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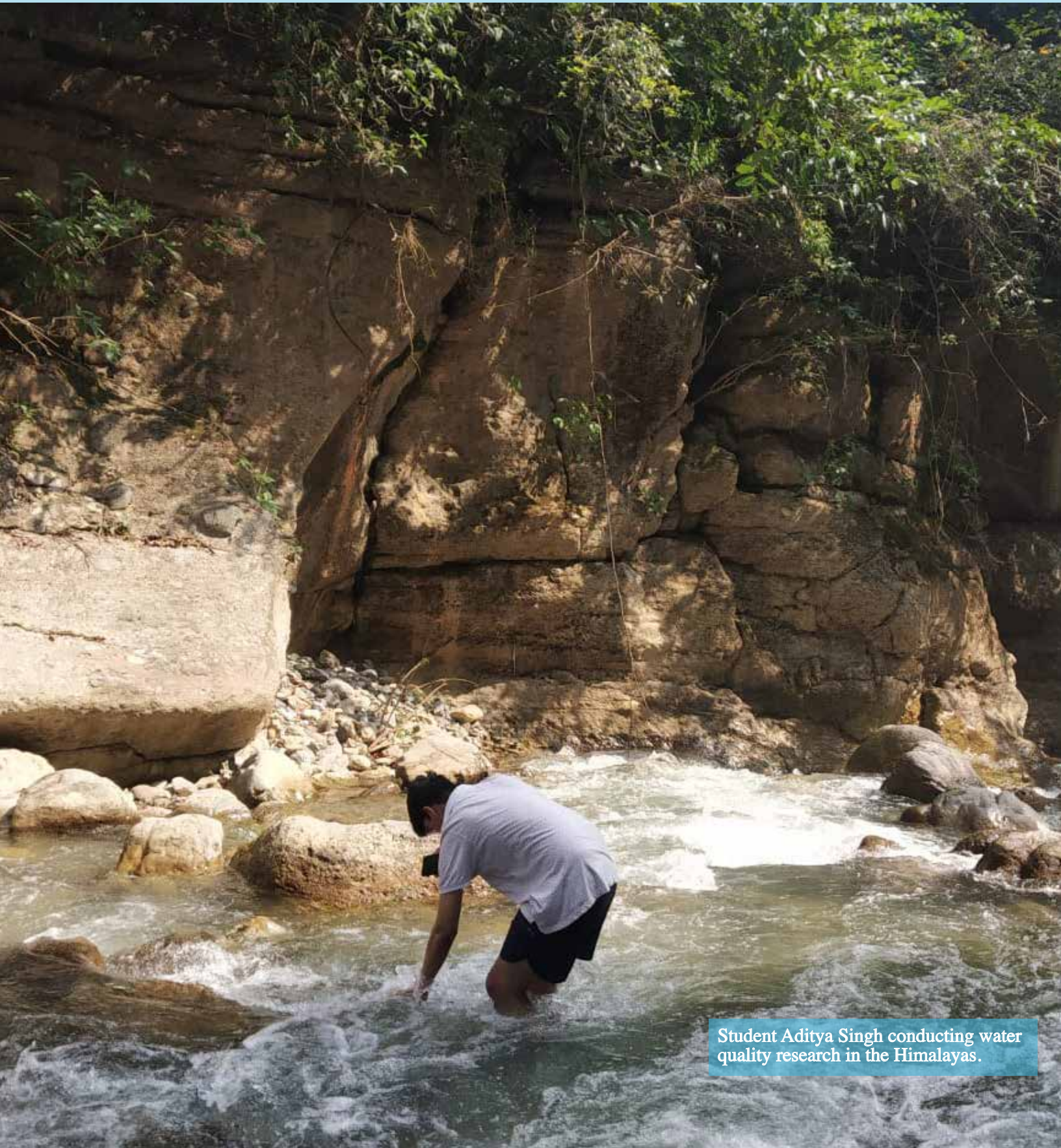
HEADWATERS SCIENCE INSTITUTE
Student Research Journal

Volume 2 • Issue 1 • Spring 2021



Student Aditya Singh conducting water quality research in the Himalayas.

Forward

The articles you see in this publication are the result of months of hard work from students in Headwaters Science Institute's 2020 Fall Research Program. While the caliber of these projects speaks loudly to these students' accomplishments, allow us to also highlight a few of the most special parts of this program here.

Part of what made this program possible was participation by a great array of professional scientists, post-docs, and graduate students from institutions across the country. These scientists not only brought great technical knowledge to the program but also shared with students their own invaluable experience in navigating the winding path of original scientific research. Thank you to all of the scientist mentors who made these students' projects possible.

One of the unique outcomes of this course was that all of the articles in this text are the students' own original ideas. While the theme of this course was Environmental Science, each student took to this task from their own unique perspectives and research interests. From there, students shaped and molded the questions they were interested in based on current scientific knowledge and access to field sites or existing datasets. Students revised their questions, collected preliminary data, and revised again, honing their scientific skills along the way. The diversity of research questions in this publication is not only evidence of the incredible creativity inside all students but also honors the wonderful job the scientist mentors did in nurturing this curiosity.

To the students who authored these papers: congratulations! All of you have worked incredibly hard to complete these manuscripts. More than having your name on this paper, be proud of all that it represents. Each one of you overcame setbacks, roadblocks, time crunches, and uncertainty to get to this point. The skills you practiced along the way will serve you well in whatever you choose to do next.

Spencer Eusden

Program Director - Headwaters Science Institute

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STUDENT RESEARCH JOURNAL

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Effects of PET Microfibers on *Ocimum basillicum*

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ABSTRACT

Of the 6300 metric tons of plastic produced globally from 1950 to 2015, 79% is located in landfill or in the natural environment. Despite PET (polyethylene terephthalate) microfibers being the most common microplastic polluting terrestrial environments, which is predicted to have from 4 to 23 times more plastic pollution than the ocean, most research conducted focuses on the effects of microbeads and other forms of plastic pollution in aquatic ecosystems. This study aims to examine the effects of increased PET microfibers concentrations present in soil on the growth of *Ocimum basillicum*, or the Thai basil, specifically on how it affects leaf nitrogen content, growth rate, and mortality rate. The results of this study suggest that as PET microfiber concentrations in soil increase, the mortality rate of the plants will increase as well. It is inconclusive whether the growth rate of plants with microfibers is significantly different from those without due to small sample sizes and incapability to track the growth rate of deceased plants. However, the presence of PET microfibers appeared to have no substantial effect on the nitrogen content in the leaves of *Ocimum basillicum*. It is hypothesized that as microplastic concentrations increase, the nitrogen content and rate of growth will decrease, though the mortality rate will increase.

INTRODUCTION

Of all the plastic produced since 1950, 9% of it has actually been recycled while 12% has been incinerated, and 79% resides either in landfills or pollutes natural environments (1). This environmental pollution has serious implications for both continental and oceanic ecosystems, though research has predominantly been conducted in marine environments while not as much has been studied in terrestrial environments despite research suggesting terrestrial systems contain anywhere from 4 to 23 times more plastic pollution than oceanic ecosystems (2, 3). In particular, not much has been studied on the effects of fibrous PET microplastic in terrestrial ecosystems, a surprising fact given that fibrous microplastic is one of the most commonly occurring microplastic (4), 70% of which is made out of PET (1). Fibrous microplastics have significant ramifications for plants, as microfibers have been found to be more abundant in terrestrial systems and have also been shown to greatly affect plants more than microbeads or micro pellets, both of which are categorized as plastics spherical in shape (5). However, despite this, most research has been done using microbeads due to their easy attainability through vendors, making more studies involving fibrous microplastics all the more vital in understanding the impacts microplastics have on plants (5).

A majority of plastic waste from Western countries like the US, Canada, and various European countries is shipped over to underdeveloped countries in Africa and Asia, where it pollutes low income communities due to the lack of room in recycling facilities overseas (6). The shipment of this waste has serious repercussions, affecting farming and fishing communities in such regions (6). Microplastic pollution in terrestrial ecosystems is extremely relevant, especially its impact on agroecosystems. Farming communities in these low income regions are disproportionately affected by plastic pollution, yet rely on plastic mulch due to its ability to retain soil moisture and temporarily increase crop yield (7). However, continued usage of plastic mulch allows a large buildup of microplastics to contaminate crops, ultimately decreasing crop yields over time as well as reducing plant height and root mass (7). A study in China done in various cotton fields showed a 6-10% decrease in cotton yield in polluted regions while various crops (peanuts, maize, potatoes, tobacco, wheat, sunflowers, and soybeans) experienced on average a 3% decrease in crop yield for every additional 40 kilograms of microplastic per acre (7). On a smaller scale, PET has been seen to decrease soil density and water content in spring onions, suggesting that although the presence of PET microplastics increases water capacity in the soil, this water was not taken up by the plants and waterlogged the soil, which could lead to decreased air supply to the plants' roots and root rot (8).

Ultimately, this experiment aims to determine the effects of fibrous PET microplastic on the growth of *Ocimum basillicum*, or Thai basil, and aims to find the effects of PET microplastic on the rate of mortality, rate of growth, and nitrogen content in plants. I hypothesize that as microplastic concentrations increase, the nitrogen content in the plants' leaves and rate of growth of the plant will decrease. Furthermore, it is predicted that as the presence of fibrous PET microplastics in the soil of basil plants increases, the rate of mortality will increase due to the larger presence of microplastics in the soil. The study will observe the effects of 0.5%, 3%, and 7% microplastic in soil on the basil plant, as well as a control group. These concentrations of microplastics are significant as certain agricultural soils have been recorded to have as much as 7% of microplastics in soil weight, or even higher (8). Clearly, this research is justified in that it explores the effects of fibrous PET microplastics on a food crop, something that has not been looked into extensively despite the commonality of fibrous PET microplastics in terrestrial environments.

METHODS

PET plastic, or plastic with the resin code identification code 1 was obtained using a cleaned out shampoo bottle. The PET was melted down into liquid, spun into thin fibers, and cut into small segments estimated to be 7 mm long and 50 µm in diameter. The trials used had 0.5%, 3%, and 7% of microplastic in soil weight as well as a control group. The experiment used MiracleGro peat potting soil. Each sample plant was to have 108 grams in total soil weight. The kitchen scale used measured to a 0.1 gram accuracy. The control soil had no added microplastics, though trace amounts of microplastics may have already been present. The microplastics and soil were mixed and sifted through to ensure even distribution of contaminants.

There were 8 samples for each trial, with 32 plants total. The basil seeds were prepared for planting by being placed in a damp paper towel for two days, then planted ¼ inch deep in the soil. Three seeds were planted in each pot in the event that some of the seeds never sprouted. The Thai basil plant was used in this experiment due to its rapid growth time of 3 to 4 weeks, allowing for a succinct study of the effects of microplastics on plant growth. The plants were put in the same area to ensure equal sunlight exposure. After sprouting, extra sprouts were removed from each sample so that only one plant remained each pot.

The plants were checked up on every three days and were watered with 100 mL of water whenever the topsoil was no longer moist to prevent overwatering. Plant heights were measured in centimeters weekly due to the small stature of the basil plants. Photos of each plant's leaves were taken weekly at 11 am PST in order to maintain a constant passive light source from the sun. All photographs were taken by an 8 megapixel iPad (6th Gen), at the same angle.

Images were then imported into the ImageJ program and cropped to isolate merely the leaf studied for each sample in all 4 trials. The same leaf of each plant was studied in order to maintain consistency. The red-green-blue (RGB) color spectrum of the isolated leaf images were analyzed, giving a numerical value to each RGB value based on the number of pixels in the image that were of that color. The green content of each leaf was taken by dividing the number of green pixels by the total number of pixels in the leaf image (9). The amount of green in a plant taken from digital image processing through RGB spectrums has been shown to be an accurate indication of the nitrogen content in a leaf by numerous studies (10, 11, 12).

RESULTS

After measuring plant height, taking photos of leaves weekly, and taking note of deceased plants, the collected data was analyzed in order to extrapolate results. The overall health of each trial group was analyzed by comparing the rate of growths between groups, the overall nitrogen content, and the rate of mortality.

