Monitoring Run-off Toxicity Using *Daphnia Magna*

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Abstract
This project investigates the toxicity of sidewalks and parking lots through a bioassay using a living organism. The *Daphnia magna*, a common water flea, is used to measure the toxicity of sidewalks and parking lots. Their heart rate and deaths are measured to determine the impact our infrastructure has on the *D. magna*. Since the *D. magna* are primary consumers, a large portion of the aquatic ecosystem in local ponds and streams is dependent on these organisms for food. In the experiment the organisms were separated into three different groups: control, simulated sidewalk run-off water, and simulated parking lot run-off water. Each of the three groups had an initial amount of 20 *D. magna*, and a total of three trials were conducted each lasting 72 hours. The heart rates and deaths were measured every 12 hours. Although the overall result for the number of *D. magna* alive in each cup for the experiment proved insignificant, the individual trials provided statistically significant results on the effect of the sidewalk and parking lots on the organisms.

Keywords
Bioassay, *Daphnia magna*, Pollution, Water Toxicity

1. Introduction
As our society further develops its infrastructure, pollution is becoming abundant affecting countless parts of our lives and the Earth's ecosystem. Our infrastructure has numerous sidewalks, streets, and parking lots that transport many harmful chemicals and plastics from one place to another. Nearby wildlife, including ponds, can be heavily impacted by the run-off waste on our roads, so how toxic is the run-off waste to the local pond life, and what part of our infrastructure is the most toxic to pond life? In this project, the live organism used is the *Daphnia magna*, a water flea often used to measure toxicity levels of different water solutions in bioassays. These creatures are common primary consumers in ponds, and therefore, they contribute to a sizable part of a pond's ecosystem. Past research on this topic includes using *D. magna* to test for chemicals in wastewater treatments (Tyagi et al 2007). The overall result of the experiment was that only the 100% use of treatment for wastewaters removed any toxicity. This experiment examines the effect of not all wastewater, but just run-off water, without treatment through the use of *D. magna.* Additionally, different concentrations of sediments are not used in the simulated run-off waters in this project.

2. *Daphnia magna* Observations
Some major steps need to be completed before experimentation. First, the experimenter must grow and cultivate a sustainable *D. magna* culture. Then, they must collect sediments from a local sidewalk and parking lot. Next, is the set-up of the groups to get ready for experimentation. Lastly, the experimenter can begin observing the *D. magna*.

In this experiment the variables under observation are the number of *D. magna* alive in the experiment and the heart rate of the *D. magna*. The number of *D. magna* are measured every 12 hours, as well as the heart rate over a 72 hour period. Three separate trials are conducted.

2.1 Project Research
Daphnia are small planktonic crustaceans ranging from 1.5 to 5 millimeters in size as adults. These organisms are easily influenced by slight changes in the water. Since they are transparent, their organs are visible under a microscope. Furthermore, *daphnia* are inexpensive and populate quickly due to their short lifespans. *Daphnia* are also essential to the aquatic biomes they are in because they are primary consumers that consume yeast and bacteria. There are different types of *daphnia*, but since *Daphnia magna* are larger compared to the other species in the

daphnia genus, they are easier to inspect in this experiment. Thus, *D. magna* are the optimal choice in a bioassay concerning local pondlife (Ebert 1970).

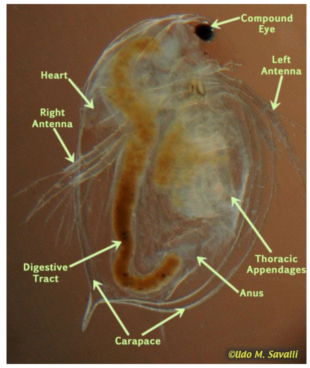

Figure 1. *Daphnia magna*

Run-off water is a less acknowledged, yet major source of water pollution. Run-off water runs across the sidewalks and streets while shifting litter, petroleum, fertilizers, and other toxic substances (National Geographic Society 2012). The sidewalks have small, fine particles that are carried into local ponds and streams by rainwater, according to the Chesapeake Bay Program. When there are too many of these sediments suspended throughout the water, the water will become increasingly cloudy which will ultimately prevent enough sunlight from reaching the aquatic plants. These plants will die due to the lack of sunlight, which will affect the other organisms that depend on those plants for food and shelter (Hebert 2019). Additionally, roads contain sealants that are made up of harmful chemicals which are known to be cancer-causing toxins (Liebl 2016).

