on increasingly resistant bacteria will be significant because it can have a dramatic impact on human health.

**Literature Cited**


Demerec M. Origin of Bacterial Resistance to Antibiotics. *Journal of Bacteriology* 1948; 63-74


ABSTRACTS

Biology 3A students complete and present posters in Fall and Spring of each year.

Fall 2008 posters were presented on 11 December 2008
Spring 2009 Posters were presented on 14 May 2009

Included here are the abstracts of those presentations
1. THE EFFECT OF CHEWING THINK GUM® OVER TIME IN MEMORY RETENTION IN HUMANS (*HOMO SAPIENS*). Nicole Barrett, Shama Bukhari, and Sarah Diamsay. *Department of Biological Science*. Saddleback College Mission Viejo, CA 92692

Many students chew gum while taking quizzes and final exams due to the belief that it enhances aspects of mental performance, including memory retention. However, no experimental evidence exists to support this argument. In this experiment, eighteen healthy individuals (mean age of 21.1 years) were selected and distributed equally into three groups, with each group having three males and three females: Group 1 was the control group that did not receive treatment; Group 2 was the gum control group that received the Wrigley’s Juicy Fruit Gum® treatment; Group 3 was the gum experimental group that received the Think Gum® treatment. Memory retention was measured by the correct answers each participant obtained upon completing two word recall tests; test 1 was conducted before the treatment, while test 2 followed five days after the treatment. The performance of each group was based upon its mean scores for Test 1 and Test 2 and the p-values from a paired left-tailed t-test, where $\alpha = 0.05$ (Group 1: 14.5, 15.8, $p= 0.00073$; Group 2: 14.7, 14.5, $p= 0.31$; Group 3: 14.3, 11, $p= 0.0021$). The p-values indicate that the Test 2 mean was significantly higher than the Test 1 mean for both Group 1 only, but not for Group 2 nor Group 3. There was a significant difference between the groups ($p= 0.00129$, ANOVA single-factor) for Test 2. Post-hoc analysis and the Bonferroni correction revealed that this significant difference occurs between Group 1 and Group 3 ($p= 0.003117$, ANOVA single-factor). Based on this, the scores in Group 1 were significantly higher than the scores for Group 3.

2. EFFECT OF MIRACLE-GRO LIQUAFEED ON YELLOW SNAPDRAGONS. Daryn Hill and Sable Tillery. Dept. of Computer Science and Business, Saddleback College, Mission Viejo, Ca 92692, United States of America.

Plant growth can depend on many different influences, included water, nutrients, sunlight and the conditions in which they are grown. Many plants only are only given water while some receive extra nutrients either naturally or artificially through store bought nutrients. Levels of growth can be measured to determine which method is the most effective increasing the size of the plants. By adding additional nutrients to plants regular diet their growth should be increased. The plants only receiving water as predicted showed an smaller average of growth in the duration of the experiment, while the plants which also received Mircle-Gro Liquafeed sized increased more rapidly.
3. COMPARISON OF RED AND BLUE LIGHT PROMOTION OF PLANT GROWTH IN ANTIRRHINUM. Hirad Azar, Tiana Dorneman and Sean Kouyoumdjian. Department of Biological Science Saddleback College Mission Viejo, CA 92692

Red light has been found to produce more plant growth than other wavelengths of light in snapdragons (Antirrhinum). These results suggest that certain wavelengths of light inhibit plant growth while others promote more efficient plant growth. A set of two dozen snapdragons plants were exposed to two different shades of light, red and blue. Each was introduced to equal circumstances of light, water, and other weather effects. Snapdragons were boxed in colored cellophane with equal opacities. The amount of light reaching the Snapdragons in either color cellophane was determined using a light meter. Measurements taken were from the base of the plant where the stem meets the soil, to the tip of the plant. These positions were marked incase packing of soil occurred. Within the duration of four weeks, snapdragons exposed to red wavelengths of light were not shown to have undergone more growth in comparison to blue wavelengths of light. Red light exposed plants grew an average of 4.6 ± 2.5 cm compared to 3.7 ± 4.0cm for blue light exposed plants, with a p value of 0.12. Therefore our tests did not verify that in comparison to blue light, red is the predominant wavelength for growth.

4. THE EFFECTS OF CAFFEINATED AND DECAFFEINATED COFFEE ON BLOOD GLUCOSE LEVELS IN NON-DIABETIC SUBJECTS. Tim Schang, Janet Cannon, Maile Mattingly, and Julianne Nelson. Department of Biological Science Saddleback College, Mission Viejo, CA, 92691

Coffee is a widely consumed beverage that can dramatically affect blood glucose levels. The caffeine in coffee increases blood sugar levels while other agents in coffee decrease blood sugar levels. In this experiment, the rise and fall of blood glucose levels were measured in eight test subjects after the consumption of caffeinated coffee with sugar, decaffeinated coffee with sugar and water with sugar. On the first day, blood glucose levels were taken from routinely coffee-consuming subjects that had fasted for twelve hours. Blood glucose levels were taken 20, 40, and 60 minutes after the subjects drank two 12 oz cups of sugared caffeinated coffee (two packets approximating five grams of sugar per cup and approximately 250 mg of caffeine per a cup). Average blood glucose levels increased a significant 23.5 mg/dL (± se, N= 4.7) above the baseline after 20 minutes and did not begin to decrease until blood glucose levels were taken at 60 minutes. At 60 minutes post consumption the blood glucose levels were still elevated to 16.25 mg/dL (± se, N= 6.1) above the baseline level. When the same procedure was replicated with decaffeinated coffee with sugar on the second day of testing, average blood glucose levels only increased 9.25 mg/dL (± se, N= 3.4) above baseline after 20 minutes, and after 40 minutes the average blood glucose levels had fallen by 5.4 mg/dL (± se, N= 3.4) below baseline. The blood glucose levels after sixty minutes continued to fall by 7.5 mg/dL (± se, N= 3.5) below baseline. When the control for the study was done for the study with water with sugar the blood glucose levels rose by 25.5 mg/dL (± se, N= 4.2) above baseline after the initial 20 minutes. After 40 minutes the average blood glucose was still elevated by 12.75 mg/dL (± se, N= 7.6) above baseline. Finally after sixty minutes the average blood glucose level had dropped by 4.38 mg/dL (± se, N= 4.7) below baseline. These results reject the null hypothesis since significant differences were found between the three groups.
5. THE EFFECTS OF CAFFEINE ON MEMORY. Heather Leimkuhler, Ary Nassiri and Ardalon Parto. Department of Biological Sciences, Saddleback College, Mission Viejo, CA 92692

In order to enhance academic performance, caffeine is commonly ingested in the form of energy drinks and coffee throughout college campuses. This study examined if a high dose of caffeine would have a positive effect on the short-term memory of humans by measuring 15 Saddleback College male and female students between the ages of 18 and 23. The subjects were measured 3 times before and after ingesting a 200 mg caffeine pill. The subjects’ memories were tested by reciting 15 words, chosen based on their frequency of use in every day language. The words were all 1 to 3 syllable concrete nouns, given to all subjects. No word was repeated for each individual. The subjects were then asked to immediately write the words they were able to recall on paper with no time restrictions. The recall abilities before ingesting the caffeine pill were significantly greater than after ingesting the pill. Before caffeine, subjects were able to recall an average of 8.8 words, and 7.0 words after (p=2.77 x 10^{-6}, one-tailed paired t-test). The significant decrease in memory recall performance rebuts the assumption that caffeine enhances academic performance.