Rate of Growth

For fig. 1, the heights of all plants in each trial were averaged and then graphed over time to compare the growth change over the course of 4 weeks. The rate of growth for each trial was plotted against the rate of growth of other trials in order to effectively compare

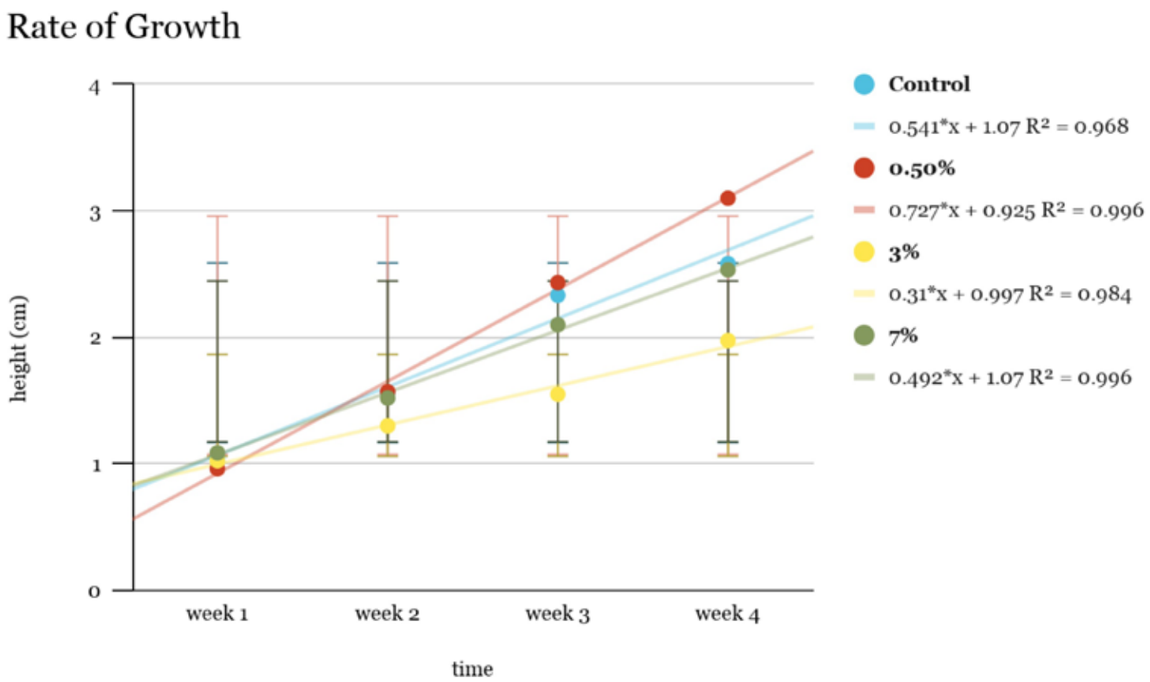


Figure 1: Averaged plant heights over time. Error bars = Standard Deviation of 1.

the changes in the growth rate. A line of best fit was applied to each trial's data set, the slope of which indicates the respective growth rate for each trial. The effects of higher microplastic concentrations on plant growth was unclear, per figure 1. It can be seen that the control with no plastic and the 0.5% group have rates that go against this pattern, as the 0.5% group has a faster growth rate compared to the control (0.727 > 0.541). This can also be seen in the 3% and 7% groups, as the 3% growth rate is significantly smaller than that of the 7% trial.

The rate of growth for each plant in all trials was also calculated, with growth rate values from each respective trial being compared against each other in a one-way anova test. The F distribution of the test shows that the null hypothesis is accepted due to overlap of growth rate values, with p-value = 0.191970 and F statistic = 1.849567. The test suggested there was no significant difference in the growth rates of each trial since the p-value is rather high and the F-statistic is comparatively small.

Greenness and Nitrogen Content

Given the method used to analyze nitrogen content observes the green percentage of the plant using digital image processing, quantitative values cannot be attributed to the nitrogen content of each plant, thus the amount of nitrogen content in each plant will be observed qualitatively based on the numerical values of the green content in the plants. Leaves with larger green content values can be assumed to have more nitrogen in their leaves based on the findings of the above cited studies.

There appears to be no significant difference in the nitrogen content between trial groups. A one-way anova test was done using average green content values for each trial group from each week. The p-value has a value of 0.439070 and the F-statistic a value of 0.969331, which showed no statistical significance between increased concentrations and nitrogen content and ultimately accepts the null hypothesis.

Mortality Rate

The mortality rate was calculated by taking the ratio of deceased plants in a trial to the number of total plants in the trial.

The data suggests that plants with lower plastic contamination have lower mortality rates. The percent of plants that died per week was averaged, with the control group having a mortality rate of 7.15%, the 0.5% group with a rate of 20.8%, the 3% group with a 31.25% rate, and the 7% group with a rate of 40.6%.

DISCUSSION

The collected data implicates a clear increased mortality rate in plants with higher PET microfiber concentrations, and while there is no correlation between higher microplastic concentrations and nitrogen content, it is unclear whether it implicates negative impacts on growth rates, though data suggests no correlation is to be found.

A study in Berlin reciprocates the results of this experiment in regards to the effect PET fibers have on nitrogen content. The study researched the effects of various microplastics on the growth of the spring onion and also found they had no significant impact on plant nitrogen (8). The similarity between the observations made in this experiment and the Berlin study suggest that in larger populations, PET fibers may not have a significant impact on the nitrogen concentration of various plants, as the basil and spring onion plants are two very different species.

While the data results for the growth rate of the plants does not conclusively suggest growth rate is negatively impacted by increased fibrous PET microplastic concentrations, the data may indicate that this aspect should be subject to further study. The results of fig. 1 did not fully display the pattern that as microplastic concentrations increase, the rate of growth decreases. The control group had a lower average growth rate than the 0.5% group and the 3% group had a lower growth rate than the 7% group, though there was a significant difference between high concentration growth rates and low concentration growth rates. Furthermore, the error bars for standard deviation in fig. 1 were rather large and the low F statistic indicated that the differences between the trials may have been random and thus statistically insignificant.

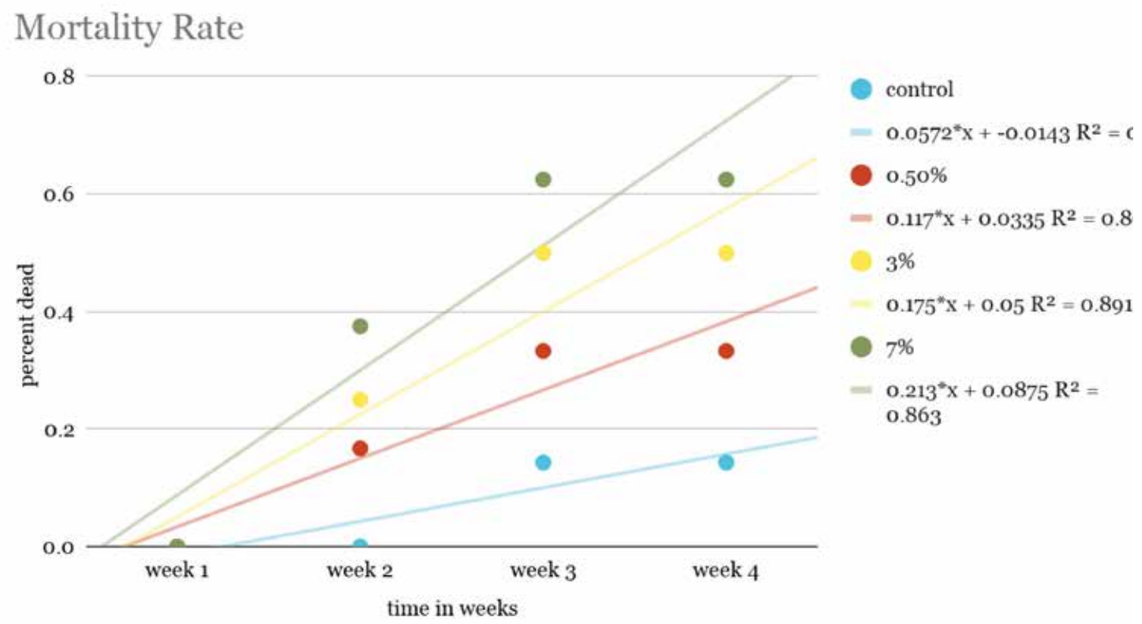
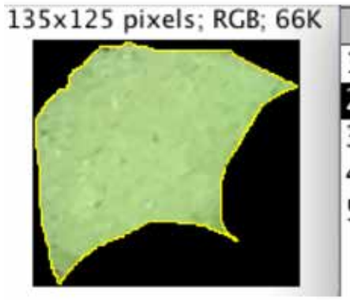


Figure 2: Linear plot shows the percent of plants that died per week.



	Label	Area	Mean	Min	Max
1	Red	9712	171.053	30	205
2	Green	9712	207.219	32	242
3	Blue	9712	132.603	10	165
4	(R+G+B)/3	9712	170.359	24	204
5	0.299R+0.587G+0.114B	9712	187.866	29	222

Figure 3: ImageJ digital image processing for plant leaves.

The results of the rate of growth may have been influenced by the small stature of the observed plants, as their light mass caused them to shift around in the dirt and get covered by a layer of soil each time they were watered. The plants had to be unearthed from under the soil in order to measure their respective heights every time measurements were to be recorded. Due to the plants' small size, it was unclear when to stop digging around the stems and thus, the portion of the plant that was considered to be the stem did not remain constant throughout the experiment. There were likely times when significant portions of the stem were still under the soil, as in some cases, plants appeared to have decreased in height, prompting me to remove more dirt from around the plant base. This error could be corrected by observing larger, heavier plants, similar to the spring onions the Berlin study observed. Plants with a larger mass would likely not be displaced by water and dirt every time watered and would hopefully be able to maintain a consistent stem height visible to the eye. Overall, the data collected was influenced by two primary sources of error: sample size and rodent interference. Future iterations of this experiment could hopefully correct for squirrels by building a tarp around the plants or by keeping the plants in a greenhouse while also increasing sample sizes. Such an endeavor would likely need more resources in order to obtain a location large enough to house a larger number of plants and to store them safely away from rodents. Although the sample size was initially 8 for each group, making 32 plants studied in total, the drastic effects of the mortality rate limited the statistical analysis for the rate of growth, as the sample size for each trial varied greatly. Retrials would better anticipate the effects of mortality on sample sizes by increasing the amount of plants studied.

The mortality rate of the plants, though accounting for the deaths by squirrels, could also be improved upon if studied in a larger sample size. The data, which observed the average percent of plants that died per week, suggested that plants with higher microplastic concentrations are more likely to die, as shown in the higher rates the 3% and 7% groups experienced compared to the control and 0.5% groups.

The data collected may prove to have significant implications for larger populations, as basil, like many other plants, are mass produced on commercially owned farms or grown in personal gardens but can also be found in the wild. Basil can be found in the wilderness in forests and meadows and may serve as a lucrative food source for wild animals and insects due to its strong odor (as shown in the squirrel consumption). Ultimately, this shows that the plastic contamination of basil and other plants commercially produced and found in the wilderness has large ramifications for both animals in such ecosystems as well as human populations that consume food grown in plastic contaminated regions. Future research would hopefully pinpoint what exactly plant components are negatively impacted by PET microfibers that would potentially cause reduced growth rate and increased mortality rates, seeing as this decrease in health cannot be confidently attributed to nitrogen deficiencies. Other potential research could also observe the long term effects consumption of crops grown in contaminated regions has on human and animal bodily systems, as the issue of PET micro plastic contamination in terrestrial ecosystems is clearly pervasive in wild and commercial settings. Repeating similar studies on the effects of PET microfibers on a larger variety of plant species (beyond basil and onions) would hopefully indicate whether the detrimental effects PET has on plant health is dependent of plant type, as this would potentially bring more attention to the plastic pollution crisis and to the effects of microplastic ingestion in organisms as current efforts have not had significant impacts on curbing the ever growing amount of plastic contaminants in both terrestrial and aquatic ecosystems.

ACKNOWLEDGEMENTS

I'd like to thank my mom for putting up with my massive plant collection and my dad for keeping an eye out for squirrels.