2.2 Hypotheses

This experiment will be examining how different solutions of simulated run-off water affects the *D. magna*'s population and heart rate. The null hypothesis for this experiment states that both the simulated sidewalk and parking lot run-off water will neither have an effect on the population of *D.* magna, nor on the heart rate of the organisms. The alternative hypothesis for this experiment is that the run-off water from the parking lot is most harmful to the pond life. Parking lots can contain dangerous oils, chemicals, and even drinks and food due to spillage. Additionally, parking lots are made out of asphalt and concrete which has been known to be dangerous to the atmosphere. Besides, the sealant used on roads and parking lots is known to be potentially damaging to human health. Therefore, parking lots are possibly more toxic to pond life. The simulated parking lot run-off water could kill the *D. magna* and affect the population. Moreover, the parking lot run-off water could perhaps decrease the heart rate due to their dire conditions in the simulated run-off water.

2.3 Materials

To conduct this experiment, the experimenter will need a culture of *D. magna* (or any other *daphnia* species of an observable size), food pellets or yeast, a tank to hold the *D. magna*, four containers with lids, a few pipettes, 3 gallons of spring water, about a teaspoon of debris from the sidewalk, about a teaspoon of debris from the parking lot, an old, rough paint brush, something to crush the debris (e.g. mortar and pestle), two ziploc bags, a microscope with a slide, a timer, a marker, some paper, and a napkin. The tank will be necessary to grow and maintain a *D. magna* culture of more than sixty adult organisms, so it is important the tank can hold more than a gallon of water. For this experiment, a 1.5 gallon tank, which came along with a *D. magna* culture kit that included the organisms and food pellets too, was used. The four containers should be of equal size and hold around ¾ of a cup of water. It is imperative to use spring water and not tap or purified water because the *D. magna* will only survive in spring water. The pipettes will be used to transport the *D. magna* to their experimenting cups and to count them. The ziploc bags and brush will be used to collect the debris that will be used to make simulated run-off water. The microscope along with the microscope slide will be used to examine the organisms' heart to calculate the heart rate. The paper, marker, and timer will be used to measure and record the data.

2.4 Set-Up and Collection of Materials

First, the experimenter must grow the *D. magna* culture enough so that there are more than 60 mature *D. magna*. To do so, the experimenter should have two 1.5 gallon tanks and 2 gallons of spring water. There must be enough spring water in the tank to fill around 70% of the tank with water. The water should be left to sit out for about an hour so the temperature of the water becomes room temperature, which is the temperature the *D. magna* will thrive in. Once the temperature of the water is set, the experimenter may add in the *D. magna* very carefully in equal amounts in both tanks. Next, the experimenter should add 2 or 3 food pellets to each of the tanks. An excess of food pellets in attempts to grow the population faster will result in the bacteria on the pellets to grow to a large quantity, infesting the water, or the *D. magna* population will overpopulate and die quickly due to the lack of space. The *D. magna* population should be left to grow for about 3 to 4 days or until there are enough mature *D. magna* to carry out the experiment.

Next, the experimenter may go outside to a random spot on the sidewalk and use the old paint brush to sweep around a teaspoon of the debris into a ziploc bag. They should repeat this step to collect parking lot sediments. The experimenter should avoid sweeping in any bugs and should label the ziploc bag to avoid confusion. If there are any large pieces of debris (e.g. rocks or twigs), a mortar and pestle (or another object that will be able to crush the debris) can be used to crush the pieces. The pieces should be around the size of sand particles. The experimenter should add ¼ of a teaspoon of sidewalk sediment to one cup, and ¼ of a teaspoon of parking lot particles to the other cup. Once again, they should label the two cups to avoid confusion. Then, they must fill three cups with spring water and mix in the debris. Nothing should be in the fourth cup, which will be used to count the *D. magna*. Once the three cups have been filled with water, they must add some of the debris found at the bottom of the tank of *D. magna.* This debris containing shedded skin and uneaten food will contain enough food for the *D. magna* for the next few days. The pipette is used to suck up 1 milliliter of the debris. Then, the experimenter must place 20 of the mature *D. magna* to each of the three cups. They must be careful and avoid squishing the *D. magna* while picking them up and avoid adding any young *D. magna*.

