# Juniata College

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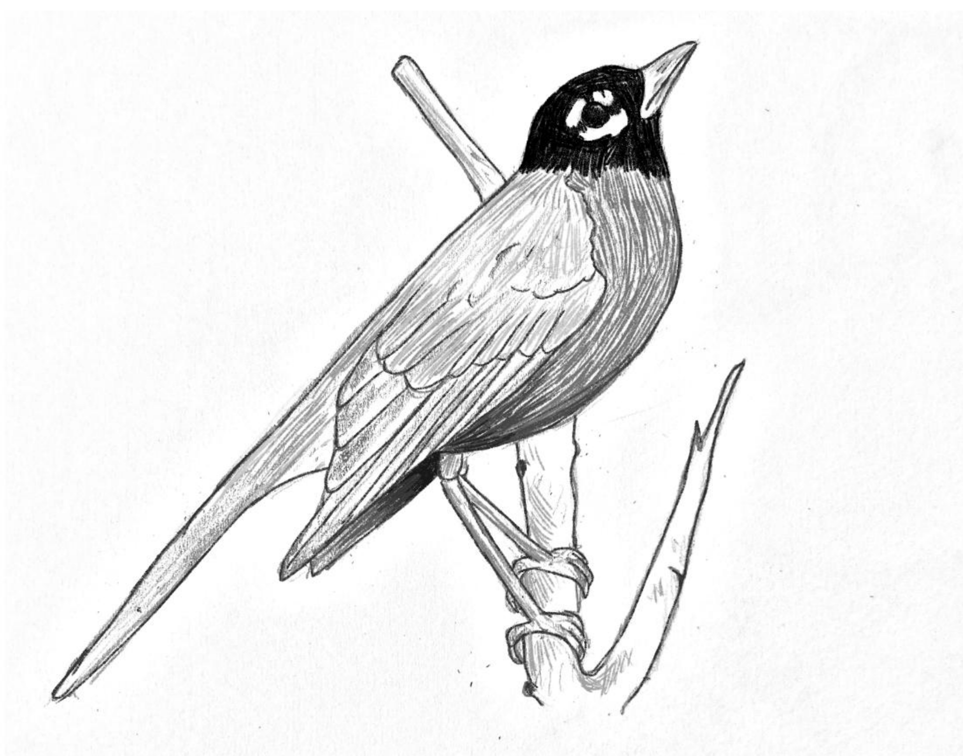
# Journal of Ecological Research



# Juniata Journal of Ecology

An annual journal of Juniata College undergraduate, graduate, and alumni research in ecology and environmental science

## **Editors**:



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## PURPOSE

The Juniata Journal of Ecology (formerly Journal of Ecological Research from 1998 - 2024) launched in 1998 as a lab component of Dr. Glazier's General Ecology course to expose Juniata students to the publication process and to archive past research projects. In 2025, we modernized its internet presence for free open-access and expanded the content beyond the General Ecology course to include any ecological research from Juniata undergraduate and graduate students and alumni. The journal focuses on research from the Mid-Atlantic and Appalachian regions, but also includes research from outside our region. The open-access policy makes the journal free for both authors and readers and increases accessibility. Journal operations are managed by a team of students with faculty mentors overseeing the project at Juniata College.

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# Journal of Ecological Research

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Cover illustration: American Robin, *Turdus migratorius*.

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# AMPHIPOD TRANSPLANT STUDY

Peyton Goodrich, Jocelyn Santos, Elizabeth Bailey, and Alexis Benner

## ABSTRACT

In this report, our research team conducted a transplant study to investigate the potential impact of water quality and the accumulation of pollutants on the survivorship of *Gammarus minus.* Given the sensitivity of this particular amphipod species to changes in the quality of water within its habitat, we hypothesized that heavy residential land use and traffic might lead to differential water quality in different sections of the Muddy Run stream, thereby affecting the survivorship of the amphipods. Specifically, ninety amphipods were transplanted in three different locations, with thirty remaining in their harvest location of Petersburg Spring and the other sixty being divided among two sites in Muddy Run, one directly outside the East Houses bridge and one the near Weis Markets parking lot. It was expected that the East Houses location would have higher pollution levels and thus lower survivorship due to heavier residential use and foot traffic. Over the course of two consecutive weeks, the survivorship of amphipods at each site was monitored by visiting once a week and counting the number of alive versus dead individuals. Our results revealed unexpected findings, with higher mortality observed among the amphipods at the Weis Parking site, contrary to our initial hypothesis. Despite this discrepancy, our study provides valuable insights into the complex interplay between water quality and the survivorship of amphipods in Muddy Run stream.

*Key words: amphipods, survivorship, transplant study, water quality*

## INTRODUCTION

Amphipods; small, elongated crustaceans within the order *Amphipoda,* are common in many aquatic ecosystems, including but not limited to springs, rivers, and lakes. The order *Amphipoda* contains many different families and genera of crustacean, but this study focuses specifically on the species *Gammurus minus.* Evidence exists to suggest that *Gammurus minus* is highly sensitive to changes in water quality, including dissolved oxygen, with a preference for high oxygen levels (Wetzel, 2001), and water temperature, with a preference for cold water (Wetzel, 2001). Previous studies have also shown that *Gammurus minus* is sensitive to the presence of several pollutants, and that survivorship can be heavily influenced by even the smallest levels of pollution (Lieb and Carline, 2000). As such, the presence or absence of *Gammarus minus* in a body of water can provide important information about water quality and ecosystem health. With this in mind, the objective of this study was to examine the survivorship of transplanted amphipods in three different locations: Petersburg Spring, a branch of Muddy Run directly outside of East Houses, and a branch of Muddy Run further downstream, near Weis. Specifically, our research team aimed to determine which location had the highest survivorship rate, and whether that survivorship was influenced by the accumulation of pollutants.

## QUESTION

Does water quality in different locations of Muddy Run affect survivorship of transplanted amphipods?

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## HYPOTHESIS

Null Hypothesis: Survivorship will not differ between sites.
Alternate Hypothesis: Survivorship will be lower at the East location due to point-source pollution from heavy residential use.

## MATERIALS AND METHODS

To investigate the potential impact of water quality on the survivorship of amphipods in Muddy Run stream, we conducted a transplant experiment that involved multiple steps. First, we visited three different locations, namely Petersburg Spring (control), East Bridge, and Weis Parking, and conducted basic water quality tests using a YSI multiparameter probe. Next, we collected a total of ninety amphipods from Petersburg Spring and distributed them into transplant containers. These amphipods were then divided into three groups, with thirty amphipods in each group: Petersburg, East, and Weis.

Over the course of two weeks, we returned to each group to count the survivorship of the amphipods. During each visit, we recorded the number of dead and alive amphipods in each group. After completing the data collection, we analyzed the survivorship of the amphipods in relation to the water quality data we had collected earlier. Specifically, we compared the survivorship rates of the amphipods at the different locations and correlated them with the corresponding water quality measurements to explore the potential effects of environmental factors on the survivorship of amphipods.

## TIMELINE

- Week 1: collect the amphipods, distribute them to each stream, do water quality testing at each stream
- Week 2: first survivorship test
- Week 3: second survivorship test
- Week 4: analyze results

## MATERIALS

- YSI multiparameter water quality probe (1)
- Transplant containers (90)
- Rubber bands (90)
- Nets (4)
- Buckets (3)
- Pipettes (4)
- Tray (1)

## FIELD SITE

- Petersburg Spring, which served as a control group habitat, is a relatively warm and neutral spring where the amphipods for the transplant study were collected.
- Muddy Run, East, is a relatively warm and neutral spring beneath a heavily trafficked area that is known for large amounts of pollutants and debris.
- Muddy Run, Weis, is the coolest and most basic spring, residing next to a grocery store parking lot and partially shaded by an overpass.

**Figure 1.**

*Bucket of transplant containers at the Petersburg spring.*

*Figure 2 was taken at the Petersburg spring.*

*Figure 3 was taken at Muddy Run (East).*

*Figure 4 shows a general representation of where we put transplants throughout Muddy Run. The blue line gives an approximate area of where Muddy Run runs and the yellow circles are approximately where we put our transplants.*

| **Date** | **Amphipods Alive** | **Amphipods Dead** | **Amphipods Missing** |
|---|---|---|---|

| | | | |
|---|---|---|---|
| 3/28/23 | 30 | 0 | 0 |
| 4/4/23 | 21 | 0 | 9 |
| 4/11/23 | 19 | 0 | 2 |
| **TOTAL** | 19 | 0 | 11 |

*Table 1 shows the survivorship of amphipods over the course of two weeks at Petersburg. On the date of 3/28/23, we put 30 amphipods into the spring, and after two weeks nineteen survived, eleven went missing, and none died.*

| **Date** | **Amphipods Alive** | **Amphipods Dead** | **Amphipods Missing** |
|---|---|---|---|
| 3/28/23 | 30 | 0 | 0 |
| 4/4/23 | 15 | 5 | 10 |
| 4/11/23 | 5 | 3 | 7 |
| **TOTAL** | 5 | 8 | 17 |

*Table 2 shows the survivorship of amphipods over the course of two weeks at Muddy Run (East). On the date of 3/28/23, we put 30 amphipods into the spring, and after two weeks five survived, eight died, and seventeen went missing.*

| Date | Amphipods Alive | Amphipods Dead | Amphipods Missing |
|---|---|---|---|
| 3/28/23 | 30 | 0 | 0 |
| 4/4/23 | 20 | 8 | 2 |
| 4/11/23 | 10 | 4 | 6 |
| **TOTAL** | 10 | 12 | 8 |

*Table 3 shows the survivorship of amphipods over the course of two weeks at Muddy Run (Weis). On the date of 3/28/23, we put 30 amphipods into the spring, and after two weeks ten survived, twelve died, and eight went missing.*

After Two Weeks

| Alive | Dead + MIA |
|---|---|
| 19 | 11 |
| 5 | 25 |
| 10 | 22 |

*Table 4. A contingency table with results from a two- week transplant study*

| Results | | | | | | |
|---|---|---|---|---|---|---|
| | Dead+MIA | Alive | | | | ***Row Totals*** |
| Petersburg | 11 (18.67) [3.15] | 19 (11.33) [5.19] | | | | 30 |
| Muddy Run East | 25 (18.67) [2.15] | 5 (11.33) [3.54] | | | | 30 |
| Muddy Run Wise | 20 (18.67) [0.10] | 10 (11.33) [0.16] | | | | 30 |
| | | | | | | |
| | | | | | | |
| ***Column Totals*** | 56 | 34 | | | | **90 (Grand Total)** |

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*Figure 2. A contingency table with results for the two-week transplant study*

| Site | ph | Water Temp | DO (mg/L) | Conductivity | Barometric Pressure |
|---|---|---|---|---|---|
| Petersburg S | 7.2 | 10.4C° | 13.65 mg/L | 147.7 SPC | 746.7 mmHG |
| East Bridge | 7.6 | 10.6C° | 19.27 mg/L | 375.7 SPC | 746.8 mmHG |
| Weis Parking | 8 | 9.9C° | 21.63 mg/L | 429.3 SPC | 747.6 mmHG |

*Figure 3. Results of water quality test*

## RESULTS

Our study began with thirty alive amphipods at each site, all extracted from Petersburg spring. After the first week, each container was checked and the amphipod inside was placed in one of three categories: Alive, Dead, or Missing in Action (MIA). If the amphipod inside was alive, it was returned to the stream for a second week of study. As shown in the contingency table above, after two weeks, nineteen amphipods remained alive in the control group at Petersburg Spring; five remained alive in the East Bridge location, and ten remained alive in the Weis Parking location. In order to calculate our results with greater significance, the dead and missing-in-action amphipods were combined. In Petersburg, eleven amphipods were dead or missing; ar the East Bridge location, twenty-five were dead or missing; finally, at the Weis Parking location, twenty were dead or missing. When these results were analyzed, it was found that the East location had the lowest survivorship, with Petersburg having the highest. However, Petersburg only served as a control group. Of the two variable locations, Weis parking had the highest survivorship. For this data set, we conducted a chi-square test, and with that test, we received a statistic of 14.2752, after running this test, we received a p-value of .000795, which results in our data being significant.

## DISCUSSION

After conducting this experiment, our results tell us that amphipods had higher survivorship in Petersburg, that was because this spring meets the ideal condition best suited for amphipods to live in such as low water temperature of 10.4 C° primary neutral pH levels of 7.4, etc. When collecting water quality samples, we noticed that the temperature in muddy run, the east location was 7.6 compared to the Weis location having a more basic pH 8. The temperature between the two muddy run locations showed us that in the east section, the more populated area has a temperature more closely like Petersburg of 10.6 C°, unlike the Weis location, its temperature was 9.9 C°. It is important that springs have plentily of oxygen for aquatic life to survive, in Petersburg the amount of dissolved oxygen of 13.65 mg/L, and both the east and Weis had higher levels of dissolved oxygen, the highest one coming from Weis being at 21.63 mg/L and east having dissolved oxygen at 19.27 mg/L. By measuring the barometric pressure, we are able to predict the short-term changes in the weather, which plays a factor in springs when harsh weathers occur, different things can enter the springs such as sticks, dirt, and plant fertilizer that can change the water quality in springs, and with these drastic changes, this can cause the aquatic life living inside of the springs do not have high survivorship in the spring. As mentioned in the paper, our hypothesis was that survivorship will be lower in the East location in the muddy run due to the point source of pollution and being a high residential area, by performing this transplant study, we were able to prove that our hypothesis was correct, in springs with both higher temperature and pH will lessen the survivorship of amphipods. What had through this two-week study was that three of the amphipods that remained in the control group, in the Petersburg spring, we happened to accidentally insert pairs of amphipods in the transplant containers, and during our second round of data collection, three of the remaining nineteen amphipods happen to had given birth, unfortunately, it only occurred in the control setting, we are not for certain that it was human error that causes them to give birth but it was still an interesting find to make during this study.

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# COLOR DETECTION IN FRESHWATER AMPHIPODS (*GAMMARUS MINUS*)

Brandon Bennett, Alexia Maxim, Amanda Puza, Avery Timko and Sydney Witter

## ABSTRACT

This study tested whether different lighting affected the color preference of the aquatic amphipod *Gammarus minus*. These amphipods were given choices between residing above either a green or blue floor under differing light conditions. Amphipods were counted on each side in a given time interval. After analyzing data, it was concluded that different lighting did affect the color preference of *Gammarus minus*. In red light, *Gammarus minus* preferred a green floor color. In soft white light, *Gammarus minus* preferred a blue floor color. The results were statistically significant $p<0.05$. Future implications for this research could include testing additional floor and light colors to see which ones the amphipods are able to detect.

*Keywords: amphipods,* Gammarus minus*, preference*

## INTRODUCTION

The *Gammarus minus*, or the Amphipod, is a crustacean-like macroinvertebrate found in fresh waters. Locally, these can be found in springs. A topic of interest for us was their eyesight, specifically the way they perceive color. According to Smithsonian Magazine, "While the vision was blurry, these eyes allowed the crustacean to see nearly 360 degrees save for a small blindspot directly behind its head. The larger eyes at the top of its head were much more focused and powerful, allowing the amphipod to see surprisingly far in the dark." However, not much is known about their ability to see color. Another species that is similar to the amphipod is the isopod. It is worth noting that isopods potentially have the ability to perceive color to a degree. Aurora Nelson from The University of Washington states "This species is highly likely to have color sensing abilities as a way of adjusting their chromatophores, so they may use those abilities to search for a substrate that they can match. I placed isopods in containers where they had access to three different colors of algae." when performing an experiment on *Lirceus* , a marine isopod species. We decided to test the ability of color recognition and preference in amphipods. Using the referenced literature, we felt that giving the amphipods the choice between a blue and a green floor would be best to test whether they had a preference in color. We tested under both direct white light and red light. We predicted that under the soft white amphipods will prefer blue, but under the red light, they will have no preference.