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Effects of Human Activity on Water Quality and Benthic Macroinvertebrate Communities

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ABSTRACT

With the growth of urbanization, different environments have grown more susceptible to human activity and pollution. Automobiles have been found to create deposits of nitric and sulfuric gas, acidifying bodies of water. This acidification of water is found to negatively influence populations of benthic macroinvertebrates, primarily those sensitive to these dramatic changes in water quality. The purpose of this study is to compare how areas of high and low human activity influence water quality, as well as benthic macroinvertebrate populations. I hypothesize that bodies of water experiencing higher levels of human activity exercise lower biodiversity of benthic macroinvertebrate populations. I measured the pH at two locations, Lake Cunningham and Dr. Robert Gross Groundwater Recharge Ponds, with seven sites at each. I then sampled benthic macroinvertebrates at these sites, using a sieve and dip net to measure (a) macroinvertebrate abundance and (b) macroinvertebrate biodiversity. The location that experienced less human activity, Dr. Robert Gross Groundwater Recharge Ponds, had a lower average pH (8.10), compared to Lake Cunningham. Furthermore, the Dr. Robert Gross Groundwater Recharge Ponds displayed two times more average diversity in macroinvertebrates compared to Lake Cunningham (3.29 vs. 1.29, respectively). The Dr. Robert Gross Groundwater Recharge Ponds had a slightly greater average macroinvertebrate abundance compared to Lake Cunningham, 27.71 and 25.86, respectively. These results suggest that human activity may possibly have a negative influence on these aquatic systems.

INTRODUCTION

Chemical components, such as pH, alkalinity, hardness, and TDS (total dissolved solids), hold influence on pond productivity and the aquatic organisms present in the pond. Fish and vertebrates tend to have an average blood pH of 7.4 (1), demonstrating that a desirable range for pond water pH is close to this value. Otherwise, fish and other aquatic organisms may become stressed when not placed in suitable conditions. Freshwater pH levels can often be found to be driven by the hydrolysis of bicarbonate (HCO3-), which results in the production of protons (H+). It can also be driven by the addition of water to carbon dioxide. This carbon dioxide can be produced as a result of natural processes, such as photosynthesis and respiration (1) in freshwaters, such as ponds. Furthermore, the addition of carbonic acid, results in smaller changes in environmental pH, as it only partially dissociates (3).

Human activities have been found to have a profound influence on the quality of water in the environment, especially in terms of pH levels. Studies have shown that acidification may result from land-use when large deposits of reduced sulfur had been exposed to the oxygen in the atmosphere by human disturbance (2). This can be seen in the case with the coal mining industry, where strong acids are used and can be leaked into bodies of water (3). Additionally, air pollutants, such as nitrogen and sulfur dioxides in the atmosphere, are released from human sources, from the burning of fossil fuels (4). These sources may include automobile exhaust, which can be incredibly prevalent in urban settings. These are examples of strong acids, such as nitric acid (HNO3) and sulfuric acid (H2SO4), which can dissociate almost fully in freshwater, which can lead to substantial drops in pH in the environment (3). These pollutants can eventually lead to the development of acidic rain, ultimately decreasing the pH of lakes, ponds, and streams. This decrease in pH inversely affects different populations of aquatic organisms, especially those sensitive to changes to pH.

Benthic macroinvertebrates are often used as biotic indicators to assess the water quality of ponds and streams (7). Benthic macroinvertebrates are small animals that live in the bottom of various bodies of water, including streams, rivers, and lakes. They can be found living among stones, logs, sediments, aquatic plants, and other places within these waters. Several of their distinguishing characteristics include containing no backbone and being large enough to be seen with the naked eye (4). These organisms have several features that allow them to be useful indicators of water quality. Their relatively long-life cycles would allow these organisms to accumulate toxins with constant exposure to the water, demonstrating the health of the water. The richness, or the abundance of species within a collected sample, has been found to be a common diagnostic to assess potential environmental impacts to the water tested (7). The large numbers of species present in the benthic macroinvertebrates' community offer a spectrum of responses to different environmental influences. (7). For instance, mayflies (*Ephemeroptera*) are found to be particularly sensitive to large changes in pH, where its abundance is significantly reduced in acidic bodies of water (6).

This paper will explore the extent to which human activity can influence the pH levels and macroinvertebrate biodiversity in urban ponds and lakes. The benthic macroinvertebrates' ability to provide information on habitat quality can indicate the effects of pH levels deviating from ideal levels can have on the biodiversity of these organisms. The two locations the experiment will take place in are Lake Cunningham and Dr. Robert Gross Groundwater Recharge Ponds (GRP). Lake Cunningham experiences relatively high human activity since it is an open space that offers a variety of activities, including fishing and boating. Additionally, it is located near an expressway, as well as a small airport. In contrast, GRP has less human activity. It is relatively secluded and is in a suburban neighborhood, nearby a local library and school. Occasional groups visit the ponds to walk or bike, as well as fishing. I hypothesize that bodies of water experiencing higher levels of human activity exercise lower biodiversity of benthic macroinvertebrate populations. Additionally, I hypothesize that waters that have high human activity would create less suitable conditions for macroinvertebrates, such as pH levels not optimal for aquatic life. In this case, Lake Cunningham should exercise less diversity in benthic macroinvertebrates compared to the GRP.

METHODS

Site Selection

The first body of water is Lake Cunningham, an artificial lake that provides citizens with a variety of activities, including sailing, boating, and fishing. Beside the lake are several docks and harbors, as well as several picnic sites scattered around the lake. Additionally, it is located alongside a local waterpark, known as Raging Waters. This lake is surrounded by many roads as well.

The second body of water is the GRP, shallow ponds that seep into the ground to refill groundwater aquifers. There are four bodies of water, where three of them are connected with a man made waterSpring. One body is secluded from the others. These ponds are not as populated in comparison to Lake Cunningham, where there were occasional visitors every hour.

Water Chemistry

Aquatic test strips were dipped in order to assess the pH, nitrite, nitrate, hardness, and alkalinity levels of the water at the fourteen different test sites. A pH meter was used to achieve a more accurate reading of the pH since the test strips used did not account for all the possible pH levels. A digital TDS meter was used to measure the total dissolved solids of the water in each of the sites.

Macroinvertebrate Diversity

To test benthic macroinvertebrate abundance and diversity at these two locations, sampling and data collection took place in seven different sites, each located in different parts of both bodies of water. Macroinvertebrate sampling had a controlled time of twenty minutes, before analyzing the different organisms collected.

The macroinvertebrates were primarily collected with a handheld sieve, as well as a dip net. When collecting macroinvertebrates, I looked in areas of vegetation and stirred up the sediment below, as they are commonly found in these areas (8). A plastic bucket, plastic spoon, and several small bowls were used to sort the macroinvertebrates. After all the macroinvertebrates were sorted, the species were identified in order to classify their levels of sensitivity to water pollution. It is important to look for distinguishing features in order to categorize the

Sensitivity to Water Pollution	Benthic Macroinvertebrate Species: Lake Cunningham	Benthic Macroinvertebrate Species: Dr. Robert Gross Recharge Ponds
Sensitive	• Mayflies (*Ephemeroptera*)	• Mayflies (*Ephemeroptera*) • Adult Riffle Beetles (*Elmidae*) • Gilled snails (*Lymnaeidae*)
Somewhat Sensitive	• Dragonflies (*Odonata*)	• Dragonflies (*Odonata*) • Orb Snails (*Planorbidae*) • Crayfish (*Decapoda*)
Tolerant	• Midge Flies (*Chironomidae*)	• Lunged Snails (*Physidae*) • Waterboatman (*Hemiptera*)

Table 1: Benthic Macroinvertebrates Species: The different species collected at the two locations, categorized by their sensitivity to water pollution. Classifications are taken from Stroud Center, West Virginia DEP (Department of Environmental Protection), and the Maryland Department of Natural Resources Chesapeake Bay and Watershed Programs Monitoring and Non-Tidal Assessment Division.

benthic macroinvertebrates. For instance, Mayflies, or *Ephemeroptera* are often identified from their two or three tail filaments and abdominal gills.

RESULTS

Benthic Macroinvertebrate Diversity

Upon collecting the benthic macroinvertebrates at the two locations, the GRP exercised a greater diversity in benthic macroinvertebrates, with a mean number of 3.29 different species collected. In contrast, an average of 1.29 different species was collected at Lake Cunningham. Out of the two locations visited, the recharge ponds had a greater

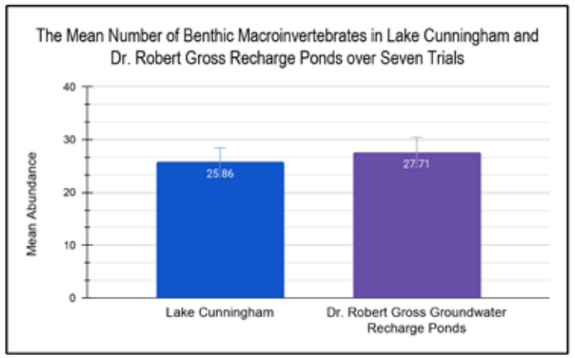

Figure 1: Benthic Macroinvertebrate Abundance: The average benthic macroinvertebrate abundance at Lake Cunningham and Dr. Robert Gross Groundwater Recharge Ponds. The recharge ponds have a slightly greater average of macroinvertebrates compared to Lake Cunningham. The error bars shown for each bar are the standard error of the mean. p-value: 0.9148. Lake Cunningham (SEM Error Bars: +/- 13.45, Standard Deviation: 35.58, N = 7). Dr. Robert Gross Groundwater Recharge Ponds (SEM Error Bars: +/- 10.39, Standard Deviation: 10.39, N = 7)

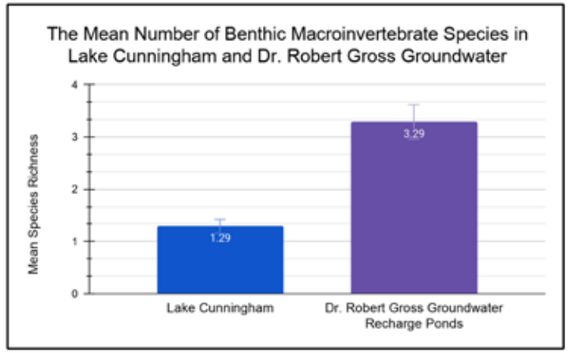

Figure 2: Benthic Macroinvertebrate Biodiversity: The average number of different macroinvertebrate species at the two locations visited, across seven different samplings. p-value: 0.0431. Lake Cunningham (SEM Error Bars: +/- 0.18, Standard Deviation: 0.49, N = 7). Dr. Robert Gross Groundwater Recharge Ponds (SEM Error Bars: +/- 0.87, Standard Deviation: 2.29, N = 7).

amount of species sensitive to water pollution and changes in water quality. Six of them were sensitive to water pollution, while the other two were somewhat sensitive or tolerant (Table 1). The standard deviation of macroinvertebrate diversity across all seven samplings at Lake Cunningham is 0.49. In contrast, the standard deviation of the samplings conducted at the recharge ponds is 2.29.(Figure 2). The p-value of the unpaired t-test is 0.0431, further indicating that the difference between the two means are statistically significant.

Benthic Macroinvertebrate Abundance

Lake Cunningham and GRP share a similar mean in the number of macroinvertebrates, with 25.86 and 27.71 macroinvertebrates, respectively. The number of macroinvertebrates collected ranged from 7 to 106 at Lake Cunningham. 7 to 20 macroinvertebrates were sampled at most of the sites, apart from one. The number of macroinvertebrates collected at the recharge ponds ranged from 0 to 77, with the amount varying every trial. The standard deviation of the macroinvertebrate abundance across all seven samplings at Lake Cunningham is 35.58, whereas the standard deviation for the recharge ponds is 10.39. The p-value resulting from the unpaired t-test is 0.9148, meaning that the difference between the means is not statistically significant (Figure 1).

pH Levels

The GRP was slightly less basic than Lake Cunningham, with an average pH of 8.1 compared to 8.98. The pH levels at Lake Cunningham generally ranged from 8.10 to 9.03. On the other hand, the pH levels at the recharge ponds ranged from 7.6 to 8.43. The standard deviation of the pH across all seven samplings at Lake Cunningham and the recharge pools were 0.0532 and 0.3164, respectively. The two-tailed p-value for pH is less than 0.0001, demonstrating that the difference is statistically significant (Figure 3).