2.5 Data Collection

Now that all the *D. magna* are in their new solutions, the heart rate for 3 organisms from each cup is to be measured. To measure the heart rate, first the experiment must use the pipette to pick up a *D. magna* and gently place it on a microscope slide. Next, they must locate the heart, which is the small clear organ pumping on the *D. magna*'s back (Figure 1. can be referenced for further guidance on locating the heart). To calculate the beats per minute, the timer will be set for a 15 second timer. The timer is then started and the marker is used to make a dot on a paper each time the heart pumps. Next, the experiment can count the number of dots and multiply by 4 to get the beats per minute. To measure the amount of *D. magna*, the *D. magna* that are actively swimming must be transported individually into the empty fourth cup. Once again, it is important to be gentle and avoid crushing the organisms while transporting. The experimenter should make sure to measure the number of young *D. magna* as well. The number of *D. magna* and their heart rate should be recorded every 12 hours for the next 72 hours.

3. Data & Statistical Analyses

Three different statistical tests are used to analyze the data. Vassarstats was used for the computation of the statistical tools. SAS JMP Software was used for ANOVA and Tukey HSD graphics.

In order to determine whether there is a correlation between the number of *D. magna* alive and the time spent in the simulated run-off water, the Spearman Rank Correlation Coefficient test is used because there is not a clear linear correlation for the Product Moment Correlation Coefficient to measure.

In pursuance of a visual depiction, a scatterplot is used to display the data from all three different trials in each of the three groups. Data is also displayed on line graphs and a bar graph represents the average *D. magna* heart rates.

To further investigate whether there is a significant difference between the populations of *D. magna* in each of the three groups, the ANOVA test is used. The ANOVA test is the most fitting option because it is a parametric test that measures the means of two or more groups of data. The Kruskal-Wallis test (non-parametric) is also another option, but a parametric test is more powerful and dependable than a non-parametric test. In this situation, a parametric test would be the optimal choice because the data is measured on the ratio scale, which is an assumption that must be met in order to use a parametric test. Two different data sets were used in the test. The first data set that was used was from each of the three individual trials. The data consisted of the number of *D. magna* alive every 12 hours. Each data point from a single group in one trial every 12 hours was added and then divided to get the average mean. That average is what was being compared with the ANOVA test.

Lastly, the Tukey HSD test is used for more information. Unlike the ANOVA test that measures significance of more than two groups, the Tukey HSD measures the significance between two groups. The Tukey HSD is usually used after the ANOVA test in order to clarify between which two groups there is a significant result and between which two groups there is not. This test is especially beneficial when a statistically significant result arises, so that the user can see whether there was a statistically insignificant result between any two groups.

3.1 Spearman Rank Correlation Coefficient (SRCC)

Once the r_s value is found, to determine the strength of the correlation, the r_s value must be closer to either +1, which is positive, or -1, which is negative, for it to be strong. The closer the r_s value to either +1 or -1, the stronger the correlation. Using the SRCC, the control produced an r_s value of 0.0901. Since the r_s value is rather far from both +1 and -1, the correlation between the number of *D. magna* alive in the control and the time spent in the simulated run-off water is weak. Next, using the SRCC, the sidewalk group provided an r_s value of 0.937. The r_s value is close to +1. Thus, the correlation between the number of *D. magna* alive in the simulated sidewalk run-off water and the time spent in the solution is strong. Lastly, with the use of the SRCC, the parking lot presented an r_s value of 0.6847. The number of *D. magna* alive in the simulated parking lot run-off water have a stronger correlation than that of the control with the time spent in the solution because the r_s value is close to +1. The r_s value for all three groups was positive, meaning a positive correlation between the variables.

3.2 Scatterplots

A scatterplot is used for each of the groups throughout all the trials. The control appears to be relatively stable, which is expected since there were no varying factors. The numbers for the control start and end up at a similar place with a little fluctuation in the middle. The numbers for the sidewalk group are very variable. This was not expected, but this could be due to a number of factors, including sex of the organisms, their health, or temperature of the water. The *D. magna* sample in the parking lot group experienced the same or very similar phenomenon.