6. EFFECTS OF OCEAN TIDES ON BACTERIA CONCENTRATIONS - COMPARING THE EFFECTS OF HIGH AND LOW TIDES ON BACTERIA CONCENTRATION USING MULTI-TUBE FERMENTATION. Will Fraker, Laura Powell, and Amber Smuts. Department of Biological Science. Saddleback College. Mission Viejo, CA 92692

It was the purpose of this experiment to show that high ocean tides can increase the amount of bacteria concentration in the water. In this experiment, bacteria samples were taken from the ocean water during specific high and low tides. After samples of various high and low tides were collected, a multi-tube fermentation analysis of the samples was taken. Bacteria concentrations at high tide were recorded at 1104 ± 354.2 (± se, N=5), similarly bacteria concentrations at low tide were recorded at 534.4 ± 466.5 (± se, N=5). These results indicate that our initial hypothesis was rejected. Bacteria concentrations at high tide were not found to be significantly different from that of the low tide concentrations (p=0.3632). Further analysis revealed that variables, which were out of our control, could have caused our null hypothesis.

7. THE EFFECTS OF SALINITY ON OXYGEN PRODUCTION IN PICKLEWEED (Sarcocornia pacifica). Kaitlin B. Beck and Vanessa N. Haber. Environmental Studies, Department of Biological Sciences, Saddleback College, Mission Viejo, CA 92692.

Marsh plants are known to be extremely tolerant of different salinities, and they provide much of the methane that contributes to the greenhouse gases. Yet, they are not very well known for contributing to a decrease in carbon dioxide or an increase in oxygen. Salinity majorly affects these photosynthetic processes of marsh plants, including pickleweed, and it was predicted that in a lower salinity, pickleweed would produce significantly more oxygen than pickleweed in a higher salinity. Pickleweed samples, taken from the Newport Back Bay, were placed into a higher salinity environment and a lower salinity environment, in order to assess this. The pickleweed samples that were placed into the lower salinity cups (5.475 ± 0.091 mg/L/g, mean ± s.e.) had significantly greater dissolved oxygen production than samples in the higher salinity (5.102 ± 0.179 mg/L/g, mean ± s.e.), with a p-value of 0.0371 for a two-tailed unpaired t-test. Due to this result, it is suspected that if the marsh areas continue to decrease in salinity due to the melting ice caps, it is possible that the marshland plants are a negative response-system used to combat the excess greenhouse gases.
8. THE EFFECT OF THE FITNESS DRINK GATORADE VS. WATER ON A PERSON AFTER EXERCISE IN RELATION TO DEHYDRATION. Stephen Gumpert, Jenny Politte and Alex Tran. Department of Biological Science. Saddleback College. Mission Viejo, CA 92692

Dehydration is one of the many problems that people exercising project. Every day, sports drink corporations throw out commercials on how their product will help prevent dehydration better than water during long term exercise, but what about short term? In this experiment, we wanted to see the effect of pre-hydrating a person before running on a treadmill to see whether water or the sports drink Gatorade is a better source of hydration during short term exercise. Eight randomly selected students were asked to take a resting heart rate, run on a treadmill for a quarter of a mile while pre-hydrating themselves with water, Gatorade, and nothing, then taking their average final heart rate. Each test was done three times. Our hypothesis disproved, as there is no significant difference in the amount of hydration between Gatorade and water during short term exercise.

9. SEX DETERMINATION OF RED-SHOULDERED HAWKS (BUTEO LINEATUS ELEGANS) IN SOUTHERN CALIFORNIA USING WINGSPREAD MEASUREMENTS AND PCR DNA ANALYSIS. Scott Thomas, Sarai Finks, and Kazuhiro Sabet. Department of Biological Sciences. Saddleback College, Mission Viejo, California 92692

Identification of sex is important for raptor studies, especially when performing mark and re-capture studies, but for some species including red-shouldered hawks (Buteo lineatus), it can be difficult to accurately determine in the field. Like many raptors red-shouldered hawks are sexually dimorphic in size, but there is no difference between sexes in plumage. Researchers in southern California often use wingspread measurements to assign sex to this species, but there has been little genetic work done to confirm this method. Field observations of paired red-shouldered hawks suggest that those with wingspans less than 100 cm are male and greater than 100 cm are female. Our goal was to determine if wingspread measurements are an accurate determination of sex. We gathered blood and feather samples in Orange, Riverside, and San Diego Counties, California and recorded morphological measurements including wingspread. Collected samples were sent to Zoogen Incorporated, Davis, CA, for polymerase chain reaction (PCR) DNA analysis to determine sex. Our predictions based on wingspan were 95.2% accurate. Using a single measurement can be useful in the field for assigning sex to color marked birds. We also observed a significant difference between males and females in wing chord measurements that could be considered when determining sex in red-shouldered hawks.

10. THE EFFECT OF HYDROGEN PEROXIDE (3% SOLUTION) AND ISOPROPYL (70% SOLUTION) ON Staphylococcus aureus. Jordan Meek and Dolly Rojo Department of Biological Science, Saddleback College, Mission Viejo, CA 92692, USA

Over-the-counter antiseptics are commonly purchased to disinfect wounds and prevent bacterial growth. Isopropyl (3% solution) and Hydrogen Peroxide (70% solution) are amongst these popular antiseptics. This experiment sought to label one of these two anti-bacterial solutions as more effective than the other in inhibiting bacterial growth. Staphylococcus aureus --common bacteria among our human everyday lives-- was spread, and soaked discs of either Isopropyl or Hydrogen Peroxide were placed on this bacteria; it was then allowed to incubate for forty-eight hours at 37°C. Results showed that inhibition zones were larger for Isopropyl than Hydrogen Peroxide (p=0.03, ANOVA), meaning that, on average, Isopropyl was able to combat a greater amount of S. aureus than Hydrogen Peroxide did.

Caffeine is known to increase energy and alertness, but how it affects memory and cognition are not well known. We tested the effect of caffeine on the cognition of house mice (Mus musculus) by timing mice running through a maze. In previous studies mice cognition has shown improvement after consuming caffeine. The experimental group was administered 4800 µg of caffeine which is about 0.0003% of their average body weight, which is equivalent to a cup of coffee to a 150 lb human. The control group received a placebo of water, both given 30 minutes before trials. The groups ran nine trials through the maze and their times recorded. The data collected showed an improvement in maze trial times for the experimental group, with an average of 31.0 ± 3.8 seconds (± SE) versus the control’s average of 42.9 ± 3.1 seconds (± SE). There was a significant difference in trials times between groups, $P = 0.02$, supporting the hypothesis that caffeine improved cognition in mice. The maze was designed to give the mice decision points on which to turn towards a dead end or to the next decision point. So, the rate at which the mice improved in their training times shows quicker decision making and better memory retention of the maze layout. However, both groups were about equal in their consistency of correct decision making, possibly indicating that the experimental group was just moving faster due to higher energy. Thus, indicating that this hypothesis needs further study to fully accept the idea that caffeine improves memory capacity and cognition.

12. THE EFFECT OF MUSIC ON SUNFLOWERS (Helianthus Annus). Felissa Silva and Sherwin Jenabian. Department of Biological Sciences, Saddleback College, Mission Viejo, California, 92656, USA.

Music has been shown to dramatically affect the physiological ecology of Helianthus Annus. Plants that have had an exposure to long periods of music have been shown to grow faster than those that have not had any exposure to music. The experiment was housed in two separate wooden containers, each having its own separate light source and ventilation. The experimental group was exposed to a constant source of music during the entire course of the experiment. The rate of growth for plants that were not exposed to music has been shown to be very consistent, but when comparing them to plants that have been continually exposed to music, a significant increase in both height and weight can be noticed. The germination rates for both groups were similar, but once placed in separate containers, the group that was exposed to music began to show a much faster growth rate. The control group showed an average height of 21.33 cm $P(T<=t) \pm 0.1909$ and an average weight of 1.24g $P(T<=t) \pm 0.0537$, while the experimental group presented an average height of 27.76 cm $P(T<=t) \pm 0.1909$ and an average weight of 2.22g $P(T<=t) \pm 0.0537$. These differences support the hypothesis and reveal that music does have a positive impact on the growth of Helianthus Annus.