## MATERIAL AND METHODS

In this experiment, our lab group used an array of different materials that aided us in getting the necessary results. The amphipods used during this experiment were collected from Linden Hall in State College, PA and were housed in a large container in an environmental control room.. This fridge was ten degrees Celcius consistently during this experiment and none of the amphipods were taken out of said environment. There were hundreds of amphipods in this large container, but only 300 random individuals

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were chosen for the entire experiment. For the main setup, a cardboard box is placed on a cart with a lamp hovering and is placed in the fridge. The lamp housed a red light bulb (60 watt) and a soft white light bulb (incandescent, 60 watt) which was used in the hope that amphipods acted differently under different light stimuli. To house the amphipods during the trial runs, they were placed in one of three flat petri dishes (3.5 in x 3.5 in). Under each tank was a sheet of paper that was half blue (dark color) and half green (bright color) to monitor color preference of the amphipods. A few other materials used in this experiment include a stopwatch, materials to record observations (pen/notebook), a plastic pipette to move/transfer amphipods, and tape (attach paper to tanks/label each tank).

In addition to materials, this experiment had several methods that were followed and never changed throughout the entire experiment. Before running each trial, the thirty amphipods in the trial runs were acclimated to both lights (red and white) for ten minutes before data was recorded in their designated tanks. Once acclimated, the number of amphipods on each color (blue/green) was recorded every thirty seconds for a total of ten minutes in each tank. Once each trial run was concluded, amphipods were released back into their original holding tank and thirty random amphipods were chosen for the next run. In total, five runs were completed under each light condition and 300 amphipods were sampled over several days.

## RESULTS

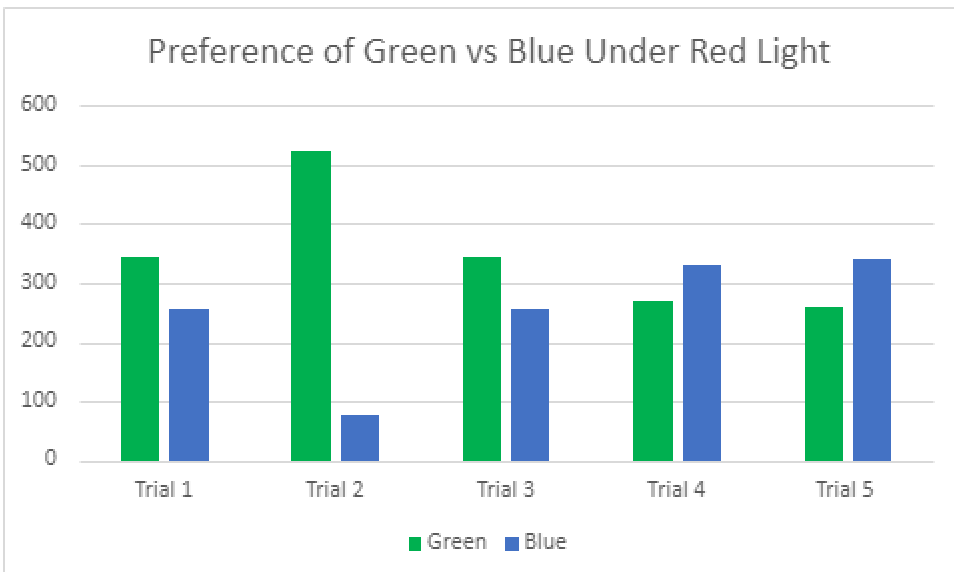


*Figure 1. Bar graph showing amphipod preference under soft white light (60W).*

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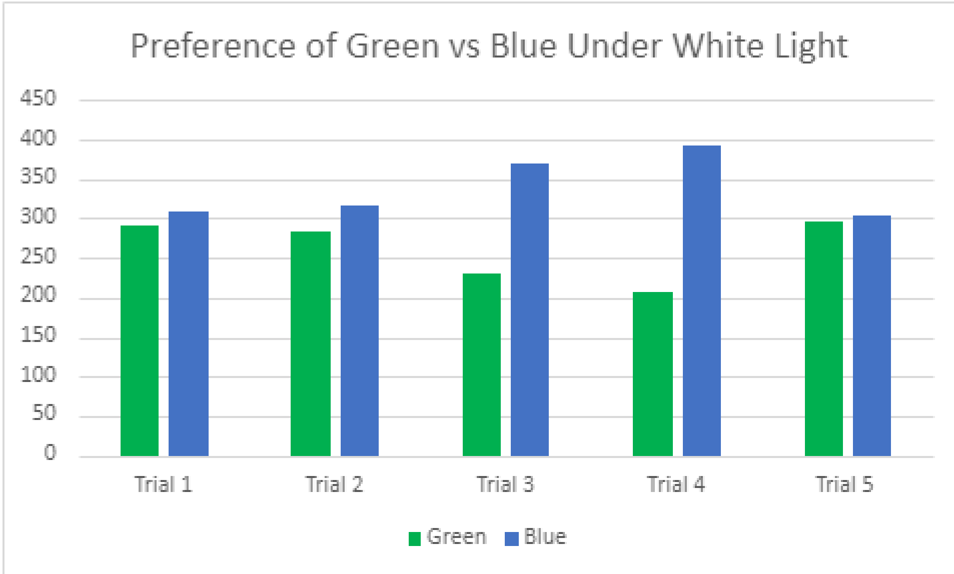


*Figure 2. Bar graph showing total amphipod color preference under red light (incandescent, 60W).*

| Results | | | | | | |
|---|---|---|---|---|---|---|
| | Trial 1 | Trial 2 | Trial 3 | Trial 4 | Trial 5 | *Row Totals* |
| Green | 344 (348.80) [0.07] | 525 (348.80) [89.01] | 344 (348.80) [0.07] | 271 (348.80) [17.35] | 260 (348.80) [22.61] | 1744 |
| Blue | 256 (251.20) [0.09] | 75 (251.20) [123.59] | 256 (251.20) [0.09] | 329 (251.20) [24.10] | 340 (251.20) [31.39] | 1256 |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| *Column Totals* | 600 | 600 | 600 | 600 | 600 | **3000 (Grand Total)** |

The chi-square statistic is 308.3648. The *p*-value is < 0.00001. The result is significant at *p* < .05.

*Figure 3. Red light (incandescent 60W) Chi Square of Independence to look at goodness of fit in green versus blue preference. Chi square statistic = 308.3648. p<0.05.*

| Results | | | | | | |
|---|---|---|---|---|---|---|
| | Trial 1 | Trial 2 | Trial 3 | Trial 4 | Trial 5 | *Row Totals* |
| Green | 291 (261.40) [3.35] | 283 (261.40) [1.78] | 229 (261.40) [4.02] | 207 (261.40) [11.32] | 297 (261.40) [4.85] | 1307 |
| Blue | 309 (338.60) [2.59] | 317 (338.60) [1.38] | 371 (338.60) [3.10] | 393 (338.60) [8.74] | 303 (338.60) [3.74] | 1693 |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| *Column Totals* | 600 | 600 | 600 | 600 | 600 | **3000 (Grand Total)** |

The chi-square statistic is 44.8708. The *p*-value is < 0.00001. The result is significant at *p* < .05.

*Figure 4. Soft white light (60W) Chi Square of Independence to look at goodness of fit in green versus blue preference. Chi square statistic = 44.8708 p<0.05*

| Results | | | | | | |
|---|---|---|---|---|---|---|
| | Green | Blue | | | | *Row Totals* |
| Red | 1744 (1525.50) [31.30] | 1256 (1474.50) [32.38] | | | | 3000 |
| White | 1307 (1525.50) [31.30] | 1693 (1474.50) [32.38] | | | | 3000 |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| *Column Totals* | 3051 | 2949 | | | | 6000 (Grand Total) |

The chi-square statistic is 127.3495. The *p*-value is < .00001. The result is significant at *p* < .05.

*Figure 5. Chi Square of Independence comparing soft white light (60W) to red light (incandescent 60W) in green versus blue preference. Chi square statistic = p<0.05*

## DISCUSSION

Our hypothesis was incorrect. Although there was a smaller preference under white light, the preference under white light was blue. There was a stronger preference under soft red light, and the preference was green. It was expected that there would be little to no preference under red light, and a preference for blue under soft white light. There was a preference for blue under soft white light, so we were partially correct.

The expected column totals were 3000. In comparison, there was a clear blue preference under white light. The amphipods had a greater color preference overall in soft white light than in red light, possibly signifying that they are better able to discern between red and blue in soft white light than in red light.

Although our hypothesis was incorrect, we were on the right track in predicting that the amphipods would have a statistically different preference under soft white light than under red light. This leads to future discussions and potential experiments. Is it more likely that the white or red light preference better reflects the amphipods preference in nature? If the amphipods seem to prefer blue under soft white light, which is similar to daylight, how would this compare to other colors? Finding a statistically significant preference is a great starting point in researching other similar topics in amphipods.

We could have looked to do more than five runs and potentially incorporated the color red in one of the trays. It would also be helpful to get a camera that snaps every 10 to 30 seconds instead of counting the Gammarus minus by eye.

## ACKNOWLEDGMENTS

We would like to acknowledge Dr. Douglas Glazier for allowing us to use his collection of amphipods from Linden Hall, PA and our teaching assistant Ryan Bennett for helping us collect the materials we needed to set up and run our experiment.

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# PRESENCE OF AMPHIPODA IN FRESHWATER SPRINGS

Riya Seshadri and Olivia Foley


## ABSTRACT

A suitable environment for the species Crustacea: Amphipoda would be in a pure water form that is perceived as moist and warm. Also, the desired habitat holds little to no involvement with the Homo sapiens species. Factors that were effective and shown to be a difference for the Amphipoda species in springs closer and farther from humans were body size, temperature, and overall presence of these organisms. The main characteristics of disparity between the freshwater spring used was the distance between itself and human contact and how congested the area was with activity from the out-of-water society. Additionally, the temperature was affected when the body of water came into close contact with the outside world. Human beings unknowingly influence the habitat of Amphipoda, which then leads to the entire ecosystem because the organism Crustacea: Amphipoda is proven as both a primary consumer and a decomposer. This research started as the study of the differences between Amphipoda in different freshwater springs, however, it adopted an accelerating growth of size and changed into what is the overall cause of those distinctions. Distinctions in question were clarified as body size, temperature of water, and presence of the desired species. Homo sapiens and their existence have a clear history of altering one's ecosystem, and the interaction between the human race and amphipods made this claim very clear. It was found the temperature is lower when more closely associated with humans and the body size of Amphipoda did decrease significantly.


*Keywords: body size,* Homo sapiens*, Amphipoda, consumer, decomposer, temperature, habitat*

## INTRODUCTION

The human race has been the reason for many habitats failing and sometimes disappearing when in contact with them. Our first question was how much does the presence of the human race effect the lifestyle and habitat of Amphipoda (see Figure 1) (Glazier, 2014). Since the general public understands how environments on land are altered by the Homo sapiens species (deforestation). Humans always have an effect on the environment when present, and we wanted to test how organisms underwater were affected as well. Our hypothesis included that the temperature would decrease and the body size of Amphipoda species within the body of water would also decrease the closer to human interactions. While having many freshwater springs surround a college campus, the group was able to test the differences between further locations and one that flows straight-through the outskirts of the campus itself. As told above, the species of Crustacea: Amphipoda can play two roles in an ecosystem: primary consumer and decomposer. Both jobs resemble the beginning steps of the cycle of life for an ecological community. Primary consumers are organisms that are apart of not the first trophic level, but instead the second level (National Geographic, n.d.). So, if the amphipods were influenced by humans, then so would the entire ecosystem made up of organisms on higher trophic levels which depended on food and other factors from the lower-level organisms. Specifically, we studied the interaction between Amphipoda and humans, and the variables that occurred when we decided to move locations from one testing site to the other. Currently, there is some information that's known about this relationship and as a result, the hypothesis which was tested will most likely follow similar patterns of deforestation and how that ecosystem was after interacting with the human race population.

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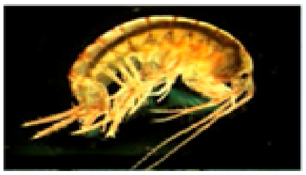

*Figure 1.* Crustacea: Amphipoda is shown above.

## FIELD SITE

Both sites examined within this study are freshwater springs that are near the Juniata College campus in Huntingdon, Pennsylvania. The first spring examined is known as Cold Springs and is approximately a twenty to thirty-minute walk from the campus, also depending on where you began the journey. The second location is known as Muddy Run and coasts through the town of Huntingdon. The location we used for this experiment was less than a five-minute walk and took place near student dorm buildings, specifically titled East Housing Residences.

For collecting data at Muddy Run we started near the bridge walkway facing the East Housing Residences buildings and then we moved locations to the under the passing of College Avenue near the neighborhoods of citizens of Huntingdon, Pennsylvania (not college-owned residences).

## METHODS AND MATERIALS

This study began once the group chose the two desired freshwater springs to collect data from, analyze, and compare. Two convenience-friendly springs were chosen because sampling was going to take place after the group walked to the locations. No other form of transportation was taken other than by foot during this experiment. Sampling occurred one day a week for five weeks. The group would be out at a location for at least an hour to two hours at a time walking up and down the stream searching for an amphipod. Before searching for amphipods, the water's temperature was recorded by the group using a thermometer upon arrival at the spring location. To search for the species, members would pick up stones and see the organisms that were found under or still latching onto the stone. Effort was also made by mixing up the sediment of the water to recycle the organisms at the bottom of the spring to the surface to be visible to the human eye for observation.

Once an amphipod was found, the organism would be pulled from the water using forceps or a transfer pipette and the length would be recorded immediately using a ruler. The amphipod would be stretched out on the ruler to obtain the most accurate measurement. Then, the organism would be released back into the water to sustain the ecological community balance.

From the weeks of observation, two out of five days was when no amphipod was found in the water, and the group left the field site after hours of searching ‘empty-handed’. On both of those occasions, the only data that was collected was the temperature of the water. Even though there were weeks that no amphipod was found to study, there was still something credible to accumulate towards the research experiment. Having no data transpired into data within itself, and it began to drive the result of our hypothesis.