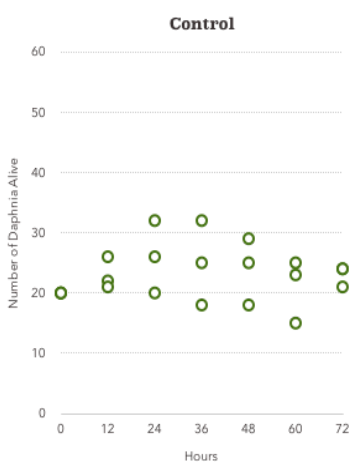

Figure 2. Scatterplot for Control

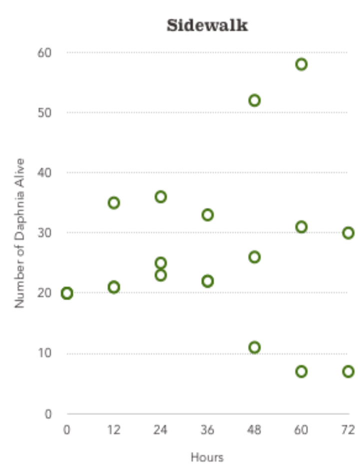

Figure 3. Scatterplot for Sidewalk

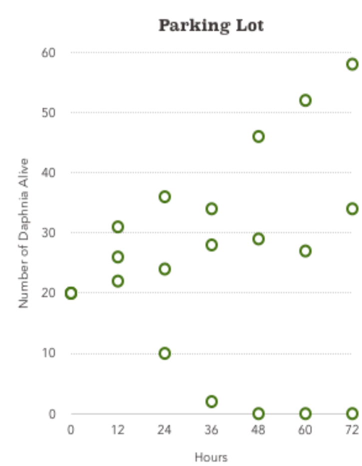

Figure 4. Scatterplot for Parking Lot

3.3 Analysis of Variance (ANOVA) & Tukey HSD

Trial 1 presented an F value of 6.9, while the degrees of freedom was 2 for the greater variance because there are 3 different groups being compared and 18 for the lesser variance because there are 7 data points from each of the 3 groups. Using the F-distribution table, the critical value using the degrees of freedom is 3.1504 at the 0.05 level and 6.0129 at the 0.01 level. Since the F value 6.9 exceeds both critical values, the result is highly significant. Diving deeper into the result of the test using the Tukey HSD Test, the mean for the control and the mean for the sidewalk group did not produce a statistically significant difference. The means for the control and parking lot group produce a statistically significant result and the means for the sidewalk and parking lot groups present a highly statistically significant result. The *D. magna* sample in the sidewalk group did not display a statistically significant difference from the control, yet the *D. magna* sample in the parking lot group did. According to this trial, this could suggest that the parking lot sediments proved to have a significant effect on the *D. magna* sample.