13. GROWTH RATE OF A PYROGEN (Escherichia coli) AT VARYING TEMPERATURES. Kristin B. Fiore, Daryl E. Fernandez, and Caitlyn L. Topper. Dept. of Biology, Saddleback College, Mission Viejo, CA, United States 92692

Invading pyrogens can indirectly reset the hypothalamic set-point temperature via the release of interleukin-1 from phagocytic cells. The deviation from normal body temperature can cause the growth rate of the invading pyrogens to vary. The hypothesis being tested is whether it would be advantageous for a host to generate a fever in its defense against gram-negative bacteria, which initiate an infection. Escherichia coli, a potential pyrogen, was cultured at two different temperatures (37°C and 41°C), to represent the deviation from normal hypothalamic body temperature. The mean number of colonies observed on 10 nutrient agar plates incubated at 41°C was 75.6 independent colonies which was greater than the mean number of colonies observed at 37°C, which was 31.7 independent colonies. ($N=30$, one-tailed unpaired t-test, $p=0.02$) Contrary to our hypothesis, the optimal-growth temperature at which E. coli reproduce is within the range of the temperatures that correlates to the time-course of a fever in the human body.
14. THE EFFECT OF SIMULATED ACIDIC RAIN ON GROWTH AND DEVELOPMENT IN SWISS CHARD CULTIVARS (Beta vulgaris). Anthony J. Avanzino, Lindsay M. Duran, Francesca T. Phan, and Michelle R. Caley. Dept. of Biological Sciences, Saddleback College, Mission Viejo, CA 92675, US.

Acid rain was simulated on two groups of Swiss Chard in a temperature and humidity controlled environment to reduce stimulation from outside variables. Plants were allowed to germinate over a period of nine days before exposure to 3.5 pH and 5.6 pH solutions. When final minus initial measurements were taken, the results showed little to no differences between the two; with the more neutral group, 22.37 ±1.45 mm, yielding slightly better mean growth than that of the more acidic, 22.30 ±1.37 mm, (p = 0.486; one-tailed, unpaired T-test). Observations on the internal health of each group provided insight into the deterioration and molecular instability in a low pH environment. Chlorophyll extractions yielded greater mean mg/L values in less acidic conditions: a difference of 0.42 mg/L. Foliar responses to pH on a morphological and macroscopic level showed no evidence of injury and, predicted results of suppressed growth and potential death, were inconsistent with those obtained. Under standardized circumstances, overall success of each plant was comparable and fairly uniform.

15. THE EFFECT OF NITRIC ACID CONCENTRATION ON GERMINATION OF SOYBEANS (Glycine max). Bishen S. Bedi, Amir P. Bahadori, and Abhinay V. Appannagari. Department of Biological Sciences Saddleback College, Mission Viejo 92692. The correlation between soybeans (Glycine max) and nitric acid can be used for an early prediction in the germination of soybean seeds. The experiment was conducted at Saddleback College Biological Sciences greenhouse. Due to the low environmental impact: temperature, pressure and humidity were controlled in the greenhouse which limited external influences on the results. Soybean seeds (n=120/tray) were germinated in a sand tray placed in grid-like fashion. Each tray was separated based on volumetric concentration of 1M nitric acid (30.0 mL, 10.0 mL, and 1.0 mL) and the last tray served as control. Based on the results obtained, 1M nitric acid did not affect the number of soybeans that germinated in each of the three experimental groups in comparison to the control group. In the control group 108 seeds germinated, and in comparison to the experimental groups, 108 seeds germinated in the 30.0 mL group, 111 seeds germinated in the 10.0 mL group and 111 seeds germinated in the 1.0 mL group. These results imply that the nitric acid component in acid rain alone has limited impact on the germination of soybean seeds.
16. THE PERCENT DIFFERENCE IN LACTATE PRODUCTION BETWEEN MALE AND FEMALE ROWERS. Jennifer Doncost, Elisha Fabrycky and Katie Hernandez. Department of Biological Sciences, Saddleback College, Mission Viejo, CA 92692, USA

During strenuous activity when not enough oxygen is present, the body produces lactic acid after glycolysis. The lactic acid is converted into lactate and delivered into the bloodstream, where the liver reverts it back to pyruvate in order to form ATP through cellular respiration. It was predicted that the percent difference of lactate in the blood preceding intense exercise and then also after intense exercise would be lower in males than in females. To determine this, blood lactate samples were collected with Lactate Scouts from male and female rowers after a brief warm up exercise and after a 2000 meter row on rowing ergometers at the UCI Boathouse. Rowers, prominently from the University of California, Irvine Rowing Team, were chosen due to the specific aerobic and anaerobic training exercises they underwent to complete the distances in competitive races. The collegiate-level training allowed them to perform the assigned distance for the study as a definite accumulation of lactate would be apparent. Males were expected to have a lower percent difference in lactate levels due to their physiological and physical enhancing differences compared to females, such as a larger lung capacity encouraging cellular respiration. Males (751 ± 29%, ±SE, n=10) had a higher mean difference than females (572 ± 63%, ±SE, n=10) supported by an unpaired t-test (p = 0.0094). The results caused the rejection of the hypothesis that men have a lower percent difference of lactate buildup. Instead, the numbers indicate that males have a greater percent difference than females. Multiple explanations contribute to why the results rejected the hypothesis including variation into physiological make-up of test subjects.

17. EFFECTS OF HERBICIDE WITH ACTIVE INGREDIENT GLYPHOSATE ON MACROCYSTIS PYRIFERA. Heather Jackson & Michelle Dehghanian. Department of Biological Science, Saddleback College, Mission Viejo, California, 92692.

The purpose of this study was to investigate the effects of accidental runoff of herbicide Roundup® from domestic homes into storm drains leading to oceans and the consequences of that runoff on marine algae found in shallow coastal waters. The objective of the study is to determine the inadvertent effects of the herbicide glyphosate, found in Roundup products, have a significant impact on chlorophyll production in M.pyrifera. Giant kelp, Macrocystis pyrifera, samples were collected off the coast of Dana Point, CA near tide pool areas where water does not mix as frequent. Each sample was tested for a baseline chlorophyll level, control 4.19 mg/L and test group 4. mg/L, and placed into either a control (1L ocean solution) or experimental (1L ocean solution and 6 ml of herbicide) environment for 24 hours. After 24 hour the samples were tested again for chlorophyll levels. The results of the chlorophyll levels, control 4.46 mg/L and test group 3.63 mg/L. The amount of chlorophyll that went up in the test group was a significant difference. Glyphosate is an enzyme inhibitor that effects photosynthesis and breaks down the pigment antenna. By breaking down the pigment more chlorophyll was able to be extracted giving a higher concentration for chlorophyll even though photosynthesis has been inhibited.
18. THE EFFECT OF EXERCISE ON RATE OF CO2 PRODUCTION OF MALES AND FEMALES (*HOMO SAPIENS*). Tiffani Lopez, Nicole Mills, Cynthia Tran, and David Truong. Department of Biological Sciences, Saddleback College, Mission Viejo, CA, 92692.

