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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.

THE LEGACY VOLUME

Since the start in 1998, there have been a total of 26 volumes created by Dr. Glazier through the Journal of Ecological Research. The Legacy Volume is a curated collection of these past 26 volumes that aims to highlight articles made by Juniata Alumni who have continued to conduct exemplary research since their time as Juniata. These alumni have gone on to become researchers and educators in their respective fields, and this volume features their past works and contributions to the Journal of Ecological Research, and now, the Juniata Journal of Ecology.

Date of printing of Volume #28 of the Juniata Journal of Ecology is xxxxx.

Cover Illustration: Common Loon Resting in a Lake (*Gavia immer*)

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DIATOMS AS BIO-INDICATORS OF NITRATE CONCENTRATIONS IN LOCAL FRESHWATER SPRINGS

Jonathan W. Baker, Charles P. Gilman, Micah F. Morton, Shannon V. Nayyar and Daryl R. Valley

ABSTRACT

We tested whether diatom abundance and diversity are correlated with differences in nitrate concentration among five freshwater springs in central Pennsylvania. We found no such correlations. We conclude that other environmental factors may be limiting diatom growth and diversity within the study springs.

Key words: Diatoms, freshwater spring

INTRODUCTION

Ruth Patrick (1966) has shown that the growth rate of diatoms is limited by hydrogen-ion concentration, amount of sunlight, water depth, stream-flow rate, and nitrate, phosphate and iron concentrations. The purpose of this study was to examine whether nitrate concentration affects diatom abundance and diversity in freshwater springs of central Pennsylvania. If so, diatoms may be used as bio-indicators of toxic concentrations of nitrate in spring water. Nitrates and nitrites are nitrogen-oxygen chemical units which combine with various organic and inorganic compounds. Once taken into the body, nitrates are converted into nitrites. The greatest use of nitrates is as fertilizers.

Why are Nitrates/Nitrites being Regulated?

In 1974, the U.S. Congress passed the Safe Drinking Water Act. This law requires the Environmental Protection Agency (EPA) to determine safe levels of chemicals in drinking water which do or may cause health problems. These non-enforceable levels, based solely on possible health risks and exposure, are called Maximum Contaminant Level Goals. The MCLG for nitrates has been set at 10 parts per million (ppm), and for nitrites at 1 ppm, because EPA believes this level of protection would not cause any of the potential health problems described below. Based on this MCLG, EPA has set an enforceable standard called a Maximum Contaminant Level (MCL). MCLs are set as close to the MCLGs as possible, considering the ability of public water systems to detect and remove contaminants using suitable treatment technologies. The MCL for nitrates has been set at 10 ppm, and for nitrites at 1 ppm, because EPA believes, given present technology and resources, this is the lowest level to which water systems can reasonably be required to remove this contaminant should it occur in drinking water. These drinking water standards and the regulations for ensuring these standards are met, are called National Primary Drinking Water Regulations. All public water supplies must abide by these regulations.

What are the Health Effects?

Over the short-term, excessive levels of nitrate in drinking water have caused serious illness and sometimes death. The serious illness in infants is due to the conversion of nitrate to nitrite by the body, which can interfere with the oxygen-carrying capacity of the blood. This can be an acute condition in which health deteriorates rapidly over a period of days. Symptoms include shortness of breath and blueness of the skin. Over the long-term, nitrates and nitrites have the potential to cause the following

effects from a lifetime exposure at levels above the MCL: diuresis, increased starchy deposits and hemorrhaging of the spleen.

How much Nitrates/Nitrites are Produced and Released to the Environment?

Most nitrogenous materials in natural waters tend to be converted to nitrate, so all sources of combined nitrogen, particularly organic nitrogen and ammonia should be considered as potential nitrate sources. Primary sources of organic nitrates include human sewage and livestock manure, especially from feeding. The primary inorganic nitrates which may contaminate drinking water are potassium nitrate and ammonium nitrate, both of which are widely used as fertilizers.

FIELD SITES

Kanesatake Spring

Kanesatake spring is located on the grounds of a summer church camp. It runs parallel to Spruce Creek, eventually meeting it at a point three feet beyond the area of sampling. Spruce creek is about 10 feet in width. There are many trees along Spruce Creek, but their shade does not extend to the spring. The spring flows out from the ground and forms a wetland area. The amount of plant coverage exposes the spring water to decaying plant material. Chemical tests showed that the pH was 7.29, and the average nitrate concentration was 7.5 ppm. During the study period, the spring had depths from one to four inches with average depth of 3.2 inches. Two slides used for diatom colonization were attached to a stick placed at a depth of 1.5 inches and where the flow rate was 0.294 m/s.

Emma Spring

Emma spring is surrounded by farm land. It flows out from rocks at the bottom of a steep sloped hill. The stream then runs under and along a road. We placed two sample slides near the spring source at a depth of 5 inches and a flow rate of .3125 m/s. Another two slides were placed about 50 yards downstream at a depth of 3 inches and a flow rate of 0.350 m/s. There were no trees or brush to shade the area where the slides were placed, though many fallen leaves were present. There were no living macrophytes in the stream. We found a frog at the mouth of the spring, and amphipods (*Gammarus minus)* were also abundant in the spring. The average pH of the spring was 6.93, and the average nitrate concentration was 0.733 ppm.

Ell Spring

Ell spring is one of the One-Hundred Springs. It is located in a forested area, but during the study period the winter exfoliated trees provided little shade. Ell Spring starts from the bottom of a hill, and has two main points where water flows from, forming an "L" shape. The two streams join to form one. The average depth was 4.57 inches. We placed slides in each beginning segment of the spring. The first stick was fitted with two slides and placed at a depth of 4 inches. The second, also fitted with two slides, was placed at a depth of 3 inches. Watercress, watercress snails (*Fontigens nickliniana*) and amphipods (*G. minus)* were abundant. The average pH was 7.51, and the average nitrate concentration was 0.44 ppm.

Petersburg Spring

Petersburg spring is a pond fed by a short brook. One slide was placed at a depth of 4.75 inches near the spring source where the flow rate was 0.333 m/s. Another slide was placed in the pond. The area was slightly shaded, but there was no shade where we placed the slides. Amphipods (*G. minus*) were numerous. The average pH was 6.94, and the average nitrate concentration was 0.29 ppm.

Blue Spring

Blue spring's source is located above a farm. A pond is formed directly after the source, then continues as a stream. We placed slides in one spot further down the stream past the bridge. There are a few trees along the stream's banks, but not enough to shade the area where the slides were placed. The dominant macroinvertebrates were isopods (*Lirceus brachyurus*); and amphipods (*G. minus*), snails, pea clams and caddisfly larvae were also numerous. The slides were placed at a depth of 16.5 inches with a flow rate of 0.345 m/s. The average pH was 6.86, and the average nitrate concentration was 0.29 ppm.

METHODS AND MATERIALS

We tested whether diatom growth (colonization rate, species diversity, and relative abundance) is related to a single limiting factor (nitrate concentrations) by attempting to keep other factors as constant as possible. We chose our study springs

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based on data on nitrate concentrations given by Glazier, Horne aand Lehman (1992) (see Table 5). Using a Hach kit, we estimated nitrate concentrations at these sites on the same day that the diatom samples from the springs were taken. We also measured pH using a Fisher Scientific ® Accumet pH Meter 915.

Using a float, meter stick, and timer we calculated the flow rate at each study site to find a relatively consistent flow rate across the study springs (2.9 seconds per meter, see Fig. 2 and Table 1). We set up the apparatus for sampling in the following manner: we obtained 10 sturdy, stationary sticks, upon which we placed microscope slides. The slides were attached to the sticks through the use of ties and wedging material. The stationary sticks, along with the attached slides were driven into the spring beds by the use of a sledge hammer. The slides were positioned either with or against the current, to evaluate whether there would be a difference in colonization of the diatoms in either case.

At study sites where there was an option between sunlit vs. shaded areas, we placed the slides in the sunlit areas. We predicted that we would find the diatom genera *Nitzschia* and *Pseudonitzschia* within the study springs. These diatoms are recently evolved forms with rapid growth rates of two individuals per day. They are classified as pennate forms which are elongated and bilaterally symmetry. They contain accessory pigments such as chlorophyll and fucoxanthin.

After a period of two weeks, we went back to each study spring and collected the slides. In the laboratory the coverslips were glued with Permount to the slides, making them permanent. A differential interference microscope was used to estimate relative abundance of each genus of diatoms found on each slide. This was difficult because the Permount caused the majority of the diatom colonies to slide ("surf") to the edges of the cover-slip.

Simpson's Diversity and Equitability Indexes were calculated for each spring, as shown in Tables 2 and 3. This was done to obtain some relative information as to how diverse the genera of diatoms were within each spring and how evenly distributed each genus was within that particular biome (study spring). We also constructed rank-abundance graphs for each of the study springs to gain insight into what might have been occurring within each of the diatom communities.

RESULTS

Nitrate concentrations in the study springs decreased in the order of Kanasatake, Emma, Ell, Petersburg, and Blue (Fig. 1). As intended, pH and flow rate were similar among all of the sampling sites (Fig. 2, Table 1).

Figure 1.

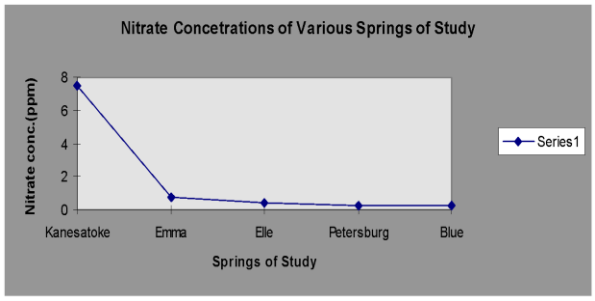

Figure 2.

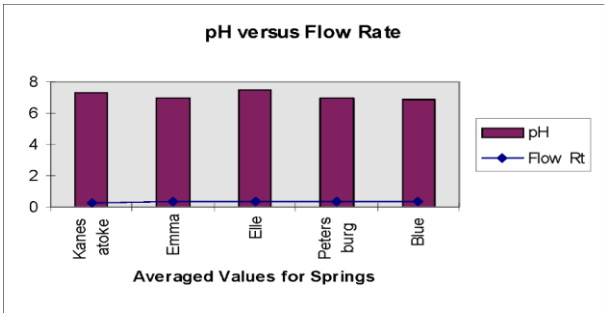

Table 1

	Kanesatoke	***Emma***	***Elle***	***Petersburg***	***Blue***
pH	7.3	6.93	7.51	6.94	6.86
Flow rate	*0.294*	*0.3125*	*0.333*	0.345	0.345

Diatom genera and their abundance in the five study springs are listed in Table 2, in order of decreasing nitrate concentrations. Dubbel spring is also included, though no data on nitrate concentration were available. Only the genera *Meridion* and *Cocconeis*/*Diploneis* occurred in all springs. Their relative abundance is shown in Fig. 3.

Table 2

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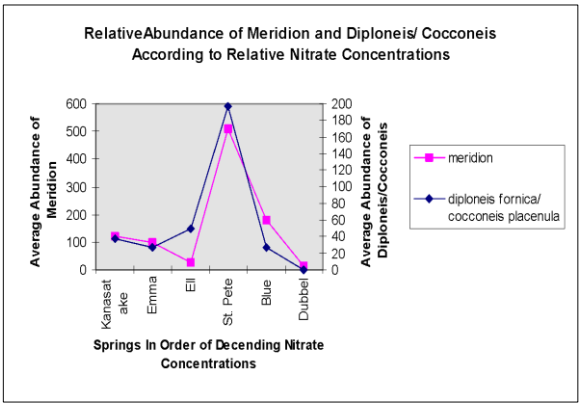
Pop Var	1872.389	1024.785	255.375	30891.8	2966.606	124.9219
Average nitrate concentrations	7.5	0.733333	0.44	0.29	0.29	
	Kanasatake	**Emma**	**Ell**	**Petersburg**	**Blue**	**Dubbel**
Meridion	123	97	28.5	509	180.5	13
Diploneis fornica/ Cocconeis placenula	37.5	27.5	50	197.3	27.5	
Navicula	26	49	5	63	32.75	
Eunotia					1	1
Diatoma		42.5	24.5	79.7	8	32
Climacosphia moniligera				9.3	7.5	
Stephanodiscus					1	
Gomphonema geminatum		5			100.3	
Nitzschia					2.25	
Synedra		1.5		1	1	
Stauroneis					1	
Desmid-*Cosmarium*						11.5

Table 3

Figure 3.



Simpson' Diversity Index (D), equitability (E), and the sums of each spring's P_i^2 are given in Table 3. Fig. 4 graphs the E values for each spring in order of descending nitrate concentrations. It shows that the equitability of the diatom community was highest in Kanesatake, Emma and Ell springs, which had the the highest nitrate concentrations. In contrast, The diatom community in Kanasatake spring had the lowest Simpson's Diversity Index (Fig. 5). Figs. 6-11 depict are rank-abundance curves for each study spring.

	D	E	åPi^2
Kanasatake	2.021	0.6737	0.4948
Emma	3.438	0.573	0.29087
Ell	2.9623	0.74058	0.33757
Pete	2.3941	0.39902	0.41769
Blue	2.9526	0.2684	0.3387
Dubbel	2.51	0.6275	0.3984

Figure 4.

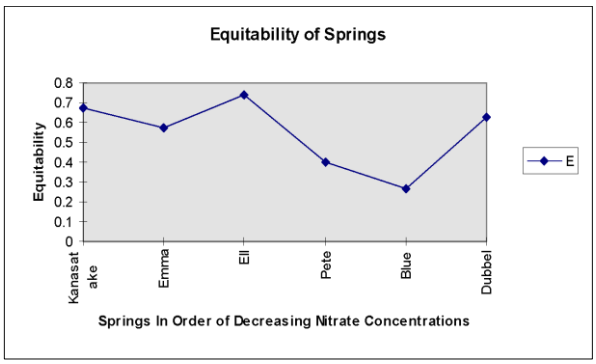


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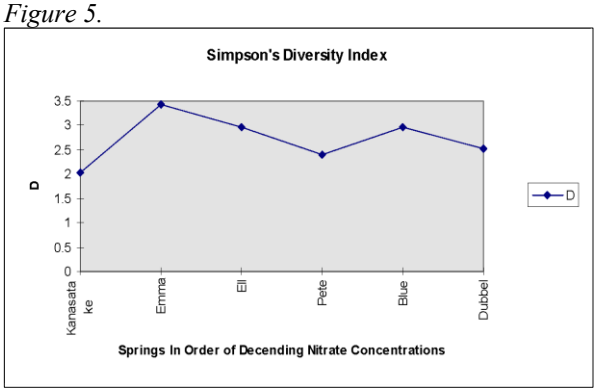
Figure 5.



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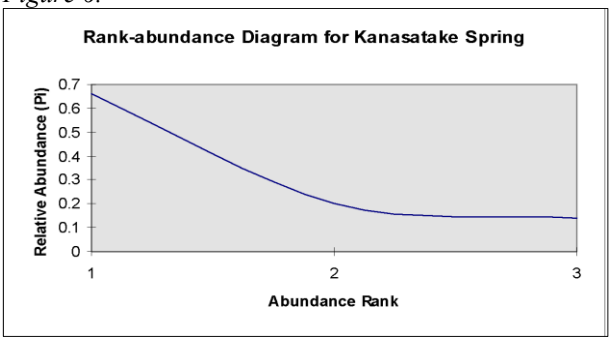

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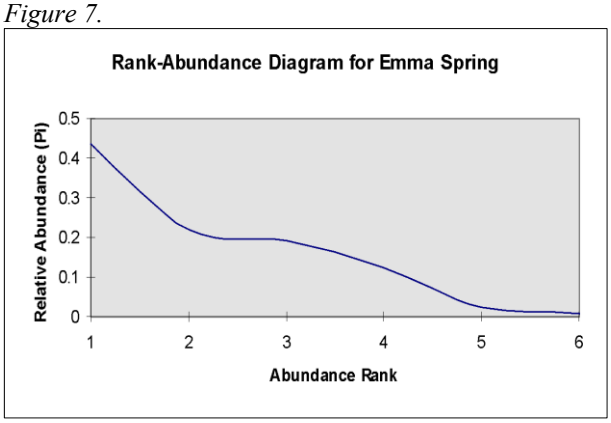

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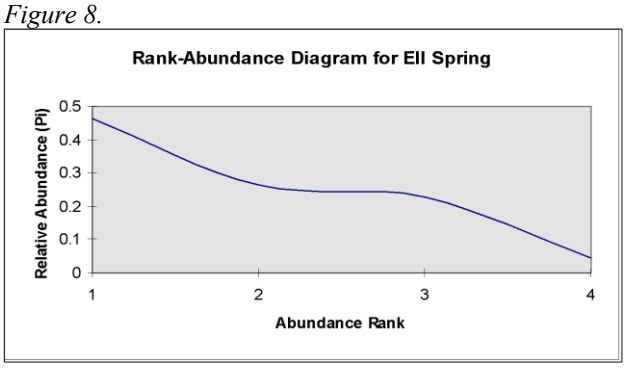

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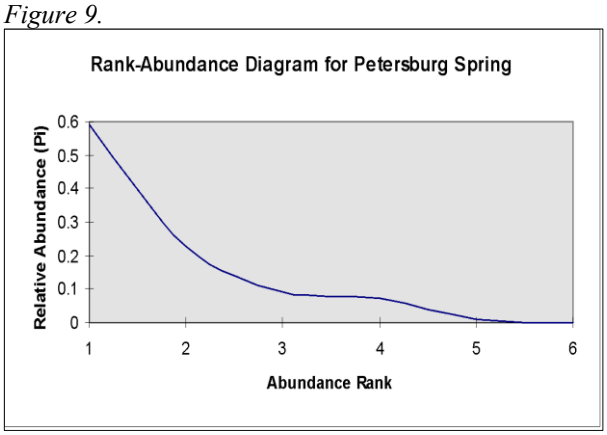

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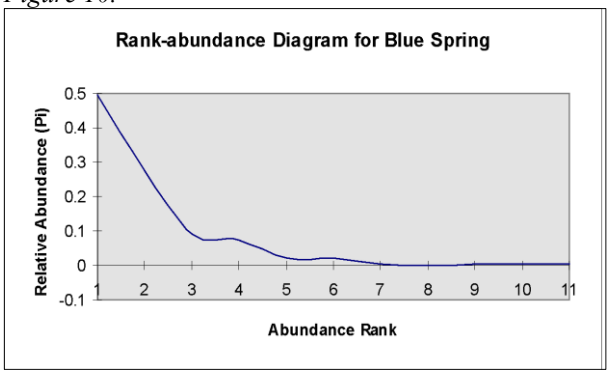

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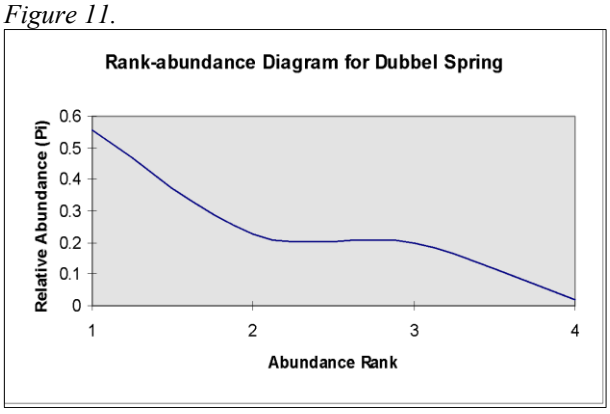

DISCUSSION

Our original hypothesis was that we would find a correlation between the relative abundance of diatoms and the nitrate concentrations among our study springs. Errors made in making permanent slides of the diatom samples led to a change in plans. We decided to look at the diversity of diatom genera on the individual slides. By looking at the slides, we obtained a relative ratio of the different types of diatom genera found within each spring (note:

species classification was not undertaken because of lack of suitable keys).

The nitrate concentrations determined in this study during the winter were compared with those carried out by Glazier et al. (1992) during the summer (see Table 5). Kanasatake and Emma springs appeared to have somewhat higher nitrate levels in the summer than in the winter, though not enough data were available to statistically test this result. Perhaps nitrate inputs to these springs were higher in the summer because of enhanced microbial activity in the marsh surrounding Kanasatake spring, and because of increased use of fertilizers on farmland surrounding Emma spring. However, the nitrate levels in the other three springs did not appear to differ much seasonally. In any case, Kanasatake spring clearly had the highest nitrate levels of all of the springs studied.

Table 5. Nitrate concentrations of study springs during the summer of 1992 (Glazier et al., 1992) and the winter of 1998 (this study).

Spring of Study	Summer '92, $[NO_3]$(ppm)	Winter '98, $[NO_3]$(ppm)
Kanasatake	*8.4*	*7.5*
Emma	*3.7*	*0.73*
Ell	*0.1-0.2*	*0.44*
Petersburg	*less than 0.1*	*0.29*
Blue	*0.3*	*0.29*

It may be no coincidence that the fewest diatom genera were sampled in Kanasatake spring. The sample slides from this spring also exhibited the lowest Simpson's diversity index (Tables 3 and 6, Fig. 5). Three genera of diatoms were identified in Kanasatake. All of the other springs, with relatively low nitrate concentrations, contained at least four or more different genera of diatoms. In addition, the equitability of the diatom community in Kanasatake spring was exceeded only by that in Ell spring (Tables 3 and 6, Fig. 4). This value is a measure of how evenly distributed the diversity of the community is among all the involved genera. Although no significant correlations were found between these community desriptors and nitrate concentration, these data suggest that such descriptors may prove to be useful as indicators of nitrate pollution. Perhaps high nitrate levels, as found in Kanasatake spring, suppress the species diversity of diatom communities.

Table 4. Comparisons of diatom-community patterns in Kanasatake spring with those in other springs with lower nitrate concentrations.
D: Simpsons Diversity Index, E: Equitability Index, $[NO_3]$: nitrate concentration in ppm.

A different picture is revealed if the abundance of specific genera is examined. For example, *Meridion* and *Diploneis/Cocconeis,* the only genera that occurred in all of the springs, were most abundant in Blue spring, which has an intermediate nitrate concentration (Fig. 3). Perhaps Shelford's Law of Tolerance can help explain these results.