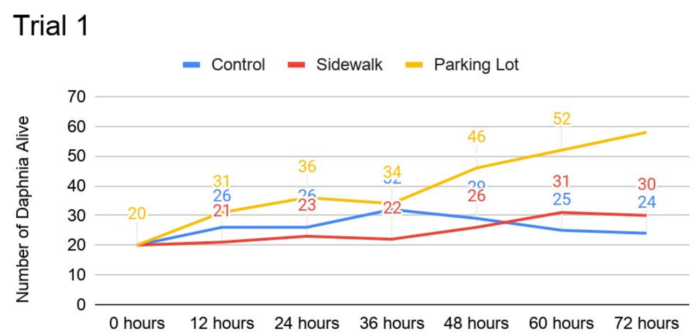

Figure 5. Daphnia Growth in Trial 1

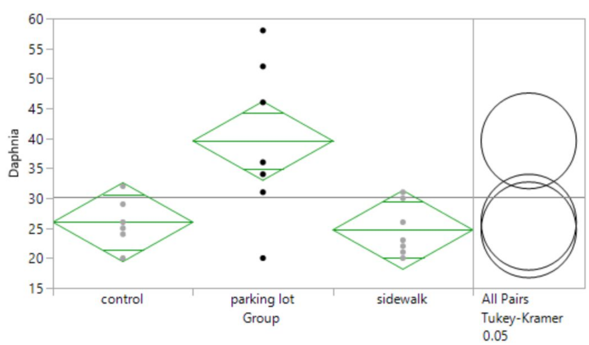

Figure 6. ANOVA & Tukey HSD Test Trial 1

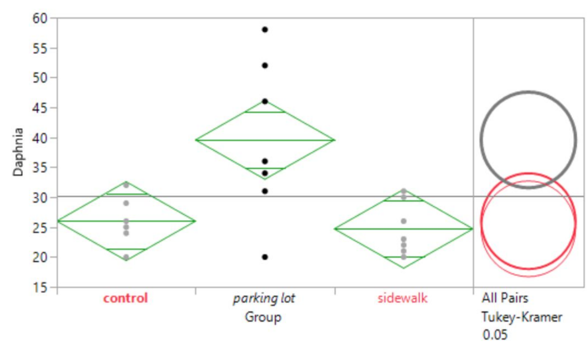

Figure 7. Tukey HSD Comparison

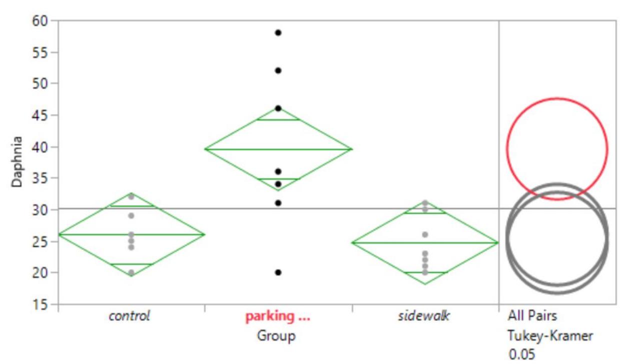

Figure 8. Tukey HSD Comparison

Trial 2 provided an F value of 4.78, with the same degrees of freedom and critical values as Trial 1. The F value 4.78 exceeds the critical value at 0.05, but not the critical value at 0.01. Therefore, the result is significant, but not highly significant. However, observing the Tukey HSD test, only the means for the control and parking lot group yield a statistically significant result, while the means for the control and sidewalk group and the mean for the sidewalk and mean for the parking lot groups produce a statistically insignificant result. Overall, Trial 2 provided a statistically significant difference among the three averages. This trial yielded a more expected result because the numbers in the parking lot and sidewalk samples declined, yet this result is starkly different from that of Trial 1. In Trial 1 the parking lot group increased in numbers drastically, while in Trial 2 almost the exact opposite occurred with the parking lot sample dying off at 48 hours. Similar to Trial 1, the parking lot group produced a statistically significant difference from the control, and the means for the control and sidewalk sample produced a statistically insignificant difference.