Respiration rate is monitored in the human body by measuring CO2 levels in the blood. When exercising, the body produces more CO2 and the medulla, part of the brain that senses this change, increases the respiration rate. The body rids itself of CO2 because as a waste product this causes a change in pH affects the ability of enzymes to function properly. The goal of this project was to measure CO2 levels in exhaled air before and after physical exercise in males and females. The prediction was that females would have a greater difference in CO2 output during exercise than males. The experiment then showed the average difference in concentration of CO2 production of males was 0.01193 ± 0.002392 mol CO2/kg/L (±se, N = 10). We then used this to determine the average male metabolic rate 0.0001443 ± 3.5 x 10⁻⁵ mol CO2/kg/L (±se, N = 10). The average difference in concentration of CO2 production of females in this study was 0.001124 ± 0.001649 mol CO2/L/kg (±se, N = 10). We then used this to determine the average female metabolic rate which was 0.0001981 ± 3.8 x 10⁻⁵ molCO2/kg/L (±se, N = 10). A one-tailed unpaired t-test revealed that the mean combined rate of CO2 production of males was significantly lower than the mean combined rate of CO2 production of females (p=0.1566). The rate at which CO2 is produced and released is directly related to the amount of energy being used by the males/females as well as the amount of O2 available to the cells doing the work.


The population of *Bufo boreas* within Laguna Coast Wilderness and Modjeska Canyon should be clinically and chemically healthy. In the experiment multiple variables were studied within a population of *Bufo boreas, Rana catasbania* and *Hyla regilla*. The environmental variables that were tested included temperatures, altitudes, and times of collection. The biological variables that were tested included sex and size. Blood samples were also taken of *Bufo boreas* and *Rana catasbania*. In order to determine chemical health, a CBC/Chem panel was ran on blood samples and were analyzed and compared to average parameters indicated by the International Species Information System (ISIS), consisting of: Albumin with a mean of 2.0 g/dl, Total proteins with a mean of 4.0 g/dl, Globulin with a mean of 2.0 g/dl, Uric Acid with a total mean of 0.2 mg/dl, White Blood Cell count with a mean of 3150, Hematocrit with a mean of 28.3%, Heterophils with a mean of 431/μL, Lymphocytes with a mean of 3617/μL, and Monocytes with a mean of 309/μL. Supporting the hypothesis, the toads collected were generally healthy with statistics of: Albumin with a mean of 1.39 g/dl, Total Protein with a mean of 3.13 g/dl, Globulin with a mean of 1.74 g/dl Uric Acid with a mean of zero mg/dl, White Blood Cell count with a mean of 4680, Hematocrit with a mean of 32.33%, Heterophils with a mean of 284.07/μL, Lymphocytes with a mean of 3553.47/μL, and Monocytes with a mean of 68.27/μL. The observed populations of the 24 toads were found to be within the general parameters of the ISIS data tables. Out of our population one toad was clinically abnormal with a swollen right hand with deformed digits and one toad was chemically abnormal with an increased level of liver enzymes. Through extensive blood testing and various professional opinions of both physical and chemical health of the specimen, the population of *Bufo boreas* that was studied was found to be healthy. The health of *Bufo boreas* reflects the stability of the environment and its capacity for supporting healthy toad populations, suggesting a significant absence of pollutants and other environmental factors that affect parasite susceptibility.
20. THE EFFECT OF INSULATION ON METABOLIC RATE OF WHITE MICE: Paula Phan and Amir Zand. Department of Biological Science, Saddleback College, Mission Viejo, CA 92692, USA.

This study examined if insulation would have an effect on the metabolic rate of white mice between upper critical and ambient temperatures. It was expected that the metabolic rate of mice with insulation would be significantly greater than mice without insulation at different temperatures (32°C and 22°C). Using the GLX CO₂ probe, The CO₂ production of eight laboratory mice were measured three times. At 22°C, there was a significant difference between the metabolic rates of the shaven and unshaven mice (p = 0 one-tailed paired t-test). At 32°C, there was a significant difference between the metabolic rate of the unshaven and shaven mice (p = 4.33 x 10⁻²² one-tailed paired t-test). Based on this study, the metabolic rate of shaven mice in room temperature was considerably less than unshaven mice while metabolic rates of shave mice were much higher than unshaven mice at 32°C. The researchers have concluded from this study that insulation allows the organism to utilize its energy more efficiently by preventing excess heat loss causing the organism to increase metabolism in order to maintain optimum body temperature.

21. THE EFFECT OF NITROGEN BASED FERTILIZER RUNOFF ON MARINE ALGAL BLOOMS. Audrey Moilanen, Kyle Meade and Russell Copeland. Department of Biological Sciences. Saddleback College. Mission Viejo, CA 92692

Algal blooms have become and increased threat to oceanic ecosystems around the world. A striking example of an algal bloom is the dead zone where the Mississippi River feeds into the Gulf of Mexico. The accumulation of dead algae has depleted the ocean of oxygen killing the organisms in that area, and affecting the surrounding ecosystems. To understand the influence of commercial fertilizers on algal blooms in the ocean, we studied the effect of different concentrations of Miracle Grow ® fertilizer on ocean water, which we collected from the local Calafia State Beach in San Clemente, California. Algal growth was directly related to higher concentrations of the nitrogen based Miracle Grow ®, whereas the buckets that received strictly water, the low concentration received ½ tablespoons of fertilizer per quart of water, and the high concentration received 1 tablespoon of fertilizer per quart of water. We concluded that the high concentration of nitrogen-based fertilizers directly influenced the growth of algae in the buckets, demonstrating the cause and affect of algal blooms in our surrounding oceans.

Abstracts from the Spring 2009 meeting
14 May 2009

1. THE EFFECTS OF LIGHT COLOR ON CHLOROPLAST MOVEMENT IN ELODEA. Kimberly Schuch and Danielle Levanas. Department of Biological Sciences, Saddleback College, Mission Viejo, CA, 92692.

Chloroplasts are organelles found in plants that conduct photosynthesis. They do this by capturing light energy to conserve free energy in the form of ATP and reduce NADP to NADPH. Two stalks were placed in a 1000 mL beaker of deionized water and placed into a box covered with either blue or red cellophane for seven days with 24 hours of continual lighting. The average time for chloroplast movement for the plant exposed to blue light was 419.7 ± 89.57 μm/sec (± SEM, N=6). The average time for chloroplast movement for the plant exposed to red light was 817.7 ± 310.9 μm/sec (± SEM, N=6). Possible explanation for why chloroplasts exposed under red light moved slower could be due to the dense and crowded chloroplasts which inhibited its movement. Blue light can improve the development of chloroplasts, and red light can increase the accumulation of photosynthesis production.
2. THE MASS AND VOLUME OF TESTICLES IN RELATION TO THE BODY SIZE OF DOGS (*Canis familiaris*). Brennan Buchan, Sarah Irish, and Cole Querry. Department of Biological Sciences, Saddleback College, Mission Viejo, CA, 92692.

Generally, dogs with a larger body mass have a larger testicular mass, while dogs with smaller body mass have a smaller testicular mass. However, a significant statistical difference was predicted between the testicle mass and volume of small dogs (10 pounds and below) and large dogs (50 pounds or above) in relation to body size when body mass is factored out, leaving a mass specific measurement. Data were collected, using an analytical balance to measure mass and a 1000 mL graduated cylinder for volume immediately following the removal of the testicles at the Affordable Spay and Neuter Clinic. Results showed that larger dogs (62.30g) do have larger testicles (p=2.62 x 10^-5) than smaller dogs (4.97g). However, there was no significant difference (p=0.17, two-tailed unpaired t-test) in the mass specific testicular volume between small (0.75 mL/kg) and large dogs (1.02 mL/kg). There was also no statistical difference (p=0.54) in mass specific testicular mass of small (1.56 g/kg) and large dogs (1.75 g/kg). These results reflect that the relationship between body size and testicular size are relatively the same between small and large dogs.