## RESULTS

In Cold Springs, we found three amphipods over three days. We first went on Sunday, April 2$^{nd}$. The water was the coldest on this day, at 57 degrees Fahrenheit and the amphipod we found was the smallest one we found, measuring 6.7 millimeters. The second time we went was Sunday, April 9$^{th}$. The water was much warmer on that day, at 68.9 degrees Fahrenheit, and the amphipod we found was the largest one we found, measuring 8.2 millimeters. We last went to Cold Spring on Tuesday, April 11$^{th}$. The water was slightly warmer than a couple days prior, at 69.1 degrees Fahrenheit, making it the warmest day so far. The amphipod we found was much smaller than the one we found two days prior, though it was larger than the one we found a week prior, measuring 7.1 millimeters. In Muddy Run, however, we did not find a single amphipod over the two days that we went, despite our thorough checking and the presence of other wildlife in the area.

| Date | Temperature of Water (degrees Fahrenheit) | Length of Amphipod (millimeters) |
|---|---|---|
| April 2nd | 57 | 6.7 |
| April 9th | 68.9 | 8.2 |
| April 11th | 69.1 | 7.1 |

## DISCUSSION

We found amphipods all three times we collected data from Cold Spring, but not a single amphipod when we went to Muddy Run. Though both streams are quite similar in terms of the wildlife that can be found there, the primary difference is their location and the proximity of their location to human settlements. Cold Spring is isolated from any human settlement, but Muddy Run is located in the middle of Juniata property, specifically being directly in front of East Houses. While surveying Muddy Run, we noticed a lot of trash scattered around the banks of the stream, submerged in the stream itself, and even caught in between the branches of the smaller trees on the stream bank. Speaking to people who lived near Muddy Run, pollution has been an issue in the area for many years now. On the surface, it might not seem like it affected wildlife that much. During our second trip, we saw a wide variety of animals, from water louses, water striders, and hellgrammites to fish, tadpoles, frogs, and even some birds and a rabbit. However, there was a complete lack of amphipods there, just like on our first trip, which was striking considering it was the warmest it had been since we started collecting data. We hypothesize that the human activity at Muddy Run is the reason for the lack of amphipods in the stream. Human activity leads to a large amount of pollution and cloudy water. While some species, like frogs, hellgrammites, water louses, water striders, and slugs can thrive in the murkier waters, amphipods prefer and thrive more in clear waters, such as Cold Spring.

## ACKNOWLEDGMENTS

We would like to thank Dr. Douglas S. Glazier for the help and guidance he provided throughout the design and development of this project.

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DOI

# EFFECTS OF DIFFERENT SHADE CONDITIONS ON THE HEIGHT OF WISCONSIN FAST PLANTS

Austin J. Baker and Emma R. Burry

## ABSTRACT

The Wisconsin fast plant, or *Brassica rapa*, is an herbaceous full-sun plant. Like other plants, it has a shade avoidance response that results in increased height. This increase in height was studied under varying shade conditions over the course of a month, which was long enough for *B. rapa* to go from seed to flower. Plants in shadier conditions grew taller earlier in the study period, but they matured slower and were overall less healthy than plants grown in full sun.

*Key words: shade avoidance,* Brassica rapa*, Wisconsin Fast Plant, growth rate, maturity rate, shoot elongation*

## INTRODUCTION

All plants need access to light to grow and reproduce. However, light can be blocked by various factors such as tall plants, geographic features, geological features, and human structures. Plants have evolved several different methods for getting around this conundrum. Many species of plants such as ferns have evolved to be shade tolerant (cite), some trees have evolved to tower over sources of shade, and some still thrive in full sun. From one of these full sun plants, a botanical lab rat was derived: the Wisconsin fast plant, or *Brassica rapa*. *B. rapa* was created in a lab in Wisconsin, and it is meant to be grown under a grow light. It is particularly useful to study, as its seeds, when purchased, come with an instruction manual. The manual outlines ideal growth conditions for the plant, along with a timeline of when certain milestones, such as flowering, should occur (Wisconsin Fast Plants® Team, 2023). This gives a good basis of comparison for study outside of those set parameters.

Any plant that thrives in full sun will try its best to be in full sun. This is called a shade avoidance response. Shade avoidance responses in plants most often take the form of extended growth in the direction of a light source, usually straight up towards the sun, but sometimes off to the side just out of the shade. This growth is regulated by the hormone auxin (cite). To see how *B. rapa's* shade response affected its height and body size, an experiment was conducted as outlined below.

## MATERIAL AND METHODS

Twenty *Brassica rapa* seeds were planted in their own small plastic pots with one seed per pot. They were planted in pre-wetted potting soil mixed with sand. Immediately after planting, they were placed in the same greenhouse under different four different shade conditions with five plants in each group. The shade conditions are defined below:

Full sun: Plants placed where they could have the most possible access to full sun

Partial plant shade: Plants placed underneath sparse cover by other greenhouse plants

Full plant shade: Plants placed in the closest possible approximation of full shade from other greenhouse plants

Full inanimate object shade: Plants placed under a pot

Every three days, we tended to the plants and recorded data. Each plant was watered with an eighth cup of water unless it was thoroughly wet. Each plant's growth was measured in centimeters with a small ruler and recorded in a data table. If the plant was drooping, it was held up against the ruler to determine how tall it would be if it were not drooping. The status of each plant -- either alive, dead, sickly, or wilted – was recorded along with the compass direction each plant was facing, if any, whether they had flowered, and any additional notes.

We performed a one-way ANOVA test using Excel on the April 7th data and the April 24th data to determine if the mean height of *B. rapa* in each shade condition differed at the beginning and end of the study period. The null hypothesis states that the means of each shade condition are equal and the alternative hypothesis states that the means of each shade condition are not equal.

## RESULTS

Anova: Single Factor

4/7/23

SUMMARY

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| Full sun | 5 | 1.7 | 0.34 | 0.038 |
| Partial plant | 5 | 5.5 | 1.1 | 0.015 |
| Full plant | 5 | 2.5 | 0.5 | 0.235 |
| Full inanimate | 5 | 9.9 | 1.98 | 0.282 |

ANOVA

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 8.272 | 3 | 2.757333 | 19.34971 | 1.43E-05 | 3.238872 |
| Within Groups | 2.28 | 16 | 0.1425 | | | |
| | | | | | | |
| Total | 10.552 | 19 | | | | |

Fig. 1: The ANOVA test results from April 7th, 2023.

Anova: Single Factor

4/24/23

SUMMARY

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| Full Sun | 5 | 49.7 | 9.94 | 31.883 |
| Partial plant | 5 | 46 | 9.2 | 3.825 |

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| | | | | |
|---|---|---|---|---|
| Full plant | 5 | 22.4 | 4.48 | 17.627 |
| Full inanimate | 5 | 0 | 0 | 0 |

ANOVA

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 320.1895 | 3 | 106.7298 | 8.004487 | 0.001751 | 3.238872 |
| Within Groups | 213.34 | 16 | 13.33375 | | | |
| | | | | | | |
| Total | 533.5295 | 19 | | | | |

Fig. 2: The ANOVA test results from April 24th, 2023.

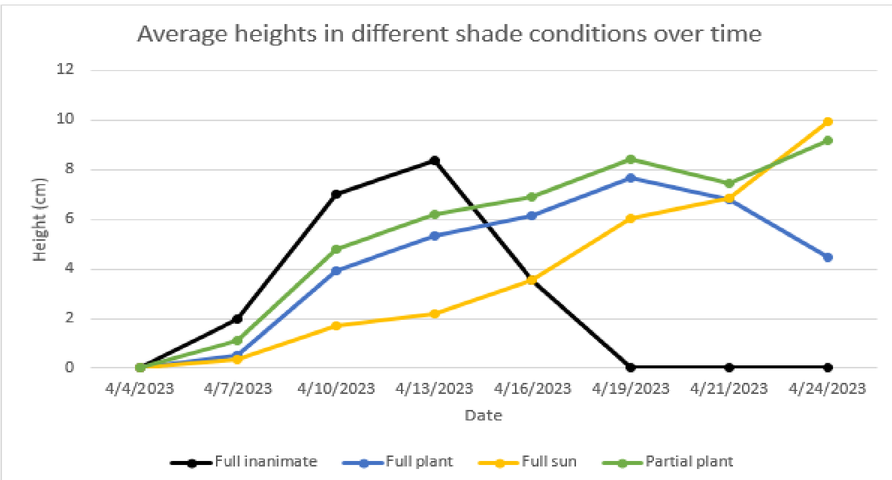


Fig. 3: A graph showing the average height of each shade response group over time.

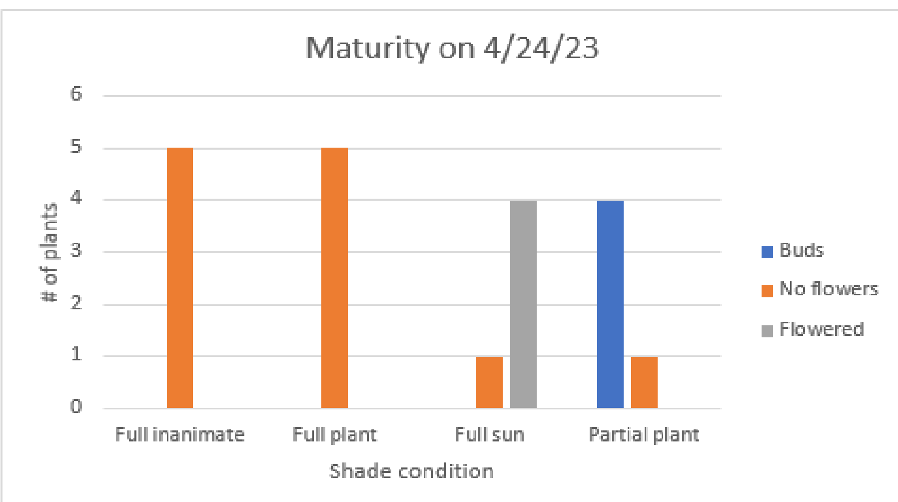


Fig. 4: A chart showing how many plants have flowered or budded in each shade condition on April 24th.

| **4/24/23 Shade Condition** | **Alive** | **Dead** | **Sickly** | **Wilted** |
|---|---|---|---|---|
| Full inanimate | | 5 | | |
| Full plant | | 2 | 3 | |
| Full sun | 5 | | | |
| Partial plant | 1 | | 1 | 3 |

Fig. 5: A table with the number of alive, dead, sickly, and wilted plants in each shade condition.

## DISCUSSION

On April 7th, $p < 0.0001$, so there was a significant difference between the average height of *B. rapa* in each shade condition (see fig. 1). On April 24th, $p = 0.002$, so there was a significant difference between the average height of *B. rapa* in each shade condition (see fig. 2). The alpha level of both tests is 0.05.

As shown in figure 3, the average height of *B. rapa* in each shade condition changed over time. The plants in full inanimate object shade grew the fastest in the beginning, but by April 19th, all the plants in inanimate object shade were dead. In partial plant shade and full plants shade, *B. rapa* grew faster than the plants in full sun. However, by April 24th, two full plant shade plants were dead, and the growth rate of both conditions appeared to be decreasing. The *B. rapa* in full sun grew the slowest, but none of the plants died off, and they were, on average, the tallest plants by the end of the study period.

*B. rapa* grows best under a twenty-four hour grow light (Carolina® Team, 2023), and our closest equivalent was the full sun plants. but it does attempt to acclimate to shadier conditions by growing tall quickly. However, the trade-off is that it does not mature as fast. When stressed, *B. rapa* tends to delay life cycle stages (Steinbrenner et. al., 2012). The plants in partial plant shade grew tall faster than the plants in full sun (see fig. 3), but they only had buds when most of the full sun plants had flowers (see fig. 4). The *B. rapa* in shade conditions (full inanimate, full plant, and partial plant) was also more likely to die; about 47% of the plants in shade conditions died, and an additional 47% were either wilted or sickly by April 24th (see fig. 5).

There were a few confounding variables in this study. The most prevalent issue was that of water. The experimental design was such that each plant would receive 1/8th cup of water once every three days, but the pots had no drainage, and there were times that, for one reason or another, they would be flooded, and so would not be watered that day. This was due to the communal nature of the greenhouse, and the fact that we, the researchers, were not solely responsible for watering all plants in said greenhouse.

Though some results may have been influenced by overwatering, the shoot elongation of the *B. rapa* in shadier conditions is more consistent with a shade response (Ruberti et. al., 2012). The shade response of the *B. rapa* allows it to survive in inadequate conditions, but it comes with the tradeoff of slower reproductive maturity. Plants' shade responses are an investment of resources that does not always get a return. Some of our plants died before they had a chance to reproduce. The best way to avoid shade may be through evolution rather than acclimation. For future study, it would be useful to grow *B. rapa* outside in natural conditions to see plants under naturalized plants have a different response to shade, or a different maturity rate.

## ACKNOWLEDGEMENTS

Thanks to Dr. Chuck Yohn, Telah Zinobile, and Dr. Ramakrishnan for letting us into the greenhouse. Thank you, Dr. Glazier, for helping us with the math, buying us seeds, and generally being a lovely man and fantastic ecology professor.

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DOI

# EXPLORING THE RELATIONSHIP BETWEEN ALTITUDE AND SOIL FERTILITY FOR OPTIMAL FAST PLANT GROWTH

Aaron Bollinger, Alex O'Harah, Devlin Foster, Hannah Moore and Mason Yeager

## ABSTRACT

Soil composition plays a critical role in the growth and development of weeds. Weeds are defined as any plant growing where it is not desired, and their presence can cause significant economic and ecological damage. The composition of the soil affects weed growth in several ways, including nutrient availability, pH, and water-holding capacity. Nutrient availability, such as nitrogen, phosphorus, and potassium, is essential for plant growth, including weeds. Soils with higher nutrient levels, particularly nitrogen, tend to promote more weed growth. In contrast, low nutrient levels, particularly nitrogen, can slow down weed growth. Soil pH also affects weed growth; some weeds thrive in alkaline soils, while others prefer acidic soils. Water-holding capacity also influences weed growth, as weeds need adequate moisture to grow and develop. Thus, soil composition is a crucial factor in managing weed populations. Understanding how soil composition affects weed growth can help farmers, gardeners, and land managers design effective weed management strategies that target specific soil conditions to control weed growth.

*Key words: Altitude,* Brassica rapa*, Correlation, Nutrient Availability, Soil-Water Content*

## INTRODUCTION

Huntingdon, Pennsylvania is found in the Allegheny mountains. As a small rural town with a large agricultural presence, we wonder how much thought is put into field placement on ones' property and how it can affect crop growth. Understanding that the soil's nutrient concentration plays a very large role in plant growth, the soil's water content is just as important of a focus. The water content starts the initial step of the plant growth process as it influences the nutrient uptake and movement by the plant (Datta, Taghvaeian, Stivers 2018). Too much water in the soil can cause the roots to rot or prevent the seed from germinating, whereas too little water prevents soil nutrients from traveling through the plant (Traunfeld 2020, Traunfeld 2023). The organism being studied, *Brassica rapa*, is a very hardy plant that grows well in a variety of soils allowing us to pay attention to the altitude and soil water content that these samples were taken from. With the background information we have collected, we expect the soil conditions of the midland environment to produce the fittest/tallest organisms and alternatively we predict the highland environment to produce the fittest/tallest organisms.

## MATERIAL AND METHODS

First off, choose four test sites, one in each altitude category (highland, midland, lowland, and wetland). Using shovels we gathered two liters of soil from each location and stored them in two separate one liter containers. Next, 100 mL of soil is transferred to a smaller 100ml container and stored in a cool and dry room in order to obtain a dry weight. With approximately equal portions of soil in each container, the fast plant seeds (*Brassica rapa*) are deposited into a depression made within the soil, two seeds at the lowest level and two more seeds are added once the original depression is half filled. These plants were watered weekly with a liter which was equally distributed among the eight separate containers. Growth progression of the eight containers is measured weekly in millimeters, and carried out for a total of three weeks. Keep plants in the same location throughout the course of the experiment to ensure that they receive the same amount of sunlight.

## FIELD LOCATIONS

The Highland location is Han's overlook (40.4311860°, -78.0173412°) with an elevation of **240m** located on top of a local mountain. The Midland location is outside of the third floor biology wing of Brumbaugh Academic Center. (40.5017941°, -78.0187916°) which is a well kept area of the college's grassland with an elevation of **214m**. The Lowland sample is a valley located along Point Road at **186m** (40.439177°,-77.981832°) which has little human interaction but soil is constantly being changed by the river causing there to be little to no topsoil. The Wetland sample is from Old Crow Wetland (40.48841°, -78.03585°) which is a wetland full of tall grasses located at **192m**. All elevations of field locations were found using source, https://www.dcode.fr/earth-elevation.