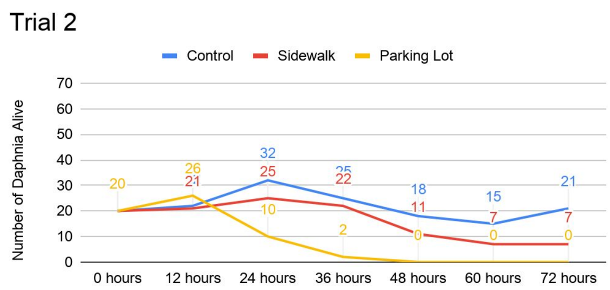

Figure 9. Daphnia Growth in Trial 2

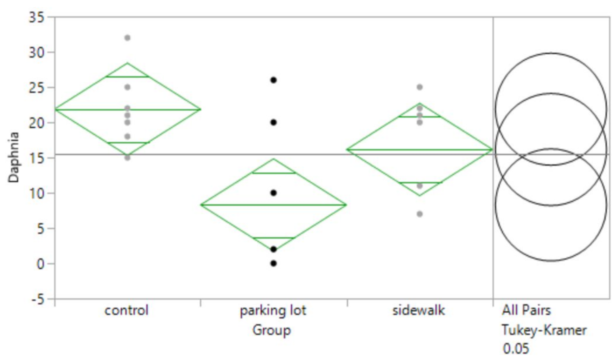

Figure 10. ANOVA & Tukey HSD Test Trial 2

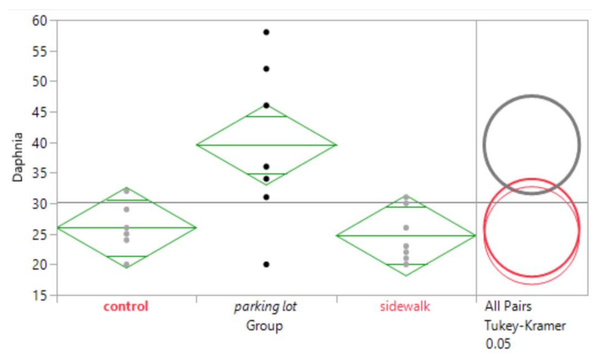

Figure 11. Tukey HSD Comparison

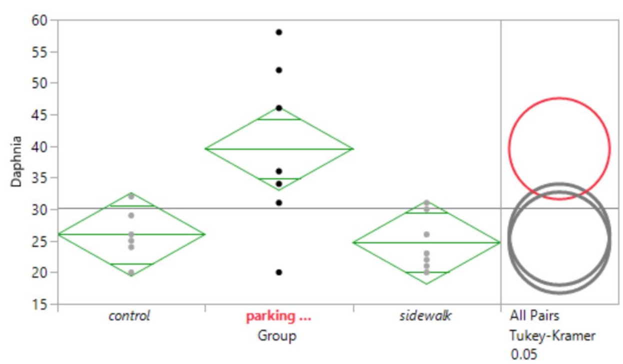

Figure 12. Tukey HSD Comparison

Trial 3 yielded an F value of 9.12, with the same degrees of freedom and critical values as the previous trials. The F value 9.12 greatly exceeds both the critical value at 0.05 and the critical value at 0.01. Investigating further into the test result, the Tukey HSD test displays that the means for the control and sidewalk group present a highly statistically significant result and the means for the sidewalk and parking lot groups produce a statistically significant result. However, the mean for the control and the mean for the parking lot provide a statistically insignificant result. Nonetheless, the difference among the different groups' means is highly statistically significant in Trial 3. This trial yielded results similar to Trial 1, except the sidewalk sample appears to have increased, while the mean for the parking lot sample displays no statistically significant difference from the control. The sample in the parking lot group seems to be relatively stable, which is not what was seen in the previous trials.