Acid rain has been shown to negatively affect certain plant species by decreasing growth and germination rates, lowering photosynthetic rates, reducing chlorophyll content, and foliar damage. Therefore, we examined the effects of simulated acid rain on the Bean Bush plant (*Phaseolus vulgaris*) over a three and a half week period, including germination. We hypothesized that simulated acid rain will significantly reduce the germination and growth rates of *Phaseolus vulgaris*. *Phaseolus vulgaris* seeds were planted in thirty 500 mL cups with 40 grams of soil. Two 5-gallon water jugs, one with tap water of a pH of 7 (control), and the other with sulfuric acid at a pH of 4 (simulated acid rain). Fifteen of these seeds were watered with the control, while the remaining fifteen were watered with the simulated acid rain. At the conclusion of the study, the plants were removed and weighed, to determine the amount of growth. Our results indicate that the mean change in biomass of the control group (4.52±0.82g) was greater than that of the simulated acid rain group (3.31±0.71g), but there was no significant difference (p=0.278). The simulated acid rain was found to have little effect on growth and germination of *Phaseolus vulgaris*. Thus, we must ponder whether or not the acid fully enters the seed with the water to simulate the restrictive effects observed in many similar studies. Overall, we conclude that simulated acid rain may possibly affect *Phaseolus vulgaris* negatively in a long-term study, but it is clear that simulated acid rain does not significantly affect *Phaseolus vulgaris* in the short term.

4. THE EFFECT OF TAURINE ON BLOOD PRESSURE IN HUMANS (*Homo sapiens*). Zachary M. Drexler, Mohammad Dadkhah and Austin D. Miller. Department of Biological Sciences, Saddleback College, Mission Viejo, CA, 92692.

Taurine is an active ingredient in most energy drinks, which is known to increase blood pressure. However, taurine can also decrease blood pressure and hypertension. The goal of the experiment was to isolate the effects of taurine from the other ingredients found in energy drinks and determine how taurine isolates affects human blood pressure. The blood pressures of 20 Saddleback College students were obtained before and after the ingestion of water and taurine in separate trials. The difference in mean arterial pressure (MAP) after the consumption of water was 0.5±0.013 mmHg (±SEM) and the MAP after consumption of taurine was -5.86± 0.023 mmHg (±SEM). There was a significant difference (p=7.0x10^-5, two-tailed paired t-test) between the trial involving water and the trial involving taurine. The results suggest that taurine decreases MAP and maybe added to energy drinks to offset the effects of other ingredients contained in energy drinks.
5. THE PREFERRED SUCROSE CONCENTRATIONS OF NATIVE SOUTHERN CALIFORNIA HUMMINGBIRDS. Hunter Bestard, Frank Leon, and Ian Smith. Department of Biological Sciences, Saddleback College, Mission Viejo, CA 92692.

Hummingbirds play an influential role in the pollination and reproduction of plant life. In Southern California, hummingbirds thrive, feasting on the abundant supply of nectar. It has been determined in prior experiments that hummingbirds process sucrose faster than glucose or fructose. The purpose of this study was to determine whether hummingbirds will favor one concentration of sucrose solution over another. Four hummingbird feeders were used to hold four different sucrose concentrations (0%, 20%, 40% and 60%). After observing the feeders for 15 hours over the course of 5 days, it was found that the mean duration of visits was 2 seconds for the 0%, 11 seconds for the 20%, 17.96 seconds for the 40% and 24.95 seconds for the 60%. Additionally, the 60% solution received the most visits from hummingbirds because it contains a higher concentration of sucrose than the others, providing more sustenance for the hummingbirds and their demanding metabolism.

6. COMPARING LACTATE THRESHOLD IN PEOPLE WHO EXERCISE REGULARLY WITH PEOPLE WHO DO NOT. Amin Najmabadi, Irina Alexandrova and Lauren Hessling. Department of Biological Sciences, Saddleback College, Mission Viejo, CA, 92692.

Human muscle cells make lactate when oxygen is scarce. This occurs as ATP production outpaces the muscle’s supply of oxygen from blood and the cell switches to anaerobic respiration. The point where lactate starts to accumulate in the blood and exceeds the lactate metabolism rate of the body can be referred to as the lactate threshold. Lactate threshold should theoretically be higher in exercisers when compared to non-exercisers. The average change of lactate levels before and after exposure to 10 minutes of cycling exercise for the non-exercising group was $4.07 \pm 0.63 \text{mM} \cdot \text{L}^{-1}$ ($\pm$SEM, N=10). The average change of lactate levels before and after exposure to exercise for the exercising group was $4.77 \pm 0.97 \text{mM} \cdot \text{L}^{-1}$ ($\pm$SEM, N=10). The one-tailed unpaired t-test revealed no significant difference in lactate levels among the people who exercise when compared to people who do not (p=0.27). These results do not support the proposed hypothesis for this study as the individuals may not have gone anaerobic and maintained aerobic respiration.

7. THE COMPARATIVE EFFICIENCY OF STERILIZATION BY CONVENTIONAL HEATING AND MICROWAVE IRRADIATION ON \textit{E. \textit{coli}}. Brian Gee and Jonathan So. Department of Biological Sciences, Saddleback College, Mission Viejo, California 92692

The efficient sterilization of pathogens is of interest to many fields. One such method suggested because of its low-cost has been microwave irradiation. It is of interest to find out if this is more efficient than conventional heating (boiling). Inoculates of \textit{Escherichia coli} were subjected to conventional heating and microwave irradiation at 2450 MHz for 60s. Samples of the treated inoculates were then streak plated and allowed to incubate for 48 hrs after which colonies were counted, recorded and analyzed via $t$-test in Microsoft Excel. An average of 1.8 colonies/Petri dish were counted on irradiated samples compared to 4.4 colonies/Petri dish after boiling. The data indicate that microwave irradiation at 2,450 MHz was more efficient (p=0.034, one-tailed unpaired t-test) at decreasing viable \textit{E. coli} than conventional hot water bathing. Microwave radiation penetrates deeper into test tubes than boiling.
8. COMPARISON OF AEROBIC METABOLISM IN COLD WATER ADAPTED AND WARM WATER ADAPTED FISH SPECIES. Krystina M. Jarema. Department of Biological Sciences, Saddleback College, Mission Viejo, California, 92692.

The metabolic rate of fish should be optimum in the adaptive temperature of the fish. Testing the validity of this assumption, tilapia (Oreochromis niloticus; warm-adapted fish) was compared to salmon (Oncorhynchus nerka; cold-adapted fish) using succinic acid dehydrogenase as an indicator of aerobic metabolic activity. Each sample was chopped and homogenized with sodium phosphate solution. Ten mL of each homogenized sample was iced in labeled test tubes with three drops of methylene blue 0.5 mL of succinic acid and placed into a 37°C incubator. The faster the sample metabolized, the faster the methylene blue was reduced to a colorless form due to the oxidation reduction process. The time it took for half of the methylene blue to be oxidized from the sample was recorded. The average combined times for tilapia (48.2 ± 3.2 minutes) was significantly faster (p=0.00034, two-tailed unpaired t-test) than the mean times of the salmon (83.8 ± 4.4 min). Thus tilapia has a faster metabolic rate at 37°C due to the adaptive temperature of the fish.


Yogurt is considered a probiotic food due to its antibacterial properties. The bacteria responsible for these properties, Lactobacillus acidophilus, is the basis of our study. The in vitro role of L. acidophilus was investigated to explore the potential inhibition of Escherichia coli growth. Investigators are interested in testing the effects of the probiotic in yogurt (L. acidophilus) on E. coli which inhabits the intestines of human. Eighteen samples of MRS broth were inoculated with E. coli. Nine of these broths were left as the control group and the other nine were inoculated with L. acidophilus and incubated at 37°C for 48 hours. All eighteen samples were streak plated onto MRS agar and incubated. After 48 hours, the MRS agars were examined for any E. coli growth. The control group had a significant amount of E. coli growth while the experimental group had no growth at all. The results confirm that L. acidophilus does have inhibition properties explaining yogurt’s ability to decrease infections.