In conclusion, no significant effects of nitrate concentration were observed on diatom communities in our study springs. Perhaps other nutrients such as silicon and phosphorus, which are known to be essential for diatom growth, are more important. Future work should consider these and other factors as potential determinants of diatom abundance and diversity in freshwater springs. It is unlikely that a single-factor analysis, as done in this report, will be sufficient.

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P-funk and the heavenly father who has given us all this wondrous beauty and inspired us to explore the natural world in which we live our daily lives. THANKYOU for making this possible.

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EFFECT OF METHYLATED MERCURY ON THE DIVING FREQUENCY OF THE COMMON LOON

Brian Olsen, Dave Evers and Christopher DeSorbo

ABSTRACT

Rising levels of atmospherically deposited mercury and its incorporation into aquatic food webs have long been a topic of concern for threatened populations of the common loon, Gavia immer, in the northeast. Although little is known about the effect of mercury on adult loon behavior, it has been shown that mercury inhibits heme production, and may affect the oxygen carrying capacity of loon blood. We examined the relationship between diving frequency and mercury concentration in common loons. We found a significant difference among the frequency of foraging dives in loons with different mercury burdens ($H = 8.75$, $df = 3$, $p = 0.033$) and a significant correlation between dive frequency and mercury level ($r = 0.136$, $N = 249$, $p = 0.032$). Additionally, the effect increased at higher concentrations ($H = 7.48$ $df = 1$, $p = 0.006$). This result prompts concern over contaminated loons' ability to forage for themselves and their offspring, especially in populations that are already stressed due to other anthropogenic disturbances.

Key words: Common Loon, methylated mercury, diving frequency

INTRODUCTION

In recent decades the level of atmospherically deposited mercury in aquatic systems has been steadily rising (Swain et al. 1992). Since the largest source of mercury in aquatic systems is due to atmospheric deposition (Swain et al. 1992, Carpi 1997), the effects in the northeast have been greater than elsewhere, due to prevailing weather systems (Evers et al. 1998). The mobilization of mercury in biological systems is also noted to be far above what occurs naturally (U.S. EPA 1997, Vitousek et al. 1997). Because of this pattern, methylated mercury concentrations in common loons, *Gavia immer*, generally increase from west to northeast (Evers et al. 1998). Although the effects of this escalating contamination on reproductive productivity have already been noted in various aquatic birds (Barr 1986, Heinz 1976, 1979, Scheuhammer and Blancher 1994, Tejning 1967), little has been recorded in loons. Nocera and Taylor (1998) found that contaminated chicks tend to brood and preen less, and Evers (unpublished DEP Report, 1998) noted that males with high mercury loads incubate eggs less frequently. Little work has been done, however, to link lower levels of mercury with adult loon behavior. This deficiency is an important gap in our understanding of the anthropogenic effects on declining loon populations (Sutcliffe 1978, Blair 1992). It has been documented, however, that methylated mercury inhibits the production of heme (Marks, 1985; Matts, et al, 1991). We hypothesized that inhibited heme production would limit the oxygen carrying capacity of loon blood, thereby decreasing diving duration. We assumed that as diving duration decreased, loons would be required to dive more frequently per foraging interval to secure the same caloric needs. We tested the hypothesis that there was no change in the diving frequency of loons at different mercury contamination concentrations.

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METHODS AND MATERIALS

Field sites

We collected data from a total of nine loon territories on six lakes in Franklin and Somerset Counties, Maine from May until August, 1999. The lakes are located in the upper drainage basins of the Androscoggin and Kennebec rivers. Both Rangely and Flagstaff lakes are dam controlled, but only Flagstaff's water level fluctuates widely. The majority of all shorelines are wooded with mixed evergreen and deciduous forest.

Approach

Loon territories were divided into four risk categories according to levels of blood mercury: extra high (> 4.0 ppm), high (3.0-4.0ppm), moderate (1.0-3.0 ppm), and low (0-1.0 ppm). We gathered diving time observations in one hour time blocks for up to 4 hours/day using one or two 15-45X spotting scopes and 10X binoculars. Observers monitored behavior continually through a spotting scope and relayed behaviors to a recorder, who noted times from a digital stopwatch and recorded observations. We gathered data from early May until late August in order to incorporate data from pre-nesting, nesting, and post-nesting intervals. Observations time were not stratified throughout the day in accordance with Evers (1994), Gostomski and Evers (1998), Mager (1999), and Paruk (1999), who found minimal or no significant relationships between time of day and behavior. During observations, we concealed ourselves as much as possible from a distance (up to 300m) to avoid any possible change in behavior due to our presence. Bradley (1985) found that this bias can influence data significantly. We collected 6 cc of blood from each loon through the methods described by Biodiversity Research Institute (Unpublished, 1999) and determined mercury concentration through cold vapor atomic absorption (CVAA).

Statistical Analysis

We calculated diving frequency as the number of dives per second foraging. We determined foraging to be a behavior state as defined by Altmann (1974), Tacha et al. (1985), and Nocera and Taylor (1998) in their time activity budget protocols. We calculated differences among treatments with the Kruskal Wallace test (Sokal and Rohlf, 1995) and Mann Whitney U (Sokal and Rohlf, 1995), and also used Pearson's Product Moment to investigate correlations between dive frequency and mercury risk category (Sokal and Rohlf, 1995). We used an alpha level of 0.05 and considered differences to be significant at $p < 0.05$.

RESULTS

Non-parametric testing determined that there was a significant difference among diving frequencies of common loons which have different levels of mercury contamination ($H = 8.75$, $df = 3$, $p = 0.033$) (Fig. 1).

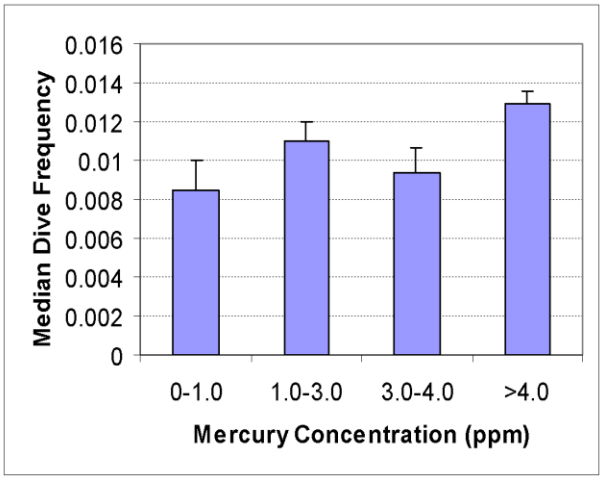

Figure 1. Median frequency of Common loon dives (+SE) among four categories of Hg contamination in loon blood during the summer of 1999 in Maine.

The frequency of diving was positively correlated with mercury load ($r = 0.136$, $N = 249$, $p = 0.032$) (Fig. 2).

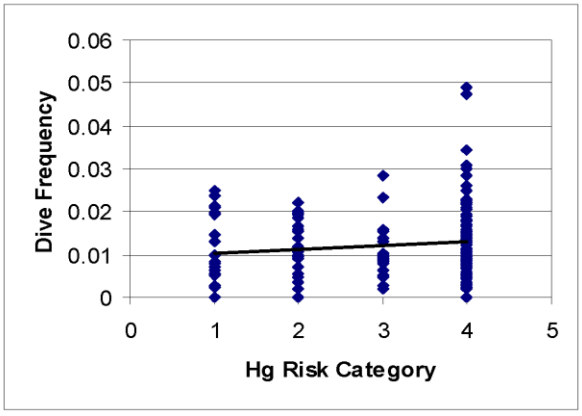

Figure 2. Frequency of common loon dives as a function of mercury contamination in the blood. Loon territories were divided up into 0-1.0 ppm , 1.0-3.0 ppm , 3.0-4.0ppm , and >4.0 ppm risk categories respectively.

This difference increases in significance as mercury loads reach the high risk category (>4.0ppm). When we pooled our first three risk categories to reduce the possible effect of low sample size, the significance of the difference increased (U = 151.0, N= 85, 164, p = 0.0063) (Fig. 3).

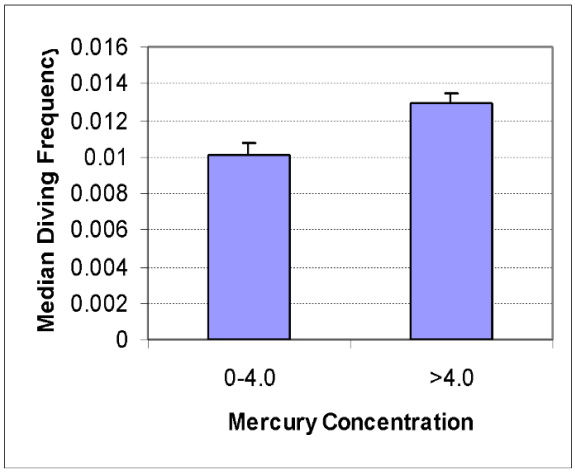

Figure 3. Median frequency of Common loon dives (+SE) of two categories of Hg contamination in loon blood during the summer of 1999 in Maine.

There was a highly significant positive difference between the diving frequencies of loons with blood concentration of 0-4.0 ppm and those with >4.0ppm.

DISCUSSION

Our findings support the supposition that there is a positive correlation between mercury contamination in loon blood and diving frequency. We hypothesize that mercury, a known heme inhibitor (Marks, 1985; Matts, et al, 1991), lowers the blood carrying capacity of the blood, thereby making it harder for loons to stay underwater as long. Shorter dives should lead to more frequent dives unless the loss in duration can somehow be compensated by foraging efficiency. In nearly exclusive piscivores such as loons, a shorter dive is presumably a less efficient dive. Thus, to meet daily caloric needs, a loon would have to dive more frequently. Although this study does not prove that mercury and dive frequency are linked, the correlation should raise sufficient concern to prompt further investigation. More frequent diving could conceivably have wide ranging effects on loon populations. Birds might have to spend more energy foraging, both because of a need for longer foraging duration, and more dives. Their ability to feed both themselves and their young might also be impacted by this effect. For many loon populations which are already under stress from shoreline development, lead poisoning, and other water quality issues, this added effect further inhibits the population's ability to rebound from disturbances and to thrive in currently established habitats.

Our conclusions are tentative, however, because of a low sample size. The moderate and high risk categories consist of only one territorial pair over the entire summer. The low category only consists of three pairs, and the extra high category consists of four territorial pairs. The high risk category's deviation from the overall pattern of increase may be because of this low sample size. However, since such a low sample size indicated a significant correlation, it is possible that further study with more sufficient sample sizes would discover a more significant relationship. It should be noted that although our correlation was not a strong one, it was a significant one. Although a number of other factors such as prey availability, water depth, and chick age may correlate more strongly with diving frequency, mercury concentration correlates significantly. Limitations of the parent data base restricted us to using diving frequency, but comparing exact diving times would yield a more direct result without further assumptions. Further study might also address the effect of increased diving frequency on foraging efficiency, caloric intake, chick feeding, and foraging duration as well as examining blood-porphyrin levels to connect the observed pattern directly to heme inhibition.

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EFFECTS OF THE HERBICIDE ATRAZINE ON RANA SYLVATICA DEVELOPMENT

Heather Galbraith, Beth Kobylarz and Julia Saylor

ABSTRACT

Newly hatched wood frog (Rana sylvatica) tadpoles were treated with sublethal concentrations of the commonly used herbicide atrazine. Tadpoles remained in dilutions of 75 ug/L and 250 ug/L for 10 d after which measurement of total length, torso length, and weight were measured. Significant differences were found in the torso length and overall length with the control organisms being smaller than those in the treatments. No conclusions can be drawn from these data without further research.

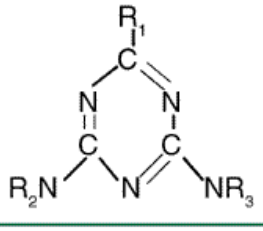
Keywords: Atrazine, body size, development, Rana sylvatica, wood frog.

INTRODUCTION

Agriculture is Pennsylvania's number one industry. There are approximately 45,000 farms in the state and Pennsylvania is second in the nation in the number of acres of farmland preserved for agricultural use (PA Farm Bureau, 2000). With this large quantity of farmland, herbicide acts as an important tool in order to boost crop production. One of the mostly widely used herbicides in the US is atrazine, which exceeds a usage of 30 kilotons each year (Raven, 1983).

Atrazine is a selective triazine herbicide (Fig. 1) that is used to control broadleaf and grassy weeds in agriculture and conifer reforestation plantings. Yet it can persist in the environment for a relatively long period of time, with a half-life of 224 days at 25°C and pH of 4-7 (Miller, 1999). Although the use of this chemical is intended to remove only unwanted vegetation, its presence in the environment can have affects on other life-forms, especially those in aquatic environments. In a 1992 survey of water quality at streamflow-gauging stations throughout the corn and soybean belt of the United States, Thurman et al. (1992) detected atrazine in 98% of post-planting water samples. Of these samples, 55% were over the Maximum Contaminant Level (MCL) set by the Environmental Protection Agency.

Figure 1 - *Structure of diamino-s-triazine.*



Despite the fact that atrazine is not the most lethal herbicide, it has been shown to have many sublethal affects in organisms. Atrazine may disrupt endocrine functions in amphibians, which are generally considered to be more sensitive to aquatic contaminants than other aquatic vertebrates, partly because of their highly permeable skin (Larson *et al*., 1998). The combination of atrazine's widespread usage, relatively long period of persistence, and sublethal effects in animals indicates that atrazine may have detrimental effects on animal life located near agricultural areas.

Larson et al. (1998) have shown that, at sublethal concentrations, atrazine can have significant effects on larval size and hormone concentration in tiger salamanders (*Ambystoma tigrinum*). Larson *et al.* (1998) suspected that interactions between stress hormones (corticosterone) and thyroid hormones (thyroxine) promoted metamorphosis. Corticosterone acts to convert thyroxine into triiodothyronine in larvae, which in turn promotes differentiation, but may

also slow growth during the time of differentiation. However, stress-hormone concentrations rise in response to stressors in the environment, as does thyroxine in stressed amphibians (Norris, 1997). Therefore, increased amounts of stressor hormone and thyroid hormones should produce increased differentiation or metamorphosis, yet the organism should appear smaller than normal due to the thyroid's ability to hinder growth. These developmental patterns was earlier recognized by Rose and Armentrout (1976), who found that amphibian larvae often have the ability to accelerate metamorphosis in response to deteriorating conditions in larval habitat. Also, their study showed that amphibians, which went through metamorphosis quicker, had a tendency to be smaller adults.

Thus, in an attempt to test the generality of these findings, we investigated the effects of atrazine on the wood frog, *Rana sylvatica*. Wood frogs are found throughout the Northeastern US, as well as Canada and Alaska. These frogs, like many others, use vernal ponds to breed, which occurs one night a year. Vernal pools contain limited amounts of stagnant water, and thus are vulnerable to high concentration effects if exposed to herbicide runoff. Therefore, we believe that vernal ponds in agricultural areas are critical systems to study for potential effects of herbicides on aquatic species. Based on the studies of Rose and Armentrout (1976) and Larson *et al*. (1998), we hypothesized that over a 10-day study, wood-frog tadpoles would experience a decrease in size and weight after exposure to sublethal concentrations of atrazine.

FIELD SITE

On March 24, 2000, *R. sylvatica* egg samples were obtained from a ditch along Route 26 heading south toward Huntingdon, PA. The water in the ditch was slow moving with a trickling drainage spout approximately 5 feet away. There was an abundance of grass, pine needles, and cattails in the water and the surrounding area. Multiple ditches similar to this were in the area, but only this one had a sufficient amount of water to contain eggs.

METHODS AND MATERIALS

The egg masses were transported back to Juniata College in plastic buckets containing the water in which they were laid. The egg masses were divided into 3 smaller groups of approximately 30 eggs, which were placed into 3 equally sized large, glass dishes and allowed to hatch at room temperature. Glass plates were placed partially over the dishes to minimize evaporation, yet still allow gas exchange.

On March 28, 2000, approximately three days after hatching, we set up our treatment solutions of atrazine. A small amount (~49 ml) of atrazine was obtained from a nearby farm in a premixed solution containing one pound (453 g) atrazine per quart of water. To facilitate accurate measurements of the treatment concentrations, we used a 10 μl syringe to dilute the original 49 mL of atrazine in one liter of water. The treatment concentrations were 75 μg/l (3.5 μl of the above base solution in one liter of sample water) and 250 μg/l (10.8 μl of the base solution in one liter of sample water). The sample water collected from our field site was used in the control group.

The tadpoles were cultured in their respective atrazine concentrations for ten days, during which they were fed fish food daily. On the tenth day, the length and mass of the tadpoles were measured. A small amount of water was placed into a clear petri dish, along with one tadpole. A ruler was placed under the petri dish, and measurements of total length and torso length were measured. The same tadpole *was* then weighed in a small weighing boat using an analytical balance. The tadpoles were weighed and measured on the same day to maximize data consistency. Analysis of variance and Tukey tests (Minitab) were used to compare tadpole sizes among the treatment groups.

RESULTS

Various size measures of the tadpoles in the three treatment groups are given in Table1. Among the treatment groups there was a significant difference in torso size ($P = 0.001$) and the overall size ($P < 0.001$) of the tadpoles, but no significant difference in the weight ($P = 0.151$) or torso length/total length ratio ($P = 0.374$). There was also no difference in the weight to length ratio ($P = 0.887$).

Tukey tests showed that torso length was significantly greater in the 250 μg/l atrazine group than in the control group ($P < 0.001$) and marginally significantly greater in the 75 μg/l atrazine group than in the control group ($P = 0.051$). However, torso length did not differ significantly between the 75 μg/L treatment and the 250 μg/l treatment ($P = 0.161$).

Similarly, total length was significantly greater in the 75 μg/l treatment than in the control ($P = 0.026$), and also for the 250 μg/l treatment compared to the control ($P < 0.001$). However, again no difference was observed between the two atrazine-treatment groups ($P = 0.076$).

Table 1. Body size measures of Rana sylvatica *tadpoles at different concentrations of atrazine.*

Control					
	Torso length	**Total length**	**Weight**	**Torso/ Total**	**Length/Weight**
Mean	6.21	18.34	0.10	0.34	191.90
Std. Dev.	0.76	1.43	0.023	0.039	47.21

75 μg/l					
	Torso length	**Total length**	**Weight**	**Torso/ Total**	**Length/Weight**
Mean	6.69	19.28	0.11	0.35	181.28
Std. Dev.	1.44	2.3	0.026	0.059	28.78

250μg/l					
	Torso length	**Total length**	**Weight**	**Torso/Total**	**Length/Weight**
Mean	7.15	20.19	0.11	0.35	204.32
Std. Dev	0.88	1.23	0.015	0.03	76.31

DISCUSSION

We hypothesized that *R. sylvatica* tadpoles in the treatment groups would be smaller than those in the control group, but instead we found just the opposite to be true. Since there was essentially no difference in tadpole weights among the treatments, perhaps the larger tadpoles treated with atrazine had less body reserves than their control counterparts. If this were the case, then atrazine may have indeed had some impact on tadpole size. However, contrary to this hypothesis, weight/length ratios were not significantly smaller in the atrazine treatment groups. This result was surprizing, but should be viewed with caution because of the large standard deviations associated with our weight and length/weight means. Procedural problems could possibly have influenced our results. First, separating frog eggs is an extremely difficult task due to the sticky jelly surrounding them. When trying to equally partition eggs into dishes, it was practically impossible to allocate an even number to each dish. As a result, our control group ended up with almost twenty more tadpoles than the treatment groups. Thus increased crowding in the control group could have inhibited tadpole growth and thus their body size.

Another problem that we encountered was that our solution of atrazine was too concentrated to accurately treat our tadpoles. On a farm, a typical solution of atrazine is formulated by mixing one pound of atrazine for every quart of water, a very concentrated solution from which to obtain 75 and 250 μg/L. The farm provided us with approximately 49 ml, which was diluted to one liter. However, even after this dilution, we still needed only 3 and 10 μl to make our treatments. The only equipment that Juniata College has to measure such small quantities is a 10 μl syringe, not a very accurate tool in comparison to an automatic pipette, for example. Therefore, it was difficult to see when there was actually a particular quantity of solution in the syringe, and more importantly, if it came out at all.

Our group also encountered a problem with weighing the tadpoles on the analytical balances. As can be seen in Table 1, tadpole weights varied greatly. This was probably due to the varying amounts of water in the weighing boats and the fact that the tadpoles themselves were wet. One tiny drop of water on a tadpole when placing it in the weight boat dramatically changed the readings that we obtained. Absorbing some of the water with tissue paper seemed to alleviate some of the problem, but it did not eliminate it.

A final problem with this study was the very limited amount of time in which to perform it. Because of the time issue, we were only able to treat our tadpoles for ten days, a very short period of time. We feel that more reliable data could have been obtained if the tadpoles were given longer exposure times. Also, the rates of metamorphosis of the tadpoles should also have been examined. It was very difficult to determine if there were any changes in the rates of metamorphosis among our groups partly because our specimens were so young.

There are many options for further research on this subject. First, this experiment could be repeated making the corrections that we suggested above to see if the results are different. In addition, these same methods could be repeated using different species such as spring peepers, spotted salamanders,

fish, or other aquatic animals. Furthermore, hormone samples could be obtained from the subjects to see if there is any correlation between hormone concentration, body size, and rate of development. Future research along these same lines could provide valuable information regarding the impacts herbicides have on the environment, and perhaps on humans, as well.

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We thank Dr. Glazier for all of his help, especially in finding and collecting the wood frog eggs, as well as Dr. John Matter for his wealth of information regarding atrazine and frog development. We also thank Jeffery Morse for his chemistry expertise in helping make the herbicide dilutions, in addition to providing us with the equipment with which to perform these dilutions.