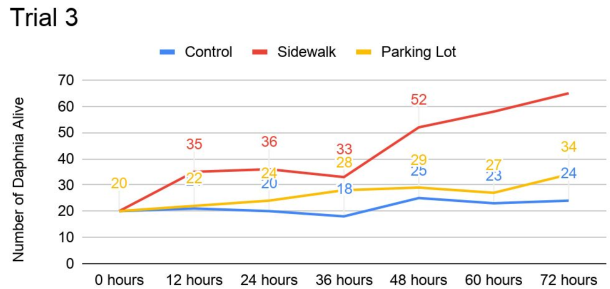

Figure 13. Daphnia Growth in Trial 3

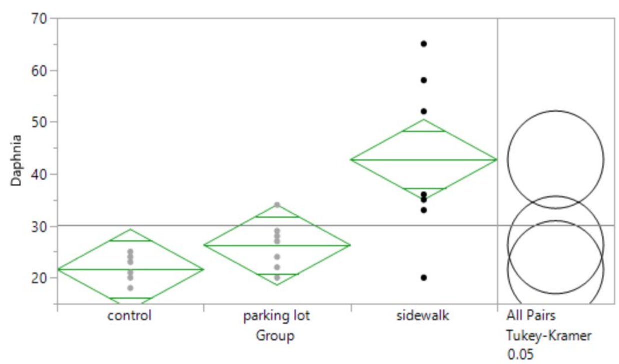

Figure 14. ANOVA & Tukey HSD Test Trial 3

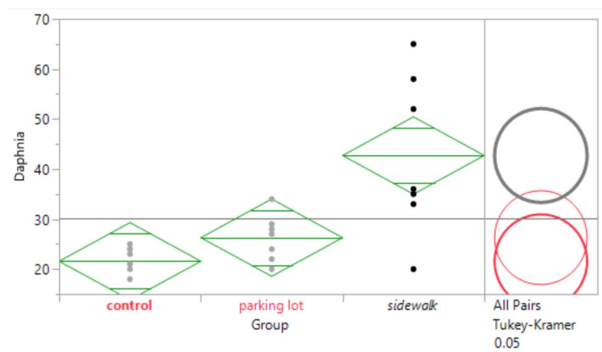

Figure 15. Tukey HSD Comparison

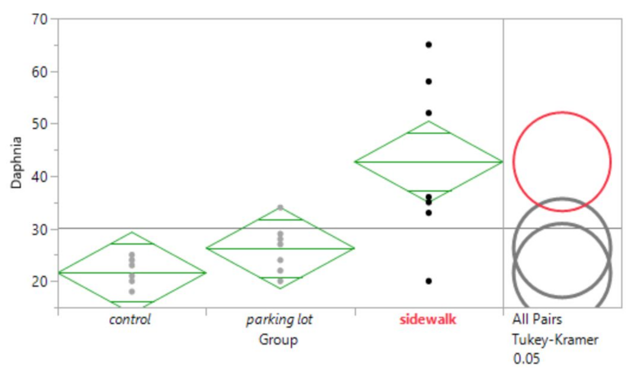

Figure 16. Tukey HSD Comparison

All three of the individual trials presented a statistically significant difference among the means of the number of *D. magna* alive on average. The second data set used to measure the difference among the means is simply plugging in all the data. Each sample will have the data from all three trials. The same ANOVA test is used, and the degrees of freedom and critical values are the same as the trials. The F value for the average from all trials is 0.71. The F value 0.71 definitely does not exceed either critical values of 3.1504 and 6.0129. Thus, the overall result from this experiment is statistically insignificant, which is rather strange, since the individual trials yielded statistically significant results and two of them produced highly statistically significant results. Although it is very likely that the results from Trial 2 cancelled out the variability in the Trial 1 and Trial 3 to ultimately produce a statistically insignificant result. However, the distinct differences may have been due to the fact that the sex of the *D. magna* was not put into consideration while placing the *D. magna* into the respective sample groups. This inconsistency could have caused the samples with more females to experience more growth. Additionally, it is difficult to tell if any of the *D. magna* placed into the different groups were pregnant. This could explain the sudden increases in numbers after 12 hours. Furthermore, some other unaccounted variables, such as temperature, age, health, could have also affected the Trial 2. *D. magna* are known to be sensitive to temperature and the particular time period could have had higher or lower water temperatures. Moreover, the *D. magna* collected for Trial 2 could have been in general older or unhealthy. It is also quite possible that the bacteria found on the food pellets given to *D. magna* grew substantially. The bacteria could have overpowered the *D. magna* samples in Trial 2, and ultimately reduce the *D. magna* numbers in the samples for Trial 2. These discrepancies could have resulted in trials that produced striking differences among each other, but the overall result cancelled out the differences to remain statistically insignificant. Figure 17., which displays the averages from each trial for each data point, provides a visual clarification of how the overall result is statistically insignificant. Both the samples from the sidewalk and parking lot groups appear to be in close conjunction with the growth of the control.

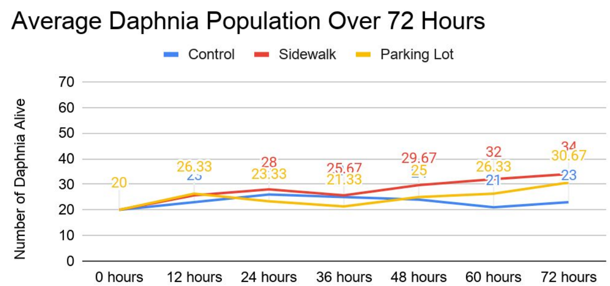

Figure 17. Average Daphnia Population

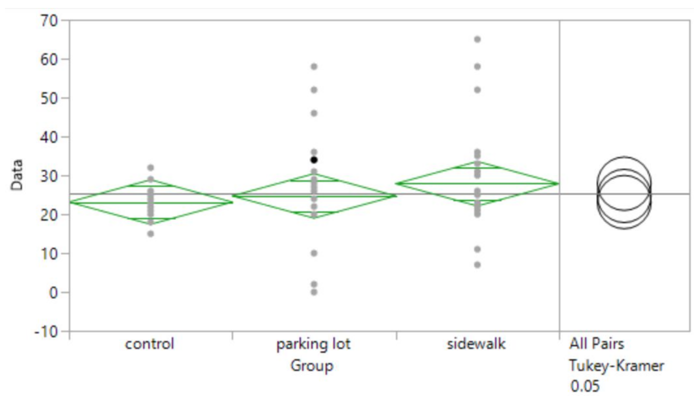

Figure 18. ANOVA & Tukey HSD Test Overall

Next, the ANOVA test will be used to determine whether the heart rate between the different groups are statistically different. By taking the averages from each group in each trial, the degrees of freedom is 2 for the greater variance and 6 for the lesser variance. There are 3 groups and 3 data points for each group (one from each trial). The F value is 0.08. The critical value at 0.05 is 5.1433 and the critical value at 0.01 is 10.925. The F value 0.08 is highly insignificant. Thus, there is no difference between the heart rates in the different solutions.

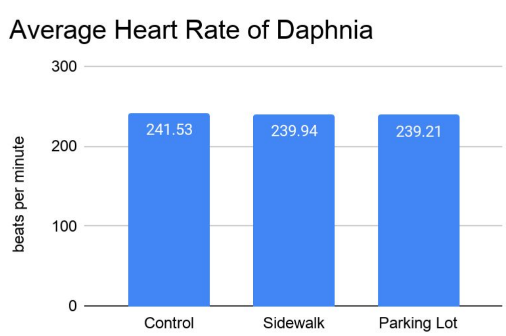

Figure 19. Average Heart Rate of Daphnia

4. Conclusions

After analyzing the data, a few important things have come to light. The correlation between the number of *D. magna* alive in the control is close to zero. Nonetheless, this makes perfect sense because the control should not have any drastic increases or decreases in population numbers over 72 hours. However, observing the correlation between the number of *D. magna* alive in the sidewalk run-off water and parking lot run-off water, it is strange that they both have positive correlations. It was expected that the numbers would decline, not increase, which is only witnessed in Trial 2. While doing further research into the matter, it appears to be that when the *D. magna* sense danger, whether that be lack of food, predators, or changes in water, they are able to reproduce parthenogenetically (La et al. 2014). This means that offspring can be produced without fertilization, and the *D. magna* are able to do this because they reproduce sexually and asexually. Thus, the number of *D. magna* increased, instead of decreased because the rate of growth exceeded the death rate. This could suggest that the sidewalk run-off water and parking lot run-off water prove to be a detectable danger to the *D. magna*, but the danger is not perilous enough to suddenly and immediately kill off the population.