DISCUSSION

Biological Results

Benthic Macroinvertebrate Diversity

The results of the conducted experiments indicate that waters with high human activity tend to exercise a lower diversity in benthic macroinvertebrates, compared to those experiencing less human activity. Lake Cunningham had a total of three different species, each of them having different levels of sensitivity towards water pollution. There was one species sensitive to water pollution (mayflies (*Ephemeroptera*)), one species somewhat sensitive to water pollution (dragonflies (*Odonata*)), and one species tolerant to water pollution (midge flies (*Chironomidae*)) (Table 1). Many of the macroinvertebrates collected at the lake were midge flies (*Chironomidae*), with a total of 177 being collected across all seven samplings. In contrast, only 1 dragonfly (*Odonata*) and 3 mayflies (*Ephemeroptera*) were collected in total. A greater number of pollution-tolerant macroinvertebrates

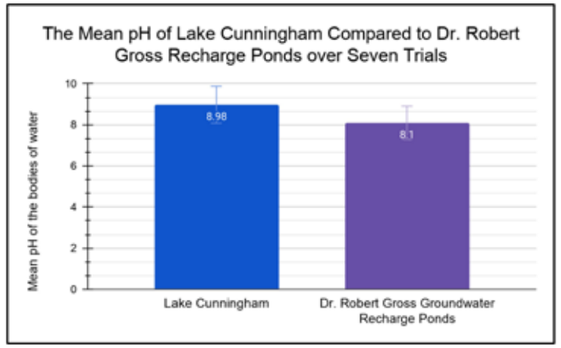

Figure 3: Mean pH Levels: Displays the average pH levels at the two locations from all seven samplings conducted. The pH of all seven sites at Lake Cunningham were found to be greater than 8, whereas two samplings had a pH between 7 and 8 at the Dr. Robert Gross Groundwater Recharge Ponds. p-value < 0.0001. Lake Cunningham (SEM Error Bars: +/- 0.0201, Standard Deviation: 0.0532, N = 7). Dr. Robert Gross Groundwater Recharge Ponds (SEM Error Bars: +/- 0.1196, Standard Deviation: 0.3164, N = 7).

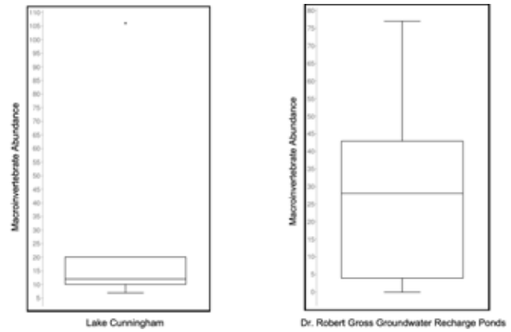

Figure 4: Benthic Macroinvertebrate Abundance at Lake Cunningham (left) and Dr. Robert Gross Groundwater Recharge Ponds (right): Lake Cunningham: The number of benthic macroinvertebrates varied across different samplings, where the highest number collected was 106 and the lowest number collected was 7. The median number of macroinvertebrates collected is 12. Dr. Robert Gross Groundwater Recharge Ponds: The number of benthic macroinvertebrates varied across different samples, where the highest number collected was 77 and the lowest number collected was 0. The median number of macroinvertebrates collected is 28.

might indicate that the quality of the water is not optimal for benthic macroinvertebrates. Many of the samplings were conducted in areas high in human activity, including picnic spots, resting areas, etc.

There were a greater number of species observed at the GRP in comparison to Lake Cunningham. A total of eight different species were collected. Three of them were sensitive to water pollution: mayflies (*Ephemeroptera*), adult riffle beetles (*Elmidae*), and gilled snails (*Gastropoda*). Another three were classified as somewhat sensitive to water pollution: dragonflies (*Odonata*), orb snails (Planorbidae), crayfish (*Decapoda*). And lastly, two species were classified as tolerant to water pollution: lunged snails (*Gastropoda*) and waterboatman (*Hemiptera*). (Table 1) The greater abundance of sensitive and somewhat sensitive benthic macroinvertebrate populations implies that the water quality at the recharge ponds may have been at optimal conditions for macroinvertebrate life. Mayflies were collected in nearly almost every trial, appearing in five out of seven of the samplings.

In Figure 2, the two locations appear to have an extreme difference in the average number of species. The calculated p-value is 0.0431. Because the p-value < 0.05, it indicates that the difference is statistically significant by conventional criteria. There may be a positive correlation between the presence of human activity and benthic macroinvertebrate

diversity, as the chances of these results occurring are very unlikely out of chance.

Benthic Macroinvertebrate Abundance

In Figure 1, the two locations appear to share similar mean abundances, where the means for Lake Cunningham and the recharge ponds are 25.86 and 27.71, respectively. This similarity can be attributed by outliers in the data set, including the 106 macroinvertebrates collected in the first trial at Lake Cunningham. Most sites fell in the range of having 7 to 20 organisms when collecting, except for one trial. According to Figure 4, the data set for macroinvertebrate abundance is skewed right, with the 106 as an outlier. This demonstrates that the 106 macroinvertebrates collected in the one trial may have had a profound influence on the mean abundance for Lake Cunningham, allowing it to be higher than it should be. According to Figure 5, the recharge ponds did not have any outliers. The data set for macroinvertebrate abundance is skewed right, thought not as much in comparison to Lake Cunningham.

pH levels

The pH levels of the two bodies of water are relatively similar, where the means for Lake Cunningham and the GRP are 8.98 and 8.1, respectively. However, in the samplings conducted at the recharge ponds, two samplings were found to have a pH between 7 and 8, which is closer to the average blood pH of fish and other vertebrates (1). The rest of the recorded pH for the recharge ponds fell between 8 and 8.5. In comparison, three samplings at Lake Cunningham had a pH greater than 9, deviating from the average pH of aquatic organisms in ponds. The remaining recorded pH levels fell between 8.80 and 8.99. The p-value for the pH data sets is less than 0.0001, meaning that the difference between the two means are statistically significant since the p-value < 0.05.

The results indicate that the pH levels in the Dr. Robert Gross Groundwater Recharge Ponds were optimal for benthic macroinvertebrate life, in comparison to Lake Cunningham. The recharge ponds had more benthic macroinvertebrates sensitive to pollution, as well as pH. For instance, the mayflies are generally sensitive to pH and are unable to withstand levels outside their ideal conditions (1). With the greater presence of sensitive species in the recharge ponds, there are implications of human activity playing a factor in the pH for the two locations. As stated previously, by conventional criteria, the p-value shows extreme statistical significance between the two means. This tells us that the results obtained from the experiment are unlikely to occur due to chance, allowing us to deduce that the pH may have been influenced by environmental factors, such as human activity.

Sampling Errors

The deviations observed in the data may have been caused by several possible sampling errors. . These possibilities include not having a true representation of each of the locations with the seven samplings, the variance in the work put into sampling, and the days at which they were sampled.

There have been instances of convenience sampling, where the sites chosen at Lake Cunningham did not cover the entirety of the lake due to several restrictions. This can also be seen with the GRP, only three out of the four bodies of water were assessed, due to fencing around the fourth. Additionally, the recharge ponds had beneficial conditions for macroinvertebrate life, where items, such as rocks and accessible sediment were found (8). In contrast, the sites at Lake Cunningham didn't have these conditions, possibly influencing macroinvertebrate abundance and diversity. The effort put into sampling may have varied as well. For instance, at the recharge ponds, macroinvertebrates were caught a minute into the course of sampling. However, at Lake Cunningham, results didn't appear until several minutes later. Additionally, the weather conditions varied when sampling at the different sites, despite the consistent time frame the macroinvertebrates were sampled in (2pm to 6pm). Several sites also had shade, allowing for the water temperature to be slightly cooled, compared to other sites. These different temperatures can have an effect on the macroinvertebrates' abundance, with its influence on development rates, metabolism, and feeding (9).

Implications

These results imply that human activity can negatively impact the quality of water, which in turn, affects the benthic macroinvertebrates present in the water. Observations using macroinvertebrates belonging to the *Ephemeroptera*, *Plecoptera*, and *Trichoptera* are often seen as good water quality indicators, due to their great sensitivity to pollution (10). The great presence of mayflies (*Ephemeroptera*) at the recharge ponds suggests that this location had healthy conditions that favored the survival of pollution-sensitive species. However, as observed at Lake Cunningham the great majority of the macroinvertebrates sampled were shown to be tolerant to pollution. Pollution tolerant species can thrive in environments that contain various environmental stressors (10).

The airport nearby Lake Cunningham may have released combustion-generated pollutants into the environment (12) , adversely affecting the water's quality. Rain and other events of precipitation can carry pollutants from the nearby attractions and automobiles into surrounding waters, deteriorating their quality as a result (11). The recharge ponds, on the other hand, are not exposed to these many sources of pollution. In order to better the quality of water in aquatic systems, there should be measures taken to reduce pollution, such reducing exhaust emissions and throwing away waste properly.

Future Directions

Though the research observed primarily macroinvertebrates, the results demonstrate the influences water has on the general ecosystem of ponds. Benthic macroinvertebrates play a role in material cycling and energy flow, as well as being food sources for organisms such as benthivorous fishes and aquatic birds (13). Further research can be conducted to assess how different macroinvertebrate species present influence the different organisms that appear in the environment. The different benthic macroinvertebrate species may influence the different aquatic organisms present as a result. Additionally, future potential experiments may include visiting more secluded bodies of water, to receive a more definite answer on the impacts human activity and pollution has on macroinvertebrate populations. Secluded locations may include closed-off lakes in forests or bodies of water far off from roads.

ACKNOWLEDGEMENTS

I would like to thank the Headwaters Science Institute for guidance and assistance throughout the entire research process, including data analysis. In addition, I would like to thank Spencer Eusden for coordinating weekly lessons essential for crafting an experimental design and writing a scientific manuscript.

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The Relationship Between Macroinvertebrates, Water Quality, and the Health of Stevens Creek

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ABSTRACT

A qualitative analysis of macroinvertebrates and water quality within Stevens Creek was conducted within this study to determine a general understanding of the health of the creek. Since federally designated threatened steelhead trout also resided in the creek, with a portion of their diet dependent on the presence and abundance of macroinvertebrates. This led to the formation of our research question: whether the abundance and diversity of macroinvertebrates found would be reflective of the creek's health. Our hypothesis was that if the conditions within the creek were found to be optimal for macroinvertebrate survival (specifically the pH range of 6.5-8.5), then there would be a higher abundance and diversity of macroinvertebrates found. Macroinvertebrate sampling and water quality testing (including pH measurements) were carried out in two locations within Stevens Creek, with seven trials being done in each location. The overall TDS as well as abundance and diversity of macroinvertebrates between the two locations were found to be statistically similar, with pollution intolerant macroinvertebrates (Orders *Plecoptera* and *Trichoptera*) being found within the 14 trials, even though the pH was observed to be on the upper limit (8.4). This indicated that the portion of the Stevens Creek sampled was likely to be healthy and relatively free from potential NPS pollution from the surrounding suburban area, boding well for the steelhead trout residing within it.

INTRODUCTION

The quality of creeks and rivers are crucial to the well-being of freshwater ecosystems, for most organisms low on the ecosystem's food chain, more specifically microbes, macroinvertebrates, and algae. Of the macroinvertebrates that can be found within aquatic systems, species in the Orders *Ephemeroptera*, mayflies, *Plecoptera*, stoneflies, and *Trichoptera*, caddisflies, tend to be used as bioindicators, as they have been found to be sensitive to pollutants compared to other organisms (1). However, sources have varying estimates on the optimal conditions for these specific macroinvertebrates, with one stating that a pH range of 6.5-7.5 (2) would be ideal while another from 6.5-8.5 (3). In fact, the latter range is used as a guideline in interpreting the relative health of surface water bodies within the Bay Area, excluding the Pacific Ocean (4). Sensitive macroinvertebrate presence generally indicates good stream health, but the absence of them doesn't necessarily mean the water quality of the creek isn't optimal (1).