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ENERGETICS OF BROODING IN THE FRESHWATER AMPHIPOD *GAMMARUS MINUS*

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ABSTRACT

Reproductive strategies among organisms vary greatly, as do the energetic investments associated with producing young. Amphipod crustaceans carry their eggs in a brood pouch and release them as juveniles. However, very little is known about the reproductive energy investments of amphipod females during the period when they are carrying their young. The goal of this research was to determine the energetic costs associated with carrying a brood in the freshwater amphipod, *Gammarus minus*. We hypothesized that there would be no difference in metabolic rate between *G. minus* females carrying a brood and females from which the brood was removed. To test this hypothesis we measured oxygen consumption of females amphipods carrying broods, debrooded females, and their removed eggs. We found no difference in metabolic rate between females carrying broods and debrooded females ($t = 0.38$, $df = 44$, $p = 0.705$). However, we found that the combined metabolic rate of debrooded females and their removed young was higher than that of females carrying broods ($t = 5.28$, $df = 44$, $p < 0.001$). These results indicate that there is no additional metabolic cost to carrying a brood, suggesting that conditions within the brood pouch lower the metabolic rate of the embryos.

Keywords: amphipods, brooding, metabolism, energetics, oxygen consumption

INTRODUCTION

The act of brooding, carrying eggs in an external pouch, is a form of extended parental care that offers an increased chance of survivorship for the young (Dick et al. 1998). However, the actual energetic input of mothers to their broods has not been thoroughly analyzed for many species. Behavioral research performed on *Crangonyx pseudogracilis*, another species of freshwater amphipod, has offered some indication of "active" pre-emergence brood care (Dick et al. 1998). They found that amphipods expand their brood pouches, allowing for pouch ventilation, increased suspension of the eggs and possible cycling of the eggs within the pouch. Females were also found to actively eject non-viable eggs from their pouches, another active mechanism of pre-emergence brood care. However, research performed on the brooding Cladoceran *Daphnia magna* revealed no extra energetic investment to carrying their broods as measured by oxygen consumption rates (Glazier, 1991). This finding indicates that mothers are able to ensure increased survivorship of their young with no additional energy costs to the female.

Gammarus minus is a species of freshwater amphipod locally abundant in cold, alkaline springs in central Pennsylvania (Pers. Obs.). *Gammarus* females like the aforementioned species brood their young. However no research has been conducted to determine the energetic requirements to carrying a brood. Therefore, we tested the hypothesis that there would be no difference in metabolic rate as measured by oxygen (O_2) consumption rate, between *G. minus* females carrying a brood and females from which the brood was removed.

MATERIALS AND METHODS

We collected brooding female amphipods from Petersburg Spring in Petersburg, Pennsylvania, brought them back to the laboratory, and allowed them to acclimate to 10°C, the average temperature of

Petersburg Spring (Pers. Obs.), for a minimum of twenty-four hours. We collected water from the spring and filtered it using GF/C filter paper to remove bacteria, algae, and fungi. This filtered water was used throughout the entire experiment. We randomly selected twelve brooding females from a holding tank and placed them in starvation chambers for twenty-four hours. Starving the females was important so that no feces were produced in the respirometer because decaying feces would consume oxygen and alter the oxygen consumption values for the amphipods. Starvation also ensured that all extraneous activities (eg. digestion) other than those required for basic survival were eliminated to reduce possible sources of variation. A starvation chamber consisted of one specimen cup with the bottom replaced by screen, placed inside another specimen cup. This screen allowed for feces to fall through making it unreachable to the coprophagic amphipod.

We used a flow-through respirometer housed in a walk-in control chamber kept at 10°C (Fig. 1). This respirometer consisted of an aerated 10-gallon carbuoy as a reservoir, a peristaltic pump, ten 5-milliliter syringes, and a wastewater collection tank. Water was pumped from the carbuoy and directed into each of the 10 syringes where the water flowed through the syringes at an adjustable rate. After the starvation period was complete, we selected eight amphipods from the twelve and placed them in the respirometer. A piece of fine netting was placed in the syringe to minimize the females' amount of movement by providing a surface to which they could cling. We did not place organisms in two of the ten syringes, which served as controls for the system. We varied the placement of the controls between runs to account for any flow-rate or oxygen concentration variability within the system.

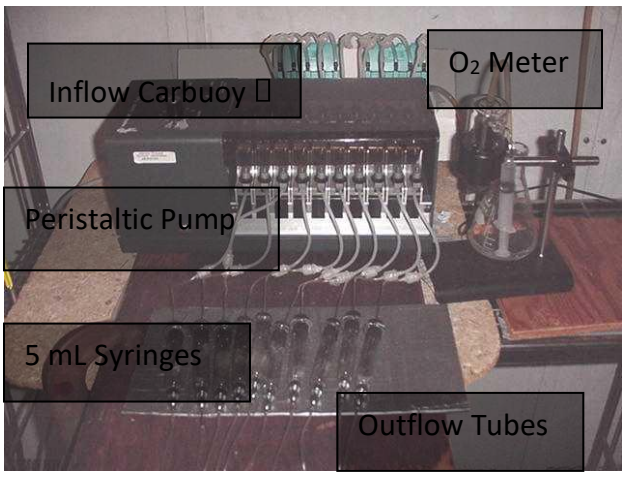


Figure 1. Picture of flow-through respirometry system

After the females were placed in the syringes, we tapped out air bubbles to ensure that no additional oxygen was being added to the system. The amphipods remained in the respirometer for approximately sixteen hours. During the sixteenth hour we connected the outflow tubes from the syringes to a Strathkelvin dissolved oxygen meter. We took readings after allowing the water to flow through the meter for three minutes to ensure the passage of any air bubbles introduced into the meter and to allow for equilibration. We repeated this process for all ten syringes during the sixteenth and eighteenth hours. Before each set of readings, we adjusted the fine calibration knob to 160 torr using water saturated with oxygen. Between the first and second readings, we determined the flow rate of each syringe by directing the discharge tubes into 10ml burets and recording the volume after 30 minutes.

After the readings were taken, we removed the females from the respirometer and briefly anes thetized them in carbonated water. They were then immobilized on a piece of dental wax using insect pins and we flushed their eggs from the brood pouch using filtered Petersburg water. We next placed the females back in fresh Petersburg water for twenty-four hours and fed them cultured elm leaves.

We counted and staged the removed eggs according to development and immediately placed them in a static respirometry system consisting of ten 2-milliliter syringes kept in a 10°C control chamber. Any eggs lost or ruptured in the debrooding process or in the transfer to and from the respirometer were noted. We removed all air bubbles from the syringes and left exactly 2 milliliters of water in the syringes. We allowed the eggs to incubate for six hours at which time the syringes were connected to the oxygen meter. Water was injected into the meter and allowed to sit for a two-minute period at which point oxygen concentrations were recorded for each brood. We then placed the broods in foil packs and stored them in a –70°C cooler.

Twenty-four hours prior to placing the debrooded females back into the respirometer, we removed them from their feeding chambers and starved them again for twenty-four hours. We placed them back in the respirometer for sixteen hours and repeated the aforementioned process of taking readings on the sixteenth and eighteenth hours. We then removed the mothers from the respirometer, euthanized them, and measured their lengths. They were stored in a –70°C cooler for later retrieval.

We collected the above measurements on 45 different females for a total of over 250 hours worth observations. Any females that died after the debrooding were removed from the data set and replaced in later runs. After we completed collecting

data, we freeze-dried all of the females and their respective broods, and measured their dry masses on a CAHN electrobalance.

To calculate oxygen consumption rates of the organisms, we used two different equations based on whether a flow-through or static system was used. Total consumption for the females was determined by $R=[(P_E-P_C)\ SAF]/m$ (Glazier and Sparks, 1991), where R is the consumption rate, P_E is the oxygen reading obtained for the organism, P_C is the control reading, S is the solubility coefficient of oxygen at 10°C, A is the volume of 1 mole of oxygen at STP, F is the flow rate of the system, and m is the dry mass of the female. Total consumption for the eggs was calculated using $R=[(P_E-P_C)\ SAV/t]$ (Glazier, 1991). Here V is the total volume of water in the syringe and t is the incubation time (6 hours for this experiment).

After testing for normality using an Anderson-Darling test and equal variances using both a Levene's and an F-test, we analyzed the data in MiniTab using either a paired t-test, a Welch's t-test, a 2-sample t-test, or a Mann-Whitney U test. We set an alpha level of 0.05 and considered differences to be significant if $p \leq 0.05$. Values between p= 0.05 and 0.10 were considered marginally significant.

RESULTS

We found no difference in metabolic rate between brooding (M= -0.3053) and debrooded (M= -0.3169) females (t= 0.38, df= 43, p= 0.705) carrying both stage I (t= -0.26, df= 21, p= 0.794) and stage II (t= 0.89, df= 22, p= 0.386) eggs (Fig. 2A). We also found that the combined metabolic rate of debrooded mothers and their removed young (M= -0.4956) was significantly higher than the rates of brood-carrying females (t= 5.28, df= 43, p< 0.001) for both stage I (W= 582.0, df= 21, p= 0.0423) and stage II (t= 5.49, df= 22, p< 0.001) eggs (Fig. 2B). We also found that eggs outside of the brood pouch (M= -0.1786) had a higher metabolic rate than those found within the brood (M= 0.0117) as calculated by the difference between the combined rate of mothers with their removed broods and the brood-carrying mothers (t= 5.64, df= 43, p< 0.001). This trend was apparent for both stage I (t= -5.91, df= 21, p< 0.001) and stage II (t= -10.27, df= 22, p< 0.001) eggs (Fig. 2C). There was a marginally higher metabolic rate in stage II eggs outside of the brood pouch (M= -1.303) than in stage I eggs outside of the pouch (M= -1.050) when examining metabolic rates outside of the pouch (W= 579.0, df= 43, p= 0.0997). However we found no metabolic difference between stage I (M= -0.0126) and stage II (M= 0.0349) eggs within the brood pouch (t= -0.78, df= 43, p= 0.439) as seen in Fig. 2D.

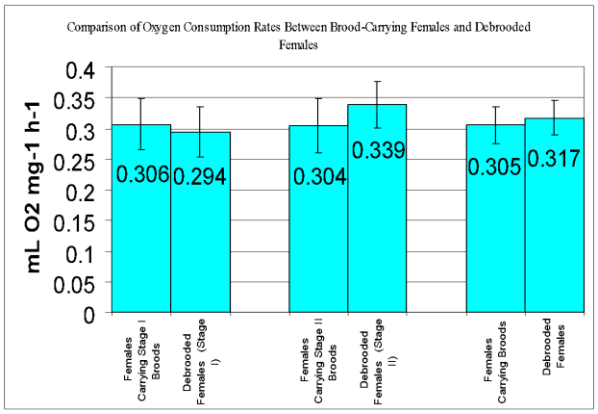
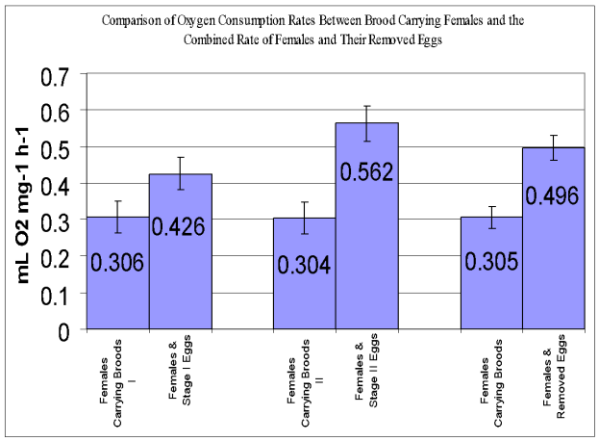


Figure 2.4



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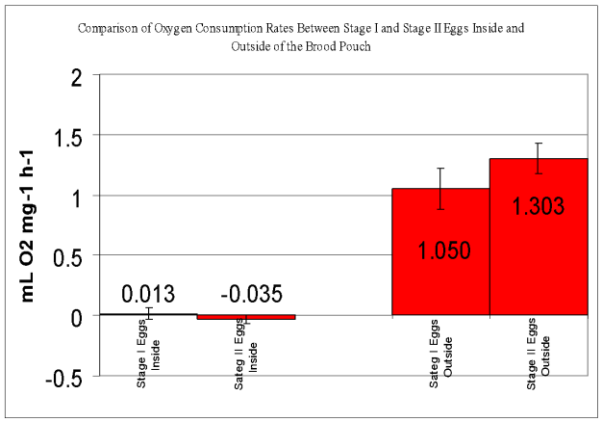

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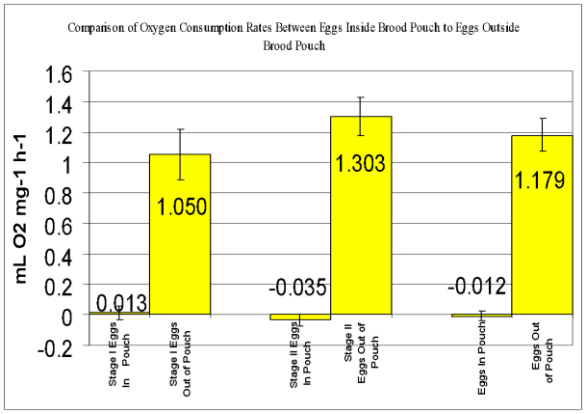

D

DISCUSSION

We failed to detect any difference in O_2 consumption rates between *G. minus* females carrying a brood and females from which the brood had been removed. This suggests that no additional energetic investment is associated with carrying young in a brood pouch. This finding appears to be a highly adaptive strategy in that females can successfully protect their young without needing to increase their energetic intake. Dick et al. (1998) observed brooding female *C. pseudogracilis* engaging in active care to improve the conditions within the brood pouch for the young. At first glance, these activities appear to contradict the findings of this study in that our data shows no increased metabolic activity in brooding females. However, it is possible that, to allow for such embryonic care, reductions are made by the female in other activities. Energy may be reallocated from active swimming, for example, and put toward brood-care activities.

We initially expected the combined rate of the debrooded females and their young (for both stages I and II) to be equal to that of the female carrying her brood. However, we found that the combined rate of debrooded females and their young was actually higher than that of the brood-carrying female. This could have been caused by an increase in the metabolic rate of the eggs after they were removed from the brood pouch or due to a slight increase in maternal O_2 consumption after the removal of the brood.

If the eggs actually do consume less O_2 inside of the brood pouch, this implies that conditions inside the pouch limit the metabolic activity of the developing embryos. One possible limiting factor could be anoxic conditions, which may exist within the crowded pouch. Another factor could be the reduced exposed surface area of the eggs in the pouch due to crowding, leading to lower O_2 exchange rates. These results contradict the findings of Glazier (1991) who found that metabolic rates of embryonic *D. magna* were not limited by conditions within the brood pouch. However this comparison to amphipods may be inappropriate given the phylogenetic differences between the organisms. Our findings suggest that not all brooding organisms conform to the same reproductive strategies.

If the metabolic rates of the females increase after debrooding, this could be linked to recovery stress associated with debrooding, preparation for another reproductive event, or increased activity associated with trying to find their lost young. Increased metabolic activity in both the eggs the debrooded females may have acted together or independently and more research must be conducted to determine the cause of this difference.

We also found that stage II eggs had a slightly higher O_2 consumption rate outside of the pouch, than did stage I eggs. However, there was no difference between the consumption rates of stage I and stage II eggs inside of the brood pouch. This may further confirm our suggestion that brood conditions are limiting the metabolic rate of the embryos.

The results of our pertain to only one population of amphipods, and further studies should include additional populations of *G. minus* as well as other amphipod species. It would be beneficial to determine any evolutionary differences in reproductive investment among freshwater, marine and terrestrial populations.

ACKNOWLEDGEMENTS

We would like to thank Dr. Douglas s. Glazier and Chuck Yohn for all of their help on this project.

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DOI

Journal of Ecological Research, **4**, 22-24 (2002)

IS SQUIRREL FORAGING ACTIVITY ASSOCIATED WITH TEMPERATURE?

Amy Skibiel, Beth Superka and Crystal Swan

ABSTRACT

Eastern gray squirrel (*Sciurus carolinensis*) foraging activity during early spring has been shown to vary throughout the day according to air temperature. We hypothesized that squirrel foraging activity should be highest in the morning when temperatures are lowest, and that foraging should be lower in the afternoon when temperatures are highest. We found that there is a negative correlation between temperature and foraging activity (r = -0.413, P = 0.036). Our data suggest that foraging activity is highest in the morning and at low to moderate temperatures.

*Keywords: Eastern gray squirrel (*Sciurus carolinensis*), foraging, temperature*

INTRODUCTION

The eastern gray squirrel (*Sciurus carolinensis*) has a distribution covering most of the eastern half of the United States, from eastern Texas to the Atlantic Coast (MacClintock 1970), including areas where temperatures are relatively moderate compared to those of the arctic and the tropics. The Eastern gray squirrel is a generalist species, capable of inhabiting a wide variety of habitats and eating a variety of foods (Halloran 1999). However, they prefer habitats with hardwood trees and primarily feed on hardwood nuts, seeds, fungi, insects, and fruits (Burt 1976). Studies conducted on squirrel foraging behavior discovered peak foraging activity in the morning about 2 hours after sunrise and about 2-5 hours before sunset (Halloran 1999). One reason for this bimodal activity may be that temperatures are more moderate during morning and evenings. If foraging activity is focused at these times rather than the afternoon, exposure to extreme temperatures is minimized (Bryce 2001).

In this study we examined whether eastern gray squirrel foraging depends on temperature. We hypothesized that afternoon foraging activity should be less than foraging activity in the morning or evenings.

Since the eastern gray squirrel is a generalist species, understanding their foraging patterns may contribute to an overall understanding of generalist species' behavior. Foraging behavior may also explain squirrel distribution patterns. If squirrels forage during cooler temperatures this might limit them to areas with moderate temperatures, such as in temperate areas.

METHODS AND MATERIALS

Eastern gray squirrels were studied in a patch of hardwood trees that line a field behind the Brumbaugh Science Center at Juniata College, Huntingdon, Pennsylvania. Beyond the study site is a residential area. Roasted, unshelled peanuts were scattered on the ground in a restricted area about 4 x 2 m in size once a day for one week before observations began so squirrels would discover the food source. Foraging activity was observed for 10 d (between April 1-18, 2002) at 3 different times during each day: morning (8am - 9am), afternoon (1pm - 2pm), and evening (6pm - 7pm). Daylight savings time occurred after the fifth day of observation so observation times were adjusted accordingly. After daylight savings time, morning observations were from 9am - 10am, afternoon observations from 2pm - 3pm, and evening observations from 7pm - 8pm. Data collected from 9am - 10am, 2pm - 3pm, and 7pm - 8pm were included with data collected from 8am - 9am, 1pm - 2pm, and 6pm - 7pm, respectively. We quantified foraging activity as the number of squirrels that captured a peanut in the feeding area. Squirrels that remained at the food site and gathered more than 1 peanut were counted only once.

Temperature (◦ C) was recorded at 20-minute intervals during the hours of observation designated above, and the mean temperature for each hour was calculated. Observers stood at a distance from the feeding area in order to decrease interference with squirrel foraging. A chi-square goodness of fit test was used to determine if there is a difference in squirrel foraging at different times of the day and at different temperatures. To determine if overall temperature is associated with squirrel foraging activity, correlation analysis was used. Null hypotheses were rejected at $P < 0.05$.

RESULTS

Number of squirrels observed at different times of the day differed significantly from expected values ($\chi^2 = 112.677$, df = 1, $P < 0.001$; see Table 1). Observed number of foraging squirrels at different temperatures differed significantly from expected values ($\chi^2 = 48.4516$, df = 1, $P < 0.001$; see Table 2). Squirrel foraging activity was significantly inversely correlated with temperature ($r = - 0.413$, $P = 0.036$; see Figure 1).

Table 1. Observed and expected frequencies of numbers of squirrels foraging at different times of the day.

Time of day	Observed	Expected
morning (8-9am)	79	31
afternoon (1-2pm)	14	31
evening (6-7pm)	1	31

Table 2. Observed and expected frequencies of numbers of squirrels foraging at different temperatures throughout the day.

Temperature (◦ C)	Observed	Expected
low (3-15)	56	31
moderate (16-28)	37	31
high (29-41)	2	31

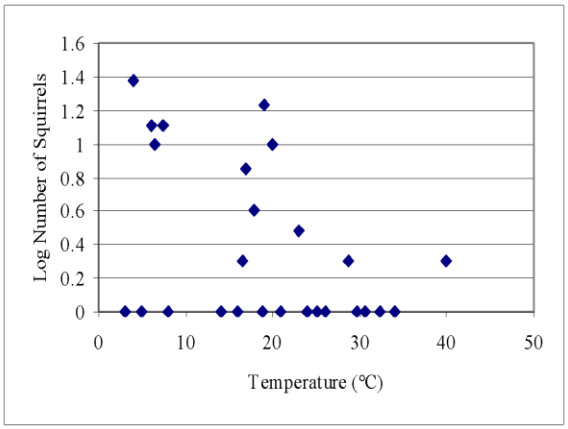

Figure 1. Significant negative relationship between log of number of squirrels observed and air temperature.

DISCUSSION

As we hypothesized, squirrel foraging activity was significantly correlated with temperature (Fig. 1). Our data suggest that there is an increase in squirrel foraging with decreased temperature, in the range observed. However, if squirrel foraging activity were observed at even lower temperatures, such as in winter, there may have been a difference in activity that would not have followed the observed trend. Furthermore the observed correlation between squirrel foraging and temperature does not take into consideration time of day. As shown in Table 1, time of day is a significant factor in the number of squirrels foraging and may have confounded our analysis of the effect of temperature on foraging activity. Our data suggest that maximum foraging occurs in the morning, consistent with the findings of Halloran (1999). However, there seems to be a decrease in activity in the evenings. Our data also suggest that squirrels forage most in low to moderate temperatures and avoid higher temperatures. This is consistent with the finding that squirrel foraging activity is decreased in the afternoon when temperatures are highest.

Our individual observations of foraging activity may not have been independent because squirrels were not identifiable, so large numbers of squirrels observed may be due to one squirrel returning to the food source. It has been found that squirrels are capable of relocating buried food after days to months (Macdonald 1997). Periodically changing the location of the feeding area is recommended to increase data independence.

Temperatures recorded may have been somewhat inaccurate because location of the thermometer caused it to be in the sun or shade at different times of the day. To ensure accurate temperature readings the thermometer should be placed in an area that is always in the shade or the sun.