## RESULTS

*Table 1. Summary of plant growth over the course of 4 weeks in soils from 4 different elevations. Growth measured in millimeters, represented with mean growth +/- standard deviation.*

| **Environment (Elevation)** | **Plant Growth** | **Variance** |
|---|---|---|
| Highland(240m) | 74 +/- 50.91 | High |
| Midland(214m) | 46 +/- 12.73 | High |
| Lowland(186m) | 1 +/- 1.41 | Low |
| Wetland(192m) | 8.5 +/- 0.71 | Low |

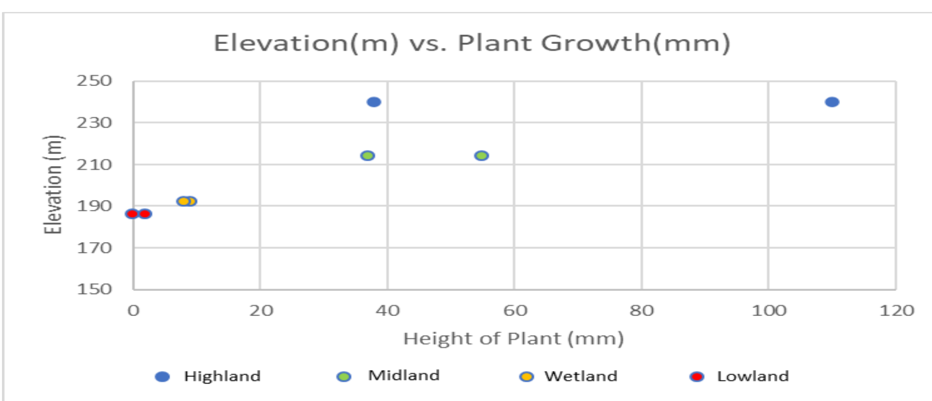


*Figure 1. Comparison of elevation in meters between four different locations versus the height of plant growth in millimeters. From largest to smallest growth in a four week period, the results show that the highland environment had the most growth followed by midland, wetland, and then lowland. A total of n=8 samples, 2 from each field location, were analyzed using correlational statistics which identify r = 0.841 and p = 0.0088.*

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*Table 2. Calculations of a soil's water content, reflected as a percentage, acquired through dry mass calculations and description of the soil's main composition.*

| | Highland | Midland | Wetland | Lowland |
|---|---|---|---|---|
| % water | 11.3% | 2.9% | 10.77% | 18.6% |
| Main comp. | topsoil | topsoil | clay | clay |

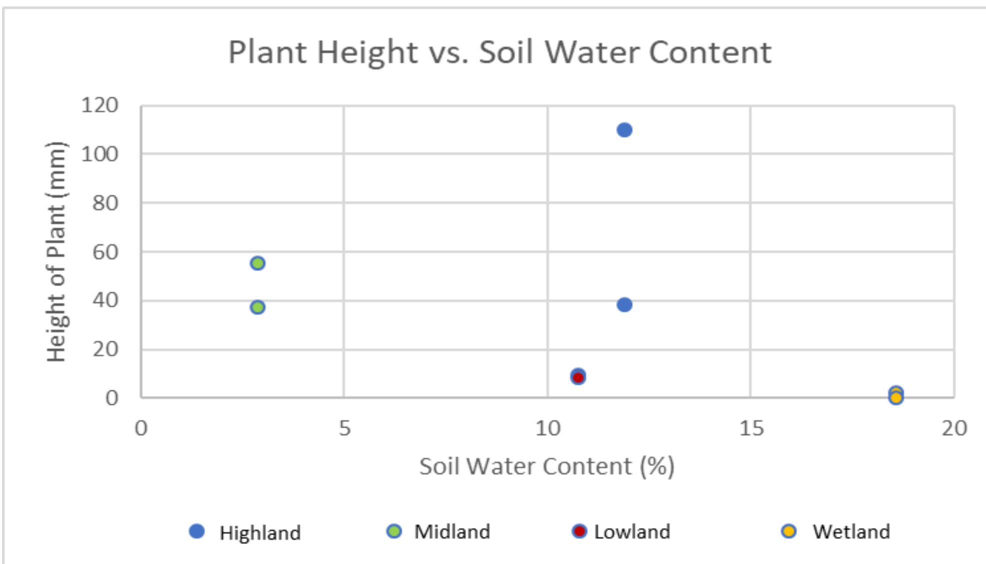


*Figure 2. Comparison of plant height grown in soils of four different locations over the course of 4 weeks, measured in millimeters, versus the water content of said corresponding soils, measured as a percent through dry mass calculations. A total of n=8 samples, 2 from each field location were analyzed to identify r = -0.394 and a p-value wasn't able to be identified.*

## DISCUSSION

Optimal soil conditions can vary due to a variety of factors. This experiment focused on the elevation that a soil sample was taken from and the height of the plant that was able to emerge from it. Figure 1, shows the comparison between these two variables and was analyzed using the Pearson's Product-Moment Correlation test as the data follows a normal distribution. This test produced an r-value of 0.841 which provides a positive linear association, meaning as the values of one variable gets higher, as does the other. The two-tailed t-test of this r value produced a p-value of 0.0088 meaning that at a 5% level of significance we have sufficient evidence to reject the null hypothesis that soil conditions don't matter in affecting the growth and survivability of plants. While the results do support our alternate hypothesis that the highland environment would produce the fittest/tallest organism, this is somewhat unexpected as we were focusing the experiment on the water content found in this soil. Figure 2 shows the comparison of plant height from the 4 different field locations to the percent water-content that is listed in Table 2. Performing correlational statistics on this data proved unsuccessful as the r-value reported and the trend of the data did not produce any type of association between the two variables. A possible explanation of why the value did follow the hypothesized trend is, as stated in table 2, that the soil samples of the highland and midland regions were mainly topsoil whereas the wetland and lowland regions had a more clay-like substance. This difference in composition could have prevented the wetland and lowland samples from obtaining much, if any, growth. Future research to expand on this topic could have more samples to measure plant growth to improve the variability factor, such as how in table 1 the highland and midland environments had high variability. Also, researchers should perform analysis on the nutrient composition of the soils as well as the water content in order to gain a better understanding of which factor plays a larger role in the growth of a plant.

## ACKNOWLEDGEMENTS

Douglas S. Glazier.

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DOI

# NORTHERN TWO LINED AND NORTHERN RED SALAMANDER LARVAE DISPERSION COMPARED WITH STREAM QUALITIES

Joshua Samuels, Tim Sullivan and Jude Smith

## ABSTRACT

Salamanders are a common amphibian found across the globe, and many studies have been conducted on their habitat and stream choice. We wanted to find out how salamander larvaes were dispersed, and what stream qualities influenced salamander larvae dispersion. Previous research on other species of salamander has indicated a preference for close proximity to the origin of the stream (Connette, Grant & Osbourn, Michael & Peterman, William 2016*)*. We studied two springs located in Central Pennsylvania, Cold Spring and Warm Spring. We divided the two streams into three sections each and collected data on stream quality as well as the salamanders we found. There were two types of salamander that were found in both springs. These were the Northern Two-Lined Salamander and the Red Salamander. We found that Warm Springs had a Two-lined Salamander majority, while Cold Springs was dominated by the Red Salamanders. Our data also indicated a preference for higher alkalinity, hardness, and lower dissolved oxygen. Both salamanders preferred to reside in vegetation, the vast bulk being watercress. Our data on Warm Spring also seemed to support Connette, Osbourn, and Petermans's study, but less so Cold Spring. Overall, Warm Spring had more salamanders, indicating a strong preference for higher temperatures.

*Key words: Salamander, Physiochemical, Springs, Population, Dispersion*

## INTRODUCTION

The first point of conducting this study was to get an accurate assessment of what species of salamander larvae were found in two different springs, determining the main species found in both springs were the Northern Two-Lined Salamander (*Eurycea bislineata*) and the Red Salamander (*Pseudotriton ruber ruber*). Each species was identified through a visual identification that looked at primarily coloration, color patterns, and body size. Any specimens whose ID could not be confirmed on scene were photographed and cross-referenced with photos of local species. The next question to answer was how are they dispersed and why are they dispersed this way. Northern Two-Lined Salamanders prefer habitats such as springs and creeks that have a sandy to small gravel substrate. They also prefer warmer water temperatures but are resilient in their ability to tolerate cold water. Red Salamanders are more cold tolerant and actually prefer colder springs and creeks, especially those with larger gravel substrates and numerous stones or other hiding places as they are a very shy species. *P. ruber* also tends to prefer softer water than the *E. bislineata*. *Pseudotriton ruber* are a larger species than the *E. bislineata* and their larvae are no different, with Red Salamander larvae being consistently larger than the Two-Lined Salamander larvae. This means that Red Salamanders need higher concentrations of dissolved oxygen due to their larger size. Because of these differences in habitat needs it was hypothesized that more Northern Two-Lined Salamanders would be found in Warm Springs and not in Cold Springs due to its preference for warm water and sandy substrates. It was also Hypothesized that the Red Salamander would be found predominantly in Cold Springs and in the later sections of Warm Springs due to the higher dissolved oxygen and the higher amounts of gravel substrates. Testing these hypotheses is worthwhile in understanding the population dynamics of each spring and understanding how the seemingly small differences in physiochemicals can have great impact on which species are found there.

## FIELD SITES

We had two field sites for this study. One was at Warm Spring and the other was at Cold Spring. Both springs were divided into three sections, labeled Section A, Section B, and Section C. Both springs were shallow and located in deciduous forested areas. Warm springs had a muddy bank and a rocky, wooded bank. Warm Springs Section A had many medium to large sized rocks and some foliage, being watercress. There were also several downed trees in and over the water. Section B contained more medium sized rocks, and lots of sand sized sediments covering the floor of the Mud Bank side. Section B also had lots of watercress covering about 70% of the section, with only parts of the Mud side not covered. Section C was a longer section, with the first 60-70% having many small to medium and large rocks covering the right side and the left side being more open. The last 30-40% was foliage, with the end of the section being covered with watercress. At Cold Springs, the water was much shallower, and the stream was far thinner. There was a bank that led to the woods and a bank covered with flowers that became muddy at Section C. Section A contained many medium to large rocks and larger grain sediment covering the floor. The stream flowed down the hill and contained a small waterfall. Section B had some medium to large rocks in the first 30-40% with the rest being covered with watercress. Section C was almost entirely covered with watercress, likely ~ 90%. There were many large and medium sized rocks concealed under the watercress.

## Warm Springs

**Section A**

**Section B**

**Section C**

**Cold Springs**

**Section A**

**Section B**

**Section C**

## MATERIAL AND METHODS

The first methods we used were out at our two Field Sites. When gathering data on the Field Site, we measured width, depth, and dissolved oxygen. We used a tape measure, yard stick, and a dissolved oxygen probe to measure this. When gathering data on salamanders, we searched for 10 minutes in each section, using an iPhone to time. While in the water we used waders or sandals to protect our feet. We captured salamanders by wading in the springs, slowly lifting rocks and scanning the bed for them. When one was spotted we used a free hand to scoop the salamanders into a small handheld net. When one of us captured a salamander, the timer was stopped and we gathered data. The salamander was then transferred to a bin where its length would be measured using a 12 inch ruler. We then weighed the salamander using a digital scale, and the distance between where the salamander was measured using a yardstick or tape measure and the nearest bank, using a tape measure. This data, along with the type of salamander and the qualities of the rock it was found under (if it was found under one), was recorded using an iPhone. We then repeated these steps until time ran out, and then we continued to the next section.

## RESULTS

We found that the Warm Springs salamander population was dominated by 81% of Two-Lined Salamander Species in the area (Fig. 3). The remainder of the salamander population in Warm Springs was 19% and it consisted of Red Salamanders (Fig. 3). The salamander population of Cold Springs was dominated by the Red Salamanders who took up 90% of the recorded population (Fig. 5). The rest of the recorded population of salamanders consisted of an equal number of Dusty Salamanders and Two-Lined Salamanders (Fig. 5). Salamanders that came out of Warm Springs tended to be larger in length (in) and weight (g) then those in Cold Springs (Fig. 1). We found that the salamanders found in both springs enjoyed residing under a variety of rock sizes and they seemed to prefer vegetation in the area (Fig. 1). Based on our water physicochemical data on Warm Springs and Cold Springs (Fig. 2) undeveloped salamanders prefer warmer water with a higher level of hardness and alkalinity in it, however it seems a lower level of dissolved oxygen is preferable for the salamanders. As we looked for salamanders farther away from the source of the springs we found less salamanders compared to the amount found upstream (Fig. 4, Fig. 6). We were not able to form a T-Test or probability test due to the limited amount of sections per spring.

| | | | |
|---|---|---|---|
| Conclusion | | | |
| Warm Springs | | | |
| | | | |
| Total Number of Salamanders | | | |
| 32 | 26 | 6 | 0 |
| Avg Length (cm) | | | |
| | 5.494326 | 6.841667 | 0 |
| Avg Weight (g) | | | |
| | 0.833441 | 2.515 | 0 |
| Most Common shelter | | | |
| | Large Moss | Medium Mossy Rock | |
| | | | |
| Cold Springs | | | |
| | | | |
| Total Number of Salamanders | | | |
| 19 | 1 | 17 | 1 |
| Avg Length (cm) | | | |
| | 1.333333 | 6.3625 | 1.216667 |
| Avg Weight (g) | | | |
| | 0.151667 | 1.414028 | 0.466667 |
| Most Common Shelter | | | |
| | Large Moss | Large Plain | Open water |

*Fig. 1*

| | | | | | | |
|---|---|---|---|---|---|---|
| Water Phiso | | | | | | |
| Warm Sprii | pH | Nitrate(ppı | Nitrite(ppn | Water Harc | Alkalinaty | Dissolved o2 |
| Section A | | | | | | |
| | 7.01 | 0 | 0 | 75 | 60 | 3.74 |
| Section B | | | | | | |
| | 7.08 | 0 | 0 | 75 | 80 | 4.19 |
| Section C | | | | | | |
| | 7.2 | 0 | 0 | 75 | 80 | 4.45 |
| | | | | | | |
| Cold Spprings | | | | | | |
| Section A | | | | | | |
| | 6.6 | 0 | 0 | 40 | 20 | 6.07 |
| Section B | | | | | | |
| | 6.39 | 0 | 0 | 30 | 10 | 5.93 |
| Section C | | | | | | |
| | 6.44 | 0 | 0 | 25 | 10 | 6.03 |