In addition to macroinvertebrates, steelhead trout (*Oncorhynchus mykiss*), which are federally designated under the Endangered Species Act (ESA) as threatened species within Northern California (5), reside within Stevens Creek, which is the creek as the focus of the study. Steelhead trout typically hatch in fast-flowing rivers and streams, with some remaining within the freshwater body (rainbow trout) while others moving into a saltwater body (a bay, ocean, etc.). The preferred diet of this organism includes "aquatic and terrestrial insects, mollusks, crustaceans, fish eggs, minnows, and other small fishes." Thus, it is seen that macroinvertebrates (which include aquatic insects) do constitute a large portion of the trout's diet. In addition, all trout return to spawn within freshwater, with any obstructions in natural streamflow presenting large problems to their migration (6).

The area of the Stevens Creek sampled is currently categorized as being under, "medium development" by the SCVURPPP's 2016 water assessment (Figure 1.1, 7). Since the study site was in a suburban setting, nonpoint source pollution, pollutants which originate from diffuse sources (agricultural fertilizer/pesticides and insecticides, oil and grease from urban runoff, sediment from construction sites, etc.), are factors potentially affecting the water quality in the creek (8). Furthermore, the construction of Stevens Creek Reservoir and spreader dams along Stevens Creek presented great challenges to the life history of the trout, resulting in reductions in the population size (9). In fact, of the 58 watersheds which drain into the San Francisco Bay estuary, only 24 still have steelhead trout residing within it (6). These fragile freshwater ecosystems are also going to be continuously threatened as humans continue to urbanize in the future. In fact, the UN has projected that by the year 2050 68% of the human population can be living in urban areas, with China, India, and Nigeria making up 35% of the projected increase (10). Numerous studies have linked this rapid urbanization to the degradation of creeks and rivers around the world, from the Huangpu River (1947-1996) in China (11), to the Han River (1960-1970) in South Korea (12), and the water system within Bucharest, Romania (13). This further increases the need to investigate whether other biological stressors may have any additional impact on these trout through a qualitative analysis of macroinvertebrates. This led to the following research question being postulated: How is the water chemistry/flow within Stevens Creek representative of the diversity and abundance of macroinvertebrates within the creek and the resulting health of the creek itself? The hypothesis for this project was that in areas with suitable water conditions, defined as the pH range from 6.5-8.5 (due to its function as the guideline for interpreting surface water health in the local area), there would be a larger abundance and diversity of macroinvertebrates.

METHODS

The test site location was the portion of Stevens Creek which ran through Blackberry Farm. Two areas, one upstream (Location B) and the other downstream (Location A) relative to a rapids area within Blackberry Farm, were ultimately decided upon. The creek bed in the upstream area was mostly filled with boulders, with the streamflow being slow relative to downstream. Further down the creek in this area, the water depth had decreased and the creek bed consisted of more small rocks and pebbles. Two of the trials at the upstream area were conducted in this location. The downstream area's streambed was also filled with boulders throughout all of the trial sites, but the depth of the creek remained consistent throughout the trials conducted at the location. The streamflow was also faster in this location relative to the upstream location.

Seven trials were performed at different places within each of the two locations, collecting both water chemistry and macroinvertebrate data. For water chemistry, the TDS content and temperature of the creek were both measured using a TDS meter by dipping the meter into the creek until the measurements for each had stabilized. Nitrate, nitrite, total hardness, total chlorine, total alkalinity, and pH measurements were recorded using water quality test strips (Tetra EasyStrips 6-in-1). For each trial, a strip was dipped into the testing location for one second, then placed onto a flat surface for one minute. After, a picture was taken of the test strip for analysis later using the freshwater comparison chart provided with the test strips.

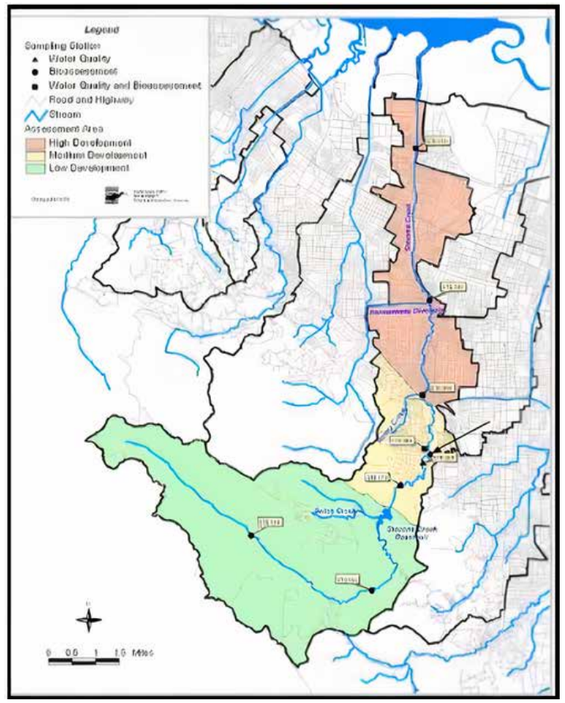

Figure 1.1: Levels of development for the Stevens Creek (SCVURPPP FY05-06 Monitoring Report). Sampling location in this study is depicted by the black arrow.

Figure 2.1: Final kicknet design.

To make the kicknet for sampling macroinvertebrates, first a 48 in. x 37 in. window screen was cut from the 48 in. x 99 in. MAGZO original product. Then, two 0.4 in. x 0.4 in. x 59.75 in. wooden dowels were lain parallel to each other on either side of the 39 in. side for the window screen, with one end of both of the dowels matching up with the corners of the window screen. After rolling the window screen one rotation around the dowels, utility wires were used to secure the rods in place. Finally, the remaining two 0.4 in. x 0.4 in. x 46 in. wooden dowels were lain parallel to each other on either side of the 48 in. side for the window screen, and the same method was used to secure these in place. This ensured the stability of the kicknet as a whole. The tips of these two dowels were trimmed off, and the final product resembled a rectangle with the top part of the two rods that were attached to the 39 in. side of the screen serving as handles (Figure 2.1). Then, the kicknet was placed a foot and a half downstream of the testing point, and the rocks and sediment on the creek bed at the point of sampling was disturbed continuously for two minutes. This was done along the creek bed parallel to the length of the kicknet (48 in.). After this period of time the kicknet was lifted out of the creek, and macroinvertebrates that were caught in the kicknet were located and photographed.

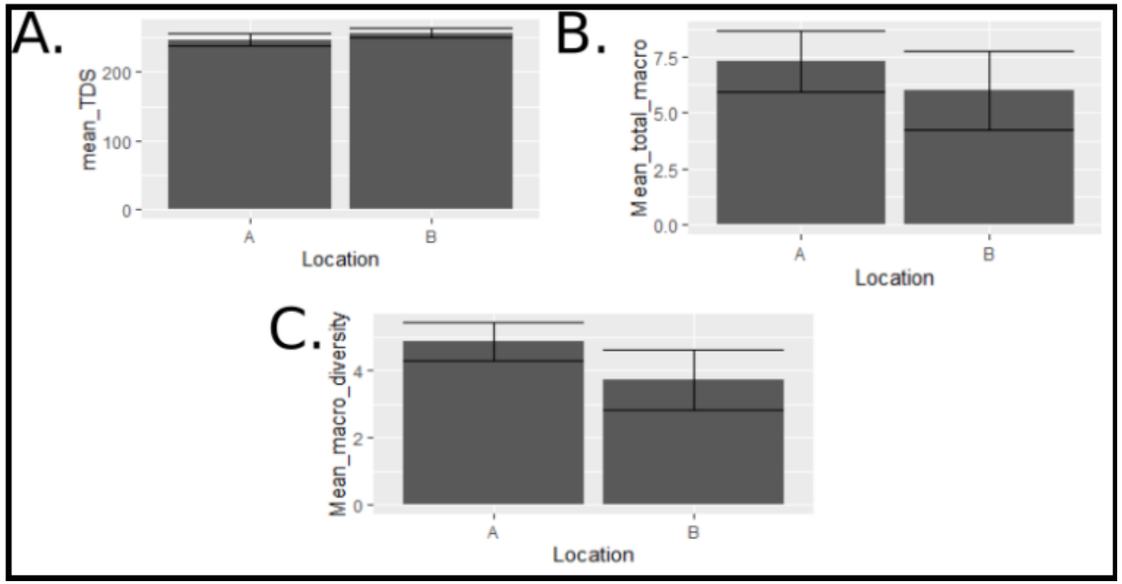

Figure 3.1: Bar graphs showing the means of TDS (A.), macroinvertebrate abundance (B.), and macroinvertebrate diversity (C.) measured at locations A and B within the Blackberry Farm segment of Stevens Creek. Error bars represent the standard error of the datasets.

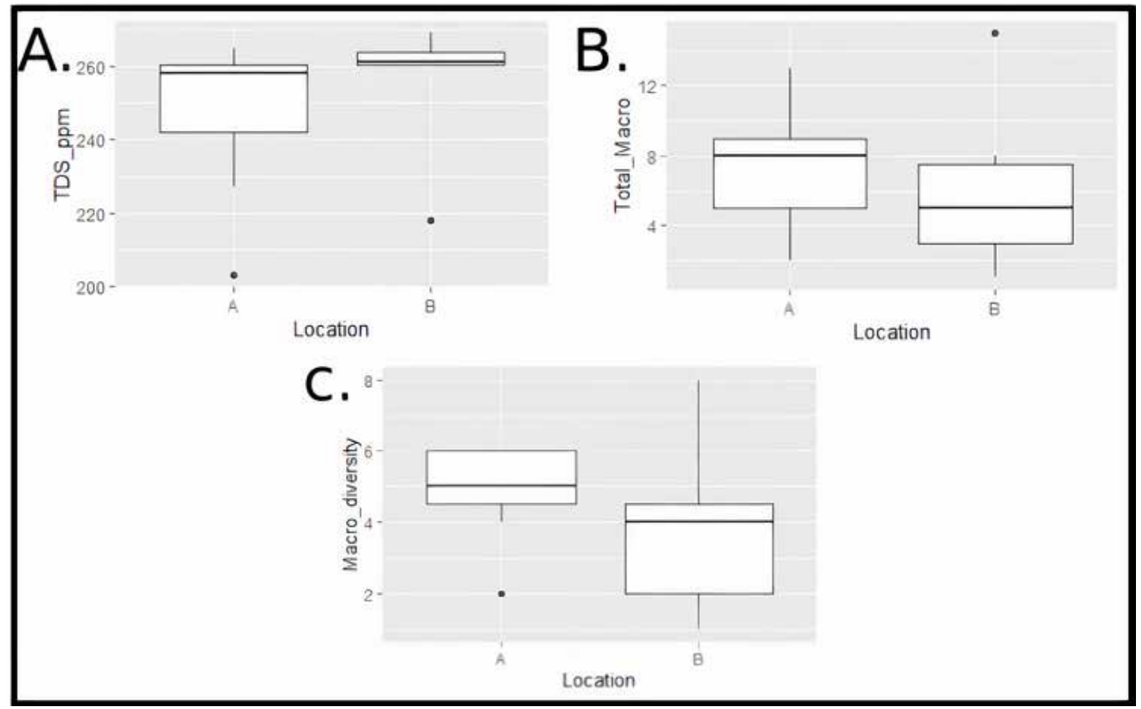

Figure 3.2: Box and whisker plots of TDS (A.), macroinvertebrate abundance (B.), and macroinvertebrate diversity (C.) sampled at locations A and B within the Blackberry Farm segment of Stevens Creek.

These photos were later analyzed to estimate the macroinvertebrate abundance and diversity on the order level, as the identification to the specific species level required expert guidance. The macroinvertebrates photographed were identified using a macroinvertebrate identification sheet (https://www.lvwash.org/assets/pdf/resources_research_macroinvertebrate.pdf). From there, the mean TDS, mean macroinvertebrate abundance, and mean macroinvertebrate diversity for all of the trials were calculated, with each category's means being placed in separate bar graphs with standard error bars (Figure 3.2). A table was then generated containing the means, standard deviations, n-values (number of trials conducted), standard error, and upper and lower limits for each of the categories (Figure 3.1). Additionally, a box and whisker plot was created for each of the three variables as well to visualize the spread of the data (Figure 3.2). Finally, a t-test was run for each of the three variables, and the resulting p-values were generated.