Another factor that could have affected squirrel foraging was the presence of humans. Since the study site was not completely remote there was some human interference. Sometimes during our observation periods a human with a dog would walk through the field or the grass in the field was being mowed. More data are necessary to determine whether temperature affects foraging activity. It would be beneficial to conduct observations throughout the year in order to increase temperature range. A study site far removed from human activity may eliminate human effects. A larger study site (more habitat) would be necessary to observe more squirrels.

ACKNOWLEDGMENTS

We thank Dr. Douglas Glazier for his guidance in developing this study. Also, we thank Dr. Vincent Buonaccorsi for his help in statistical analysis.

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DOI

DIFFERENTIAL HABITAT USAGE BY THE EASTERN COTTONTAIL (*SYLVILAGUS FLORIDANUS*) AS ESTIMATED BY FECAL COUNTS

Eric M. Butler, Gaia R. Eirich, Mark P. Barnsley and Karl W. Justice

ABSTRACT

We conducted a study to determine if Eastern cottontails differentially utilized scrub and field microhabitats within a mixed woodland/field habitat. Previous studies conducted by others led us to hypothesize that the rabbits should spend more time in the scrub, thereby depositing more feces in that area. Relative usage of the microhabitats by the rabbits was determined by counting fecal piles within predetermined areas. There was no statistically significant evidence to support the hypothesis. This leads to the conclusion that rabbits utilize both of the microhabitats with approximately the same frequency.

Keywords: Eastern cottontail, fecal droppings, field, microhabitat use, woodland

INTRODUCTION

Mixed woodland/field habitat, accompanied by heavy shrub growth, has become increasingly important in many Northeastern states following the regrowth of significant portions of cleared woodlands. Analyzing the interactions of the species inhabiting these habitats can be complex, however, due to the patchy nature of the mixing. While a species may be present in all microhabitats in the mixed zone, its use of these microhabitats may not be equal. If a species prefers one microhabitat, it is quite likely that its effect on that microhabitat will be more significant than its effect on other microhabitats in the mixed zone.

The Eastern cottontail rabbit (*Sylvilagus floridanus*) is one species found in mixed woodland/field habitat. Previous research has shown that cottontails show microhabitat preferences in mixed shelterbelt habitats in the Midwest, making it likely that they would show microhabitat preference in Northeastern mixed woodland/field habitat (Swihart and Yahner 1982). Cottontails are opportunistic herbivores, and are fed upon by many species of carnivore, suggesting that they can play an important role in the mixed habitat ecosystem (Hockman and Chapman 1983) (Hamilton and Neill 1981).

Previous studies (Swihart and Yahner 1982) have indicated that *S. floridanus* shows a preference for scrub microhabitats in the Midwest. We hypothesized that this preference for scrub microhabitats would be the same in the Northeastern mixed woodland/field habitat.

FIELD SITE

The field site is located in the Baker-Henry Nature Preserve of the Juniata College campus in Huntingdon, Pennsylvania. The field microhabitat is dominated by a mix of grasses and a small amount of greenbrier, with a random scatter of Autumn Olive and one or two small trees. The small amount of bush and tree provide approximately 10-15% cover. The scrub microhabitat is similar in that the ground cover is the same mix of grasses, but with a much greater concentration of greenbrier. However, there are large numbers of Autumn Olive, Viburnum, and Auburn Honey Suckle, with a swath of Hawthorn near the fringe. These bushes provide a 50-60% cover. There is an extensive network of rabbit trails in the area, both in the field areas and the scrub. Rabbit feces are abundant in both microhabitats. Visual sightings confirmed that the rabbit species in the area was the Eastern cottontail.

MATERIALS AND METHODS

To estimate the amount of feces in each respective microhabitat, we first measured out five 3 x 3 m squares in both the field and the brush microhabitats. Side lengths were measured with a pre-measured string, and the four corners marked with orange flagging tape. Locations for the sampling

squares were chosen at random as representative of cover for each microhabitat. The number of individual piles of feces was then recorded during the period March 15-21, 2003. The number of piles was recorded every two days noting the number of additional piles. In addition, a medium mammal trap was used, baited with a variety of vegetables, to attempt to trap a rabbit and confirm the specific species.

RESULTS

The data were collected, organized (Table 1), and then analyzed using the Chi-Square test, which tests for significance within the data range. The Chi-Square test was used on both the total increase of scat in each area (Table 2) and the total density of scat for each area (Table 3). No significant differences between microhabitats were observed for either increase of scat or scat density.

Summary Table of Scat Data

	Total Density	**Total Increase**
Field	24	14
Scrub	30	16

Table 1. Total density and total increase of scat in each microhabitat at the end of the study.

Table 2. Chi-squared test comparing the frequency of additional fecal piles of cottontail rabbits in field (1) and scrub (2). (P > 0.05).

Chi-Square Test for Total Increase

Expected counts are printed below observed counts

	C1	C2	Total
1	14	15	29
	14.50	14.50	
2	16	15	31
	15.50	15.50	
Total	30	30	60

Chi-Sq = 0.017 + 0.017 + 0.016 + 0.016 = 0.067

DF = 1, P-Value = 0.796

Chi-Square Test for Total Density

Expected counts are printed below observed counts

	C1	C2	Total
1	24	27	51
	25.50	25.50	
2	30	27	57
	28.50	28.50	
Total	54	54	108

Chi-Sq = 0.088 + 0.088 + 0.079 + 0.079 = 0.334

DF = 1, P-Value = 0.563

DISCUSSION

We hypothesized that the rabbits should utilize the scrub microhabitat more than the field microhabitat. Our results suggest that eastern cottontails utilize both microhabitats equally. Thus, our hypothesis is not supported.

Possible sources of error include both human and natural factors. We visually confirmed the count of feces and relied entirely on our own perception to determine the number of scat piles. In addition, a judgment call was required to determine what constituted separate piles. Some scats may have also been concealed under vegetation. A final source of error was the relatively short time span over which the study was conducted. There were three counts over six days. The duration of the study was short enough that abnormal weather patterns or other short-term factors, not common to the area, could have affected the rabbits' behavior.

This study, while brief, presents the possibility for several related studies. This would include further testing over a longer period of time; testing sites along rabbit trails and comparing to sites away from trails; testing of a larger microhabitat size; testing of a broader range of microhabitats; and testing comparisons of pure and mixed habitats.

While our data do not indicate preferred utilization of scrub habitat, Swihart and Yahner's data indicate that cottontails do, in fact, prefer habitats with significant areas of scrub (Swihart and Yahner, 1982). It is possible that cottontails in the northeast show similar preferences, but do not display increased utilization of the scrub habitat. Testing rabbit densities in scrub habitats, field habitats, and mixed habitats would help determine the effect of scrub on rabbits' use of the habitat as a whole.

ACKNOWLEDGEMENTS

We thank Dr. Douglas Glazier for providing the equipment for our study, as well as background information through his teaching of the General Ecology course.

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DOI

HIGH RISK FEEDING AND FOOD PREFERENCE IN THE EASTERN GRAY SQUIRREL, *SCIURUS CAROLINENSIS*

Janette Hartney, Lynn Rassel and Kimberly Sebrasky

ABSTRACT

Foraging Eastern gray squirrels (*Sciurus carolinensis*) were observed for a period of three weeks to analyze their risk behavior, as well as their food preference. We set up a grid of 20 or 25 feeding sites on a lawn bordering a small woodlot on the southern side of the Brumbaugh Science Center of Juniata College (Huntingdon, Pennsylvania). The study area was chosen because of prior sightings of grey squirrels there. Our data showed that the squirrels would collect peanuts at varying distances from cover (distance from the woodlot) with equal frequency. The squirrels also preferred peanuts over sunflower seeds.

Keywords: Food preferences, grey squirrels, optimal foraging, risk of predation, Sciurus carolinensis

INTRODUCTION

The eastern gray squirrel (*Sciurus carolinensis*) is one of eight species of tree dwelling squirrels that inhabit the United States, and one of hundreds of species worldwide. The eastern gray squirrel can be found as far north as Maine, south into Florida and Texas, and as far west as the Dakotas. Normally they grow to be about 18 inches long and weigh between 12 to 26 ounces. Males and females are similar in size and color. They build arboreal nests consisting of leaves, twigs, moss, and sticks and other materials. The eastern gray squirrel may live 15 to 20 years in captivity, but often survive only one year in the wild. Deaths can be attributed to disease, malnutrition, and predation by red-tailed hawks, crows, weasels, foxes, owls, raccoons, cats, dogs, cars, and humans (Ackerman 1995).

The diet of the eastern gray squirrel consists of nuts, acorns, flower shoots, seeds, truffles and other fungi, fruit, insects, tree buds, baby birds, and carrion. Squirrels prefer to eat in the safety of a tree, as they are much more vulnerable on the ground. They most often carry their food to a low branch and eat while holding their food with both hands and keeping an eye on the ground (Lipske 1997). Eastern gray squirrels have a highly adapted sense of sight, even in dim light, as well as a wide field of vision. This trait along with their tough curved claws and the ability to reverse their hind foot 180 degrees makes them highly adapted for climbing trees. They are excellent climbers and can leap considerable distances using their powerful hind limbs (Campbell 1999).

Foraging activity of gray squirrels peaks in the morning, about 2 hours after sunrise, and in the evening, about 2-5 hours before sunset (Halloran 1999). One reason for this bimodal activity may be that temperatures are more moderate during mornings and evenings. If foraging activity is focused at these times rather than the afternoon, exposure to extreme temperatures is minimized (Bryce 2001).

Gray squirrels are non-territorial, with overlapping home ranges that average 5 hectares in size. This is where the squirrels do most of their foraging for food, making nests, and rearing young. Due to an increase in their range during the breeding season, males have a slightly larger range than females, but there is little territorial behavior and many home ranges overlap. Individual squirrels are often seen feeding close to each other without any aggressive activity, but this is due to their acceptance of others which is dependent upon food supply and squirrel population density. These densities are highest in habitats where the numbers of tree species that produce food are highest (Campbell 1999).

We hypothesized that since gray squirrels live in relatively safe wooded areas where it uses its keen climbing ability to escape predation, they should prefer to feed closer to wooded areas than farther away in open areas. We also hypothesized that the squirrels would prefer large peanuts offer smaller sunflower seeds and that they would less likely venture into an open area for sunflower seeds than for peanuts.

METHODS AND MATERIALS

A squirrel foraging area was set up prior to the first day of our experiment, which was conducted from March 27 to April 17, 2003. We set up a grid of twenty feeding sites each 25 to 30 ft. apart (Fig. 1) on a lawn bordering a small woodlot on the southern side of the Brumbaugh Science Center of Juniata College (Huntingdon, Pennsylvania). The grid bordered a woodlot where gray squirrels had frequently been observed, as well as various potential predators, including crows, hawks, dogs, humans, and raccoons. Each feeding area contained two shelled unsalted *Fowlers Famous* roasted peanuts. We marked each sight with blue ribbon tied to sticks placed in the ground at all feeding sights. We observed squirrels from 7:00am till 8:00am and again from 3:00pm till 4:00pm Monday through Friday near the Juniata College Observatory. During these observations we placed two new peanuts if they were eaten and collected data from the feeding sights by noting where the peanut were missing. We also noted squirrel behavior during these times.

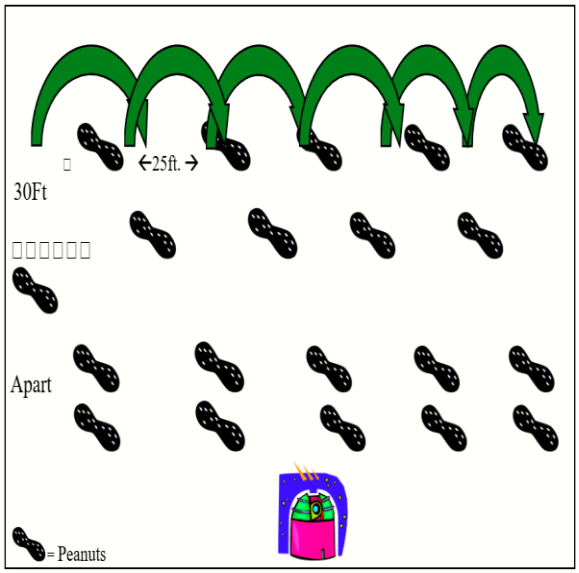

Figure 1. During week one, the squirrel feeding grid behind the Brumbaugh Science Center of Juniata College (Huntingdon, Pennsylvania)

During the second week, started on April 3, 2003 we increased the distance between feeding sites to 50 ft and added another row of feeding sights, for a total of twenty-five sites (Fig. 2). We assumed that increasing the distance between the feeding sites also increased the risk of foraging at them.

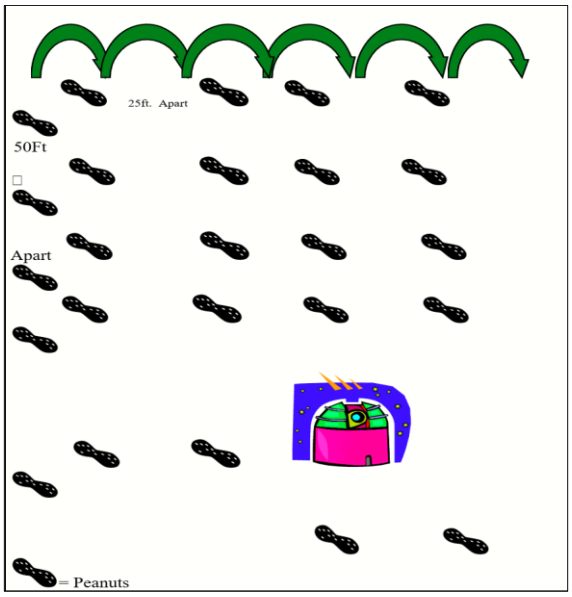

Figure 2 . During week two, the squirrel feeding grid behind the Brumbaugh Science Center of Juniata College (Huntingdon, Pennsylvania)

During the third week, which started on April 24, 2003, we tested the food preference of the squirrels. Three of the rows were switched from peanuts to sunflower seeds (Fig. 3). All other procedural methods stayed the same.

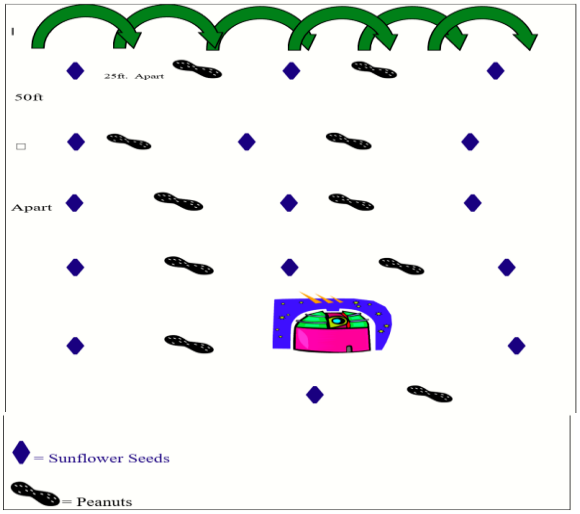

Figure 3. *During week three, the squirrel feeding grid behind the Brumbaugh Science Center of Juniata College (Huntingdon, Pennsylvania)*

RESULTS

During our first week of observation (Table 1), the squirrels did not show a preference for low risk peanuts close to the woods. They missed the lowest risk peanuts, located in the cover of the edge of the woods for the first few days, and were observed running out to the furthest feeding site and then slowly making their way back stopping at each sight to eat peanuts. The average number (out of a possible five at each distance) of sights visited by squirrels was 4.2 at 0 ft, 4.9 at 30 ft, 4.8 at 60 ft, and 4.9 at 90 ft from the woods. Our null hypothesis that squirrels would consume the same amount of peanuts at each different distance, could not be rejected ($\chi^2 = 0.386$, $P = 0.945$).

Table 1. Week one data of squirrel foraging behavior: number of feeding sites visited each day at various distances from the woods (out of a possible 5 sites for each distance).

	Number of feeding sights visited by squirrels at various distances						
	0ft	**30ft**	**60ft**	**90ft**	**Total**	**Mean**	**Standard Dev.**
Thursday 7:00 AM	1	5	3	4	13	3.25	1.71
1:00 PM	3	4	5	5	17	4.25	0.96
Friday 7:00 AM	3	5	5	5	18	4.50	1
1:00 PM	5	5	5	5	20	5.00	0
Monday 7:00 AM	5	5	5	5	20	5.00	0
1:00 PM	5	5	5	5	20	5.00	0
Tuesday 7:00 AM	5	5	5	5	20	5.00	0
1:00 PM	5	5	5	5	20	5.00	0
Wednesday 7:00 AM	5	5	5	5	20	5.00	0
1:00 PM	5	5	5	5	20	5.00	0
Total	42	49	48	49			
Average	4.2/5	4.9/5	4.8/5	4.9/5			
Standard Dev.	1.4	0.32	0.63	0.32			

The squirrels did not show a preference for low risk peanuts close to the woods even after the risk factor was significantly increased in our new set up during the second week of our observations (Table 2). The squirrels ate 100% of the peanuts at each distance everyday of the week.

Table 2. Week two (increased risk factor) data of squirrel foraging behavior: number of feeding sites visited each day at various distances from the woods (out of a possible 5 sites for each distance).

	Number of feeding sights visited by squirrels at various distances							
	0ft	**50ft**	**100ft**	**150ft**	**200ft**	**Total**	**Mean**	**Standard Dev.**
Thursday 7:00 AM	5	5	5	5	5	25	5	0
1:00 PM	5	5	5	5	5	25	5	0
Friday 7:00 AM	5	5	5	5	5	25	5	0
1:00 PM	5	5	5	5	5	25	5	0
Monday 7:00 AM	5	5	5	5	5	25	5	0
1:00 PM	5	5	5	5	5	25	5	0
Tuesday 7:00 AM	5	5	5	5	5	25	5	0

1:00 PM	5	5	5	5	5	25	5	0
Wednesday 7:00 AM	5	5	5	5	5	25	5	0
1:00 PM	5	5	5	5	5	25	5	0
Total	50	50	50	50	50			
Average	5.0	5.0	5.0	5.0	5.0			
Standard Dev	0.0	0.0	0.0	0.0	0.0			

Week three data showed that the squirrels preferred peanuts over sunflower seeds (Table 3; $\chi^2 = 17.73$, $P < 0.001$). However, having a less preferred food source present did not change the risk behavior in squirrels. The seeds appeared to be taken by squirrels only at the high risk feeding sights at 150-200 ft from the trees.

Table 3. Week three data of squirrel foraging behavior: number of peanut feeding sites visited (possible of 2) each day as compared to the number of sunflower seed sites (possible of 3) visited each day at particular distances.

	Number of feeding sights visited by squirrels (possible number of peanuts = 2, seeds =3)									
	0ft		**50ft**		**100ft**		**150ft**		**200ft**	
	peanuts	**seeds**	**peanuts**	**seeds**	**peanuts**	**seeds**	**peanuts**	**seeds**	**peanuts**	**seeds**
	2	0	2	0	2	0	2	1	2	0
1:00 PM	2	0	2	0	2	0	2	0	2	1
Friday 7:00 AM	2	0	2	0	2	0	2	1	2	1
1:00 PM	2	0	2	0	2	0	2	1	2	1
Monday 7:00 AM	2	0	2	0	2	0	2	1	2	0
1:00 PM	2	0	2	0	2	0	2	1	2	1
Tuesday 7:00 AM	2	0	2	0	2	0	2	0	2	0
1:00 PM	2	0	2	0	2	0	2	1	2	1
Wednesday 7:00 AM	2	0	2	0	2	0	2	0	2	1
1:00 PM	2	0	2	0	2	0	2	1	2	1
Total	20	0	20	0	20	0	20	7	20	7
Average	2	0	2	0	2	0	2	0.7	2	0.7
Standard Dev.	0	0	0	0	0	0	0	0.48	0	0.48

DISCUSSION

Our data show that gray squirrels in our study area foraged for peanuts at all sites regardless of possible differences in predatory risk. They did not prefer to feed closer to cover than out in the open, even though potential predators had been observed in the vicinity. They also appeared to show no fear toward people, as they fed within a few feet of us, even when we were walking about replenishing food on the feeding grid.

However, the grey squirrels preferred to eat peanuts rather than sunflower seeds. Perhaps the squirrels perceived the seeds as not being as worth the risk to collect in an exposed habitat than were the larger peanuts. However, even the sunflower seeds were consumed by squirrels in high-risk areas (150 ft and 200 ft from the woods).

More data are needed to determine whether squirrels in our study area actually take into account predatory risk while foraging, as predicted by optimal foraging models (Ricklefs and Miller 2000). Future studies should consider ways of increasing the risk factor. It is possible that we didn't create a situation of high enough risk. Another possibility is to perform this study on squirrels that do not live in a highly human populated area. Because the squirrels live on a college campus, high-risk behavior may be beneficial

for their survival. Humans are messy with their food and give risk-taking squirrels many opportunities for obtaining a meal.

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EFFECT OF MOUND DIAMETER ON MOUND SPACING IN A *FORMICA EXSECTIODES* POPULATION IN CENTRAL PENNSYLVANIA

Eric Butler and Amara Camp

ABSTRACT

Several studies have suggested the existence of a "kill zone" around ant colonies that prevents other colonies from settling within it. In this study, data were collected on mound diameter (assumed to be a measure of colony size) and mound spacing in an attempt to assess the existence and strength of any relationship between colony size and size of "kill zone" in *Formica exsectiodes*. Sampling was conducted exhaustively in a clearing in Huntingdon, PA, in an area of dense *F. exsectiodes* population. A regression test was run on the data, and produced a non-significant p-value of 0.088. This provides no evidence to suggest the existence of a relationship between colony size and spacing.

Keywords: Formica exsectiodes, *territoriality, ant mounds*

INTRODUCTION

Formica exsectiodes, a mound-building ant found in Pennsylvania, can occur quite densely in certain areas. Previous research on other mound-building species indicates that ant mounds can have a significant effect on the vegetation of an area (King 1977, Coffin and Lauenroth 1990). Ants appear to affect the distribution of certain species of plants by hoarding their seeds, which, in turn, causes these plants to grow on ant mounds (Howe and Smallwood 1982). The effects of ants at the autotrophic level are potentially significant, as the secondary and tertiary trophic levels are influenced by the composition of the autotroph level. Therefore, the factors that influence ant communities are significant to the understanding of the ecosystems of which they are a part.