Although the three trials observing the number of *D. magna* alive in each of the groups present statistically significant results in the difference of the means, after plugging in all the numbers from every trial into a single test, it produced statistically insignificant results. Despite the controversy, this would make sense because the trials displayed stark differences. In Trial 1, the population in the cup with simulated parking lot run-off water increased drastically, yet in Trial 2, the population in the same cup (different set of *D. magna*) died off after 36 hours. Furthermore, in Trial 3, the population of *D. magna* in simulated sidewalk run-off water flourished, while in Trial 2 the population was much smaller. The test cancelled out these discrepancies, and thus, provided a statistically insignificant result. Therefore, as a whole, the population of the *D. magna* was not statistically significant, which is made clear in Figure 17.

If the results were examined by trials, two out of three trials displayed a statistically significant difference between the control and parking lot group, while only one of the trials produced a statistically significant difference between the control and sidewalk group. These individual trials suggest that the parking lots are more dangerous to the *D. magna* compared to sidewalks. However, more trials would be necessary to confirm that claim.

These results could propose that the run-off water from either parking lots or sidewalks did not pose enough of a danger to gravely harm or alter the *D. magna* population. This result may have surfaced because of the conditions the sediments were collected in. The time period in which the sediments were collected was when a shelter in place order was in place. This could have impacted the amount of pollutants, such as microplastics from littering, because of the reduced number of people travelling on roads and sidewalks. It is also possible that the areas the sediments were collected from have relatively low levels of pollutants.

Some other inconsistencies could be the lack of account for the number of each sex found in each group. A lack of females could mean that there are no chances for the *D. magna* sample to grow, while the abundance of females results in increased numbers. Furthermore, it is challenging to observe which *D. magna* are pregnant and which ones are not. These inconsistencies could be fixed in future experimentation with painstaking preciseness. Additionally, unknown factors such as temperature, age, and health of the *D. magna* could have severely affected Trial 2, leading to the discrete results.

The data from the heart rates of the *D. magna* showed not to be significant, but highly insignificant. This may be because the factors that could have changed the heart rate of the *D. magna* were constant (e.g. water temperature). Thus, the lack of difference between the heart rates of the *D. magna* in different groups is no surprise.

Since the statistical tests present statistically insignificant data, the null hypothesis is accepted. The alternative hypothesis is rejected because the parking lot did not appear to affect the overall *D. magna* population in a statistically significant manner. The heart rate of the *D. magna* did not change due to the supposedly dire conditions the *D. magna* in the simulated sidewalk run-off water and simulated parking lot run-off water were exposed to. The run-off waters were noticeable hazards to the *D. magna*, but did not turn out to be life threatening for the population.

Overall, it is fair to conclude from this experiment that the run-off waters do affect the *D. magna*, but not in a statistically significant fashion. This project suggests that the run-off water from our infrastructure does not gravely affect the nearby local ponds' and streams' aquatic ecosystems, but there were a few discrepancies that could have possibly altered the results. More trials would be necessary to obtain a more clear conclusion.

5. Future Experimentations

If this experiment were to be repeated, it would be an optimal choice to include more trials to get more samples and more data. Additionally, the experimenter could pay close attention to the sex of the *D. magna* and whether the females are pregnant or not. This could allow the trials to be more consistent. The experimenter could also be more consistent with the amount of food given to the organisms because a precise amount of food was not given to each group (1 milliliter of the debris could have been entirely shedded skin or only food), which could be a possible source of error. More sediments from multiple locations, instead of a single location, or maybe a location closer to a pond could also be a better option. Moreover, if an experiment similar to this were to be carried out again, the experimenter could possibly fluctuate the number of initial *D. magna* in each of the groups to see if the amount of space has a factor in the change of population, or vary the amount of sediments put in to simulate run-off water to create different concentrations of sediments. Maybe experimenting with different water temperatures or changing the amount of time the *D. magna* spend in the simulations could also be interesting for further investigations.

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Biography

Saloni Patel is a student at Stanford Online Highschool. She has earned awards in local science and engineering fairs, as well as innovation challenges, including Second Place in the Alameda County Science and Engineering Fair. Saloni's research interests include pollution and its impact on our world.