RESULTS

The segment of Stevens Creek sampled contained no nitrate or nitrite, but a total hardness and total alkalinity of 300 ppm were observed in each of the fourteen trials. The pH of the creek was 8.4 across all of the trials, while chlorine levels fluctuated between

Location	mean_TDS	mean_total_macro	mean_macro_diversity	sd_TDS	sd_total_macro	sd_macro_diversity	se_TDS	se_total_macro	se_macro_diversity
A	247.29	7.29	4.86	23.27	3.59	1.46	8.8	1.36	0.55
B	256.71	6	3.71	17.34	4.69	2.36	6.55	1.77	0.89

Table 3.1: Summary table depicting the means, standard deviations, and standard errors for TDS, macroinvertebrate abundance, and macroinvertebrate diversity sampled at locations A and B within the Blackberry Farm segment of Stevens Creek. n=7 across all variables within locations A and B.

0.1 and 1 ppm within the fourteen trials conducted. There was also an overall higher mean TDS in the upstream location B than the downstream location A, higher mean macroinvertebrate abundance in the downstream location A than the upstream location B, and a higher mean macroinvertebrate diversity in the downstream location A than the upstream location B (Figure 3.2). However, between the two areas of Stevens Creek there wasn't a statistically significant difference in mean TDS, mean macroinvertebrate abundance, or mean macroinvertebrate diversity (p=0.4081987, 0.576118, 0.3017922 respectively). Macroinvertebrates spanning from stoneflies and caddisflies (pollution intolerant) to damselflies (in between group) to leeches and midges (pollution tolerant) were identified from the pictures taken.

DISCUSSION

The total hardness measured in all fourteen trials was on the higher end of the measurement scale (in the "very hard" category), indicating that there was a large amount of dissolved solids, mainly calcium and magnesium, present within the creek (14). However, the measurements were also consistent with the levels typically found in surface waters in California (100-300ppm hardness, 15). The total alkalinity was also found to be "high" in all trials, implying that the creek had a relatively large buffering capacity against inputs of acid (16). The pH of the creek was basic, and leaned greatly towards/exceeded the upper limit of tolerance for mayflies, stoneflies, and caddisflies, using the threshold issued by Waterboards in California (6.5-8.5). Finally, total chlorine measurements fluctuated between "safe" and the lower end of the "danger" category.

The macroinvertebrates photographed spanned all three categories in the identification chart according to pollution tolerance. This indicates that even though there were pollution tolerant macroinvertebrates such as leeches and midges within the creek, pollution intolerant and very sensitive stoneflies and caddisflies were also present, meaning that the conditions within the stream were likely healthy for sensitive macroinvertebrates were able to survive within it. This is also despite the relatively high pH present, boding well for predators within the creek, including the endangered steelhead trout. The notion that potential sources of NPS pollution within the section of the creek sampled (which was located within an area under medium development) may be mostly nonexistent is also supported.

Also, the absence of statistically significant differences between the two locations pertaining to TDS, macroinvertebrate abundance, and macroinvertebrate diversity indicated that these variables remained similar between the two locations, even when a potential disrupting factor (the rapids) separated the two locations. This signifies that the results of the qualitative analysis of these aspects of the creek could be applied to a much broader portion of the creek than just the two locations where the sampling occurred.

Limitations of this study include the water quality test strips used, which used a color code system to determine the measurements of different aspects of water quality. It is this reason that the readings of these test strips were highly subjective, and the color displayed for each of the water chemistry variables could have been affected by the differing amounts of sunlight exposure between trials. Another limitation for the test strips was that the freshwater comparison chart used to decode the colors on the test strips had discrete color codes, meaning that shades which didn't quite match with those present on the chart had to require estimation. Furthermore, the chart also didn't account for values of the water quality variables which were higher than the color codes present, resulting in uncertainty surrounding the exact value of water quality variables when they fell on the upper color codes. For example, the measured pH had a value of 8.4 using the freshwater comparison chart, but it was also the highest value the chart contained, so the actual pH of the creek could have been higher than the chart's capacity without the test strip showing otherwise.

Next steps for this research would include repeating the water quality testing and macroinvertebrate sampling at more locations within Stevens Creek, and using the same analysis of that data to gain an even better understanding about the water chemistry of Stevens Creek as a whole, as well as the health of the creek through the type and abundance macroinvertebrates found. Also, a historical approach in analyzing the health of Stevens Creek would place these recorded measurements in relation to previous measurements done, offering a point of reference in seeing if the health of Stevens Creek had improved or declined over the time period of study. Finally, a historical analysis of a similar layout of other surface waters within the Bay Area can offer insight into the effects of urbanization within the Bay Area as a whole on the health of these surface waters and freshwater ecosystems within the region.

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ACKNOWLEDGEMENTS

I would like to take this moment to thank Spencer Eusden for putting together, running, and assisting with the Headwaters Fall Digital Research Program, and for creating a fun environment every lesson on Tuesdays. I am also grateful for the other students who were also a part of this research program who helped me find a research topic and peer edited my manuscript. Finally, I am eternally in debt to the Headwaters Science Institute and its donors for making this research program possible in the first place.

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The Effect of Aquatic Plants on Limiting Freshwater Acidification

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ABSTRACT

As our dependence on fossil fuel combustion solidifies, many environmental impacts of carbon emissions are exasperated, including freshwater and saltwater acidification. These processes result in acidifying water ecosystems, invoking many negative changes in ecosystem conditions for organisms. Existing research identifies the potential of aquatic plants on limiting ocean acidification. However, research relating to freshwater acidification is minimal, so I attempted to explore the impact of aquatic plants, *Anubias Congensis* and *Anubias Nana*, on minimizing changes in pH in my local ecosystem in Livermore, California. To examine this hypothesized effect, I exposed creek water samples with the selected aquatic plants and without plants to dry ice to stimulate carbon emissions and monitored the pH over an 8 hour period. I hypothesized that the pH of the water samples with aquatic plants in them would have a smaller change in pH than water samples without aquatic plants. The data obtained was utilized to quantify the capacity of aquatic plants to serve as a buffer in limiting pH. Nearly statistically significant differences were identified between the water samples with plants and without using a t-test. There was approximately a 25% increase in the observed molar hydrogen ion concentration for the water samples with plants in comparison to those without plants, suggesting aquatic plants have the potential to limit acidification to some extent. These findings can guide future research to further understand the viable partial solution of aquatic plants in combating freshwater acidification in varying ecosystems.

INTRODUCTION

TAs our world becomes more reliant on fossil fuel combustion for energy and transportation, the impacts of carbon pollution on the environment will only continue to drive many environmental issues. Carbon emissions are on the rise as the EPA estimates an increase in 5.9 percent since 1990. These emissions are trapped in the atmosphere, which allows bodies of water to absorb carbon dioxide pollution, fueling freshwater and saltwater acidification.

Acidification occurs when multiple chemical reactions take place between water and carbon dioxide, resulting in an increased concentration of hydrogen ions or a decreased pH level (1). Since the industrial revolution, the pH of the ocean has dropped from 8.2 to 8.1, representing a 25% increase in acidity (2). Ocean acidification has many implications on saltwater ecosystems as organisms require specific pH conditions to prosper in, meaning that when these conditions are manipulated, they face a wide range of complications. These negative implications primarily affect calcifying organisms that rely on their calcium carbonate shells and skeletons for structure and protection as those carbonate atoms are used in chemical reactions with carbon dioxide instead of calcification (3). Growth in organisms is impaired as changes in acidity forces organisms to use more energy for maintaining their body fluid at the expense of other body functions (4).

Research from 2016 suggests that specific aquatic plants like kelp and eelgrass have the potential to effectively minimize the impacts of ocean acidification. A researcher examining the long term impacts of ocean acidification at Cheeca Rocks in Florida, found a 0.38 difference in pH levels between inshore waters with much seagrass growth and the average Florida reefs, highlighting the potential of aquatic plants in combating ocean acidification (5).

While research exists in relation to ocean acidification, research pertaining to aquatic plants in relation to freshwater acidification remains very limited. Unlike saltwater, freshwater ecosystems are affected by many varying conditions that lead to freshwater acidification like acid rain, nutrient runoff, decomposing matter, and anthropogenic pollutants in addition to carbon dioxide emission, meaning freshwater acidification varies from ecosystem to ecosystem (6, 7). Because of this lack of research, I was interested in discovering if a similar effect of aquatic plant life on acidification existed in a freshwater ecosystem in my local area.

In the east bay of Northern California, namely Livermore, the freshwater ecosystem I would be examining is impacted mostly by the proximity to human developments like vehicles or buildings, meaning the ecosystems I was examining may potentially be exposed to carbon dioxide from vehicle or building carbon emissions. Consequently, I attempted to mimic these carbon emissions using dry ice in the experiment that I would perform.

Based on the background information I had about ocean acidification, the research I hoped to conduct was created with the aim of answering the question, "Can plants serve as a buffer in limiting acidification in freshwater ecosystems?". Given that aquatic plants had the capability of limiting acidification in marine ecosystems, I hypothesized that the pH of the water samples with aquatic plants in them would have a smaller change in pH than water samples without aquatic plants as I expected the plants to absorb some of the carbon dioxide.

METHODS

The effect of aquatic plants on acidification was examined by exposing water samples with and without plants to carbon dioxide and monitoring the pH levels over an 8 hour period.

Water was collected from the Arroyo Valle creek in Livermore, California and tested for water quality. The water quality test included testing the water's nitrate, nitrite, total hardness, total chlorine, total alkalinity, TDS, temperature levels and pH using Tetra Easystrips (nutrient test strips), a Lxuemlu Professional TDS and temperature meter, and a Vantakool pH meter. Aquatic plants of *Anubias Congensis* and *Anubias Nana* species were selected

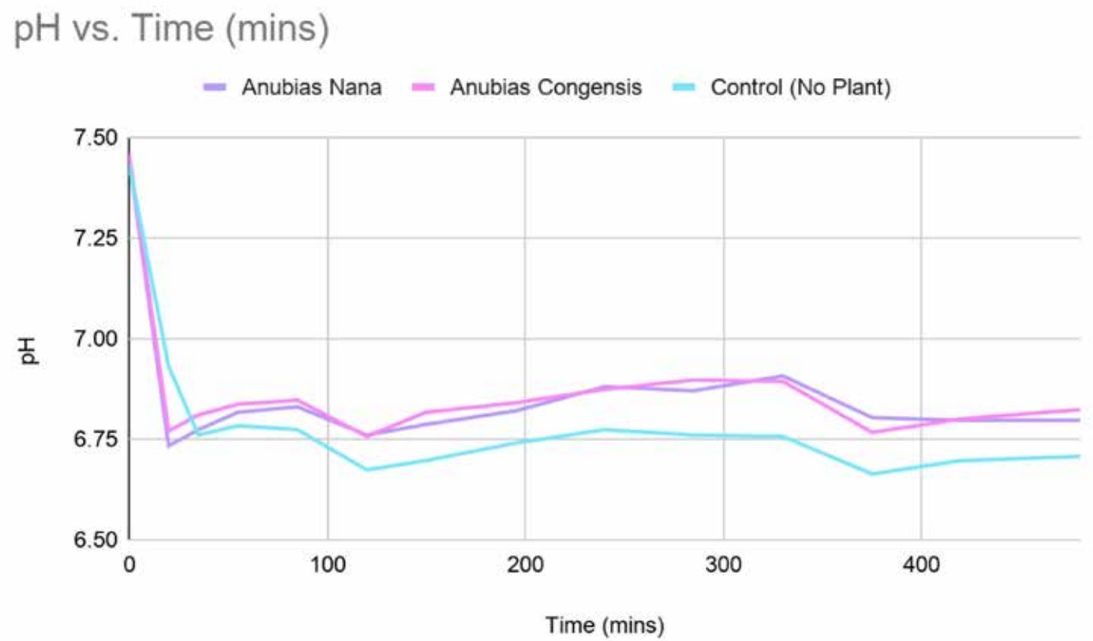

Figure 1: line graph of mean pH of 3 samples per plant throughout an 8 hour period with 0 minutes representing pH before. In general, the control pH was lower than the *Anubias Nana* and *Anubias Congensis* pH levels, which were both comparative to each other.