*Fig. 2*

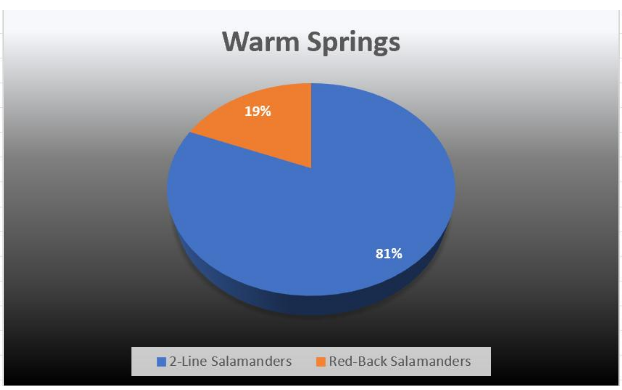


*Fig. 3*

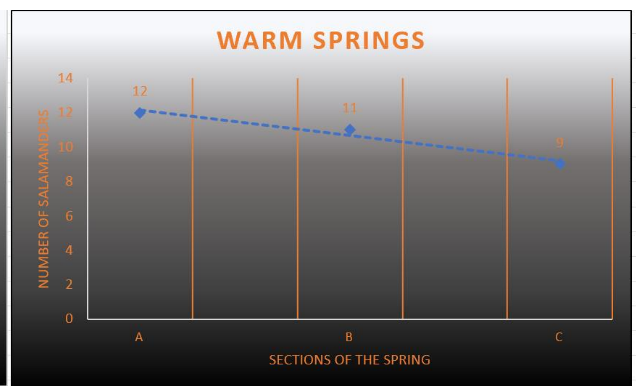


*Fig. 4*

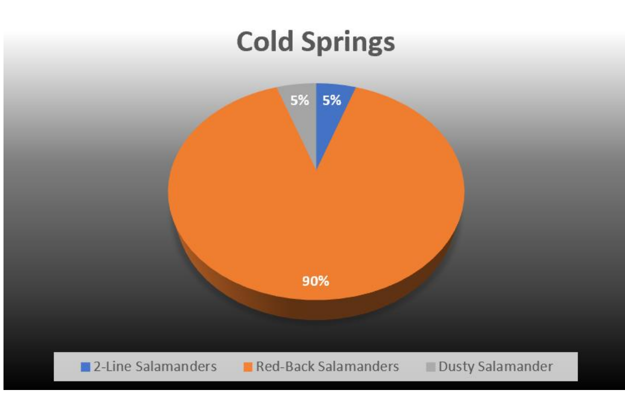


*Fig. 5*

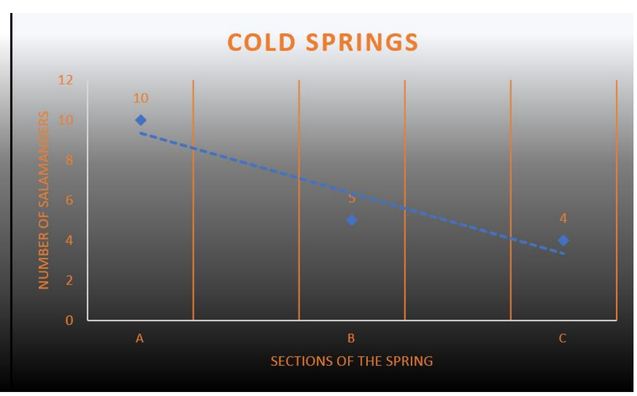


*Fig. 6*

## DISCUSSION

The results confirm and prove the hypotheses, *P. ruber* was the most common and abundant species in Cold Springs while also being found somewhat readily in the later two sections of Warm Springs. *Eurycea bislineata* was, conversely, the most common species found in Warm Springs. Data from Cold Springs showed that very few salamanders were found in the spring itself, especially the last two sections, yet when we flipped rocks near the spring out of curiosity after packing up our equipment, we found an abundant number of salamanders. What is interesting is that we found more adult Northern Two-Lined Salamanders than Red Salamanders. Data was not recorded for the adults however, I believe we found upwards of ten Two-Lined Salamanders and five Red Salamanders. This is odd since we only found two larval *E. bislineata* in the spring itself. Further research could be done to look into why there are so few larval salamanders in Cold Springs but lots of adults. It would also be worthwhile to conduct research that looked into why there are so few larval *E. bislineata* even though there were enough adults in the surrounding area for there to be an established breeding community. Research could also be done to explore if water temperature and changes of it explains any of this interesting data since we did not account for temperature play a serious role in species dispersion since the temperature in these springs remains relatively constant year round. It is also worth mentioning that our data does not include salamanders that escaped/evaded capture. About 50% of the salamanders we found managed to escape across all sections and springs so while the trends in the data likely would remain the same, a large amount of data was not able to be recorded. In Warm Springs, our data also shows that the salamander population decreases as we get further from the stream origin. This lines up with the study mentioned in the abstract. “95% of Ocoee Salamanders are distributed within 79 m of their stream of origin and that relative abundance should decline exponentially with distance into terrestrial habitat” (Connette, Grant & Osbourn, Michael & Peterman, William 2016*)*. While this study was on the Ocoee Salamander, the two salamander species we studied, there is overlap and they have similar breeding habits and habitat choice. As stated earlier, the data from Warm Spring supports their conclusions, but Cold Springs was not the same. However, we had a small sample size and more research would have to be done to be conclusive.

## ACKNOWLEDGEMENTS:

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Dr. George Merovich

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DOI

# STREAM HEALTH ASSESSMENT BASED ON MACROINVERTEBRATE DIVERSITY

Kamden Clapper, Ella Gerhardt, Alison Superka and Barrett Walters


## ABSTRACT

Our study was done in Crooked Creek, Muddy Run, and Standing Stone Creek by collecting macroinvertebrates, measuring steam characteristics such as pH, oxygen content and temperature, and measuring the EPT% index. These streams showed three different stream types and areas. We found that Standing Stone Creek had the highest EPT% index followed by Crooked Creek and then Muddy Run. These macroinvertebrates also showed four different functional guilds. Muddy Run had the most species abundance but the fewest number of species while Crooked Creek and Standing Stone Creek were dominated by one species. Standing Stone had the most amount of individual organisms collected followed by Crooked Creek and then Muddy Run. Before the study was started, we assumed that Muddy Run would have the lowest stream health due to its location and visible pollution. We had assumed that Standing Stone and Crooked Creek would be similar in water quality and species richness and abundance due to their location and appearance of stream quality. A chi-square test proved that the difference in the EPT% index of Standing Stone and Crooked Creek from Muddy Run was significant. In conclusion, the stream health of Muddy Run was significantly worse than Standing Stone and Crooked Creek.


*Key words*: *EPT% Index, Species Abundance, Urbanization, Macroinvertebrates, Functional Guild*

## INTRODUCTION

Waterways are extremely important ecosystems that carry a lot of ecological value. The health of waterways in our immediate areas not only affects human lives but impacts the lives of organisms within the waterway itself. Clean and healthy streams provide quality drinking water for humans and organisms and allow for recreational uses. For this study, our group wanted to test the EPT% Index in three different streams and see how they ranked. The three streams that we collected our samples from were Crooked Creek, Muddy Run, and Standing Stone Creek. We had hypothesized that the EPT% index at Muddy Run would be significantly lower than that of Standing Stone and Crooked Creek which would have higher EPT% indexes. We also looked at the functional guild of each macroinvertebrate that we found. The different functional guilds that we focused on were predators, scrapers, collectors, and shredders.

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Muddy Run has the most urbanization around it and is the smallest of streams by width. Crooked Creek has the second most water flow and width. It also has some urbanization but less than Muddy Run. Standing Stone Creek is the highest water flow and the largest width. There is also less urbanization than in the other streams. The purpose of this study was to see how urbanization affects stream health and contributes to the macroinvertebrate abundance of these streams. These streams had a good distribution of these attributes which contribute to stream health.

A study done in Kansas City, Missouri, found that the EPT% index in urban areas was lower than in rural areas. This can be attributed to the rise of the human population in the area which leads to increased soil erosion, changes in hydrologic characteristics, and loading of contaminants associated with urban runoff due to urbanization. These changes in the stream were the main cause of degradation in the aquatic life which included the loss of fish and aquatic macroinvertebrate species which led to these species being replaced with more tolerant organisms. The results of the study stated that 217 macroinvertebrate taxa were collected while only 34 of these taxa were EPT. The EPT richness ranged from 1-14 across all study sites. The non-urban control sites had the highest EPT% each year of the study followed by the control storm sewer system site, then the transitional sewer site, and lastly the urban sewer site. This study shows how over time the transition from rural to urban areas of the stream greatly impacts the EPT richness of the stream and its overall health. (Poulton, 2019)

In another study in Hulu Terengganu, Terengganu used the EPT% index to see if the human and agricultural presence had an effect on macroinvertebrate type and abundance. An upstream site with little to no human/agricultural presence was sampled and a downstream site with human/agricultural presence near and upstream was also sampled for macroinvertebrates. The study concluded that upstream had more abundance and diversity of ETP (Ephemeroptera, Plecoptera, Trichoptera) and fewer pollution sensitive species whereas downstream had less diversity and abundance of ETP and more diversity and abundance of pollution sensitive species such as Chironomidae. This study concludes that the ETP% index can be used to determine if and how much pollution is present in waterways (Wahizatul, 2011).

## FIELD SITE

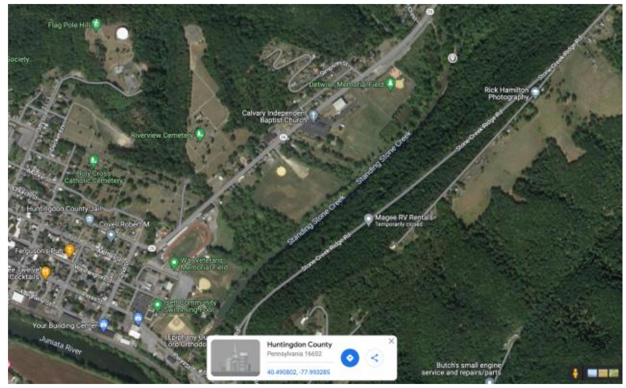

*Figure 1. Aerial photo of where macroinvertebrate and water qualities were approximately collected from Standing Stone Creek.*

*Figure 2. Aerial photo of where macroinvertebrate and water qualities were approximately collected from Crooked Creek.*

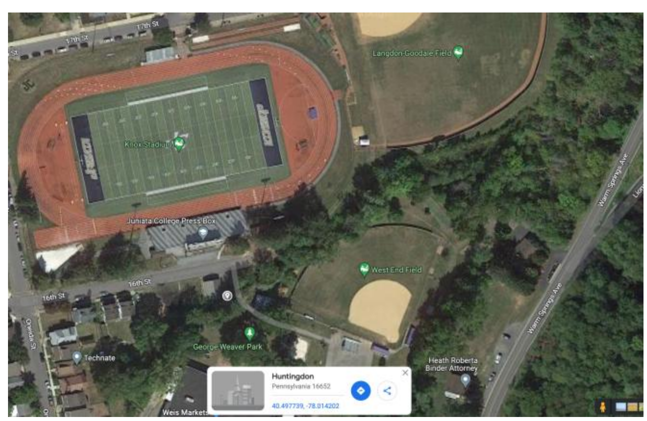

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*Figure 3. Aerial photo of where macroinvertebrate and water qualities were approximately collected from Muddy Run.*

## MATERIAL AND METHODS

The materials for testing water qualities included a pH reading device, a temperature reading device, and an oxygen content reader. These were taken at each of the three sites. For collecting macroinvertebrates, kick nets, three collection jars, and a sorting tub. At each of the three sites, 10 samples were collected randomly from the locations above. One individual held the kicknet while the other individual stirred up the stream bed roughly 1 meter by 1 meter in front of the net. After each sample was collected, it was carefully sorted in a tub and all visible macroinvertebrates were placed in one of the jars. Later in the lab, the macroinvertebrates within the three jars were carefully sorted, identified, and counted using a website.

## RESULTS

| | Temperature (C) | pH | O2 Content (mg/L) | Total Number of Individuals | PTI | EPT Index (%) |
|---|---|---|---|---|---|---|
| Crooked Creek | 9 | 7.51 | 10.93 | 76 | 22 | 78.9 |
| Standing Stone | 9.1 | 7.24 | 10.83 | 98 | 23 | 86.7 |
| Muddy Run | 9.5 | 6.86 | 11.08 | 45 | 12 | 0 |

*Table 1. Quantifications of the characteristics recorded for each field site.*

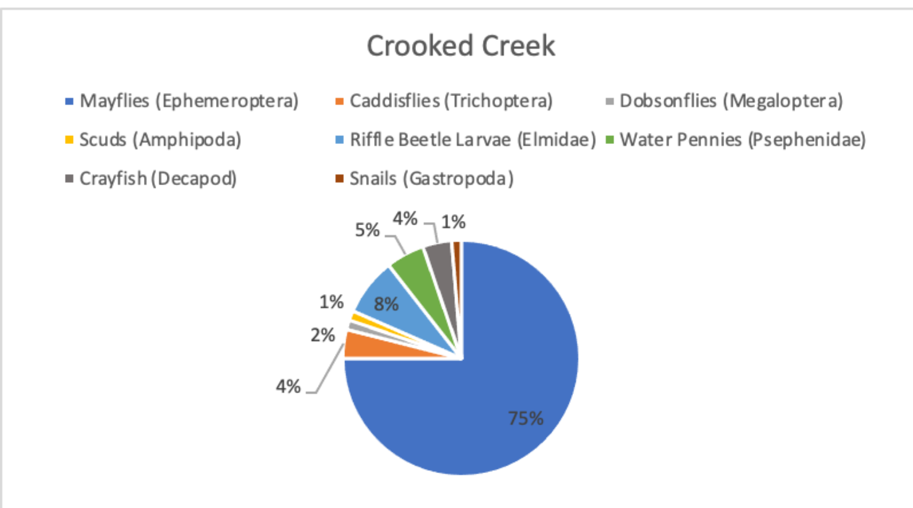


*Figure 4. Chart displaying the proportion of different macroinvertebrates found at Crooked Creek.*

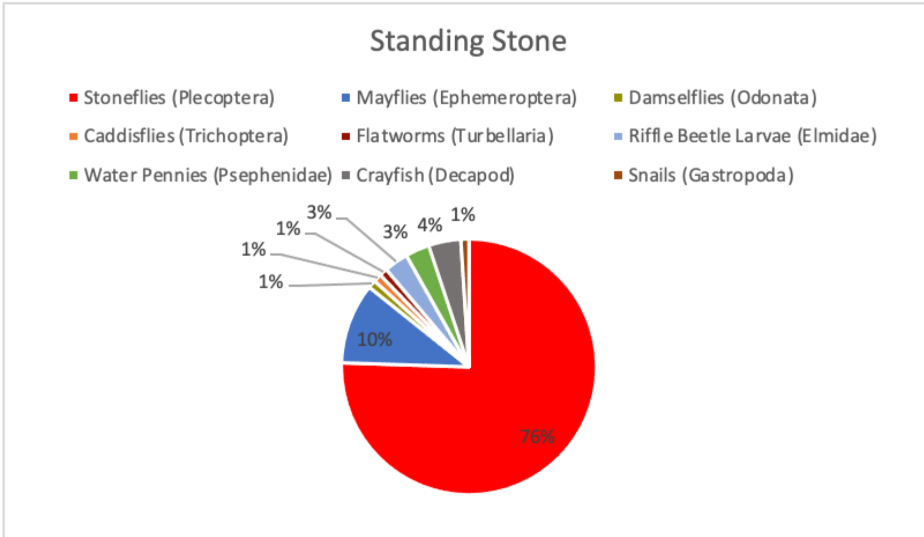


*Figure 5. Chart displaying the proportion of different macroinvertebrates found at Standing Stone Creek.*