Previous research on other ant species suggests that established colonies affect population density by creating a "kill zone" around the nest, in which new colonies cannot settle (Ryti and Case 1992, Gordon and Kulig 1996). The size of this kill zone should, we hypothesize, be linked to the territory size of the colonies. In a study on fire ants, Tschinkel et. al (1995) showed that larger colonies control a larger territory. Therefore, we hypothesized that the size of the colony should be proportional to the distance to its nearest neighbor.

To conduct this study, however, a measure of colony size was needed. Several studies have dug up entire colonies and counted individuals. This was judged as far too labor-intensive and disruptive, and so some of the conclusions from these other studies have been utilized to provide an easier measure of colony size. In the fire ant study cited above, and in another study conducted on *Formica ulkei*, it was observed that larger mounds tended to contain larger ant colonies (Dreyer 1942). Although Andrews (1929) has disputed this link, it was based on a very small sample size taken under a variety of conditions. It should be noted, however, that no study assumes a perfect correlation between mound size and colony size, as large colonies sometimes suffer die-backs without a resulting decrease in mound size. This will not always affect the visible results of the kill zone, as it may take time for new colonies to take advantage of the reduced kill zone.

Taking all of these factors into account, we hypothesize that the spacing of *Formica exsectiodes* colonies (and, consequently, mounds) is influenced by the size of the colony, such that the size of an ant mound should be directly proportional to the distance from it to the nearest other ant mound. Furthermore, this effect should be most noticeable under higher

population densities. This conclusion is drawn from studies on a variety of different organisms, including foxes (Trewhella et al. 1998), bluehead wrasses (Warner and Hoffman 1980), and a general paper on territory and its relationship to population density and food availability (Hixon 1980). All of these studies agree that territorial interactions are increased by population density.

METHODS AND MATERIALS

We tested our hypothesis on a population of *Formica exsectiodes* in Huntingdon, PA. During November 2003, the population was located in a section of a field (about 30 m wide) which had been cleared from the surrounding forest, in order to put up electrical poles. Ant mounds were scattered throughout the area at varying densities. As we could not determine the reason for these differences, which may have included food density or soil characteristics, we chose as our population of study the colonies in an area where the mounds were most densely distributed. Sampling across an even ant food gradient is desirable so that, if territory size is effected by food availability, mounds in an area with less food, and correspondingly larger territories for their size, are not being compared to mounds in food-rich habitats with smaller territories. By exhaustively sampling where the mound distribution was densest, we hoped to avoid sampling across such random, unmeasured fluctuations in habitat, or sampling across areas that ants could not colonize for other reasons. Our chosen population of study was composed of 50 active ant mounds in an area approximately 150 meters long. Active mounds were determined by the presence of ants on the mound.

In sampling a mound, we measured the diameter of the base of the mound and the distance from the center of that mound to the center of its closest neighbor using a 35 meter measuring tape. Both measurements were estimated within 5 cm, because of the difficulty in locating a precise edge to an ant mound and the difficulty of keeping the tape absolutely straight across long distances and around obstacles like shrubs. Each data point included the diameter of a mound, and the distance to that mound's closest neighbor (regardless of size). If mounds were not circular, we tried to measure a diameter that we estimated to be between the widest and the narrowest measurement of the mound.

Because we assumed that, if a relationship existed, the distance between mounds would depend on the diameter of the mound, we plotted distance to nearest neighbor versus diameter of mound, and used the regression test to determine if the dependence of distance to nearest mound on the diameter of the mound was significant.

RESULTS

Preliminary tests on the data were run to ensure that the data fit the assumptions for regression testing. A plot of residuals versus fits was constructed, which indicated equal variance. A normality plot of residuals was also constructed, and the accompanying Anderson-Darling normality test gave a p-value of 0.284, indicating that our y-variable residuals were normally distributed. The regression test yielded an r^2 value of 0.059 and a non-significant p-value of 0.088. Furthermore, a visual examination of the scatter plot of our data does not suggest a strong dependence of distance between mounds on diameter of mound.

(Fig. 1).

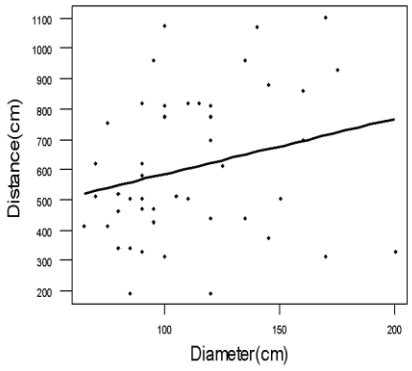

Figure 1. Regression of distance to nearest ant mound versus the diameter of 50 selected ant mounds.

DISCUSSION

Our hypothesis was that larger ant mounds would be farther from their neighbors. This, however, was not supported by our regression test. Because this research question was designed to examine possible territorial effects, the lack of significance in our results leads us to conclude that we have no evidence to support territoriality in *Formica exsectiodes*. However, several of our assumptions about the link between the data we collected and

territorial effects can be questioned, and further research should investigate these assumptions.

We have assumed that there is a positive correlation between mound diameter and colony size. If this relationship does not exist, our research will not have addressed the question of territoriality. Furthermore, territorial effects should not occur between closely related colonies, or multiple mounds that are inhabited by a single large colony. We assumed that each mound was inhabited by separate, unrelated colonies when framing our research question. To properly correct for this, the genetic relatedness of ant mounds in the study should be examined. Both of these factors could be investigated in further research to ensure that we did not simply miss territorial effects in our study through poor design.

If, however, our assumptions are valid and our results are correct, further research is still recommended. As stated before, the factors that influence colony spacing in *Formica exsectiodes* are important to discover for a better understanding of the ecosystems in which the organism occurs. Therefore, further research should be directed at discovering what other factors may regulate mound spacing. Possible factors might include habitat differentiation or predation. Studies should be conducted on both of these factors.

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EFFECTS OF TEMPERATURE ON THE DEVELOPMENT OF THE WOOD FROG, *RANA SYLVATICA*

Jessica Darrow, Andrea Nulton and Danielle Pompili

ABSTRACT

Eggs of the wood frog *Rana sylvatica* were collected from a temporary pond and placed into three controlled chambers with temperatures of 9°C, 21°C and 26°C. As a control, eggs were also placed in an outside container with an average temperature of 11.2°C. The eggs remained in the four containers for 21 days. Their development was regularly monitored and their average size and hatching time recorded. Significant differences were found in the growth rates of the frogs placed at the different temperatures when compared to the control, with the frogs placed in the cooler temperature having a slower growth rate, and the frogs placed in the warmer temperatures having a faster growth rate than the control. There were also noticeable differences in hatching time between the four temperatures. These data suggest that temperature does play a role in the development of the wood frog.

Keywords: development, global warming, Rana sylvatica*, temperature, wood frog*

INTRODUCTION

Since the 19th century when the Industrial Revolution began in the United States, global warming has been a growing concern. Global warming is defined as "the world wide trend towards warmer and warmer temperatures" (Mayer 248). Global warming is believed to be caused primarily by increased levels of greenhouse gases such as carbon dioxide, ozone and chlorofluorocarbons in the atmosphere. Carbon dioxide is the most prevalent greenhouse gas and so its prevention is of the highest concern. A rise in CO_2 levels is caused primarily by the burning of fossil fuels, which is one reason why alternative energy is such a crucial necessity (Mayer, 2001).

The prevention of global warming is being investigated by over 25,000 scientists worldwide. However, recent temperature analyses have shown a drastic increase in temperature over the last 100 years (Figure 1). This rise in temperature poses serious environmental threats to many animals and plants, and could have drastic effects on their development and life styles.

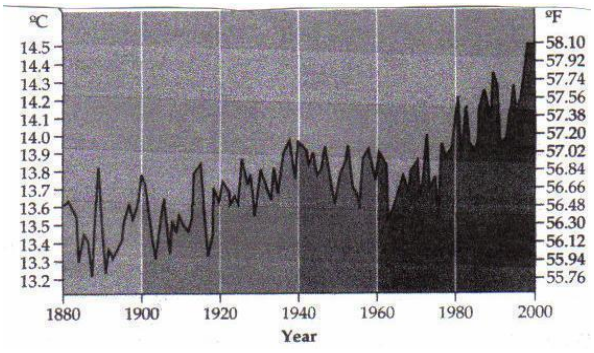

Figure 1. Average global temperature between 1880 and 1998 (Mayer 254)

Ectothermic mphibians are particularly affected by global warming and extreme temperatures

since their body temperature is not internally regulated but changes with the environment. Additionally, amphibians depend on ephemeral ponds for breeding. Due to their small size, these ponds are easily affected by changes in the climate. A rise in temperature can cause them to become extremely warm, which can affect their ability to sustain life (Halliday, 2002).

To test the effects of temperature on amphibian development we chose to conduct an experiment that tested the effects of different temperatures on the development of the wood frog. Wood frogs are common in Pennsylvania and are distributed throughout much of the northern United States, as well as Canada and Alaska. They are known to breed in both temporary and permanent bodies of water, but prefer to use temporary ponds since there are fewer predators present. Due to their reliance on these ephemeral ponds the wood frog is extremely susceptible to temperature changes. This can be detrimental to its existence since optimal growth has been found to occur at a temperature of approximately 22◦C and temperature extremes have been found to be deadly (Censky, 2001). Therefore, we hypothesized that over the course of our twenty-one day study, the frogs placed in a container with a water temperature close to 21◦C will have a higher growth rate and earlier hatching time than the eggs placed in the two extremes.

FIELD SITE

On March 19, 2004, *Rana sylvatica* egg samples were obtained from a puddle along the power lines located near the Raystown Field Station in Huntingdon, Pa. The water in the puddle was approximately six inches deep, muddy and stagnant. There was very little vegetation present, only patchy areas of grass and algae. There were multiple puddles in the area, but this one was the only puddle that contained eggs.

METHODS AND MATERIALS

The eggs were extracted from the puddle using a bucket, and transported back to Juniata College where they were placed in three temperature-control chambers containing approximately 2L of water, which was obtained from Warm Springs, a neutral fresh water spring in Huntington Pa. The control chambers were set at 9◦C, 21◦C and 26◦C. There was also a container filled with 2L of water left outside to simulate the frog's natural environment, from which we obtained our expected values for growth rate and hatching time. The eggs were then separated into approximately four equal masses by use of a spatula and placed into the four containers. Two liters of water from the puddle in which they were found was also poured into the containers.

The eggs were checked for average size, and the date when the first eggs from each group began to hatch was recorded. The water was checked for the dissolved oxygen level and temperature. Any other observations were also noted. Data were collected on a daily basis except for on the weekends. When the dissolved oxygen level was low, fresh water was added and a bubbler was placed in the container. Once the eggs hatched they were fed algae pellets as needed, and there activity was recorded. A t-test was then conducted comparing the slopes (regression coefficients) of body size vs. time at the different temperatures (Zar, 1984).

RESULTS

Among the different temperatures there was a noticeable difference in the day of hatching (Table 1). The t-test showed that the growth rate of the fogs placed in a temperature of 9◦C was significantly less than the growth rate of the frogs placed in the control, and that the growth rate of the frogs placed at a temperature of 21◦C was significantly greater than that of the control frogs (Table 2). The tadpoles' growth rates are shown in Figure 2, and a regression analysis of their development in relation to temperature is shown in Figure 3, with the slope values recorded in Table 3. It was also found that as the temperature of the water increased, the average dissolved oxygen level of the water decreased (Table 4).

Table 1. Hatching time of Rana sylvatica *at different temperatures*

Temperature	9◦C	21◦C	26◦C	Control (outside)
Day Hatched	Day 15	Day 13	Day 4	Day 11

Table 2. T-tests comparing the mean slopes of the frogs placed at 9◦C and 21◦C in relation to the slope of the control

Temperature	9◦C	21◦C
t-value	- 3.45	8.086
P-value	<0.001	<0.001

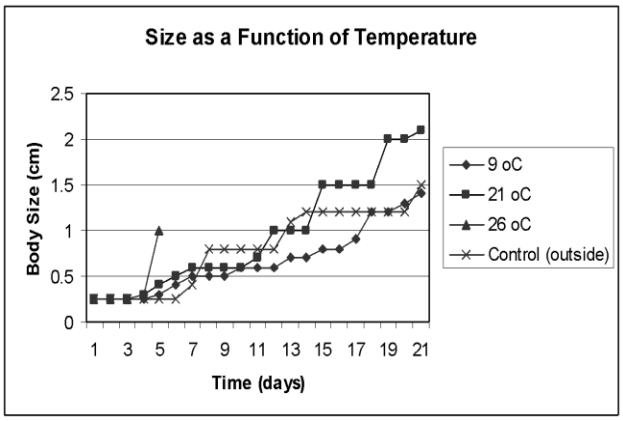

Figure 2. Tadpole growth at different temperatures

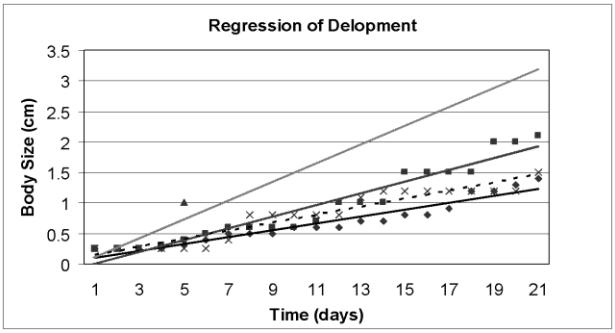

Figure 3. Regression analysis of Rana sylvatica *development in relation to temperature*

Table 3. Slope calculations from the regression chart

Temperature	9◦C	21◦C	26◦C	Control (outside)
Slope	0.056	0.096	0.155	0.066

Table 4. Mean concentrations of dissolved oxygen at the different temperatures

Temperature	9◦C	21◦C	26◦C	Control (outside)
Mean dissolved O_2 level	9.78	7.52	4.20	10.1

DISCUSSION

As expected from our hypothesis, temperature does play a role in the development of the wood frog. The frogs placed at 9◦C had a growth rate that was significantly lower than that of our control group, which had an average temperature of 11.2 ◦C, and the frogs placed at a temperature of 21◦C had a growth rate significantly higher than that of the control group. The growth rate of the frogs placed at a temperature of 26 ◦C was not statistically analyzed, since they did not live the entire duration of the experiment, and therefore could not accurately be compared. However, for the time period that they were alive, it was noted that their growth rate was much higher than that of the control. There was also a noticeable difference in hatching time, when comparing the control to the frog eggs placed at 26 ◦C.

Our results partly refuted our hypothesis that the frogs placed in the intermediate temperature would have a higher growth rate and an earlier hatching than the frogs placed in the two extremes. In contrast, we found that the frogs placed in the warmest temperature, 26◦ C hatched first, and also had the highest growth rate. However, these frogs died within two days of hatching, thus supporting Huey (2004) who found that temperatures of approximately 27 ◦C and higher to be detrimental to the frogs survival.

One reason why an increase in temperature is detrimental to the frog's survival is because as the temperature of water increases, its ability to hold oxygen decreases (Ward 2003). In order to survive, most organisms need dissolved oxygen levels of 5ppm or higher (Washington University 2004). This was a problem in our experiment. The warmer the water was, the faster that its oxygen level dropped. When we first placed the water into the four containers, it had a dissolved oxygen level of 11.2ppm. However, after the first weekend, the eggs placed in the water with a temperature of 26◦C had already hatched and had a dissolved oxygen level of 0.97ppm. This rapid decrease in the oxygen content may also have been due to the increased activity level of the frogs after hatching. The water in the container was quickly changed in an attempt to save the frogs. However, the frogs were all dead by the next afternoon.

Another problem that we encountered during our research, which may have affected our data, was a malfunction in the heating unit of the 21 ◦ C tank, which caused the temperature in the tank to drop to 10 ◦C. It remained at this temperature for approximately three days until the problem was corrected. If this had not occurred, we predict that the frogs would have hatched a few days sooner and would have had an even higher growth rate, thus showing an even stronger

correlation between temperature and developmental rate.

The biggest problem that we encountered during our research was not having enough data to properly analyze our results. This occurred because instead of taking multiple random measurements, we only recorded the average size and hatching time of our frogs. This turned out to be highly problematic because most statistical tests require multiple measurements. For this reason we were not able to statistically analyze the hatching time of our frogs, since no test is available for compare only averages.

There are many opportunities to conduct further research on this subject. First, our experiment could be repeated taking multiple random measurements instead of just averages. This would produce more accurate data and allow for the use of more statistical tests. Also, this experiment could be conducted for a longer period of time, comparing all the major developmental stages of the frog, as well as the life span of the frogs in each of the temperature groups. After death, the frogs could also be dissected to determine if temperature had any effects on organ development. Our experiment could also be performed using other amphibians such as salamanders. This additional research could help determine if temperature plays a significant role in the development of amphibians, and if so, help humans predict what effects global warming may have on amphibians.

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EFFECTS OF ABIOTIC FACTORS ON THE ABUNDANCE OF EASTERN SKUNK CABBAGE (*SYMPLOCARPUS FOETIDUS*) IN TWO CENTRAL PENNSYLVANIA SPRINGS

Erin V. Satterthwaite, Brad C. Simpson, and Beth A. Woodhouse

ABSTRACT

This study investigated the abundance of the skunk cabbage (*Symplocarpus foetidus*) at Warm Spring and Cold Spring. We tested whether abiotic factors such as spring water pH, spring water temperature, soil pH, soil moisture, and soil temperature affected the abundance of skunk cabbage. We examined ten plots at each site, five on each side of each spring brook and conducted total counts within each plot. We found that skunk cabbage abundance was significantly positively correlated with soil moisture along both Warm Spring ($r_s = 0.5971$, $P < 0.009$) and Cold Spring ($r_s = 0.5993$, $P < 0.009$). Soil moisture appeared to have a stronger effect on skunk cabbage abundance than did the other abiotic factors examined.

Key words: abiotic factors, pH, soil moisture, springs, Symplocarpus foetidus

INTRODUCTION

Winters in central Pennsylvania are taxing on the flora and fauna of the region and few species have been able to adapt to rise from the ground before the traditional spring bloom. During the harsh winter months, plants encounter the problems of water loss through transpiration and difficulty photosynthesizing due to weak, limited sunlight. Many plants overcome these difficulties by becoming dormant during these winter months, essentially hibernating, until spring. Low growing plants are more likely to have the ability to survive in warm depressions created under the snow. The plants then wait for the snow to melt so that they can begin their growth period (Fish and Wildlife).

Another, rarer adaptation of plants is thermogenesis. Thermogenesis is where a plant has the ability to produce its own heat internally. Even more uncommon, is the physiological regulation of flower temperature, where heat production increases at lower environmental temperatures. This type of regulation is known in only two species (Seymour 1998). One of the unique native species that is able to perform this type of regulation is *Symplocarpus foetidus*, known as skunk cabbage, and is the topic of our study.

Skunk cabbage, from the family Arum (Araceae), is typically found in wet woods, marshes, and streamsides (Connecticut 2005). The spathe, which protects the floral spike, begins to poke through the snow and ice covering the ground in late winter. This is due to the endothermic nature of the skunk cabbage, which releases heat from the flower head (Holdrege 2000). At two local springs, Warm and Cold, we observed the skunk cabbage poking through the snow in late February.

In observing the blooming of this plant, we noticed that the areas where skunk cabbage is most prevalent appeared to be in relatively water-saturated areas near the springs. This led us to investigate the correlation of abiotic factors with skunk cabbage

abundance. The abiotic factors studied were soil moisture, temperature and pH, in addition to the spring water temperature and pH. We hypothesized that the greatest abundance of skunk cabbage should be found where the environmental conditions indicate high soil moisture and the pH is 4 and 7.

FIELD SITE

For this study, we concentrated on two springs located in Huntingdon, Pennsylvania. Both Warm Spring and Cold Spring are located just off of Cold Springs Road. Warm spring is aptly named because the average temperature is slightly higher than that of a typical spring in central Pennsylvania. The average water temperature is 18.5° C and the average water pH is 6.80. This spring is both wider and deeper at the source than at Cold Spring. At the source of Cold spring, you can see that it has been altered by man, lining the banks with rocks most likely to prevent erosion if the water level was to rise. The average water temperature at Cold Spring is 12.4° C with an average pH of 5.06. Both springs have wet and dry sections along their banks, with most of the drier sections occurring where the ground level is well above the height of the surface waters of the spring. Vegetation at both springs is similar, with a mix of trees, mosses, and thorny plants.

METHODS AND MATERIALS

At both Warm Spring and Cold Spring, we divided the springside area using a 5-m by 5-m grid system. After the five 25-m² plots were marked out along each side of each spring brook, we marked every other plot as our sample sites, alternating on the other side. Out farthest plot reached back 60 m from the source with a five plots along each bank and a total of ten plots at each spring. See Fig. 1 for clarification of sample site setups.

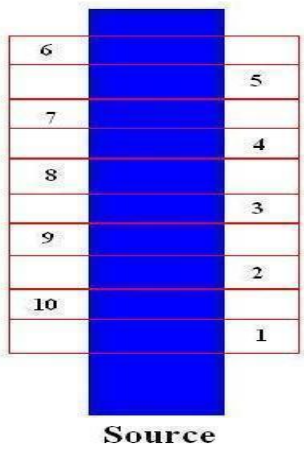

Figure 1. Layout of sampling plots for both springs.

To gather environmental data, we used a probe thermometer for soil temperature, a mercury thermometer for water temperature, and a Markson Model 88 pH meter for water and soil pH. Soil pH was estimated from equal samples of soil from each plot that had been mixed with 100mL of de-ionized water. Soil-moisture data were gathered by taking samples from each plot and packing them evenly into equal sized containers and weighing them. After drying in the oven for a week, the samples were weighed again and after subtracting the mass of the container, the soil moisture could be calculated.

Population counts were gathered by using a total count technique where every plant in the plot was counted. We defined one plant by looking at the main stem. Everything that branched off of the one main stem was considered one plant.