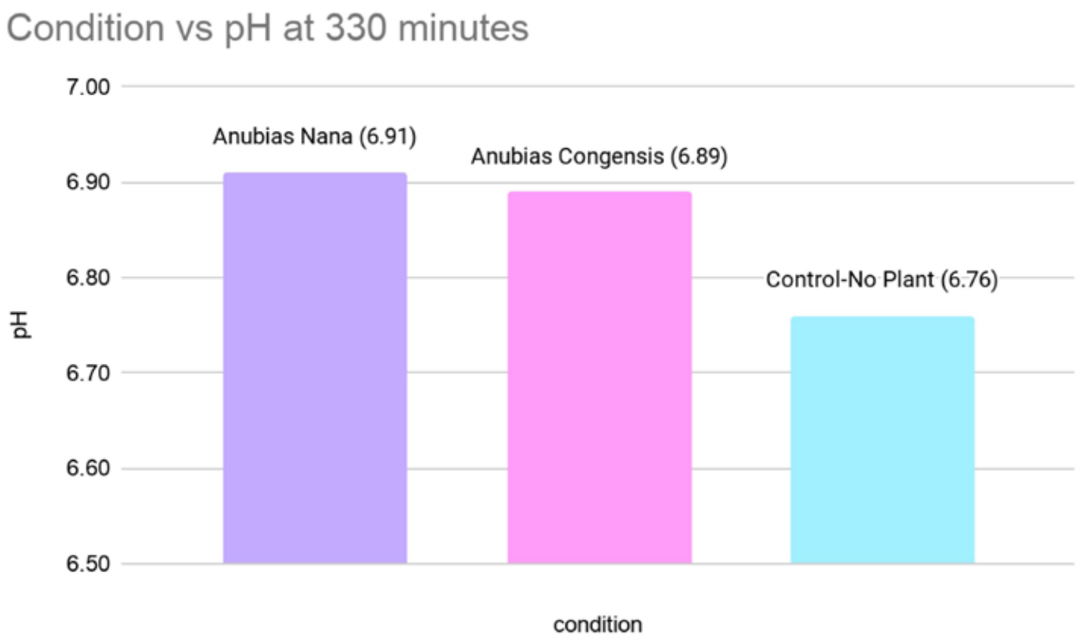

Figure 2: bar graph comparison of mean pH of 3 samples per selected plants at 330 minutes based on plant type. At 330 minutes, a p-Value of 0.022177 was obtained using a t test of the *Anubias Nana* and *Anubias Congensis* vs the control.

for the experiment because of convenience. In this way, all materials were acquired and all preliminary data was recorded for my experiment.

In order to begin the data collection process, I set up my experiment using the water samples collected and the selected plants. These plants were exposed to direct sunlight for approximately an hour on the morning of the experiment in order to ensure photosynthesis was taking place. Measurements including mass of plants and volume of water sample were recorded, while observations of each sample and plant were taken. Each preselected plant was placed into a sample container, while a select few samples remained without plants to serve as the controls. 460-480 mL of water was added to each sample vial and the water quality test was repeated for each sample. The temperature was recorded to ensure the water temperature was optimal for the aquatic plants selected. Using the pH meter, each water sample's pH was recorded.

Approximately 0.75 grams of dry ice were massed using a Weigh Gram Digital Pocket scale and placed into each water sample, while the cap of each sample was screwed on partially to allow partial ventilation for safety purposes. The pH of each sample was recorded every 15 to 20 minutes for the first hour. In the following hours, the pH of each water sample was measured every 30 to 40 minutes. A total of 9 trials were performed simultaneously, with 3 containing *Anubias Nana* aquatic plants, 3 containing *Anubias Congensis* aquatic plants, and 3 serving as controls without any plants.

Using a t-test, p values were obtained that compared trials with plants and trials without plants at each recorded time to determine the statistical significance between the two groups. A t-test was selected for the data analysis rather than an ANOVA test due to limitations in sample size, meaning both *Anubias Congensis* and *Anubias Nana* plants were grouped together as the plant group. In order to quantify how much aquatic plants could limit acidification, the recorded pHs were used to calculate the increase in molar hydrogen ion concentration for trials with plants and without plants.

RESULTS

In general, the average pH levels were slightly higher throughout the trial time for both water samples with *Anubias Nana* aquatic plants and *Anubias Congensis* aquatic plants in comparison to water samples without plants. The average pH levels over an 8 hour period for each plant condition are shown in figure 1. An average difference of 0.11 pH units between the average pH of the samples with plants and the average pH of the samples without plants after 120 minutes quantifies the depicted difference seen in figure 1. Figure 2 portrays the comparative average pH levels of the *Anubias Nana* and *Anubias Congensis* plant trials at 330 minutes. Contrastingly, the control pH level is about 0.10 pH units lower than both plant trial averages.

Given p-values of close to 0.05 or below 0.05 as shown in Figure 3 for pH values after 120 minutes, the data obtained from this experiment is nearly statistically significant, especially given that only three trials were performed per each plant type.

Time (mins)	p-Value of plant vs no plant conditions
0	0.220094
120	0.084806
150	0.058886
195	0.087518
240	0.066264
285	0.040548
330	0.022177
375	0.037208
420	0.048678
480	0.044657

Table 1: table of p-Values of plants vs no plant samples for each time using a t test from 0 minutes to 480 minutes. 0 minutes is indicative of the pH levels recorded before exposure to carbon dioxide. N= 3 no plants, 6 plants (3 *Anubias Nana*, 3 Anubias Congensis). Red indicates a not statistically significant p value, yellow indicates an almost statistically significant p value, and green indicates a statistically significant p value.

Based on the p-values in Figure 3, there was 91.5% to 97.8% that the results were not obtained by random chance, indicating that there was a significant difference in pH levels between water samples with plants and without plants.

DISCUSSION

The variation in pH between water samples with aquatic plants and without aquatic plants can likely be attributed to the consumption of carbon dioxide during photosynthesis. When the water samples are exposed to carbon dioxide, their reaction forms carbonic acid that will decompose into bicarbonate and carbonate, which releases a proton in the process, lowering the pH of the water as a result. However, if the plants absorb some of the carbon dioxide that would typically be utilized in that chemical reaction, the plant can serve as a buffer in preventing a change in pH to the typical extent. Aquatic plants require carbon dioxide to perform photosynthesis to produce glucose and oxygen, meaning the water samples have a lower supply of carbon dioxide that could possibly be used in reactions that would decrease the pH level of the sample. Therefore, it is reasonable to explain the difference in pH levels by plants absorbing the carbon dioxide based on fundamental chemical reactions.

An important aspect of my experiment is identifying the influence of the water quality of each water sample on the experiment conducted. Since all samples have water collected from the same location, the water quality likely has a similar influence on the results. In general, nitrates, nitrites, total chlorine, total hardness levels, and temperature have minimal impacts on changes in pH when exposed to carbon dioxide. Total alkalinity and pH are strongly impacted by each other as alkalinity is a measure of the water's ability to resist a change in pH based on the ability of bicarbonates, carbonates, and hydroxides to neutralize acids. Specifically, the water from the sample site had a total alkalinity measurement of 240 ppm, which is demonstrative of a somewhat high alkalinity level as the average total alkalinity for freshwater ecosystems ranges from 20 ppm to 200 ppm (8). A high total alkalinity indicates that the water samples had a strong ability to stabilize the pH level of the water as it was resistive to changes in pH such as exposing the sample to carbon dioxide. Due to a higher than average total alkalinity level, the freshwater ecosystem I was examining may not be reflective of all freshwater ecosystems. Therefore, water samples of other freshwater ecosystems could

possibly have more drastic changes in pH when exposed to carbon dioxide if they had lower total alkalinity levels.

Based on a difference in increase in mean hydrogen ion concentration between trials without plants and with plants of 3.98 × 10-8 M or 0.11 pH units, plants have the potential to minimize freshwater acidification to some extent. Comparably, similar research conducted with seagrass meadows in coral reefs in the Indo-Pacific Ocean and Halimeda Cuneata alga in marine coastal ecosystems in Brazil found variations in pH up to 0.38 pH units and 0.18±0.08 pH units, respectively, for limiting saltwater acidification (9, 10). In this experiment, the aquatic plants were able to limit freshwater acidification by 3.98 × 10-8 M, which is approximately 25% of the increase in the observed molar hydrogen ion concentration for the water samples with plants. However, there was still a significant change in pH observed with aquatic plants indicated by the 1.198 x 10-7 M increase in mean hydrogen ion concentration for water samples with plants. As shown in figure 1, all water samples began with a pH level around 7.45, but none of the mean samples were able to reach that original pH level throughout the experiment after their carbon dioxide exposure. Therefore, the aquatic plants were not able to consume all carbon dioxide from their exposure, suggesting that aquatic plants can only minimize acidification rather than impede acidification. Because of this observed potential in aquatic plants, they are a viable buffer for limiting the rates of acidification in freshwater ecosystems driven by carbon emissions to a minimal extent.

In order to further examine the potential of aquatic plants limiting acidification, long term and wide scale research is required, which could possibly have substantial outcomes in combating acidification. Since the aim of my research was to mimic the effects of carbon emissions, any changes in pH from carbon emissions would occur over a long period of time. Therefore, conducting research that monitors pH levels over a period of years would be more significant for understanding the role aquatic plants play in relation to long term freshwater acidification. Exploring the effect of carbon emissions on multiple samples from different freshwater ecosystems will provide more clarity about variation in pH changes between ecosystems. A limitation of my experiment was the lack of multiple trials, meaning having a larger sample size would increase the meaningfulness of the statistical tests in determining if a difference between water samples with plants and without plants exists. With further research, aquatic plants may be a viable partial solution minimizing drastic changes in pH levels in freshwater ecosystems. Therefore, as rates of anthropogenic carbon emissions surge, identifying methods, including aquatic plants, to mitigate the implications of climate change is vital for sustaining our planet.

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Eggshell consumption in different reproductive stages and broods of the Western Bluebird, *Sialia mexicana*

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ABSTRACT

Calcium is an essential micronutrient to egg production and reproductive success in birds. Its availability at the start of the reproductive cycle is especially important to passerines since they do not store calcium when not reproducing. To date, no data has been published to illustrate whether Western Bluebirds would consume provisioned eggshells, at what phases and to what degree in the reproductive cycle consumption might occur, and whether the amount consumed would vary between broods in a single season. I hypothesized that Western Bluebirds would consume the highest amount of eggshells during nest-building and egg-laying and that consumption would be greater during the first brood. Western Bluebirds breeding in nest boxes hanging in trees were provided eggshells in small containers attached to the roof of the box. Comparison of average consumption between reproductive stages was significant. Greatest consumption was seen during nest-building, egg-laying, and incubation, and less during the nestling phase. This was likely in preparation for and to replenish calcium needed during egg-laying in both broods. Average consumption between both broods produced by a single breeding pair was compared and was not significantly different. However, there was significant variation in the amount of consumption between breeding pairs. This high variation suggests that birds have different strategies for obtaining and consuming calcium.

INTRODUCTION

Calcium is a vital mineral for wildlife, including reptiles (1), mammals, and birds (2). It is essential for biological processes such as growth and maintenance of the skeleton and teeth, eggshell development, and muscle contractions (3). Calcium can come in many forms in the environment, including mollusk shells, eggshells, calcium-rich soil, and vertebrate bones (2). Recently, available calcium sources, e.g calcium-rich soils, have become increasingly scarce for birds, especially near urbanized areas due to increased acidity (4, 5).

For birds, calcium plays a crucial role during the breeding season in the form of egg development and initiation of breeding (6). Great Tits (*Parus major*) provided with snail shells or eggshells abandoned the nest less frequently and had fewer shell abnormalities as compared to non-supplemented females in a calcium-poor environment (4). In addition, European Pied Flycatchers (*Ficedula hypoleuca*) and Great Tits provided with snail shells or eggshells exhibited increased egg volume, eggshell thickness, and earlier start of breeding compared to supplemented birds in calcium-deficient soils (7). Calcium can also affect reproductive success. For example, calcium supplemented house wrens (*Troglodytes aedon*) laid more eggs per clutch than non- supplemented birds. (8).