10. THE EFFECT OF CREATINE MONOHYDRATE ON WESTERN FENCE LIZARDS (Sceloporus occidentalis). Sean Parsa, Kristin Perkoski and Heeva Ghane. Department of Biological Sciences, Saddleback College, Mission Viejo, California, 92692

Creatine is a protein in the form of glycine and arginine. Glycine promotes muscle building and strength gain by slowing the process of muscle tissue breakdown. Arginine increases the body’s ability to produce lean muscle mass. The purpose of this study was to see what effects creatine monohydrate would have on the mass of western fence lizards. Researchers hypothesized that creatine monohydrate will increase the mass of western fence lizards. Sixteen lizards were caught at Saddleback College, and separated into two groups. Eight were the control group, which were fed regular crickets and the other eight were the experimental group, which was fed crickets covered with creatine monohydrate. After 11 days the control group increased in weight to .53±0.25g (±SEM, n=8) and the experimental group increased in weight to .09±0.22 (±SEM, n=8). These results show that the data did not support our hypothesis. One possible explanation for these results is that creatine monohydrate does not effect reptiles in the same way it effects humans.

Metabolic rate is the amount of oxygen dissolved in a given time divided by the weight of the organism tested. Metabolic rates in 10 different hermit crabs (*Pagurus hirsutiusculus*) in two different saltwater concentrations (32‰ and 38‰) were compared for this project. The proposed hypothesis was that the metabolic rate would be greater in the 38‰ saltwater than the control concentration (32‰). The researchers took two groups of ten hermit crabs and ran each of them in the two saltwater concentrations. For the first group each crab was placed in a 25mL beaker containing saltwater with a salt concentration of 32‰, and (Xplorer GLX probe) measured the amount of oxygen dissolved in the beaker, for ten minutes. Mass specific metabolic rates were determined by finding the slope of the dissolved oxygen divided by the weight of the crab. The procedure was repeated for the 38‰. The weight specific metabolic rate for the 32‰ group resulted to 0.261, while the weight specific metabolic rate for the 38‰ group resulted in 0.822. The 38‰ saltwater groups had a higher mass specific metabolic rate than the 32‰ group. A paired t-test was run, and the mass specific metabolic rates were compared. Due to an increase of salt in the saltwater concentration (6‰) there was a significant difference in the metabolic rates of the hermit crabs (P=0.869).

12. THE EFFECT OF FREQUENT BLOOD DONATION ON PHYSIOLOGICAL RESPONSE TO BLOOD LOSS. Stephanie Melton and Amanda Tarney. Department of Biological Science. Saddleback College. Mission Viejo, CA 92692

Several physiological compensation mechanisms occur during blood loss. A change in blood pressure is one such mechanism. A blood donor loses approximately ten percent of total blood volume when donating blood. Ten percent is a blood volume significant enough to stimulate compensation mechanisms. This study was conducted to test the hypothesis that there is a significant difference between the pre- and post-donation blood pressures in first-time donors, compared to frequent donors. The objective of the study was to evaluate if donors physiologically adapt to blood loss if blood loss occurred frequently. The study took pre- and post-blood donation blood pressures from ten frequent donors and ten first-time donors. Our results indicate that there is not a significant difference in the change in systolic and diastolic blood pressures pre- and post- donation in frequent donors compared to first-time donors. These results suggest that frequent donors do not adapt to blood loss.

13. THE CHANGES IN METABOLIC RATE OF GOLDFISH (*Carassius auratus*) IN AN ENVIRONMENT INVOLVING AQUATIC PLANTS AND NO AQUATIC PLANTS. Michelle Garcia and Jessica Garcia. Department of Biological Sciences, Saddleback College, Mission Viejo, California 92692

Plants and animals have always gone together in an environment. They need each other in order to perform respiration and create energy. In a small sample experiment including goldfish and aquatic plants the metabolic rates will be different than goldfish that live in a tank without plants compared to a tank abundant with plants. The metabolic rate for goldfish without plants was higher than that of the goldfish living in the plant environment, because the plants were competing for precious oxygen in the tank with vegetation. These differences are due to the fact that fish in the plant environment are conserve oxygen since there is less to consume, while the fish in the environment without plants had no outside force that needed them to conserve oxygen.
14. THE EFFECTS OF HUMAN ACTIVITY ON THE DENSITY OF *ANTHOPLEURA SOLA* POPULATIONS IN SOUTHERN CALIFORNIA. Sara Rose and Alrene Santome. Department of Biological Science. Saddleback College Mission Viejo, CA 92692

An ecosystem is characterized by the organisms and abiotic components that are a part of it. We measured and analyzed population densities of sunburst anemone (*Anthopleura sola*). Data were collected from four beaches throughout south Orange County, CA. The beaches studied included those with low human foot traffic as well as high human foot traffic. The measurements were taken from each of the three tidal zones. Our results suggest that the beaches with high human traffic had a significantly smaller number of sea anemones in comparison to beaches with low human traffic. The research data suggest that there is no statistical difference between the high tide zones of the beaches. This is significant because human foot traffic, on any beach, is highest in the high tide zone. This leads to the conclusion that factors besides human activity have an impact on the distribution of *Anthopleura sola*.

15. EFFECTS OF pH ON GOLDFISH (*Carassius auratus*) METABOLIC RATE. Andrew Hernandez, Kasra Sadjadi and Kristianne Salcines. Department of Biological Sciences. Saddleback College. Mission Viejo, California 92692

Environmental factors are expected to affect metabolic rate. In aquatic organisms, water pH levels may impact metabolic reactions. We hypothesize that adding hydrogen ions into a solution will increase the metabolic rate of the goldfish, *Carassius auratus*, because increased levels of carbon dioxide and decreased pH result in a decrease in hemoglobin's affinity for oxygen, thereby promoting oxygen release to the tissues. An increase in the rate of cellular respiration produces more carbon dioxide, therefore more hydrogen ions in solution. Metabolic rate was determined by the frequency of their opercular pumping rate. When exposed to high pH water, we found a significant increase in opercular rate when compared to fish exposed to neutral or basic pH, which was associated with the lowest level of metabolic rate. Our results thus demonstrate that pH significantly affects the metabolic rate of *Carassius auratus*. Our results have implications for predicting the consequences of environmental changes caused by pollution or acid rain in the metabolism of aquatic organisms.


In the citric acid cycle succinate is produced, because of this we were able to use succinic acid to catalyze the reaction rate so that we could measure the levels of SDH in the beef. The tests were conducted between 80% ground beef to 20% fat and 93% ground beef to 7% fat. Methylene blue was added as the color indicator and Succinic acid was used to help speed up the reaction time. The samples were incubated 37°C, to simulate normal human body temperature. It was found that the 7% fat ground beef sample had more enzymatic activity, fully changing color to its original 6 minutes sooner than the 20% fat content beef. The 20% fat content beef ended with a mean time of 45 minutes, while the 7% fat content beef ended with a mean time of 39 minutes (SEM ± 1 minute). Our hypothesis of the leaner meat having more enzymatic capacity was proven wrong, with no statistical difference between the two samples. (P=0.000202499 Two tailed-unpaired)
17. THE EFFECT OF PH ON CYANOBACTERIA ANABAENA’S GROWTH. Andrew Lazenby, Yaowen Chang, and Andrew Naillon. Department of Biological Science, Saddleback College, Mission Viejo, CA 92692.