RESULTS

Fig. 2 shows the abundance of the skunk cabbage at the two springs examined. Skunk cabbage was present in all of the ten plots at Warm Spring, though it was missing from plots 6 and 7 at Cold Spring. The total number of skunk cabbage at Warm Spring is on average much higher than that at Cold.

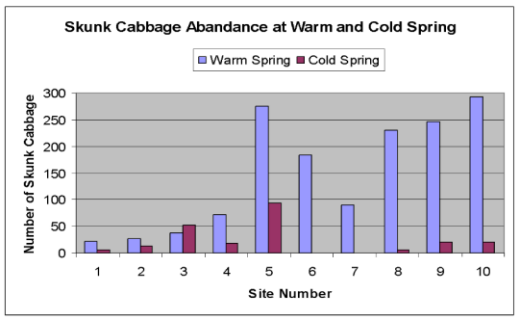

Figure 2. Graph of the abundance of skunk cabbage at the ten sampling sites from Warm and Cold Springs.

Fig. 3 shows the percentage of soil moisture from the ten plots at both springs. On average, the soil moisture is higher at Warm Spring than Cold Spring (55.66% versus 40.83%). The soil moisture peaks at 81.76% for Warm Spring and at 71.44% for Cold Spring and has a minimum of 27.84% at Warm Spring and 22.20% at Cold Spring.

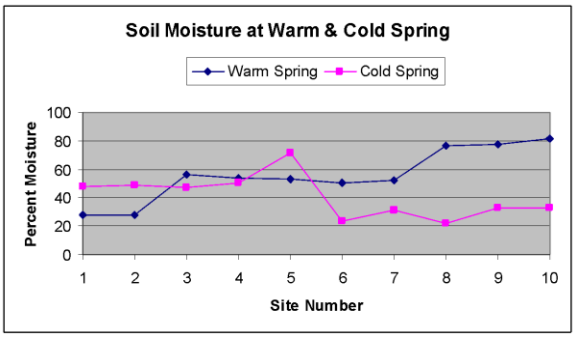

Figure 3. Graph of the soil moisture percentage at the ten sampling sites from Warm and Cold Springs.

Fig. 4 shows the soil pH from the ten plots at both springs. On average, the soil pH is slightly higher at Warm Spring than Cold Spring (4.66 versus 4.57). The soil pH peaks at 5.90 for Warm Spring and at 5.75 for Cold Spring and has a minimum of 3.98 at Warm Spring and 4.13 at Cold Spring.

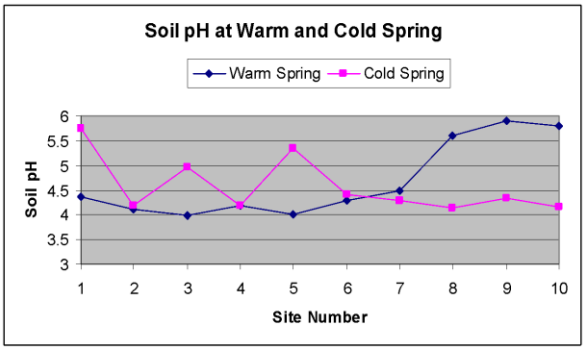

Figure 4. Graph of the soil pH at the ten sampling sites from Warm and Cold Springs.

Fig. 5 shows the correlation between the percentages of soil moisture and the number of skunk cabbage at each of the 10 sample sites for both springs. On average, Warm Spring has a higher percentage of soil moisture and a greater number of plants, but this does not necessarily correspond to a greater correlation. The r^2 value for cabbage number in relation to percentage soil moisture at Warm Spring is 0.5971 ($P = 0.009$). The r^2 value for Cold Spring is 0.5993 ($P = 0.009$).

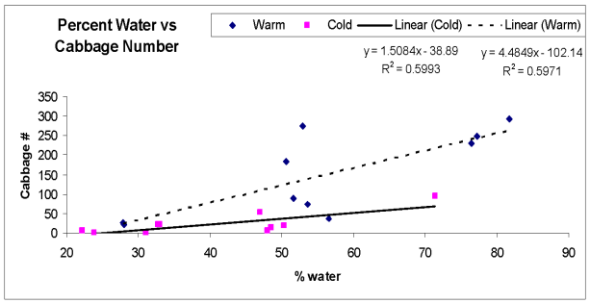

Figure 5. Graph of number of skunk cabbage plants versus soil moisture percentage at the ten sampling sites from Warm and Cold Springs.

Fig. 6 shows the correlation between the soil pH and the number of skunk cabbage at each of the 10 sample sites for both springs. On average, Warm Spring had a higher soil pH and a greater number of organisms. The r^2 value for Warm Spring is 0.4256 ($P = 0.041$). The r^2 value for Cold Spring is 0.2195 ($P = 0.172$).

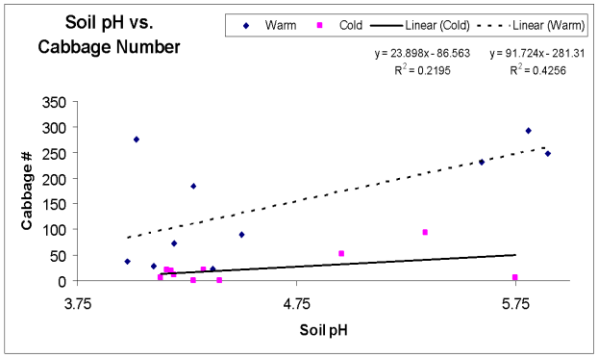

Figure 6. Graph of number of cabbage versus soil pH at the ten sampling sites from Warm and Cold Springs.

Table 1 shows the variation in the water temperature at the two springs, average 18.5° C (range 17 to 19° C) at Warm Spring and 12.4° C (range 12 to 14° C) at Cold Spring. The water pH of Warm Spring ranges from 6.59-7.02 with an average of 6.797, and the pH of Cold Spring ranged from 4.97-5.20 with an average of 5.055. Both the soil temperatures for Warm and Cold Springs ranged from 11 to 13° C with an average of about 12° C.

Table 1. Other environmental factors examined at both springs

Environmental Factors	Spring	Min	Max	Average
Water temperature	Warm	17 °C	19° C	18.5°C
	Cold	12°C	14° C	12.4 °C
Water pH	Warm	6.59	7.02	6.797
	Cold	4.97	5.20	5.055
Soil Temperature	Warm	11°C	13° C	12°C
	Cold	11°C	13° C	12.1°C

DISCUSSION

We found that the environmental factors of soil temperature, water temperature, and water pH of the springs had no significant effects on the abundance of skunk cabbage due to their relatively unchanging nature between both springs (Table 1). Soil moisture and pH were the most variable factors, and they both appeared to affect the abundance of skunk cabbage. Skunk cabbage density was statistically significantly positively correlated with soil moisture percentage at both Warm and Cold Springs. Skunk cabbage density was also correlated positively with soil pH, but the correlations were weaker and at Cold Spring it was nonsignificant.

To tease out the relative importance of soil moisture and pH, we performed a multivariate regression analysis on the abundance of skunk cabbage versus both soil moisture percentage and soil pH. The *P*-value of this test was 0.001, which is close to the *P*-value of the regression analysis for soil moisture percentage alone. Therefore, there is no better fit when soil pH is taken into account, thus the soil pH data has virtually no affect on the significance of the data. From these regression tests we can conclude that soil moisture is probably the environmental factor which most greatly affects the abundance of skunk cabbage.

The literature describes the range of pH tolerance for skunk cabbage as being between 4 and 7 (USFWS). The overwhelming majority of our pH values occurred within that projected range so even though there is variation in pH along the ten test sites at each spring, this variation can be tolerated by skunk cabbage and should therefore not have a large effect on their abundance.

The range of soil-moisture values tolerated by skunk cabbage is not well documented in the literature, but most sources state that saturated, marshy areas are the most suitable location for this species. Thus, soil moisture is a key factor in the abundance of skunk cabbage, just as we had suspected.

Our findings could be verified by performing further replicates of our test. We only obtained one set of data at each spring due to timeline and weather constraints. This accounts for possible sources of error within our findings.

AKNOWLEDGEMENTS

We thank Dr. Douglas Glazier for providing us with the tools and support necessary to carry out our research, Marian Orlousky for her help in the lab and her unwavering moral encouragement, and the owners of Warm Spring for allowing us to park on their property.

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APPROACHABILITY OF EASTERN GRAY SQUIRRELS

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Karla G. Rodriguez and Nisha S. Pulimood

ABSTRACT

The Eastern Gray squirrel (*Sciurus carolinensis*) is known to forage in the morning and evening hours. In this study we hypothesized that Eastern Gray squirrels on the campus of Juniata College should be more approachable in the evening hours because they are less alert to the presence of potential predators than they would be in the morning while they are foraging. We believe that they would be more careful while they are feeding in the morning, and more relaxed in the afternoon. This study took place on the Juniata College campus (Huntingdon, Pennsylvania) where large numbers of gray squirrels can be found. We studied the squirrels at different hours of the day, using the same two people to approach them for every data collection event. The squirrels were more approachable in the late afternoon and evening hours; in the evening, the mean distance of approachability before a squirrel fled (2.249 ± 1.519 meters) was found to be smaller than that of the morning distance (3.718 ± 1.519 meters).

Keywords: Eastern Gray Squirrel (Sciurus carolinensis), foraging, habitat, vigilance behavior

INTRODUCTION

The Eastern Gray squirrel (*Sciurus carolinensis*) is the most common species of squirrel found in Pennsylvania, followed by the fox, red, and flying squirrels. The adult gray squirrel weighs 1 to 1.5 pounds, has a length of 18 to 20 inches, and is predominantly gray in color. Their diet consists mainly of acorns, hickory nuts, and walnuts, but in early spring they focus their diet on tree buds and flowers, which are a high energy food source. They only see in shades of black and white, and they are most active early in the morning and during late afternoon.

Squirrels that live in the kind of environment provided by a college campus have a high rate of regular human interaction and hence might be more

likely to allow humans to approach them. The Eastern Gray squirrel has already been found to be a highly social species, and even wild squirrels have been sighted eating food from human hands (Steele, 2001). In this investigation, human approachability of gray squirrels was measured at different times of the day. The degree of approachability in the early morning was compared to that of the late afternoon or evening.

We hypothesized that Eastern Gray Squirrels on the Juniata College campus should be more approachable in the evening. In the morning, the squirrels are very alert and hungry after a long night of rest. When approached, they are easily frightened and skittish. Conversely, in the evening, the squirrels are more relaxed. Having just eaten a few hours prior to their long rest in the afternoon, which we found to be common among Eastern Gray squirrels, it is not imperative for them to find food at this time. In the evening, we hypothesize that the squirrels will be more approachable because they will be less focused on finding food and will be more likely to manifest their natural curiosity through human interaction.

FIELD SITE

The study was conducted on the campus of Juniata College in Huntingdon, PA. For every data collection event, the squirrels were located near both trees and college buildings, allowing them equal opportunity for safety and equal exposure to human presence.

MATERIALS AND METHODS

The squirrel and the human tester were captured on video in a single frame, and optimal zoom for the clearest viewing. The model of the digital camera used was a Canon Powershot DS850 IS. The height of the person in the video was taken as the known, to provide the constant measure of distance needed for quantification. After this point, the camera was not zoomed in or out, to ensure constancy of distance covered towards the subject squirrel. The attire of the person interacting with the squirrel was controlled, so that this could be eliminated as an experimental variable. Dull solid colors, with no loud or bright patterns that might alarm the squirrel were worn.

The human tester walked slowly and steadily in the direction of the squirrel, trying to remain unnoticed and not alarm the squirrel for as long as possible. The behavior exhibited by the squirrel as the gap between the human and itself closed was observed and the final distance between the two subjects before the squirrel darted away was also measured. The video recording was stopped after the squirrel had noticed the tester and had run away, and this was considered as one raw data point for further analysis.

In order to accurately quantify this distance, we used a computer software program called Data Point, which takes distance measurements using the pixels from the video footage and the known height as a measure to give us comparable numerical data for each different data collection event. Statistical analysis of the quantified data was conducted using Minitab.

RESULTS

The mean distance of approaches obtained for the morning data was 3.718 ± 1.519 meters and for the afternoon data the mean was calculated to be 2.249 ± 0.859 meters. A single tailed, two sample t-test was used to analyze the data that were collected using the Data Point software. Two sample t-tests are used to infer whether a difference existed between the two sets of data. In this study, we tryed to determine if we could approach squirrels more closely in the afternoon as compared to the morning or vice versa. A single tailed test was utilized because the original hypothesis infers directionality; i.e., we predicted that we would be able to approach squirrels more closely in the afternoon as opposed to the morning. The raw data and Minitab calculations are attached in the Appendix.

It was determined that the test statistic for the two groups of data was 2.32 and the critical t-value was determined to be 2.145 ($df = 14$, and $\alpha = 0.05$). In this case, the null hypothesis, which states that a difference between means does not exist, can be rejected because the critical value is less than the test statistic, which is supported by the P-value of 0.019 that was obtained. In other words, the means are different enough to statistically say that there exists a significant difference in approachability of the squirrels in the morning in comparison to in the afternoon.

In the interest of thoroughness, we thought it was important to see if a statistical difference existed between the two investigators, Tommy and Nisha. We did this to eliminate the possibility that the different testers obtained different results because of their appearance/stature. To do this, another two sample t-test was preformed to compare the mean distance calculated from Tommy's data to that calculated from Nisha's data. This was done for the morning and afternoon data sets. For the morning data, a P-value of 0.564 was calculated, and for the afternoon data a P-value of 0.434 was calculated. Due to the fact that both of these P-values are above 0.05, the null hypothesis was not rejected. This means that there was no statistical difference between the mean distances of the two testers in either the morning or the afternoon data.

DISCUSSION

Squirrel approachability was tested to determine whether there was a difference in how close one could get to a squirrel at different time periods of the day. We hypothesized that there was such a difference and that squirrels could be better approached during the afternoon period. This could be because the squirrels forage for their food in the morning (Steele, 2001), and thus are more alert to predators and anyone that passes by. In the afternoon, squirrels are foraging less, if at all, and although still alert to predators, they seem to be more relaxed and less frightened by any human interactions.

The mean distance for the afternoon was found to be 2.249 ± 1.519 meters. This finding supports the hypothesis in which the mean distance was found to be smaller than that of the morning distance (3.718± 1.519 meters). This meant that the testers were able to get closer to the squirrels in the afternoon when compared to in the morning. This result was further confirmed through statistical analysis of the raw data, in which a P-value of 0.019 was attained. This P-value was seen as significant because not only was it smaller than 0.05, but it established that the means were statistically different, reconfirming the hypothesis that squirrels are more approachable in the afternoon.

Squirrel approachability is surely affected by human interactions. Thus, the data collected by both testers in the morning and afternoon were also analyzed in order to eliminate any direct effects they may have had on the squirrels themselves. The two testers had some significant differences which could affect the outcome of the data. Tommy is not only male but also taller and of a lighter skin tone. On the contrary, Nisha is female, of a shorter stature and has a much darker skin tone. After analyzing and comparing their data, it was calculated that the P-values for both the morning (0.564) and afternoon (0.434) yielder a value that was much higher than 0.05, meaning the data collected by both testers is statistically not dissimilar. This means that the differences between the two testers did not affect the squirrels and thus in the end, did not alter the data.

With this elimination, we are confident that squirrels are more approachable in the afternoon rather than the morning. We believe this is so because the squirrels are not preoccupied with finding food, as they are in the morning. It is clearly visible that squirrels are exposed more to humans during the afternoon on the Juniata College campus, rather than in the morning. Unlike squirrels, the students here seem to be more active in the afternoon, and for this reason, the squirrels may have a natural adaptation to being more accustomed to human interactions at that time.

A possible next step to verify the findings would be to calculate the human population on the Juniata College campus, and recreate this study in a remote area where squirrels interact less with humans. This would help in verifying the afternoon approachability hypothesis more and would aid the conclusion that higher levels of human presence and interaction in the afternoon on the Juniata Campus results in the squirrels being more adapted to their presence and thus more approachable.

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APPENDIX

Raw Data:

Tester	Location	Distance (m)	Distance (ft)	Time of Day
Tommy	Museum	1.830	6.004	Morning
Tommy	Museum	5.360	17.585	Morning
Tommy	Museum	6.312	23.990	Morning
Tommy	Museum	4.570	14.993	Morning
Tommy	Sunderland front	2.057	6.747	Morning
Nisha	Sunderland front	4.582	15.029	Morning
Nisha	Museum	2.157	7.075	Morning
Nisha	Museum	3.146	10.319	Morning
Nisha	Museum	2.977	9.765	Morning
Nisha	backyard	4.192	13.750	Morning
	Average	**3.718**	**12.526**	
	Std Dev	**1.519**	**5.667**	

Tester	Location	Distance (m)	Distance (ft)	Time of Day
Nisha	Beside Von Lebig	1.323	4.340	Afternoon
Nisha	Beside Von Lebig	0.706	2.315	Afternoon
Nisha	backyard (?)	1.984	6.510	Afternoon
Nisha	backyard (?)	1.963	6.439	Afternoon
Nisha	backyard (?)	2.295	7.528	Afternoon
Nisha	backyard (?)	3.048	10.000	Afternoon
Nisha	Steps by Halbritter	2.742	8.996	Afternoon
Nisha	Steps by Halbritter	2.364	7.756	Afternoon
Tommy	Beside Von Lebig	2.285	7.495	Afternoon
Tommy	Beside Von Lebig	3.784	12.412	Afternoon
	Average	**2.249**	**7.379**	
	Std Dev	**0.859**	**2.819**	

Statistical Analysis:

Two-Sample T-Test for morning and afternoon

```
           N  Mean  StDev  SE Mean
morning   10  3.72   1.52     0.48
afternoon 10  6.00   2.71     0.86


Difference = mu (morning) - mu (afternoon)
Estimate for difference:  -2.277
95% CI for difference:  (-4.382, -0.173)
T-Test of difference = 0 (vs not =): T-Value = -2.32  P-Value = 0.036  DF = 14
```

Two-Sample T-Test and CI: Nisha-am, Tommy-am

```
Two-sample T for Nisha-am vs Tommy-am

          N   Mean  StDev  SE Mean
Nisha-am  5  3.411  0.976     0.44
```

```
Tommy-am  5   4.03   2.00     0.89


Difference = mu (Nisha-am) - mu (Tommy-am)
Estimate for difference:  -0.615
95% CI for difference:  (-3.174, 1.944)
T-Test of difference = 0 (vs not =): T-Value = -0.62  P-Value = 0.564  DF = 5
```

Two-Sample T-Test and CI: Nisha-pm, Tommy-pm

```
Two-sample T for Nisha-pm vs Tommy-pm

          N   Mean  StDev  SE Mean
Nisha-pm  8  2.053  0.754     0.27
Tommy-pm  2   3.03   1.06     0.75


Difference = mu (Nisha-pm) - mu (Tommy-pm)
Estimate for difference:  -0.981
95% CI for difference:  (-11.089, 9.127)
T-Test of difference = 0 (vs not =): T-Value = -1.23  P-Value = 0.434  DF = 1
```

DOI

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HABITAT PREFERENCE OF *PEROMYSCUS*

Jonathan Borrelli, George Braun, Nicole Lundberg, Jess Michelangelo and Elaine Pang

ABSTRACT

Mice in the genus *Peromyscus* are widely distributed throughout North America. Two species, *Peromyscus leucopus* and *Peromyscus maniculatus*, are able to live in a variety of different habitats. Past researchers have found that in general these mice prefer to live in habitats characterized by forest or shrubby structure. We wanted to know whether or not *Peromyscus* in the vicinity of the Maya Lin Peace Chapel in Huntingdon PA had this same preference. To test the hypothesis that *Peromyscus* prefer to live in forest type habitats, we set up 100 Sherman live traps on a hillside near the Peace Chapel. Three habitat types were covered including grassy areas, shrub, and forest. Our results indicate that there is a definite preference of *Peromyscus* to be in shrub and forest type habitats. No mice were captured in grassy areas, three mice were captured in shrub areas, and two mice were captured in forested areas. It is probable that the protection offered by the overhanging coverage from aerial predators is the reason behind this preference.

Key words: Habitat preference, home range, Peace Chapel reserve, Peromyscus

INTRODUCTION

Mice in the genus *Peromyscus* are some of the most common and widely distributed mammals in North America (Linzey et.al., 2008). *Peromyscus leucopus,* the white-footed mouse, is probably the most abundant mouse in the eastern United States, and has a geographical range from southern Canada to southern Mexico (Linzey et.al, 2008). *Peromyscus maniculatus*, the deer mouse, is also a common mouse of Pennsylvania, and also has an extremely diverse geographical range, extending from mid- and northern Canada to the south of Mexico, and spanning from the east to the west coast of the United States (Linzey et.al, 2008). *P. maniculatus* is extremely robust and has been known to live in habitats ranging from tundra to swamps, forests, prairies and deserts (Linzey et.al, 2008). Both *P. maniculatus* and *P. leucopus* live in central Pennsylvania and are ecologically important for more than just being a food source for larger predators. Studies have demonstrated that *Peromyscus* populations are reservoirs for several diseases, including Hantavirus (Netski et. al., 1999) and *Borrelia burgdorferi*, the agent of Lyme disease, which migrated west from New England (Lord et. al., 1992).

Peromyscus leucopus is a brownish or grayish rodent commonly found in mixed deciduous and coniferous forests in the eastern United States. They range in length from 150-205 mm and weigh (on average) between 15-25 g (Rafinesque 1818). White-footed mice range from parts of Southern Canada and two-thirds of the Eastern United States down to Northern Mexico (Fig. 1) (Rose et. al., 2005). It is the most abundant rodent in Pennsylvania (Merritt 1987). Identification of *P. leucopus* is usually based upon external characteristics (Fig. 2), with the exception of identification and comparison to *P. maniculatus, P. polionotus,* and *P. gossypinus* (Hall, 1981).