Obtaining sufficient calcium is a time-consuming behavior, even in calcium-rich environments (5), so there may be an incentive for birds to consume readily available calcium instead of searching for other sources. Songbirds typically only retain enough calcium for routine metabolic needs and do not store calcium in their medullary bones as with other species (9, 10). External sources of calcium are also crucial to insect-eating birds as arthropods are not a sufficient source of calcium for eggshell formation, amounting to about 5-10% of the needed calcium even in calcium-rich areas (11). Therefore, it is likely birds may need additional calcium via external sources during the breeding season. One study of Eastern Bluebirds (*Sialia sialis*) reported that females sometimes eat eggshells of their own hatchlings (12). The same study reported that some

remove broken eggshells from hatchlings and deposit them some distance from the nest. The clutch size of first broods (4.94 eggs) is usually significantly larger than second brood clutches (4.06 eggs) (13), and more eggs would require more calcium consumption for eggshell formation. However, it is unknown when in the breeding process birds need to consume this additional calcium and if consumption is greater in one the broods.

External sources of calcium are then essential for avian reproductive success, especially during egg development. While research has been done on calcium supplementation in passerines and, more specifically, insect-eating birds and its effects on egg success, differences between consumption in different reproductive stages and broods have not been studied. Therefore, I investigated how eggshell consumption differs throughout the reproductive stages and broods of the Western Bluebird (*Sialia mexicana*). This passerine species is ideal for the study because they readily use nest boxes and reproductive stages can be easily observed and identified. By providing Western Bluebirds with supplemental eggshells throughout their entire breeding season, I hypothesized that most eggshell consumption by the Western Bluebird would take place before and during egg-laying because the female would be preparing for and replenishing calcium; and, more consumption would be seen during the first brood compared to the second because a greater clutch size in the first brood would require more calcium.

METHODS

This study was conducted from March to August 2020 on 52 nest boxes in 9 cities in Orange County, California. Volunteers from the Southern California Bluebird Club assisted in data collection. Chicken eggs were commercially purchased, rinsed, air dried, and baked at 121°C for 10 minutes to sterilize the eggshells and safeguard the birds' health. After eggshells cooled, they were crushed to small pieces, roughly 0.25 cm^2.

Eggshells were provided in a small white plastic container with a dime-sized hole cut on the clear lid to allow for easy access of eggshells by bluebirds but to limit larger birds and possibly mammals from tampering with eggshells. A thumbtack was used to create four evenly spaced pinholes on the bottom of the container as drainage. Western Bluebirds were observed investigating and consuming the eggshells. Volunteers were requested to not put mealworms on or near the box to limit variables and avoid attracting other birds or animals. As a general practice, monitors avoid putting boxes where non-avian predators are known to exist. No other bird or mammal was seen on the boxes and eggshell containers were not altered, damaged, or missing. In boxes where no birds nested (n=6), no consumption greater than the margin of error (0.05g) was seen. Further research, possibly with cameras positioned to watch the top few nest boxes throughout the breeding season, is needed to prove that no other bird or animal consumes or tampers with the eggshells.

The container was affixed to the top of each nest box using two pieces of velcro. Each container had 5 g of eggshells. Eggshell consumption was measured each week using a scale (Brifit Digital Mini Scale with a precision of 0.01 g) supplied to each volunteer. Afterwards, the eggshells were replenished to 5 grams. Although bluebirds were not expected to consume this much in a single week, a surplus ensured that birds would have more than enough to consume. Western Bluebird nest boxes were monitored to determine the reproductive stage (based on nesting material, laying of eggs, hatchlings, and evidence of successful fledging) and nesting success. Nests were checked between dawn and dusk. All volunteers used standardized nest boxes with dimensions pre-established by the North American Bluebird Society, Cornell Nestwatch Project, and National Audubon Society to keep out invasive species (Bluebird Nestbox Plans, North American Bluebird Society, 2020).

Statistical Analysis

For statistical modeling, all tests were run with MATLAB (R2019b, MathWorks, Natick, MA, USA). Individual nests that consumed an average amount of eggshells below the margin of error (0.05) throughout the entire period of data collection were excluded from the analysis (n=4). Sample sizes for each group are outlined in Table 1. Significance was taken at $\alpha = 0.05$. A Bonferroni correction was applied on the p-values. Three sample groups were used. Brood 1 consisted of all nests in brood 1 (n=40), Brood 1s consisted of all nests in brood 1 with a corresponding brood 2 nest (n=13), and Brood 2 consisted of all nests in brood 2 (n=13). The five reproductive stages were lumped into nest-building, incubation and egg-laying, and nestlings and fledglings stages for the analysis.

Raw data was log-transformed. All models met assumptions of normality using an Anderson-Darling test. Individual variation in eggshell consumption of nests in each of the sample groups was tested using One-sample T-tests. A Paired T-test was used to compare average eggshell consumption between the two broods within nests and two broods across different stages. Using a two-sided Wilcoxon signed rank test, average eggshell consumption between stages was compared.

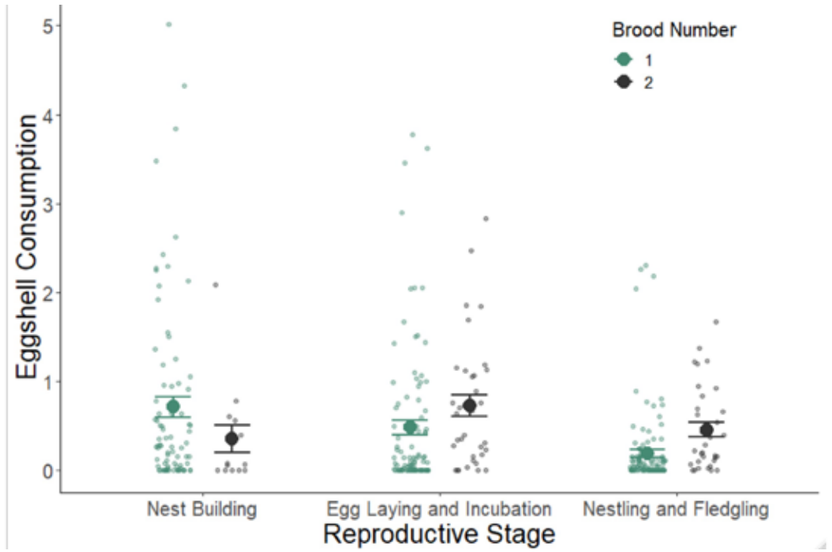

Figure 1: Means of consumption in grams of different stages in the two broods with standard error bars and raw data overlaid.

RESULTS

Average consumption between nests in Brood 1 was highly variable (Table 2). Some individuals consumed almost no eggshells, whereas others consumed a moderate amount. A few individuals consumed very large amounts, up to 12 grams through the course of an entire brood.

Average consumption was not variable for Brood 1s nests (Table 2). This could be due to small sample size as Brood 1 nests were highly variable. There was high individual variation in the average amount of consumption in each nest in Brood 2 (Table 2). This is somewhat expected due to the high variation seen in Brood 1 (Table 2).

There was no difference in eggshell consumption between the two broods within nests (Table 2). Also, there was no difference in eggshell consumption between the two broods across different stages (Table 2). This indicates that brood number has very little if any impact on eggshell consumption.

Eggshell consumption between stages in Brood 1 were different (Table 2). There was no difference in average eggshell consumption between stages in Brood 1s and Brood 2 (Table 2).

DISCUSSION

Eggshell consumption of Western Bluebirds was measured across broods and stages to determine when birds would consume eggshells during the breeding season. The results showed that consumption differed between stages but not between broods.

Sample Group	Data included	Sample Size
Brood 1	all nests in Brood 1	40 nests
Brood 1s	nests with a corresponding Brood 2 data	13 nests
Brood 2	all nests in Brood 2	13 nests

Table 1: The data included and sample size of each sample group.

Test	Significance
Brood 1 individual variation	p-value < 0.001
Brood 1s individual variation	p-value = 0.0567
Brood 2 individual variation	p-value = 0.0153
Comparison between Brood 1s and 2	p-value = 1
Brood comparison within stages	p-values = 1 and 0.9531
Stage comparison within Brood 1	p-value = 0.0117
Stage comparison within Brood 1s	p-value = 1
Stage comparison within Brood 2	p-value = 1

Table 2: A summary of the statistical tests and results. A One-sample T-test was used to determine individual variation. Comparisons between broods used a Paired T-test; comparisons between stages used a two-sided Wilcoxon signed rank test. The p-values were corrected using a Bonferroni correction.

The results showed that there was high individual variation in the average amount of eggshells consumed and some variation in the timing in which the eggshells were consumed. Also, some birds chose to take no eggshells at all. This means that they may have relied on other sources of calcium. Other birds may have obtained a fraction of their needed calcium from the offered eggshells, but mostly obtained calcium elsewhere. Whereas a few birds could have heavily relied on the offered eggshells for calcium, as indicated by the few nests with high average consumption. This could imply that birds from each nest have different strategies in terms of when to consume calcium, which calcium sources to choose, and how much calcium is obtained from each source.

The birds' decision to choose a particular strategy may be heavily influenced by the pair's experience, the location of the nest box (which affects the availability of calcium and likely changes what and where calcium sources are), and individual choice. For example, some birds may tend to stay away from calcium provided by humans, whereas others consume offered eggshells because they may have limited sources. Birds with limited choices may choose to consume provided eggshells because they would otherwise lose valuable foraging time searching for calcium (14). Additionally, birds that reside in places with more acidic soils may choose supplemented eggshells. Some studies have shown that natural calcium sources can be severely reduced by increased acidity of the soil (15) and can cause birds to lose both the calcium-rich soil and snail shells as sources. Some sources of calcium may be more easily eaten and absorbed or have more calcium content than other sources (16, 17). Furthermore, consumption from a novel container high off the ground may have been a deterrent for some birds. This possibility can be extrapolated from one study (18) which illustrated that thrushes which typically forage on the ground show a tendency to seek calcium sources found on the ground when given a choice between containers on the ground or platforms. Further study is needed to determine under what circumstances birds are more inclined to consume human offered eggshells and which strategy, if any, is more successful than the others. Reproductive stage had a large effect on eggshell consumption. This is likely because the timing of consumption within a brood was of greater importance to the quality of the egg than it was to the specific brood in which the egg was produced.

The highest eggshell consumption was seen during nest building, egg laying, and incubation. Consumption seen during nest building was likely in preparation for the egg laying stage. It is reasonable to assume that consumption seen in egg laying was either to partially replenish calcium from the last egg laid or to prepare for laying the next egg. The low consumption seen in the nestling and fledgling stage may have been due to caretaking demands on the female. This provides a plausible explanation for an increase in eggshell consumption at the onset of a second brood. Coincidentally in the nestling phase, it is not well documented whether females (and males) feed their own eggshells to nestlings or if nestlings consume the eggshells themselves, in addition to incidental calcium present in arthropod prey which provide 5-10% of calcium needed for skeletal growth (11). Given the lack of available data from larger sampling, it is also unknown if females that lay a second brood consume a greater amount of eggshells during the nestling and fledgling stages of the first brood than was demonstrated in this small study.

Contrary to my second hypothesis, the brood number had little, if any effect on the average amount of eggshells consumed. The original hypothesis that greater consumption would be seen in brood 1 was based on a study showing that the average clutch size of first broods (mean= 4.94 eggs) is usually significantly larger than the average size of second brood clutches (mean = 4.06 eggs) (13).

Summary

Western Bluebirds mainly consumed calcium in the nesting, egg laying, and incubation stages. Brood number had little effect on when calcium was consumed. High individual variation was seen in consumption between nests, which implies that individual birds may have different strategies for obtaining calcium. This may be due to the birds' individual experience, the location of their nest box, and what other calcium sources are easily accessible.

ACKNOWLEDGEMENTS

Thank you to the Southern California Bluebird Club for providing funding and to the 12 volunteers who collected data for this project. Thank you Dr. Timothy J. Bradley for project advice and consultation. Thank you Dr. Matthew McHenry for providing resources for statistical analysis. Thank you to Kimball Garrett for reviewing the manuscript. Thank you to the Headwaters Science Institute, Megan Seifert, and Spencer Eusden for their guidance. Thank you to my mentors, parents, Lindsey and Cathy Groves, Lena Hayashi, Susan Gilliland, Trude Hurd, and my teachers, Holly Danielson, Kristopher Cook, and Oscar Flores for their support.

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