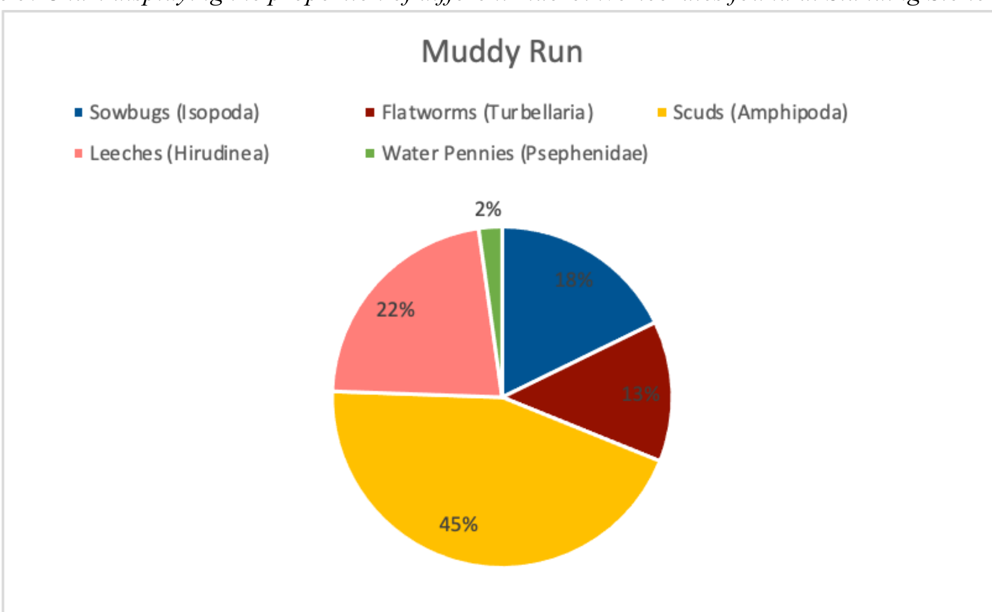


*Figure 6. Chart displaying the proportion of different macroinvertebrates found at Muddy Run.*

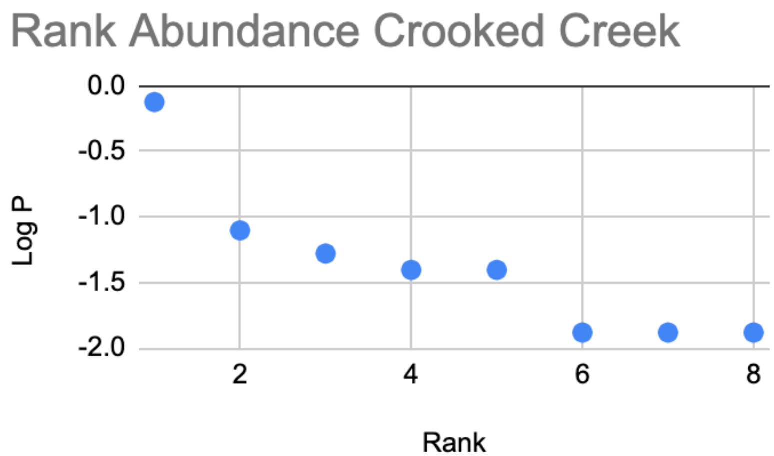


*Figure 7. Graph displaying the rank of species (according to the number found at the field site) by the log of P (the proportion of total taxa that the individual species makes up) for macroinvertebrates collected from Crooked Creek.*

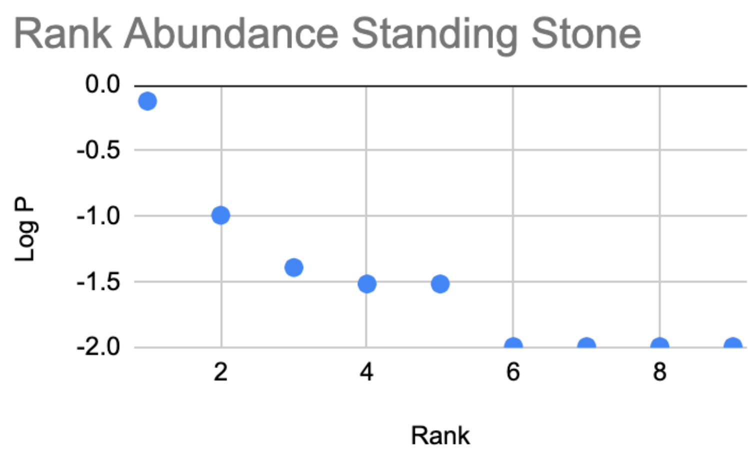


*Figure 8. Graph displaying the rank of species (according to the number found at the field site) by the log of P (the proportion of total taxa that the individual species makes up) for macroinvertebrates collected from Standing Stone Creek.*

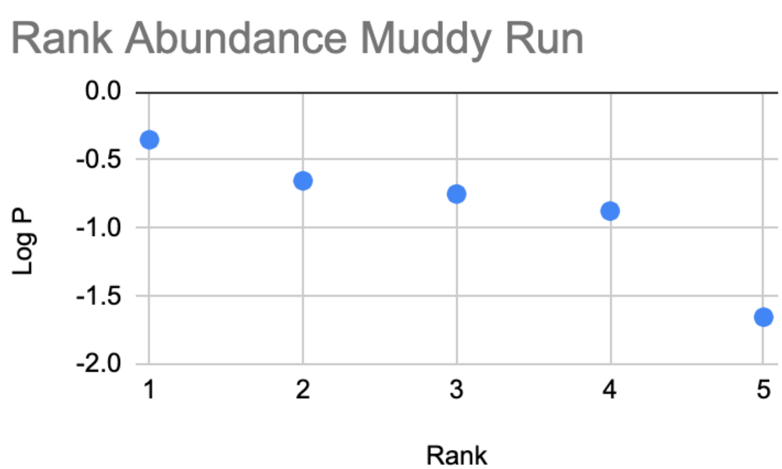

*Figure 9. Graph displaying the rank of species (according to the number found at the field site) by the log of P (the proportion of total taxa that the individual species makes up) for macroinvertebrates collected from Muddy Run.*

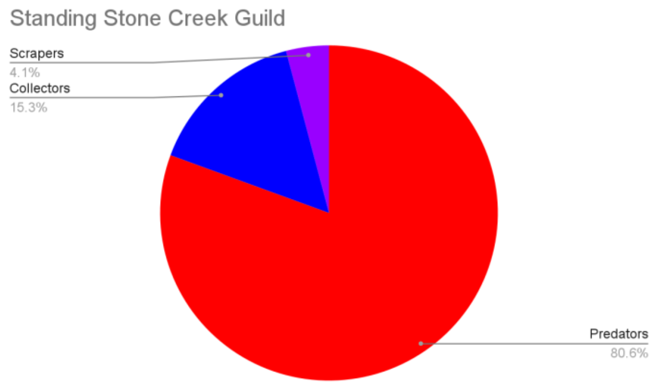


*Figure 10. Chart displaying the proportion of taxa from Standing Stone that fall into each feeding guild.*

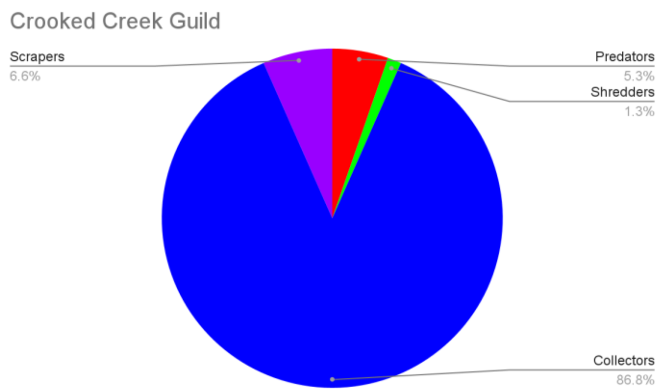


*Figure 11. Chart displaying the proportion of taxa from Crooked Creek that fall into each feeding guild.*

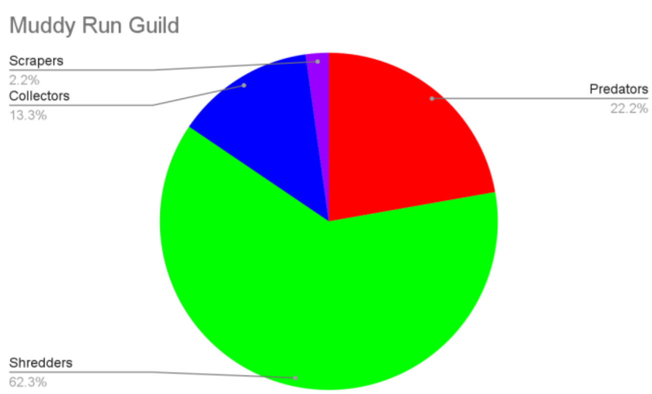


*Figure 12. Chart displaying the proportion of taxa from Muddy Run that fall into each feeding guild.*

| | EPT Individuals | Non-EPT Individuals |
|---|---|---|
| Crooked Creek | 60 | 16 |
| Standing Stone | 85 | 13 |

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| | | |
|---|---|---|
| Muddy Run | 0 | 45 |

*Table 2. Count of taxa that qualify as EPT and Non-EPT at each field site.*

To determine if there is a significant difference in the number of EPT individuals collected from Standing Stone and Crooked Creek compared to Muddy Run, a chi-square test of independence was performed using the values found in Table 2. The test produced a $X^2$ value of 112.14 and a p-value of ~0.000000.

## DISCUSSION

Based on observations of taxa and stream characteristics of Standing Stone Creek, Crooked Creek, and Muddy Run, we can infer that the more rural streams (Standing Stone and Crooked Creek) are healthier than the urban stream (Muddy Run). Standing Stone and Crooked Creek had EPT Indexes of 86.7% and 78.9% respectively while Muddy Run had an EPT Index of 0%. These values tell us that the rural streams possess more macroinvertebrates that are critical for good stream health than the urban stream. The chi-square test that was performed on the count of EPT individuals at each stream produced a p-value of approximately 0.00000 (an extremely small value). At a 5% significance level, this p-value allows us to reject our null hypothesis, in which the rural and urban streams will have similar EPT indexes.

Not only does the EPT index provide an indication of stream health, but assumptions of stream health can be made based on other characteristics of the stream. The pH of a stream can be used to determine stream health. None of the streams studied possessed excessively acidic or basic waters; all of the pH values fell within a ±0.5 range from 7 (the standard pH of water). In general, more acidic water can lead to corrosion in the water which limits available food sources for organisms in the stream.

When comparing the proportion of different feeding guilds of each stream based on the pH of the streams, there are noticeable differences. The more basic streams (Standing Stone and Crooked Creek) possessed a much smaller proportion of shredder species than the acidic stream (Muddy Run). Shredders are organisms that feed on pieces of large organic matter such as the sowbugs and scuds found in Muddy Run. Other feeding guilds include collectors, predators, and scrapers. Collectors are organisms that consume small organic matter such as the mayflies and caddisflies that make up a large proportion of the species found in Crooked Creek. Predators are organisms that feed on other invertebrates in the stream such as the stoneflies and crayfish that make up a large proportion of the species found in Standing Stone. The final guild is scapers, which are organisms that scrape algae off of different surfaces in the stream such as water pennies and snails which were found in smaller proportions at all three streams (Lewis, 2014).

## ACKNOWLEDGMENTS

We would like to acknowledge and thank Dr. Douglas Glazier for putting in the time and effort to guide and teach us this semester. We would also like to thank Teacher Assistant Conner Frey for helping us during the lab and Juniata College’s Environmental Science and Studies Department for letting us borrow the supplies necessary to conduct the study.

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DOI

# COLOR PREFERENCE IN THE BROWN-HEADED COWBIRD

Nathaniel Harris, Margaret Haigney, Moira McAninch, Shawn Mills and Sydney Weichler

## ABSTRACT

This study investigated the color preference of the Brown-headed Cowbird. The cowbird is an intelligent species that exhibits parasitic behavior upon the nests of songbirds, so further understanding of their behavior is important in the conservation of songbird populations. Previous studies have shown that color can affect the feeding patterns of birds in captivity, but less is known about color preference in wild birds. This study hypothesized that Brown-headed Cowbirds would prefer to feed from a blue bowl and was based on previous studies. The Brown-headed Cowbirds were observed through trail cameras over a period of two weeks. Each color bowl was filled with the same amount and type of food, with a black bowl filled with water in the middle to further attract birds. After two weeks, the photos were analyzed with individual feeding events being recorded and translated into Excel. The data was then analyzed using a Chi-Square test, where the p-value was found to be well below the 0.05 required for the result to be significant. The results indicated that Brown-headed Cowbirds prefer to feed from blue bowls, and the null hypothesis was rejected.

*Key words: Color preference, brown-headed cowbird, color vision, feeding*

## INTRODUCTION

Vision is an important sensory channel for communication and perceiving the environment. Most bird species rely heavily on vision and are well-adapted to take full advantage of this sensory channel. They can see light wavelengths ranging from 315 nm to 750 nm. Compare this to humans, whose eyes can only perceive from 380 nm to 740 nm. It is not just that birds can perceive all these wavelengths, they also have an effect on the bird's behavior. A study conducted on broiler chickens found that blue (450nm) and green (550nm) light had a positive effect on body weight increase of the birds and red (700nm) light was found to negatively affect body weight increase and caused an increase in aggressive behavior and activity (Soliman and El-Sabrout, 2020). In captivity color can affect the feeding behavior of birds, but how does it affect the behavior of wild birds with more choice of where they feed? A species of particular interest to us was the Brown-headed Cowbird. This species exhibits a litany of remarkable behaviors that show that further research into their intelligence is warranted. For example, not only do Brown-headed Cowbirds parasitize other nests but they also have been recorded destroying the nests of other birds to create additional opportunities to parasitize the nest when it is rebuilt (Hoover and Robinson, 2007). This behavior happens much more frequently to nests that had previously had a Brown-headed Cowbird egg laid in them, but it was removed by the host birds (Hoover and Robinson, 2007). It is additionally important to better understand the behavior of this species because it is an obligate parasite that can have a negative impact on other songbird populations, especially those that are already endangered due to habitat loss. The least Bell's vireo and southwestern willow flycatcher are two examples of birds that rely on outside control of cowbird populations to survive in their endangered state (Peer et al., 2020). If Brown-headed Cowbirds show significant preference or dislike towards certain colors it may provide more information on how we might keep them from parasitizing nests of endangered birds. Based on previous studies, we hypothesize that Brown-headed Cowbirds will prefer to feed from the blue bowl.

## MATERIAL AND METHODS

Our experiment was conducted in Hustontown, Pa at one of our group members, Shawn's, house. Due to cowbirds' preference for fragmented environments where the field meets forest, an area between two trees on the edge of the field was chosen for the study, both to appeal to local cowbird populations and to provide adequate cover to any bird that attempts to eat.

To gather data, a metal rack around four feet long was hung five feet off the ground, in between two trees. On this rack were four bowls, three of which were filled with identical parrot food, and the fourth containing water. The three bowls containing food each had a different color: orange, pink, and blue. Trail cams were hung on trees positioned on either side of the rack and set to take photos whenever motion was detected. After two weeks of observation and occasional checks to see if the cameras were properly aligned, the SD cards were removed and the data on them was analyzed.

When looking through the pictures, cowbird feeding events were recorded on paper and translated into an Excel workbook. A feeding event was recorded any time a cowbird was seen actively eating at or perched on the side of one of the bowls. If a cowbird was seen at the same bowl over multiple pictures, additional feeding events on that bowl would not be recorded. If a cowbird was seen moving from one bowl to another, a feeding event would be counted on both bowls.