*Figure 1. White-footed mouse (*Peromyscus leucopus*).). Smithsonian Institution National Museum of Natural History.*

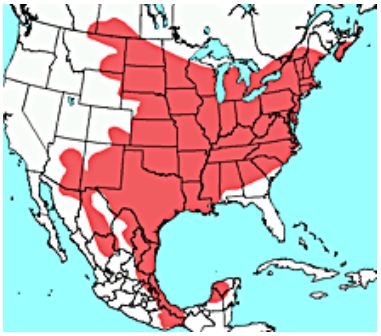

*Figure 2: Distribution of white-footed mouse (*Peromyscus leucopus) *Smithsonian Institution National Museum of Natural History.*

White-footed mice are primarily nocturnal but can be active at dawn and dusk (Zollaner et. al., 1999). *P. leucopus* are semi-arboreal and inhabit both brushy and woody regions (Lackey et. al., 1985). They spend most of their active periods on the ground (Madison, 1977). White-footed mice usually select nest sites above the ground, however, nests have been found near or at ground level in logs, rock piles, stumps, under trees, and in ground burrows (Mumford et. al., 1982). Highest densities of the white-footed mouse are found in brushy fields and woodlots containing large quantities of deciduous trees. Lowest densities are found in grassy fields (Hamilton et. al., 1979). Past studies comparing habitat preference at Raystown Field Station performed by Olsen et al. (2000) and Black et al. (2002) have also concluded that *P. leucopus* preferred forest habitats over fields. The average home range for the white-footed mouse is approximately 0.1ha. However, males normally have a larger area than females, and home ranges fluctuate seasonally (Maier, 2002). They may also be affected by food availability, age, and population density (Stickel, 1968). *P. leucopus* tend to feed on insects, starchy matter (such as mast and seeds), green vegetation, and fruit (Hamilton, 1941). The home range may also be influenced by moonlight, which may affect the white-footed mouse's perception and therefore influence their ability to find food (Zollaner et. al., 1999).

Another species of *Peromyscus* commonly found in Pennsylvania is *P. maniculatus*, or the deer mouse. Deer mice are very widespread, ranging from grasslands to forests and woodlands (Sullivan, 1995). It is the most widely distributed and abundant mammal in North America (Fig. 4) (Hygnstrom et al., 1994).

Figure 3. Deer mice (Peromyscus maniculatus). Smithsonian Institution National Museum of Natural History.

*Figure 4. Distribution of Deer Mouse (*Peromyscus maniculatus*). Smithsonian Institution National Museum of Natural History.*

Deer mice can vary in length from 120-155 mm and can weigh anywhere between 10-30 g (Wagner, 1845). Like all other individuals of the *Peromyscus* species, deer mice have white feet, white undersides, brown upper surfaces, and relatively long tails. The deer mouse's tail is more sharply bi-colored than that of the white-footed mouse (Fig. 3) (Hygnstrom et al., 1994)

Much like the white-footed mouse, deer mice are nocturnal animals that feed opportunistically upon any food source that is available, specifically seeds, nuts, fruit, berries, insects, and even other animal matter. As previously stated, deer mice will inhabit a variety of habitats, such as grasslands, shrub lands, woodlands, and forests. They are agile climbers, and will find shelter anywhere that is available (Wagner 1845). They construct their nests of an assortment of materials, such as stems, twigs, leaves, and roots and may be line with feathers, fur, or shreds of cloth. They primarily build nests underground in areas like the roots of trees, beneath logs, or in other rodents' burrows, but have also been known to facilitate aboveground sites such as hollow logs or fence posts. *P. maniculatus* occupies a home range that can be as small as 1/3 of an acre and as big as 4 acres or larger (Hygnstrom et al., 1994). Most studies have found that the size of the deer mouse's home range correlates with food availability and varies with the seasons; and there exists an inverse relationship between the population density of *P. maniculatus* and the size of the home range (Sullivan, 1995). They also use several home sites within the home range and will travel in between them, based on habitat changes and loss or gain of conspecific neighbors. Adults may also shift their home ranges in response to disturbances, and one adult female that was caught 4 times within a 75-foot radius shifted her home range as much as 1,000 feet (Sullivan, 1995).

Our study aimed to study the habitat preference and home range of *Peromyscus* at the Maya Lin Peace Chapel in Huntingdon, PA. Based on past research (e.g. Hamilton et. al., 1994; Olsen et. al., 2000) we expected that *Peromyscus* would prefer forested habitat over grass or shrub habitat. Forested habitat should be preferred because it offers a great deal of cover for the mice for protection against predators. We tested this hypothesis by setting Sherman live traps in grass, shrub, and forested areas near the Peace Chapel and using mark-recapture techniques to determine the density of mice in the area as well as in which areas they frequented.

FIELD SITE

We selected the area around the Juniata College Peace Chapel, Huntingdon, Pennsylvania as the site for our study. We chose to survey the habitat adjacent to the trail leading up to the Peace Chapel and next to the trail leading up to the area known as Meditation Point. The field site spanned three different habitats, our trap lines went from a grassy area to a shrubby area to grassy and forested habitat. The field site was located on a gentle hill with the grassy area in the low lying area and the forested habitat at the top of the incline, with shrubby habitat in between.

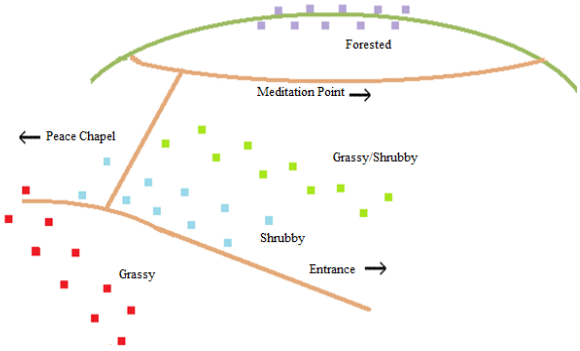

Figure 5. Diagram of field site. Dotted lines represent transect lines of ten traps each. The red dots represent grassy habitat, the blue dots represent shrubby habitat, the green dots represent a mix of grassy (lower transect line) and shrubby (higher transect line), and the purple transect line represents forested habitat.

MATERIALS & METHODS

Sherman live traps were set up in sets of four transect lines, containing ten traps each. Transect lines were set up horizontally across the incline. A combination of peanut butter and corn was used to bait the traps. Previous studies have shown that *P. leucopus* tends to be an opportunistic eater and will consume a wide variety of foods (Anderson et. al., 2009). We began our collection period on the twenty-seventh of March 2010 and continued to the eleventh of April 2010. At the onset of our study traps were checked twice a day, once in the morning and once in the afternoon. However we reduced our visits to the traps to only a morning visit, due to an increase in temperature, and concern of disturbing the traps too much. Traps were checked at approximately 8 a.m. each morning to ensure that the mice would not be stressed too long from being kept in the traps, or overheated in the course of the day.

When a mouse was captured, it was weighed using a spring mass scale. After being weighed the mice were measured for body length from nose to the base of the tail, tail length. The sex of the mouse was also determined for each mouse captured. Once all

data was collected, the mouse was released at the site where it was captured. The trap was then cleaned out, re-baited, and reset at its previous location. At the end of the study, tape measures were used to determine the distance between traps in order to chart out the relative location of each trap. Population size for the area was estimated using the Lincoln index. The Lincoln index was defined as the number of mice marked (M) multiplied by the number of mice caught the second time (n), divided by the number of mice that were marked and recaptured (m).

RESULTS

Results indicate that the *Peromyscus* spp. preferred shrub and forest habitat. Three individuals were caught in shrub-dominated habitat, while two were caught in forest habitat (Fig. 6). Furthermore, of the five individuals that were caught, fourteen recaptures were made in shrub habitat, and three were made in forest (Figure. 7).

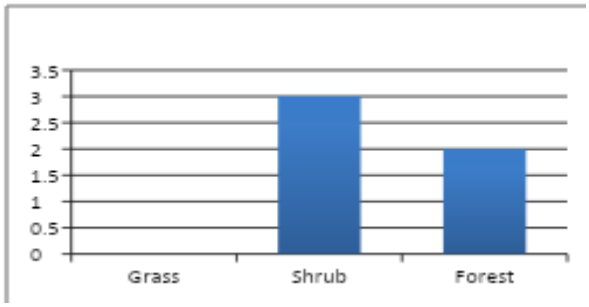

Figure 6. Number of Peromyscus *spp. individuals caught in each type of habitat.*

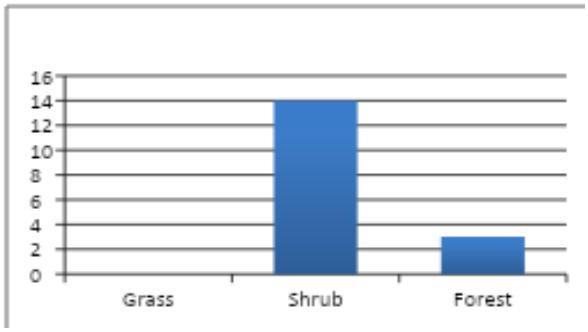

Figure 7. Total number of Peromyscus *spp. captured in each habitat type.*

Based on the Lincoln index for mark and recapture studies, we determined that at most there could be six mice living in our study area, or as few as two earlier on in the study. On Day 9 based on the captures made the Lincoln index calculated is infinite, because no mouse was recaptured that day making the denominator of the index zero.

Table 1. Lincoln index for the number of Peromyscus *spp in our field site where M is the number of animals marked, n is the number caught the second time, and m is the number of marked animals recaught. The row with an asterisk indicates that the mouse tagged 94 may be the same as the one with tag 37. We believe the 37 tag fell off of the mouse. This would change the Lincoln index for the 14th capture because it means that we re-caught 3 mice instead of only 2.*

Recapture	M	n	m	Lincoln Index
1	1	2	1	2
4	2	1	1	2
5	2	1	1	2
7	2	1	1	2
8	2	1	1	2
9	2	1	0	Infinite
10	3	1	1	3
11	3	1	1	3
12	3	1	1	3
13	3	2	1	6
14	4	3	2	6
14*	4	3	3	4
15	4	2	2	4

Since several of the mice we caught were recaptured multiple times we were able to track changes in body mass, and body length, as shown in Fig. 8 for the mice tagged 177 (caught 5 times) and 178 (caught 8 times). Furthermore in those two mice, body length was plotted against body mass as well (Fig. 9). Mouse 178 showed some increase in body mass with little to no increase in body length. A plot

of length vs. mass shows that as body mass increased there was a slight decrease in body length for mouse 178. Mouse 177 showed a trend of increasing body mass and length. A plot showing the locations of each trap where mice 177 and 178 were caught (Fig. 10) shows that mouse 178 moved around the field site more than mouse 177.

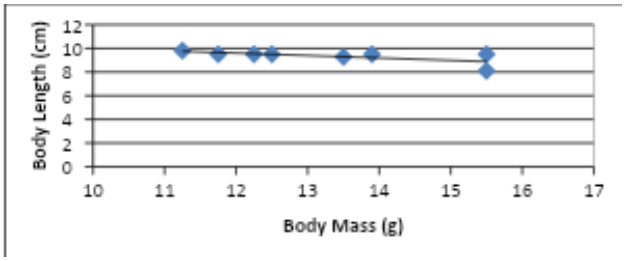

Figure 8. Graphs showing body mass and length measured during our mark and recapture study for mice tagged 178 (top) and 177 (bottom).

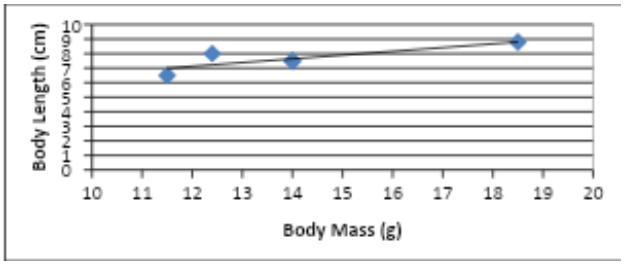

Figure 9. Length vs. mass plotted for mouse 178 (top) and mouse 177 (bottom).

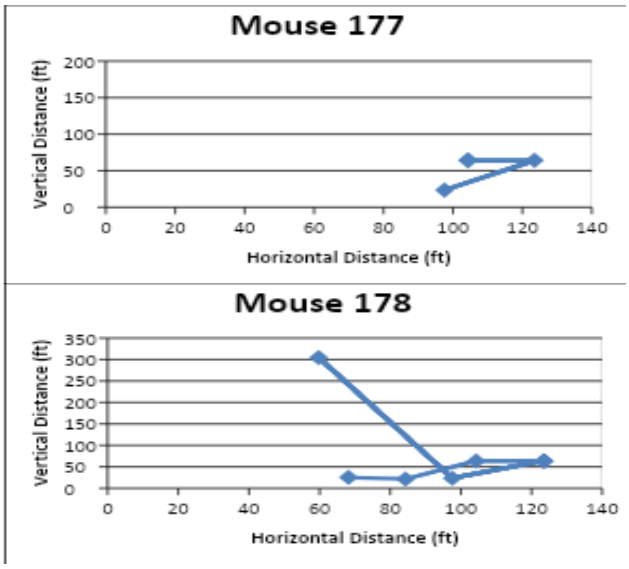

Figure 10. Plot showing the location of each trap where mouse 177 (top) and mouse 178 (bottom) were caught within the field site. The line connecting the points represents the order in which the mice were caught at each trap. For some points the mice were caught multiple times.

DISCUSSION

Despite having a small number of individuals captured in this study, a clear preference is exhibited by *Peromyscus*. No mice were caught in areas with only grass for structure, which is an observation that conforms to previous studies done by Hamilton et. al. (1994) and Olsen et. al. (2000). However, several mice were caught in shrub and forest habitats. The data suggest that the mice have a preference for both shrub dominated and forest dominated areas, perhaps because they both provide adequate amounts of cover to hide from predators. These results agree with the findings of Hamilton and Whitaker (1979), who showed that the lowest densities of *P. leucopus* were in the open grassy fields, and their highest densities occurred in the shrubby areas. When looking solely at number of catches in each habitat type we see a much higher number of captures in the shrub habitat.

Although the amount of data collected was small, we were able to calculate an estimated population size based on the Lincoln index for our experimental site. Towards the end of the study the numbers for population size given by the Lincoln index were likely more accurate, as trapping efficiency increased.

Looking at our most frequently captured mice, numbers 177 and 178, we were able to track body mass, body length, and tail length data collected throughout the course of the study (Figure 8). Both mice showed some increase in body mass over the two weeks of data collection. However, only mouse 177 showed any real increase in body length, while mouse 178 remained relatively constant. One possibility is that mouse 177 is a juvenile, leading to a higher rate of growth than mouse 178, which may have been an adult. In the plot of body length vs. body mass it seems as if mouse 178 increased in mass without increasing in size, meaning that he was getting fatter, while mouse 177 increased in both length and mass, meaning that she was getting larger.

Based on the location of the traps where the mice were caught it seems that mouse 178 had a much larger home (foraging) range than mouse 177. While 178 at one point crossed the boundary of

grassy area to forage approximately halfway up the hill, mouse 177 remained at the base of the hill in shrubby coverage. Since the only capture of 178 halfway up the hill was the first capture we cannot be certain whether or not he came from the top of the hill down, or if he wandered up from the bottom of the hill. Because he went down to the bottom afterwards, and then remained there, it is likely that that is where he originally came from. It is unclear exactly why 178 would increase his range, but it could be related to the cold weather and the need to find food. In general, this finding is consistent with the findings of Maier (2002), who showed that male *Peromyscus spp.* have greater home ranges than females, as males will have greater home ranges in order to find as many mates as possible.

There were two issues that arose in this study, and that may have confounded our results. First, it is possible that the mice captured in the wooded habitat were actually the same mouse. The second time a mouse was captured in the woods it had a tear in its ear where a tag may have ripped out. Because of this, our total estimated population size would be reduced to 4, with only one mouse caught in the woods. Second, it is possible that our two most frequent captured mice may have learned to tolerate being handled in exchange for a reward of peanut butter every night. If this is the case, it could indicate that mice were not any more active in the shrub habitat than in the wooded habitat were it not for the learned behavior. This study could be improved upon in the future by both using multiple other similar sites, as well as by expanding the scope, including a larger area covered by more traps. Using multiple sites would allow for a more robust exploration of habitat preference in *Peromyscus*. By expanding the scope we would be able to better understand the extent of the home ranges used by the mice.

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POND SNAIL (*PHYSELLA HETEROSTROPHA*) MORTALITY IN RESPONSE TO GYPHOSATE AND ORGANIC BASED HERBICIDE CONTAMINATION

Lauren Brandenburg, Erica Cichetti, Ethan Habbershon and James Kollinger

ABSTRACT

Herbicides are commonly used around the world to prevent loss of crops but many have devastating effects on the surrounding terrestrial and aquatic ecosystems. One of the most commonly used active ingredients that is used in herbicides is glyphosate, which was applied on over 80 million acres in 2001, and has broad effects on many broadleaf plants (EPA 2001). Pesticides that are prone to runoff may contaminate aquatic ecosystems. Since glyphosate is potentially toxic to unintended targets, an organic alternative using citric acid is explored. Pond snail mortality over hour period in varying treatments of glyphosate or citric acid is compared.

Keywords: glyphosate (N-(*phosphonomethyl*)*glycine*)*, herbicides, organic herbicides, pond snail* (Physella heterostropha).

INTRODUCTION

Herbicides play an important role in society by aiding in the protection of crops against unwanted plants. However, the lethality and effects it has on terrestrial and aquatic ecosystems is overlooked by many. It is imperative to ask how the use of herbicides could be affecting daily life. The herbicides runoff into nearby water systems has been studied for many decades (Trichell, 1968); yet, some herbicides are rarely studied before being put into use by farmers around the globe.

In particular, Glyphosate (*N*-(phosphonomethyl)glycine) is a popular choice; it is the most widely used herbicide in the world, and one of the most commonly used herbicides to use glyphosate is RoundUp some would hail it as a miracle herbicide due to its ability to kill a broad range of broad-leaf plants (Gasnier, 2009; Duke and Powles, 2008). Others debate its toxicity on non-target species (Busse, 2001; Ayoola, 2008). Although the toxicity of glyphosate is a topic of debate, one proven merit of its use is that it has a relatively low half-live when compared to many other herbicides commonly used today. Glyphosate is one of the least persistent herbicides on the market, with a reported half-life ranging between 7 and 25 days (Duke et al., 2003; Battaglin, 2005). This greatly reduces the risk of an organism encountering glyphosate with such frequency as to impose a high possibility of chronic health problems. However, the risk of acute exposures during spraying seasons is still possible.

On the other side, the anti-GMO politics and yet uncertain toxicity of glyphosate has some pushing for more organic pesticides. In that case, acids and oils are popular choices for organic farmers (Franck et al., 2009). Citric acid lowers the pH of the environment, which facilitates aluminum uptake in plants. The acid itself is not especially toxic to the plants; it is the aluminum that kills plants near these water systems (Delhaize and Ryan, 1995). While comparatively expensive, clove oil is also an effective herbicide (Franck et al., 2009). The consumer herbicide Burn Out makes use of both of these ingredients and is a potential organic alternative to the use of glyphosate.

Snails are a common choice when looking at the health of an aquatic ecosystem, in particular the flora health. Snail shells are highly sensitive to pH changes and their tissues are known to store certain toxic heavy metals which makes them excellent

bioindicators for aquatic ecosystem health (Dallinger et al., 1997). The pond snail (*Physella heterostropha*) was chosen for its dependence decaying organic matter (which typically lies on the sediment where herbicides are in their highest concentration), especially flora matter, its local abundance in an agricultural area, and its small body size (which means they have a lower body burden), making it particularly susceptible to acute exposures that may occur in the height of agricultural production each year. In this study, we compared the acute effects of two pesticides, KleenUp (a RoundUp knockoff that features the same main ingredient) and Burn Out on *Physella heterostropha* (an organic based herbicide) to determine if there is a significant difference in using a glyphosate based herbicide or an organic based herbicide mortality(commonly thought by many people in the general population to be safe). This study was a two pronged approach that looked at the lethal dosage at 50% mortality and dosage of each chemical and duration of exposure.

FIELD SITE AND SNAIL COLLECTION

Pond snails were collected in Petersburg Spring (Figs 1-3), a cold spring (~ 10◦C) with an abundance of macroinvertebrates including *P. heterostropha*. This site was chosen for this study because it does not neighbor agricultural land thus allowing for a population not currently being affected by pesticides. Snails were primarily found in the lentic portion of the spring near the end of the brook (*Fig.* 1).

Figure 1. Picture of the field site, Petersburg Spring in Petersburg, Pennsylvania.

Figure 2 and 3. Photos of two different habitats (rocks and watercress) in Petersburg Spring where pond snails were collected. Spring located in Petersburg, Pennsylvania.

Figure 4. Lab setup of the pond snail bins as stored in Juniata College Ecology Department's laboratory fridge near native temperatures (~10◦C). The bins in the left tray contained different concentrations of Kleen Up weed and grass killer and the bins in the right tray were different concentrations of Burn Out weed and grass killer.

MATERIALS AND METHODS

Study Methods

The pond snail *P. heterostropha* was collected from Petersburg Spring in Petersburg, PA because of it high population abundance which allowed for high collection volume thus

allowing for a greater range in body sizes (N ≈ 450). Spring pH was measured within 5 meters of the source with a Markson digital pH Meter Model 88 (Markson LabSales, Honolulu, HI). Snail were collected with dip nets and placed in 1 L plastic containers with native spring water with no more than 50 per container to allow for normal dissolved oxygen concentrations.

One glyphosate herbicide, Kleen up (40% w/v glyphosate),and one organic based herbicide, Burn Out (8.9% and 2.6% w/v citric acid and clove oil, respectively), were mixed into 0.25 L of natural spring water using a static model of exposure in the following doses: 0.010, 0.027, 0.030, 0.055, 0.070, 0.109, 0.150, 1.75, 3.5, 5.2, 6.9 and 8.9 mL The pH of each exposure treatment (see *Table 1*) was then measured using a Markson digital pH Meter Model 88 (Markson LabSales, Honolulu, HI). A control sample, using only native spring water, was used for each dose. Snails were randomly placed into each exposure dose and controls (N = 15). Mortality was recorded after a 24 hour period for each sample stored in a Chase refrigeration unit (Chase Industries, Cincinnati, OH) at 10°C (*Fig.* 4).