Cyanobacteria’s ability to convert atmospheric nitrogen into a useable form for plants, such as ammonia, as well as convert CO₂ into O₂ has played an important role in the building of our earth’s atmosphere. *Cyanobacterium Anabaena* as a species has survived many of earth’s calamities, but this experiment will try to determine whether a change in pH will have any effect on their growth. Studies have shown that the ideal pH for *Anabaena* to grow is 7.5 and growth was limited when in a pH of 5 or less. To test this hypothesis, samples of *Anabaena* were placed in nutrient rich water at pH’s of 8 and 5, then were left to cultivate outdoors for a week. The initial count of *Anabaena* in the basic solution was 0.60 ± 0.221 per 0.009 ml (±SEM, N=10), whereas the acidic sample had 1.2 ± 0.389 per 0.009 ml (±SEM, N=10). After a week, the final count was 0.10 ± 0.0429 per 0.009 ml (±SEM, N=50) for the basic and 0.54 ± 0.115 per 0.009 ml (±SEM, N=50) for the acidic solution. The final counts of the basic solution were significantly lower than the acidic solution after a week of cultivation (p=2.64 x 10⁻⁴, one tailed unpaired t-test). The data does not support the original hypothesis and this could be attributed to the conditions of the water.

18. IS THERE A LINEAR INCREASE ON HEART RATE DUE TO THE CONSUMPTION OF A RED BULL ENERGY DRINK? Isaac Lam, Michael Barr and Stephanie Cruz. Department of Biological Sciences, Saddleback College, Mission Viejo, CA 92692

Increased heart rate may result from physiological responses to several environmental factors. Energy drinks are popular beverages with high contents of caffeine, sugar, and other substances that may tend to increase heart rate. Since sustained elevated heart rates may be associated with increased risk of cardiovascular disease, it is of general interest to understand the physiological effects of such beverages on individuals. Here, we study the pattern of changes in heart rate after consumption of an energy drink (Red Bull). We hypothesized that the human body would respond gradually to the presence of caffeine and we thus test whether Red Bull consumption produces a linear increase in heart rate within the first 30 minutes of consumption. Heart rates were taken from selected individuals for 30 minutes after initial consumption of 250ml of Red Bull at 10 minute intervals. Our results show that, while consuming Red Bull does increase your heart rate, such increase is not linear within the first 30 minutes after consumption.

19. FREQUENCY OF LACTOSE INTOLERANCE IN MIDDLE EASTERN AMERICANS AND ASIAN AMERICANS. Grace Naddour, Andres Mendoza, Ida Jelveh and Jay Cloyd. Department of Biological Sciences Saddleback College Mission Viejo, CA 92692

Lactase is an enzyme that breaks down lactose, a sugar found in milk. In this study, Asian Americans and Mediterranean Americans were asked if they were lactose intolerant, a condition that causes abdominal pain, diarrhea and cramps. There is a high frequency of lactose intolerance in Asians, but little is known about the frequency of lactose intolerance in Middle Easterners. Given the close geographic proximity between these two human populations, we hypothesized that the frequency of lactose intolerance would be similar between them. We tested this hypothesis by comparing the observed frequency of lactose intolerant individuals in samples of Asian Americans and Middle Eastern Americans with the expected frequencies based on previous studies on Asians. The observed frequency of lactose intolerant Asian Americans was significantly different from the expected frequency (X² = 3.968, df=1, p=0.0464). In Middle Eastern Americans the expected frequency was significantly opposed (X²=15.260, df= 1, p=0.0001).The study came to an astonishing conclusion when much less than expected people of Middle Eastern ethnicity were extremely tolerant to lactose. Although results for Asian Americans supported what we had expected from the opening of the paper.

Soil porosity, composition, and amount of organic matter are a few of the key factors that affect the growth of plants. We focused on these three variables to test plant growth of California poppies (*Eschscholzia californica*) in clay and sand. We hypothesized that sand would favor increased plant growth due to its porous texture, which would allow for easier absorption of nutrients. A soil sample of clay was taken from Hannah Ogren’s Backyard and a sample of sandy soil from Califia Beach, California to test the soil porosity and organic matter and to determine their effects on plant growth. Ten pots were filled with sand and ten pots with clay and California poppy seeds were planted in each pot. Our hypothesis was only partially supported, with the sandy soil producing quicker growth, but the plants surviving longer in clay.

21. THE EFFECT OF POTASSIUM NITRATE ON GROWTH RATE OF FRENCH MARIGOLD. Azariah Bezer, Daniel Chavez and Brandon Abernethy. Department of Biological Sciences. Saddleback College, Mission Viejo California 92692

An experiment was conducted to investigate the effects of the growth rate of French marigold (*Tagetes patula*) when continuously irrigated with water containing low concentrations of potassium nitrate. Irrigation took place over a 22 day period with growth rate observed and documented. The plants were grown in Burpee “Eco-Friendly Seed Starting” seeding tray kits, in a temperature controlled greenhouse (23 ± 5° Celsius). The geographic location was 33.63 latitude and -117.65 longitude. There were 25 seeding for both the control and experimental groups. The control group was irrigated with tap water supplied by the Mission Viejo City Water District, at the Saddleback College greenhouse. The same water was used for the experimental group, only containing 1% potassium nitrate by weight. After seeding, irrigation was immediately executed for the control and experimental groups. The data was then transferred to Microsoft Excel, version 2001, and an analysis conducted. It was revealed that the average lateral stem length grown for the control was 25 ± 1 mm (N=18) with the experimental group being 19 ±1 mm (N=5). The N value was less for the experimental vis-à-vis the control due to death. A two tailed t-test revealed that p=0.04. Because the p-value was less then 0.05, it was logical to conclude that irrigation with 1% potassium nitrate demonstrated a significant effect to the growth of *tagetes patula*. Also noted, potassium nitrate not only had ceased the growth process, but had also terminated 6 out of 11 of the individual species.

22. EFFECTS OF PH ON GOLDFISH (*CARASSIUS AURATUS*) METABOLIC RATE. Andrew Hernandez, Kasra Sadjadi and Kristianne Salcines. Department of Biological Sciences. Saddleback College. Mission Viejo, California 92692

Environmental factors are expected to affect metabolic rate. In aquatic organisms, water pH levels may impact metabolic reactions. We hypothesize that adding hydrogen ions into a solution will increase the metabolic rate of the goldfish, *Carassius auratus*, because chemical reactions would be required to reestablish the ionic balance in the organism’s blood (homeostasis). Metabolic rate was determined by the frequency of opercular pumping rate. When exposed to high pH water, we found a significant increase in opercular rate when compared to fish exposed to neutral or basic pH, which was associated with the lowest level of metabolic rate. Our results thus demonstrate that pH significantly affects the metabolic rate of *Carassius auratus*. Our results have implications for predicting the consequences of environmental changes caused by pollution or acid rain in the metabolism of aquatic organisms.

Lactate can accumulate in high levels during vigorous exercise. The purpose of this experiment was to measure the difference in blood lactate levels due to increasing exercise distance. Our experiment consisted of male swimmers (n=10) between the ages of 18 and 21 swimming sprint distances of 25 yards, 50 yards and 100 yards. The mean lactate for the 25 yard sprint was 0.004±0.004 mM/Kg. The mean for the 50 yard sprint was 0.05±0.01 mM/Kg, and the mean for 100 yard sprint was 0.05±0.01 mM/Kg. There was a difference in the lactate levels between the three distances (p=0.0002, ANOVA). The Bonferroni/Dunn comparison showed that there were differences between 25 yards vs. 50 yards, 25 yards vs. 100 yards, but not for the 50 yards vs. 100 yards. This suggests that there is a difference between increasing the distance and the amount of lactate builds up in the blood. This is because when swimming shorter distances a person may convert the lactate quickly enough so that it is not accumulating in their blood. Swimming a longer distance such as 100 yards would cause the person to have an excessive build-up of lactate in which they cannot convert quickly enough; therefore, resulting in higher blood lactate concentrations.

24. THE EFFECTS OF CAFFEINE CONSUMPTION ON HEART RATES OF INDIVIDUALS IN DIFFERENT AGE GROUPS. Rose Park, Derek Silvers and Laura Chan. Department of Biological Sciences, Saddleback College, Mission Viejo, CA 92692 USA.