## RESULTS

| | Blue | Orange | Pink | Total observations |
|---|---|---|---|---|
| Observed | 24 | 8 | 7 | 39 |
| Expected | 13 | 13 | 13 | |

*Fig 1. Raw data; count of cowbird feeding events observed at each bowl*

| | Observed | Expected | Difference | Difference Sq. | Diff. Sq. / Exp Fr. |
|---|---|---|---|---|---|
| Blue | 24 | 13 | 11.00 | 121.00 | 9.31 |
| Orange | 7 | 13 | -6.00 | 36.00 | 2.77 |
| Pink | 8 | 13 | -5.00 | 25.00 | 1.92 |
| | | | | | |
| | | | | | |
| | | | | | 14.000 |

The Chi^2 value is 14. The $p$-value is .00091. The result is significant at $p < .05$.

*Fig 2. Chi-square goodness of fit test calculated on social science statistics*

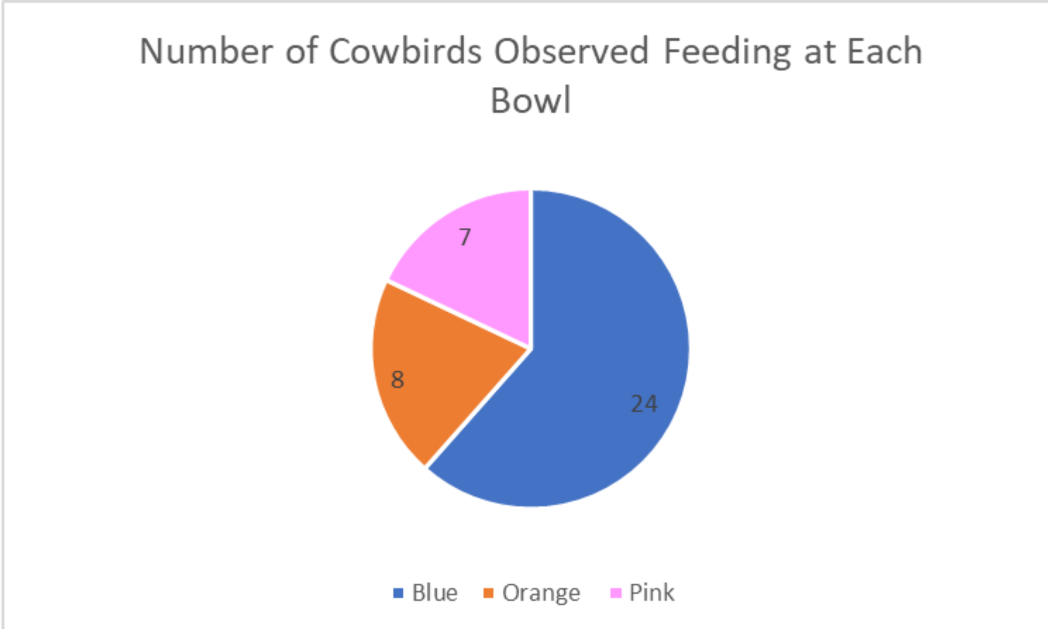


*Fig 3. Pie chart breaking down how many feeding events were recorded at each bowl*

Based on the Chi-Square having a p-value well below .05, the null hypothesis that the cowbirds would have no preference towards any one color can be rejected. From the raw data and the pie chart, it can be clearly seen that the birds studied showed a strong preference for food from the blue bowl.

## DISCUSSION

According to Texas Parks and Wildlife, the brown-headed cowbird is a direct threat to 225 known species of North American songbirds, including endangered species such as the Kirtland's Warbler and the Black-Capped Vireo. To effectively limit the impact of an animal like the cowbird, it is important to understand as much about it as possible. Understanding what colors Cowbirds are the most partial to can possibly be used to make bird feeders in areas with fragmented forests less appealing and this could possibly limit the resources available to this devastating brood parasite. Removing the blue bowl from the rack and starting another observation period to see how the Cowbirds respond to the lack of their preferred color and if this impacts how many end up feeding in the observed area could have provided valuable insight and would be a good place to start for further study on this topic. Additionally, further study on how Cowbirds react and see other colors could help make bird feeders as unappealing as possible to Cowbirds without negatively impacting other birds that are reliant on human-supplied food. Until we better understand the cowbird color preference, the data gathered from this study suggest that people interested in setting up bird feeders or bird houses in areas with a high populations of cowbirds should avoid using the color blue, as cowbirds do seem to have a strong preference for it.

## CONCLUSION

This study aimed to investigate the color preference of Brown-headed Cowbirds in terms of their feeding behavior. The Brown-headed Cowbird is an interesting species that exhibits parasitic behavior upon the nests of other songbirds, which can have a negative impact on the conservation of endangered species. Previous studies have shown that color can affect the feeding behavior of birds in captivity, but less is known about color preference in wild birds. The data was then analyzed using a Chi-Square test, which showed that the Brown-headed Cowbirds had a strong preference for feeding from the blue bowl, thus rejecting the null hypothesis that there would be no preference towards any one color. Understanding the behavior of the Brown-headed Cowbird is important for the conservation of songbird populations, particularly those that are already endangered due to habitat loss. The results of this study provide valuable information on how color can influence feeding behavior in wild birds, which may help in developing strategies to prevent Brown-headed Cowbirds from parasitizing the nests of endangered birds.

## ACKNOWLEDGMENTS

- Dr. Glazier: provided guidance and support throughout the length of the study.
- Leroy and Elroy Mills: provided emotional support.
- Shawn’s Mom: provided the metal rack and allowed the experiment to occur on her property.
- Dr. Yohn and Dr. Ramakrishna: provided the trail cams and SD cards for data collection.
- Harold the Mouse: provided ¼ of the pictures gathered by the trail cams.

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DOI

# THE FORAGING HABITS OF AMERICAN ROBINS (*TURDUS MIGRATORIUS*) AND EASTERN GREY SQUIRRELS (*SCIURUS CAROLINENSIS)* IN AREAS OF VARYING TRAFFIC CONDITIONS THROUGHOUT THE JUNIATA COLLEGE CAMPUS

Sarah Halteman, Dorinda Russo and Duncan Urquhart

## ABSTRACT

The effect of anthropogenic noise pollution on natural ecological systems and animals is a complex topic that is actively trying to be understood by scientists worldwide. In this experiment, it was hypothesized that if the activity of traffic and noise around a location decreased, the feeding occurrences observed of eastern gray squirrels and American robins would increase across the Juniata College Campus. To conduct this experiment, researchers counted feeding occurrences of squirrels and robins across three locations of various noise and traffic levels. It was found that the high foot traffic area had the most feeding occurrences of robins and squirrels. Surprisingly, no feeding observations were made in the forest area with no surrounding traffic or anthropogenic noise pollution. Given the amount of wildlife observed near traffic, this study supports the idea that more of an effort should be made to mitigate the negative effects this traffic has on the abundant wildlife species, such as through automobile collisions and habitat fragmentation. Therefore, these results also provide a sound foundation for the argument of increasing natural habitats for terrestrial and avian species across the Juniata College campus.

*Key words: Foraging habits, American robins,* Turdus migratorius*, Eastern Gray Squirrels,* Sciurus carolinensis

## INTRODUCTION

While on a hike in the remote woods, it would overall not be uncommon or unlikely to see wildlife such as deer, squirrels, or birds foraging for food or in search of shelter. However, the same cannot be said of more populated or busy areas. Generally, many animals avoid or are typically not seen in areas with a lot of activity or high noise levels. This phenomenon of anthropogenic noise affecting the behavior of animals has been shown to have significant environmental consequences. As a form of noise pollution, it must be considered a serious mode of environmental change as it affects aquatic, avian, and terrestrial species (Kunc & Schmidt 2019).

Many studies have proven this by showing the negative effects roads have on wildlife due to many factors such as population fragmentation, pollution, habitat loss, noise, and direct collisions with cars. Also, the factors of road mortality and traffic noise have been studied regarding their important effects on many wild avian species (Husby 2017). Overall, roads, and the traffic on them, decrease the populations of many species because of this range of factors (Fahrig & Rytwinski 2009). In addition, given that several hundred million terrestrial and avian animals are killed in collisions with cars every year, it is expected that bird and squirrel densities be lower in areas closer to roads with considerable amounts of traffic.

Overall, a species' vulnerability to traffic conditions is influenced by its ecological traits, adaptations, and even behavioral responses like reproductive rate and mobility. While the material and surface of the road might cause animals to avoid areas of high traffic, other disturbances from traffic like chemical emissions, noise, and light also show significant effects on animal behavior and abundance (Rytwinski & Fahrig 2015).

More specifically, it has been shown that mammals, such as squirrels, with lower reproductive rates are more susceptible to road mortality. As a result, they are more likely to avoid roads and experience habitat and population fragmentation. Also, birds with larger territories, high body masses, and that fly at low levels are more susceptible to road mortality (Fahrig & Rytwinski 2009).

To expand, many scientists have recently become intrigued by how species' populations and abundances are negatively affected by their experience of anthropogenically driven environmental changes, whether it be noise from physical human movement or automobile movement. Just as human health can be negatively impacted by noise pollution, the same can be said of terrestrial and aquatic wildlife. Various species across several different taxonomic groups show a significant response to noise. For example, anthropogenic noise negatively impacts bats hunting efficiency (Kunc & Schmidt 2019). However, the effect of several types of anthropogenic noise and human activity on the foraging behavior and abundance of specific species has yet to be studied.

To study this trend, experimentation can be done in the branch of ecology called foraging ecology. Foraging ecology is the branch of scientific study and behavioral ecology that answers these questions of how and why an animal behaves the way it does. Specifically, foraging ecology deals with theories and explanations as to how an animal is best able to forage or find the food necessary for its survival and reproduction (Libretexts 2022).

Through the lens of foraging ecology, the behavior of a species tells a lot about its niche in its environment and community. In general, natural selection favors species that have as little negative competition as possible with other species and those that find and use food most effectively. Therefore, ecological communities are structured by the competition for food and the passage of food through trophic levels and energy chains from prey to predators.

In addition, human foot traffic can potentially increase predator-prey interactions, among other far-reaching ecological effects. This is caused because, as animals attempt to avoid people, they can adapt their behavior by becoming more nocturnal or more arboreal. They might also begin avoiding trails on which humans commonly travel (Griffiths & van Schaik 1993). These behavioral changes not only affect their competition with other species but also an increased potential to interact with new predators or stressors.

Therefore, in this experiment, the foraging habits of eastern gray squirrels and American robins according to the amount of nearby human and automobile traffic was tested. It was hypothesized that if the activity of traffic around a location decreased, the feeding occurrences observed of eastern gray squirrels and American robins would increase because they will experience less fear and stress from the loud surroundings. Contrarily, the null hypothesis states that if the activity of traffic around a location decreased, the feeding occurrences observed of eastern gray squirrels and American robins would not be affected or change.

## FIELD SITE

Observations were held at three contrasting locations throughout the Juniata College Campus. Location #1 was The Quad on Juniata Campus which is a high foot traffic area. This means that this area typically has higher noise levels as a result of human activity and movement. Location #2 was outside of the Brumbaugh Academic Center and in front of Moore Street on the Juniata College campus. This area is characterized by a lot of noise resulting from automobile traffic. Location #3 was the Peace Chapel which is a low-traffic area. This area is a forested location where the noise from both automobile traffic and foot traffic is not as present as in the other locations (Figure 1). Each observation location had trees and patches of grass and are therefore potentially suitable habitats for American robins and Eastern gray squirrels.

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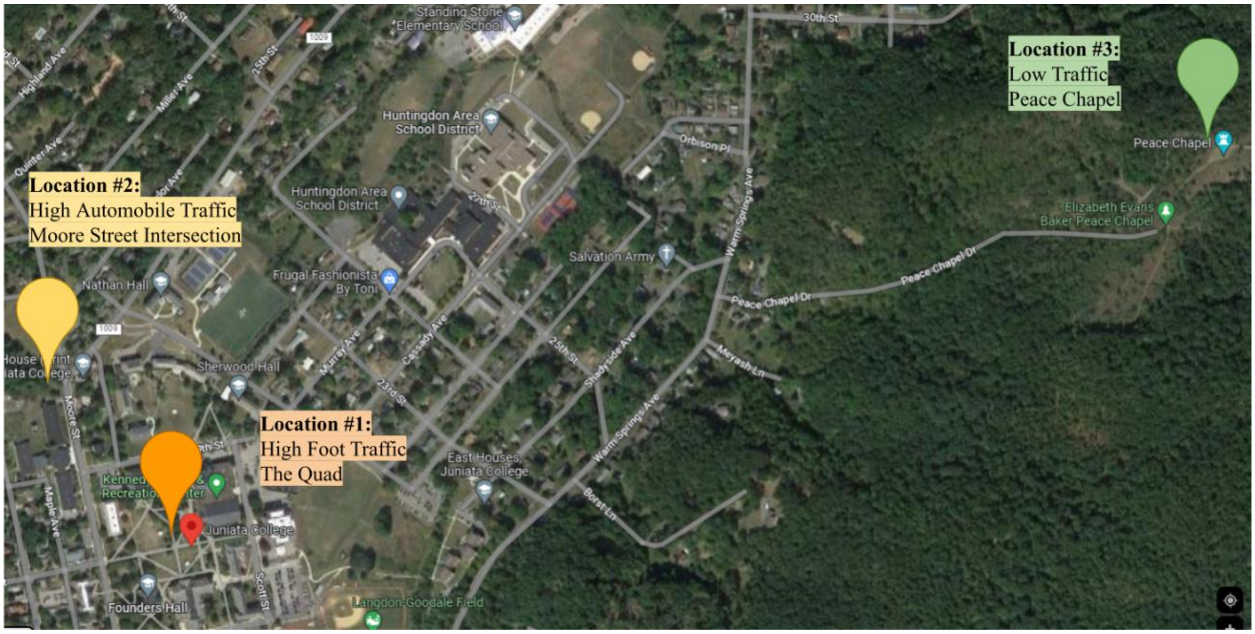


*Figure 1. Google Maps satellite image of the Juniata College campus with the three field sites labeled: High Foot Traffic Area, High Automobile Traffic Area, and Low-Traffic Areas*

## MATERIAL AND METHODS

To begin the experiment, three plastic dishes were filled with “Feathered Friend” Striped Sunflower Seed Wild Bird Food, which is marketed as a high energy, high protein seed to attract a variety of larger birds. At the same time and day, one researcher was dispatched to each location detailed in Figure 1 to simultaneously collect data. The plastic dishes of sunflower seeds were placed at the foot of a tree at each location. Then, the feeding occurrences of robins and squirrels from these dishes were simultaneously observed from a large distance and recorded at each of the three location sites for the duration of between a half hour to an hour. This was repeated over the span of several days in 2023. In this experiment, a feeding occurrence was defined as when an animal touched or interacted with the striped sunflower seeds from the plastic dish.

Overall, data was collected over a total of eight non-consecutive days. The dates over which data collection occurred were March 29$^{th}$, March 30$^{th}$, April 3$^{rd}$, April 4$^{th}$, April 5$^{th}$, April 10$^{th}$, April 12$^{th}$, and April 13$^{th}$. Data was first collected between 1:30-2:30 PM on March 29th, but each subsequent session was held in the hours around dusk (7:30-8:00 PM) to increase the probability of squirrel and robin activity. This is because squirrels are most active in late afternoons (*Squirrels*). Also, robins are most commonly seen foraging in flocks at night (Kaufman 2019).

At the beginning of each data collection session, the outdoor temperature was also recorded. Following the completion of data collection, several statistical analysis tests were run to determine the significance of any gathered information.

## RESULTS

The only location where squirrels were observed foraging from the sunflower seed dishes was in the high foot traffic location. This area had the most observed feeding occurrences of both robins and squirrels. No feeding activity was observed at all in the low-traffic, forested location (Table 1). These results were reorganized in Table 2 to afford a more concise table on which statistical analysis could be performed. Table 3 was utilized to perform Pearson’s product-moment correlation testing for the feeding total of the high foot traffic location to the high car traffic location.

Regarding this statistical analysis, the Pearson’s product-moment correlation statistical test for total feeding occurrences at the high foot traffic location versus total feeding occurrences at the high automobile traffic location showed a strong direct association. Another Pearson’s product-moment correlation test for feeding instances of robins at the high foot traffic location versus feeding instances of robins at the high automobile traffic location showed a strong, direct association (Table 4). Then, a Kruskal Wallis Rank Sum statistical test for the independence of Robin feeding instances versus Squirrel feeding instances at the high foot traffic location indicated that these variables are independent (Table 5).

In addition, a chi-squared statistical analysis comparing robin feeding occurrences and location resulted in a p=.000233. Another chi-squared statistical analysis compared feeding instances of squirrels and location which resulted in a p=.032752. Both results are significant at $p < .05$ (Table 6).

Various two sample T-Tests were performed to determine if there were any significant differences between the feeding instances of robins and grey squirrels and both high foot traffic and high automobile traffic areas (Table 7).

*Table 1. Feeding occurrences of robins and squirrels observed at a high foot traffic location, high automobile traffic location, and a general low-traffic area in Huntingdon County.*

| | **High Foot Traffic Area (The Quad)** | | **High Automobile Traffic Area (Moore Street)** | | **Low-Traffic Area (Peace Chapel)** | |
|---|---|---|---|---|---|---|
| **Date Time Temperature** | **Robins** | **Squirrels** | **Robins** | **Squirrels** | **Robins** | **Squirrels** |
| **3/29/23 1:30-2:30PM 53°F** | 0 | 0 | 0 | 0 | 0 | 0 |
| **3/30/23 7:30-8:00PM 49°F** | 3 | 3 | 3 | 0 | 0 | 0 |
| **4/3/23 7:30-8:00PM 69°F** | 1 | 4 | 1 | 0 | 0 | 0 |
| **4/4/23 7:30-8:00PM 71.6°F** | 1 | 0 | 0 | 0 | 0 | 0 |
| **4/5/23 7:30-8:00PM 78°F** | 6 | 1 | 1 | 0 | 0 | 0 |
| **4/10/23 7:30-8:00PM 79°F** | 15 | 2 | 4 | 0 | 0 | 0 |
| **4/12/23 7:30-8:00PM 77°F** | 2 | 0 | 1 | 0 | 0 | 0 |
| **4/13/23 7:30-8:00PM 76°F** | 1 | 1 | 6 | 0 | 0 | 0 |
| ***Totals*** | *29* | *11* | *16* | *0* | *0* | *0* |

*Table 2. Concise organization of data from Table 1 used for statistical analysis.*

| | Temperature | Date | Robin_1 | Squirrel_1 | Total_1 | Robin_2 | Squirrel_2 | Total_2 | Robin_3 | Squirrel_3 | Total_3 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 53.0 | 3/29/23 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 49.0 | 3/30/23 | 3 | 3 | 6 | 3 | 0 | 3 | 0 | 0 | 0 |
| 3 | 69.0 | 4/3/23 | 1 | 4 | 5 | 1 | 0 | 1 | 0 | 0 | 0 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **4** | 71.6 | 4/4/23 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| **5** | 78.0 | 4/5/23 | 6 | 1 | 7 | 1 | 0 | 1 | 0 | 0 | 0 |
| **6** | 79.0 | 4/10/23 | 15 | 2 | 17 | 4 | 0 | 4 | 0 | 0 | 0 |
| **7** | 77.0 | 4/12/23 | 2 | 0 | 2 | 1 | 0 | 1 | 0 | 0 | 0 |
| **8** | 76.0 | 4/13/23 | 1 | 1 | 2 | 6 | 0 | 6 | 0 | 0 | 0 |
| **9** | *NA* | | 29 | 11 | 40 | 16 | 0 | 16 | 0 | 0 | 0 |
| | *Key:<br>Underscore 1 is the High Foot Traffic area in the Quad<br>Underscore 2 is the High Automobile Traffic area near Moore Street and Cold springs Road<br>Underscore 3 is the Low-Traffic Area at peace chapel* | | | | | | | | | | |

*Table 3. Pearson's product-moment correlation data for statistical test Feeding Total of High Foot Traffic vs High Car Traffic.*

| | *Total 1 (High Foot Traffic)* | *Total 2 (High Car Traffic)* |
|---|---|---|
| *1* | *0* | *0* |
| *2* | *6* | *3* |
| *3* | *5* | *1* |
| *4* | *1* | *0* |
| *5* | *7* | *1* |
| *6* | *17* | *4* |
| *7* | *2* | *1* |
| *8* | *2* | *6* |
| *Total* | *40* | *16* |

*Table 4. Pearson's product-moment correlation statistical tests for association between two variables*

| |
|---|
| Feeding Total of High Foot Traffic vs. High Car Traffic<br><br>Pearson's product-moment correlation<br>data: AD$Total_1 and AD$Total_2<br>t = 5.5976, df = 7, p-value = 0.000818<br>alternative hypothesis: true correlation is not equal to 0<br>95 percent confidence interval: 0.6005778 0.9798727<br>sample estimates: cor 0.9040951<br><br>There is a strong, direct association between the instances of feeding in the high foot traffic vs high automobile traffic area. |
| Feeding Instances of Robins at High Foot Traffic vs. High Automobile Traffic<br><br>Pearson's product-moment correlation<br>data: AD$Robin_1 and AD$Robin_2<br>t = 4.7226, df = 7, p-value = 0.002151<br>alternative hypothesis: true correlation is not equal to 0<br>95 percent confidence interval:0.4952273 0.9728681<br>sample estimates: cor 0.8724183 |

There is a strong direct association between the instances of robins feeding at high foot traffic and high automobile traffic.

*Table 5. Kruskal Wallis Rank Sum statistical tests for the independence of Robin Feeding Instances vs Squirrel Feeding Instances at High Foot Traffic Areas.*

Kruskal-Wallis rank sum test: Robin Feeding Instances vs Squirrel Feeding Instances at High Foot Traffic Areas

data: AD$Robin_1 by AD$Squirrel_1
Kruskal-Wallis chi-squared = 5.7931, df = 5, p-value = 0.3269

The total feeding instances of Robins and Squirrels at the high foot traffic area are independent.

*Table 6. Results of the two-chi-square statistical analysis.*

| **Robin Chi ^2 Results** | | | | |
|---|---|---|---|---|
| | **Robin High Foot Traffic** | **Robin High Car Traffic** | **Robin Low-Traffic** | ***Row Totals*** |
| Observed | 29 (22.24) [2.05] | 16 (15.67) [0.01] | 1 (8.09) [6.21] | 46 |
| Expected | 15 (21.76) [2.10] | 15 (15.33) [0.01] | 15 (7.91) [6.35] | 45 |
| ***Column Totals*** | 44 | 31 | 16 | **91 (Grand Total)** |

The chi-square statistic is 16.7278. The *p*-value is .000233. The result is significant at $p < .05$.
*The feeding instances of Robins is related to the amount and type of traffic in the area.*
**Please note that in the table above the low-traffic observed data was changed to a value of 1. This is to allow the calculations to run and does not change the overall understanding of the data.*

| **Squirrel Chi ^2 Results** | | | | |
|---|---|---|---|---|
| | **Squirrel High Foot Traffic** | **Squirrel High Car Traffic** | **Squirrel Low-Traffic** | ***Row Totals*** |
| Observed | 11 (7.80) [1.31] | 1 (2.60) [0.98] | 1 (2.60) [0.98] | 13 |
| Expected | 4 (7.20) [1.42] | 4 (2.40) [1.07] | 4 (2.40) [1.07] | 12 |
| ***Column Totals*** | 15 | 5 | 5 | **25 (Grand Total** |

The chi-square statistic is 6.8376. The *p*-value is .032752. The result is significant at $p < .05$.
*The feeding instances of squirrels is related to the amount and type of traffic in the area.*
**Please note that in the table above the observed values for the low-traffic and the high car traffic squirrel data were changed to a value of 1. This is to allow the calculations to run and does not change the overall understanding of the data.*

*Table 7. Results of Various Two Sample T Tests. None of the tests were found to be significant, and we accept the null hypothesis*

Welch Two Sample t-test: Robin Feeding Instances at High Foot Traffic Vs High Car Traffic

data: AD$Robin_1 and AD$Robin_2
t = 0.79512, df = 12.117, p-value = 0.4418
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval: -5.018817 10.796595
sample estimates: mean of x mean of y 6.444444 3.555556

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| There is no significant difference between the feeding instances of robins between the high foot traffic areas and high automobile traffic areas. |
| Welch Two Sample t-test: Squirrel Feeding Instances at High Foot Traffic Vs High Car Traffic<br><br>data: AD$Squirrel_1 and AD$Squirrel_2<br>t = 2.0929, df = 8, p-value = 0.06971<br>alternative hypothesis: true difference in means is not equal to 0<br>95 percent confidence interval: -0.2489423 5.1378311<br>sample estimates: mean of x mean of y 2.444444 0.000000<br><br>There is no significant difference between the feeding instances of squirrels between the high foot traffic areas and high automobile traffic areas. |
| Welch Two Sample t-test: Total Feeding Instances at High Foot Traffic vs High Car Traffic<br><br>data: AD$Total_1 and AD$Total_2<br>t = 1.1673, df = 10.481, p-value = 0.269<br>alternative hypothesis: true difference in means is not equal to 0<br>95 percent confidence interval: -4.78419 15.45086<br>sample estimates: mean of x mean of y 8.888889 3.555556<br><br>There is no significant difference between the total feeding instances at high foot traffic areas and high automobile traffic areas. |

## DISCUSSION

The results of this experiment were unexpected. As shown in Table 1, there were no observed feeding occurrences of robins or squirrels at the low-traffic area which was originally predicted to have the most observed feeding occurrences. Instead, the area of high human foot traffic was observed to have the most occurrences. Location #2 of high automobile traffic also showed robin feeding occurrences (Table 2). As shown in Table 6, the feeding occurrences of robins, squirrels were significantly different from the expected between the high automobile traffic location and the high human foot traffic location. Therefore, the two variables, traffic level and feeding instances are not independent of each other. However, the Two Sample T-test, which measures if two populations are equal, was not found to be significant. This could be changed with a larger study and more data points which would make the data more normal.

Given the results of Pearson's product-moment analyses, there is a strong, direct association between the instances of feeding in the high foot traffic versus the high automobile traffic area. There is also a strong direct association between the instances of robins feeding at high foot traffic and high automobile traffic. This means that there is a strong linear relationship between the variables of traffic level and feeding instances. As traffic level changes so will the number of animals that feed in the area. An increase in automobile traffic in a neighborhood can affect how many squirrels or robins will feed in the area. Also, the total feeding instances of robins and squirrels at the high foot traffic area are independent of each other and were not found to be significant (Table 5). Therefore, the number of robins feeding in an area does not affect the number of squirrels feeding in the area. The two organisms' feeding habits, in this case, are unrelated.

Because of this, the hypothesis that if the activity of traffic around a location decreased, the feeding occurrences observed of Eastern Gray squirrels and American robins would increase because they will experience less fear and stress from the loud commotion is rejected. There was no data from the low traffic area, but in a longer study with more data points that could change. Based on the results of this study, if the activity of traffic around a location increases, the feeding occurrences observed of Eastern Gray squirrels and American Robins also increase. Given the results of this study, it is possible that with a larger sample size at more locations, the results could be different, and the data could become more normal.

These trends might also possibly be explained by different traits in the squirrel and robin populations in the three locations. While it is known why animals avoid high automobile traffic areas, the difference in animal abundances between areas of foot traffic and areas of automobile traffic has yet to be distinctly compared. However, we can extrapolate that the robins and squirrels in the populations of both high-traffic locations could have acclimated to the noise and activity of humans and automobiles in the area.

It has previously been cited in literature that, at roads where the density of traffic is higher and moving at a faster pace, the bird abundances are lower (Husby 2017). A reason for this trend could be the decreased amount of vegetation surrounding larger roads. However, studies indicate that birds show an active avoidance behavior of loud cars by either increasing their flight height or avoiding the roads altogether because, when comparing two roads of the same size and the same amount of surrounding vegetation, birds were less likely to be observed around the road with more automobile traffic. Also, for many species, traffic noise is one of the greatest factors leading to differences in breeding densities (Husby 2017).

Other differences in behavior could also account for our results. For example, it could be theorized that wildlife in forested areas away from human activity would be less adventurous in their foraging habits and take less risks. Higher-traffic environments have both fewer predators and more irregular sources of food, such as trash cans or litter, and so encourage active and constant exploratory behavior to utilize their resources. In contrast, the forest presents the risk of predation, which discourages careless foraging, and has sources of food which may be more fixed and regular.

The relatively short data collection periods may have exacerbated these behavioral differences. The squirrels and robins in and around campus would be more accustomed to human activity and would thus not be as cautious when presented with it, while forest inhabiting individuals would be more disturbed by the intrusion of researchers. This may have caused the low-traffic site to not record any feeding events, as the wildlife would have been either driven from the area or scared into inactivity and not have had time to return and begin.

Also, the daily weather could have affected the observed trends of robin and squirrel abundance. During this experiment, peak robin feeding occurrences were observed on April 10$^{th}$, 2023. This likely occurred because of the rainstorm that had occurred the day prior. Rainy weather makes it easier for robins to forage for food because insects and worms, which are their preferred foods, are easier to find in rainy weather (Garrett 2019).

It has been proven that roads used for automobile traffic have fragmented the natural habitats and changed the behavior of many avian and terrestrial species. Given that there are not a lot of natural habitats left for these present populations to find shelter, they may currently be forced to use these less-than-ideal locations as their homes. Crossing structures could be installed to improve habitat connectivity, keep wildlife off the roads and decrease the number of inevitable automobile collisions with them (Rytwinski & Fahrig 2015).

The fact that these species are using these locations as their habitat could have long-term effects on their natural competitors, too. A study has shown that the effect of intense human foot traffic on wildlife has been shown to mirror the effects of hunting (Griffiths & van Schaik 1993). The presence of humans eventually can reduce predation on the habituated species by their natural predators, which could ultimately reduce the populations of any competitor species from resource domination (Griffiths & van Schaik 1993).

Overall, the high populations of wildlife inhabiting these busy areas are concerning given that there are many negative effects that roads and humans have on wildlife, such as vehicle-caused mortality (Husby 2017). Therefore, the long-term implications of this study may be far-reaching. While they do not indicate the negative effects of human-generated anthropogenic noise on wildlife population abundance, they support the notion that more can be done to mitigate the effects of habitat fragmentation caused by the construction of Juniata College.

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Given the amount of wildlife observed near traffic, this study supports the idea that more of an effort should be made to mitigate these negative effects that traffic has on the abundant wildlife species like automobile collisions and habitat fragmentation. Therefore, these results also provide a sound foundation for the argument of increasing natural habitats for terrestrial and avian species across the Juniata College campus to provide them with space away from traffic to inhabit.

Overall, when interacting with nature, conducting field research, or even just hiking on a trail where one might only have a minor interaction with wildlife, it is important to evaluate the environmental impact human intervention has on natural habitats.

Though, it may be ever more important to be conscious of human development in previously natural environments. Even a simple sidewalk through campus is an indication of the fragmentation humans have created in wildlife habitats.

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