In this study, we evaluated if caffeine consumption could be a contributing factor in developing cardiovascular disease. We tested the increase in heart rate from before consuming caffeine to after consuming caffeine in two different age groups of men. Our reasoning for this was that if there was a higher increase in heart rate for the 55 to 85 year olds than the 18 to 30 year olds, caffeine consumption could contribute to heart disease being more prevalent in elderly persons than younger persons. Our hypothesis was that there would be a significantly higher increase in heart rate among the older age group than in the younger age group. We tested our hypothesis by recording heart rates of 53 subjects, who belonged in one of our two age groups, before and after drinking coffee. The mean increase in heart rate for men between 18 and 30 years old was 1.84±2.16 beats per minute (±S.E.M., N=25), while the mean increase for the 55 to 85 year old group was 2.68±3.57 bpm (±S.E.M., N=28). The results supported the null hypothesis that there is no significant difference in heart rate increase between both age groups (p=0.30). If caffeine affected both groups similarly, we cannot conclude that caffeine consumption is a factor in developing heart disease in the elderly.

25. THE EFFECT OF THE COMBINATION OF SUGAR AND CAFFEINE IN ROCKSTAR ENERGY DRINK ON HUMAN (Homo sapien) SHORT TERM MEMMORY. Yousif Astarabadi, Gonzo Mosquera, and Aaron Bitare. Department of Biological Sciences, Saddleback College, Mission Viejo, CA 92692.

Energy drinks are becoming common and are being consumed by people everywhere. In this experiment, the short-term memory of a human was tested after ingestion of sugar water solution and Rockstar energy drink. The hypothesis that energy drinks give human memory a temporary boost was assessed by testing subjects with a short-term memory test; recording the amount of words they recalled in two time intervals under the influence of the two aforementioned pre-measured solutions. At the three minute interval, the average amount of words remembered for the Sugar Water and Rockstar was 12 ± 1 (±SEM, n=20) and 14 ± 1 (±SEM, n=20), respectively. A one tailed, paired t-test revealed that the words remembered with Rockstar is significantly greater than Sugar Water for three-minute and six-minute intervals (p=3.02x10⁻⁴ and p=8.11x10⁻⁶, respectively). At the six minute interval, the average amount of words remembered for the Sugar Water and Rockstar was 11 ± 1 (±SEM, n=20) and 14 ± 1 (±SEM, n=20), respectively. There was significant evidence to support the claim that combination of sugar and caffeine in Rockstar energy drink gave humans a short-term memory boost.
26. TRANSPIRATION AND BACTERIAL MOVEMENT IN PLANTS (*Apium graveolens*). Chelsea Roche and Zachary Hargreaves. Department of Biological Sciences, Saddleback College, Mission Viejo, CA, 92692.

Safeguarding vegetation from bacterial contamination is a vital issue when addressing public safety in regards to food consumption. The hypothesis being investigated predicts that through transpiration, pGLO bacteria should not travel up the xylem to the upper portion of *Apium graveolens*. Ten celery stalks were obtained and nine were placed into 250 mL beakers with pGLO transformed *E. coli* and one control was place in a solution of water and red food dye. The number of pGLO colonies were: 57.1 ± 8.7, 30.0 ± 5.1, 7.1 ± 2.5, and 0.8 ± 0.3 (Mean ± S.E.) at zero, two, four and six centimeter heights respectively. The frequency of pGLO bacteria at different stalk heights was statistically significant (p=8.9 x 10⁻⁹, two-tailed ANOVA) and post hoc testing, exhibited a statistical difference between 0 cm and all other heights; as well as statistical difference between 2 cm and 4 cm values. The frequency of bacteria reduced as celery stalk height increased because capillary action and negative forces were not strong enough to pull large amounts of bacteria in the time allotted, but the presence of small amounts indicate the bacteria was small enough to be transported in the xylem.

27. A COMPARISON OF *AMOEBA PROTEUS* MORTALITY IN DEIONIZED WATER AND A CONTACT LENS SOLUTION, OPTI-FREE. Jessica Ochoa, Jamie Kim, and Crystal Shum. Department of Biological Science. Saddleback College. Mission Viejo 92692

This paper investigates the efficiency of the contact lens solution, Opti-free in preventing contamination of amoebas. In this study *Amoeba proteus* were placed in deionized water and contact lens solution, Opti-free, for six hours to determine whether there would be significant difference amoeba mortality. Using a Nikon microscope, the number of live and dead amoebas was recorded. The results showed that there was no significant difference between the percentage of amoebas found dead in deionized water and the percentage of amoebas found dead in contact lens solution (P=.37, Two-tailed, unpaired T-test).

28. EFFECTS OF EXERCISE ON THE LACTATE LEVELS IN THE TROTING HORSE (*Equus caballus*). Anahita A. Ariarad and Allison S. Lindsay. Department of Biological Sciences, Saddleback College, Mission Viejo, CA, 92692.

During glycolysis, L-Lactate is produced from pyruvate via the enzyme lactate dehydrogenase (LDH) during normal metabolism and exercise. Due to increased recruitment of skeletal muscle during exercise, it was predicted that lactate levels in the horses would increase after a set duration of sustained trotting. To test this, lactate levels of seven horses during rest and after exercise were taken. Lactate levels after trotting (0.0006 ± 0.0002 mM/l/kg) were not statistically different (p=0.31, paired one-tailed test) from levels before trotting (0.0005 ± 5.9 x 10⁻¹⁰ mM/l/kg). This no difference in lactate was most likely due to the short duration of exercise which did not force lactate to accumulate in levels beyond the animals normal processing rate. Because of the short time of exercise, it is possible that horses were not pushed into an anaerobic state and were still processing lactate aerobically.
29. THE EFFECTS OF TEMPERATURE ON METABOLIC RATE OF CHICKEN (*Gallus gallus*). Trevor McDermott. Department of Biological Sciences, Saddleback College, Mission Viejo, CA, 92692.

Metabolism is the process by which energy and materials are transformed within an organism and exchanged between the organism and the environment; therefore, metabolic rate is the speed at which metabolism occurs or the rate at which organisms transform energy and materials. It was hypothesized that the metabolic rate of a baby chick would rise as temperature rose from ambient temperature (21.5°C) to 3.5°C higher (25°C). Four four-day old chicks were used in this experiment, and they were placed inside of a Fox Box by Sable Systems (gas analysis) in order to determine the rate of O₂ consumption. The average metabolic rates at room temperature (4.14±0.04, 5.64±0.06, 3.86±0.03, 4.90±0.03) were consistently lower than those at 25°C (2.37±0.03, 3.52±0.02, 2.47±0.03, 2.96±0.03). This is due to the fact that the chicks’ metabolic rate is at a minimum at a definite temperature of 35°C for baby chicks and increases with the lowering of temperature, as well as the increase of temperature above the minimum metabolic rate.

30. EFFECTS OF AQUEOUS CAFFEINE ON TOMATO PLANT GROWTH RATE. Beau Gentry, JD Walters, and Patrick Schafer. Department of Biological Sciences. Saddleback College, Mission Viejo, CA 92629

Plots of land where coffee and tea has been grown exhibit elevated caffeine levels in the soil, yet its effects on the growth rates of other agricultural plants remain largely unknown. We decided to test the effects of a dilute aqueous caffeine solution on the growth rate of hybrid tomato plants. Two sets of tomato plants were grown in a greenhouse in Dana point, CA, with one group being watered by a dilute solution of caffeine. The mean stem growth rate for the control group was 5.51 cm and the mean stem growth length of the experimental group was 7.2 cm. Plants grown with caffeine were not taller than plants grown without caffeine (p = 0.053, one tailed unpaired t-test). This ran contrary to our hypothesis that caffeine would reduce the growth rate of the plants. However, our results do show a small difference in the stem growth of the two groups, and if our experiment was extended it is possible that a statistically significant difference might have been observed.