Statistical Analysis

Linear analysis was conducted between the mortality vs time for the initial concentrations of Kleen Up and Burn Out. LSRs were graphed using MiniTab version 17 and mortality rates were compared using an ANOVA analysis. Dose response curves were generated using Sigma Plot version 9. The LD_{50} of each dose response curve was graphically calculated using Rstudio dose.p function for a general linear model (GLM) under the MASS library package. Standard errors were converted to standard deviations to determine the significant differences or similarity for the LD_{50}'s of Kleen Up and Burn Out.

RESULTS

Dosage and Time

Significant relationships were found between dosage and time groups ($P < 0.05$) of each LSR and there was significant differences among the slopes (*ANOVA:* N = 4, $P = 0.006$, $F = 21.28$). However, though there were also significant relationships among each LSR ($P < 0.05$), when compared to time and herbicide type no significant difference was found ($N = 4$, $P = 0.229$, $F = 1.88$).

LD_{50} Analysis

LD_{50}'s were calculated on Rstudio using a GLM (see *Fig. 6 A and B*) and error was represented by the calculated one standard deviation for Kleen Up (DF = 13, $LD_{50} = 1.91 \pm 0.71$ mL) and Burn Out (DF = 13, $LD_{50} = 1.81 \pm 0.75$ mL). The LD_{50}'s showed no significant difference at one standard deviation over a 24 hour exposure period.

Table 1.Shows the pH of each individual bin tested. All measurements recorded on April 5th, 2016: seven days after setup of the Kleen Up and Burn Out bins with 1.75 and 3.50 mL of herbicide, five days after setup of the Kleen Up and Burn Out bins with 0.880, 0.440 and 0.200 mL of herbicide, same day as the setup of the Kleen Up and Burn Out bins with 0.109, 0.055 and 0.027 mL of herbicide. pH measured using Markson Digital pH Meter Model 88

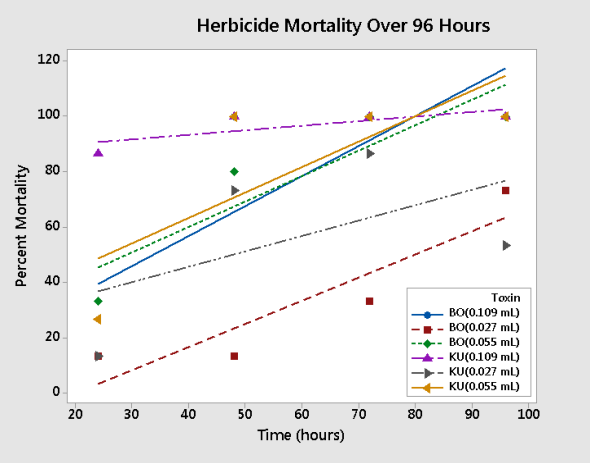

Figure 5. Slopes were calculated using a LSR method in MiniTab 17 (BO = Burn Out, KU = Kleen Up). Slopes indicate the rate of mortality over a 96 hour period and were compared using an ANOVA with simultaneous Tukey test which shows that only dosage and not herbicide type affects mortality.

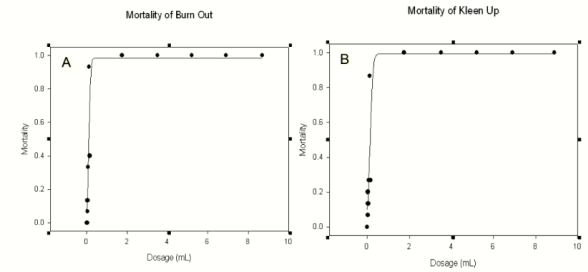

Figure 6. Dose response curves of Burn Out (A) and Kleen Up (B) experimental trials. Data displayed were collected after a 24 hour exposure period using a static exposure model. No significant difference between the LD_{50} of each herbicide was found when using Rstudio dose.p function from the MASS library for a GLM model.

DISCUSSION

ANOVA testing shows no statistical difference in mortality over time when comparing the mortality rates over time between Kleen Up and Burn out. However, there was a significant difference when comparing the dosages, which has been widely documented among toxicological studies. However, we found no significant difference when comparing the LD_{50} of each herbicide. Due to the generality previous studies have shown in glyphosate herbicides, and there relatively high mortality on other test species, we thought that the Kleen Up would have higher mortality rates at lower dose levels. Though this was not shown in the results, we think that pH (see Table 1) could have been a factor in having similar mortality.

We hypothesize that the lowered pH could have been a confounding factor that could have altered the biochemical detoxification pathways; especially for the organic pesticide, that is intentional as part of the pesticide mechanism of action. Since the snails generally prefer alkaline conditions (though they were collected from a slightly acidic spring,) the severe decrease in pH alone may have been enough to cause mortality (NYSDEC). The heavy decomposition of snail shells was also observed and may be related to the pH decline, rather than to the toxicity of the pesticides. We mention pH decline because it is possible that calcium may be leached from calcium containing shells to stabilize hydronium atoms, and free radicals like H^{-1}, causing less calcium to be present for gastropods and other macroinvertebrates.

Further studies into the potential benefits of alternative pesticides is recommended. Pesticides are not likely to go away any time soon, and in that case, research into their effects are imperative to the health of not only snails, but to all species. When safer alternatives to any pesticide, even already relatively safe ones, are found, a concerted effort must be put forth to make the switch for the sake of our future.

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BIOTIC AND ABIOTIC INDICATORS OF WATER QUALITY IN MUDDY RUN

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ABSTRACT

As many organisms need water for habitat and physiological functions, it is important to evaluate its quality. Water quality can be evaluated abiotically through alkalinity, dissolved oxygen, hardness, pH, and temperature measurements, or biotically through analyzing the amount of fecal coliform bacteria present in the water. Abiotic and biotic water conditions can create an unhealthy environment for both the aquatic and terrestrial biota, including humans. Types of macroinvertebrates found in water bodies serve as another biotic indicator of water quality. In fact, several biotic indices exist to classify stream water quality based on the macroinvertebrates residing in them. We hypothesized that water bodies with higher numbers of fecal coliforms would have fewer pollution intolerant macroinvertebrates. To test our hypothesis, we analyzed the water quality of Muddy Run, a stream in Huntingdon, Pennsylvania, by completing a qualitative study of the macroinvertebrates present in it. We also gathered abiotic water quality measurements to give us a broader understanding of the water quality including alkalinity, dissolved oxygen, hardness, pH, and temperature measurements. Our qualitative analysis of macroinvertebrates categorized Muddy Run as a moderately polluted stream, however we did not find a correlation between macroinvertebrate pollution tolerance and number of fecal coliform colonies. Overall, our abiotic parameters of Muddy Run reveal that its water quality is adequate for aquatic organisms, however, it is not suitable for human recreational use.

Keywords: *Fecal coliforms, macroinvertebrates, water quality, pollution tolerance, Huntingdon*

INTRODUCTION

Water quality is a very important topic, as many organisms need water for physiological functions and (or) habitat. Thus, it is important to evaluate water quality accurately. This is done using several common indicators of water quality, which include various biotic and abiotic factors.

Abiotic measurements of water quality include alkalinity, dissolved oxygen, hardness, pH, and temperature. Alkalinity affects the body of water's resistance to changes in pH; a high alkalinity helps prevent rapid changes in pH, protecting organisms in the water (Oram 2014a). Hardness is a measure of calcium and magnesium ions dissolved in the water (Somridhivej and Boyd 2016). Moreover, calcium is necessary for aquatic organisms' bone and exoskeleton formation, blood clotting and other metabolic reactions (Wurts, W. A. 2014). Like alkalinity, hardness also affects pH since calcium is a base. pH is the concentration of hydrogen ions in the water, with lower pH's being more acidic, due to a higher concentration of hydrogen ions (EPA 2016). Dissolved oxygen describes the amount of oxygen that is in the water (Sánchez et al. 2007). The level of D.O. in the body of water can affect the survivability of aquatic organisms. Temperature affects water chemistry, especially DO; colder water holds more

DO than warm water (Cox 2003). Moreover, the flow can also influence the level of DO (the faster the flow is, the higher the DO level). Some taxa need more dissolved oxygen than others, but most need at least 4 to 7 mg/L (Cary Institute of Ecosystem Studies 2016).

One common biotic indicator for health of a body of water is the amount of fecal coliform bacteria in a water body. Although fecal coliforms generally do not cause disease themselves, they are good indicators of pollution, as most enter water bodies with fecal matter (EPA 2012). Fecal coliform contamination comes from runoff from lawns where pets are exercised, home septic systems that are not working properly, and fecal material from wild and domestic animals (Drohan, Sharpe and Smith 2004). Fecal coliforms are considered one of the primary indicators of water quality for recreational water in the United States (EPA 2012). Fecal contamination in recreational waters is associated with an increased risk of transmission of infectious diseases, such as gastrointestinal (GI) illness and less often identified respiratory illness, as harmful bacteria are often present if fecal coliforms are present (Office of Water 2012).

Macroinvertebrates are another indicator used to assess water quality, as these organisms are continuously exposed to the steam's conditions (Oleson 2013). Several biotic indices exist to classify the quality of streams based on the macroinvertebrates found in them (Oleson 2013). For instance, the presence of pollution-intolerant macroinvertebrates, such as mayflies, stoneflies and caddisflies, indicates healthy stream conditions. Consequently, we were wondering if there was a correlation between the number of fecal coliform colonies and the types of macroinvertebrates found in a stream. We hypothesized that areas of the stream with higher numbers of fecal coliform colonies would have fewer pollution intolerant macroinvertebrates than areas with a lower number of fecal coliform colonies.

We decided to study the quality of the water in Muddy Run, the stream that goes through Juniata College, by doing a qualitative study of the macroinvertebrates present in it, and by testing the water for the presence of fecal coliforms. To give us a better understanding of the water quality, we also gathered abiotic measurements of water quality, i.e. alkalinity, DO, hardness, pH, and temperature. We wish to test these hypotheses because the aquatic ecosystems can support many recreational activities, and we needed to make sure that the human population is not at risk of getting infectious diseases when they get in touch with this water.

We were expecting to find a worse quality of water upstream than downstream; the most upstream site is close to the East dormitories, and because of the littering we were expecting the water there to be more polluted, meaning we would likely find more fecal coliforms and less pollution-sensitive macroinvertebrates.

FIELD SITE

Muddy Run is located on the outskirts of the Juniata College campus in Huntingdon, Pennsylvania. It flows through residential homes northeast of campus and continues behind Weis Market. We divided the stream into four separate sites (Figure 1). We began our data collection at the East Apartments bridge heading towards Ellis Hall (Figure 2). We collected our data on the side of the bridge rather than underneath due to differences in sunlight and temperature. This section of Muddy Run consisted of mid-thigh high, murky water with darkened leaf matter and litter along the edges. Besides macroinvertebrates, we observed Green Frogs, *Lithobates clamitans*, and blacknose dace, *Rhinichthys atratulus*. Sediments in the stream comprised mainly of gravel and silt.

The second site was located further downstream behind the baseball field. This section of the stream was extremely shallow with small boulders, large cobbles, gravel, and sand (Figure 3). The flow of water created ripple marks in the sediment underwater. Vegetation consisted mainly of watercress along the edges of the stream and moss on the larger rocks.

The third examined section of Muddy Run was located at the end of the baseball field (Figure 4a). We noticed a corrugated drainage pipe came from the side of the stream (Figure 4b). This section also consisted of boulders and cobbles along the sides and floor of the stream. Vegetation mainly included algae with no other observed aquatic flora. Other noticed biota included the blacknose dace, *Rhinichthys atratulus*, and tadpoles of the Green Frog, *Lithobates clamitans*.

The last site we examined was located downstream of the bridge behind Weis Markets (Figure 5a). A small building with bathrooms was located next to the stream with a drainage tunnel draining into the stream (Figure 5b). Similar to our second site, our fourth site consisted of gravel and sand. Vegetation included small patches of watercress and algae. The water is visibly clear enough to see the ripple marks created in the sand. We also found a blacknose dace, *Rhinichthys atratulus*.

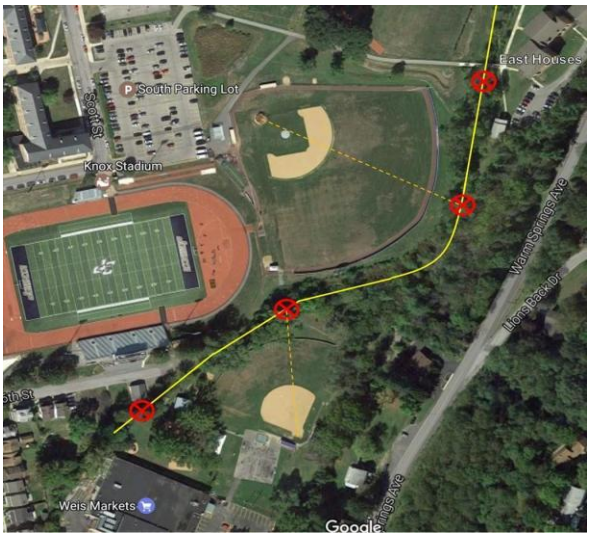

Figure 1. Map of Muddy Run indicating the locations of our sites, beginning at East Houses and ending downstream at the bridge behind Weis Markets. The yellow line indicates Muddy Run, and the red crossed circles represent our sampling sites.

Figure 2. Site 1 at East Houses.

Figure 3. Site 2 behind the baseball field.

Figure 4a. Site 3 at the end of the baseball field.

Figure 4b. Site 3 Corrugated Drainage Pipe

Figure 5a. Site 4 behind Weis Markets.

Figure 5b. Site 4 Drainage Tunnel.

METHODS AND MATERIALS

On March 25, we collected macroinvertebrates and measured fecal coliforms and hardness for sites 1 and 2. The weather conditions on this date were cloudy and a high of 71°F, according to

the weather channel iPhone app. On March 29, we collected macroinvertebrates and measured the pH, hardness, and fecal coliforms for sites 3 and 4. On the same date, we measured the alkalinity for all four sites. The weather was sunny with a high of 59°F. On April 2, we measured temperature and dissolved oxygen for all sites. The weather conditions on April 2 were 60°F and partly cloudy with some spurts of sun. On April 12, we collected macroinvertebrates for all sites as well as the pH measurements for sites 1 and 2. The weather conditions on April 12 were sunny and 67°F. We determined the number of fecal coliform colonies in the petri dishes on March 26 for sites 1 and 2 and on March 30 for sites 3 and 4.

Alkalinity, dissolved oxygen, hardness, temperature, pH, and fecal coliforms were measured for each of the four sites. We took two water samples at each site by rinsing two cylindrical containers in the stream water three times prior to collecting and sealing the water. Using HACH water test kits, we determined alkalinity and hardness within twenty-four hours of the samples being taken (Hach, Loveland, Colorado). We determined pH using a Markson Digital pH meter model 88 (Markson, Henderson, NC). Dissolved oxygen and temperature measurements were taken with a YSI dissolved oxygen and temperature probe model 55 (YSI, Yellow Springs, Ohio). Temperature and dissolved oxygen were measured three times at each site then averaged. Likewise, our results for alkalinity, hardness, and pH are averages of the two water samples for each site.

We quantified the number of fecal coliform colonies using Coliscan Easygel kits, following the recommended protocol (Microbiology Laboratories, Goshen, IN). We used 1 mL of water from each sample and converted the resulting number of fecal coliform colonies to number of colonies per 100 mL after allowing the samples to incubate for twenty-four hours at 35°C. Then we averaged the number of fecal coliforms for each site.

At each site, we took two qualitative samples of macroinvertebrates; we spent thirty minutes collecting them, using sieves and nets. We later identified them using a dichotomous key (Kellogg 1994), which gave common names and orders for most of the macroinvertebrates we found. Then we classified each of the identified macroinvertebrates by pollution tolerance, according to a biotic index (Sharpe, Kimmel, and Buda).

We used the Kruskal Wallis test to determine if the numbers of fecal coliform colonies per site were significantly different and if the number of taxa found for each pollution tolerance varied among the sampling sites for the two qualitative assessments. All Kruskal Wallis tests were done with 3 degrees of freedom. Additionally, we plotted the number of fecal coliforms with the number of taxa for each pollution tolerance for the different sites to see if there was a correlation between them, and we used a two-tailed linear regression t-test to see if the correlations were significant, using a TI-83 graphing calculator.

RESULTS

Many taxa were present in at least two sites (Table 2 and Table 4). Consequently, most of the streams were found to be moderately polluted, except for site 3, which was considered grossly polluted according to the results of our first qualitative assessment and Sharpe, Kimmel, and Buda's biotic index (Table 3 and Table 5). Notably, crayfish, a pollution sensitive taxon, were only found at sites 1 and 4, and mayflies, another pollution sensitive taxon, were only found at site 2.

However, the number of taxon found for each pollution tolerance was not statistically different among the sampling sites for either qualitative assessments. The H test statistic for the Kruskal Wallis test on the first qualitative assessment was 0.57, with a p value of 0.903. Similarly, the H test statistic for the Kruskal Wallis test on the second qualitative assessment was 0.667, with a p value of 0.881.

Most of the scatterplots with number of fecal coliforms and number of taxa had a negative slope for the regression line (Figures 8, 9, 11, 12, and 13). However, some of those regression lines had very low R^2 values, meaning the line captured little of the variation among the sampling sites (Figure 9 and Figure 13). Moreover, none of the R^2 values were greater than 0.3253 (Figure 10 and Figure 12). The differences in the number of fecal coliform colonies per 100 mL of water were not found to be significant among the sampling sites, as the H test statistic was 4.019, and the p value was 0.259 for the Kruskal Wallis test. Moreover, none of the correlations were significant. Moreover, the number of fecal coliforms did not increase or decrease going downstream, instead they varied by section starting at the East Houses with 400 colonies per 100 mL and sites two, three, and four with 100, 300, and 350 colonies per 100 mL respectively (Table 1).

According to our abiotic water quality measurements the temperature throughout the studied section of Muddy Run remained constant (Table 1). However, the concentration of dissolved oxygen

increased moving downstream from the first site at East Houses to the fourth site at Weis Markets. The concentration of dissolved oxygen in Muddy Run is well above Environmental Protection Agency's, EPA, standard for Pennsylvania streams (Figure 14). The pH of the stream varied from site to site, but remained within EPA standards (Figure 15). The alkalinity of the sites also varied between site locations. However, the alkalinity of all four sites are under the EPA's minimum standard for alkalinity in Pennsylvania streams (Figure 16). The hardness for the sites also varied, however, the fourth site at Weis Markets had a noticeably lower measurement of 190 mg/L of calcium and magnesium, whereas the first three sites had a hardness of 230, 210, and 220, reported in site order (Figure 17).

Table 1. Water quality measurements for the four Muddy Run sampling sites.

Sampling Site	Alkalinity (mg/L of $CaCO_3$)	Dissolved Oxygen (ppm)	Fecal coliforms (Colonies per 100 mL)	Hardness (mg/L of Ca and Mg)	Temperature (°C)	pH
Site 1	11.2	11.87	400	230	13.1	6.92
Site 2	9.6	12.50	100	210	12.9	7.42
Site 3	11.0	12.72	300	220	12.9	7.31
Site 4	10.4	12.76	350	190	12.9	7.41

Table 2. Macroinvertebrate taxa identified in the first qualitative sample for the four Muddy Run sampling sites in Huntingdon, Pennsylvania. Common names are in parentheses.

Taxon	Site 1	Site 2	Site 3	Site 4
Order: Amphipoda (amphipod)		X	X	X
Class: Bivalvia Family: Corbiculidae (asian clam)			X	
Order: Decapoda (crayfish)	X			
Order: Diptera Family: Chironomidae (non-biting midge)	X	X		X
Class: Gastropoda Subclass: Prosobranchia (operculate snails)	X	X	X	X
Class: Gastropoda Subclass: Pulmonata (non-operculate snails)	X	X	X	X
Order: Hemiptera Family: Gerridae (water strider)				X
Class: Hirudinea (leech)	X	X	X	
Order: Isopoda (isopod)		X		X
Order: Odonata (damselfly)	X	X	X	X
Order: Odonata (dragonfly nymph)	X			X
Class: Oligochaeta (aquatic worm)		X		X
Class: Turbellaria (flatworm)		X	X	X

Table 3. Pollution sensitivities for the first qualitative sample for the four Muddy Run sampling sites in Huntingdon, Pennsylvania (Sharp, Kimmel, and Buda).

Sampling Site	Pollution sensitive macroinvertebrates (number of taxa)	Moderately tolerant macroinvertebrates (number of taxa)	Pollution tolerant macroinvertebrates (number of taxa)	Stream Classification
Site 1	1	2	3	Moderate pollution
Site 2	0	3	4	Moderate pollution
Site 3	0	2	3	Gross pollution
Site 4	0	4	4	Moderate pollution

Table 4. Macroinvertebrate *taxa identified in the second qualitative sample for the four Muddy Run sampling sites in Huntingdon, Pennsylvania. Common names are in parentheses.*

Taxon	Site 1	Site 2	Site 3	Site 4
Order: Amphipoda (amphipod)	X	X	X	X
Class: Bivalvia Family: Corbiculidae (asian clam)				
Order: Coleoptera Family: Haliplidae (crawling water beetle)	X	X	X	X
Order: Hemiptera Family: Corixidae (water boatman)	X	X		
Order: Decapoda (crayfish)	X			X
Order: Diptera Family: Chironomidae (non-biting midge)	X	X	X	X
Order: Ephemeroptera (mayfly)		X		
Class: Gastropoda Subclass: Prosobranchia (operculate snails)	X	X		
Class: Gastropoda Subclass: Pulmonata (non-operculate snails)	X	X	X	
Order: Hemiptera Family: Gerridae (water strider)	X	X		X
Class: Hirudinea (leech)	X	X		
Order: Isopoda (isopod)		X	X	X
Order: Odonata (damselfly)	X	X	X	X
Order: Odonata (dragonfly nymph)	X	X	X	X
Class: Oligochaeta (aquatic worm)	X	X	X	X
Class: Turbellaria (flatworm)		X	X	X

Table 5. Pollution sensitivities for the second qualitative sample for the four Muddy Run sampling sites in Huntingdon, Pennsylvania (Sharp, Kimmel, and Buda).

DOI

Sampling Site	Pollution sensitive macroinvertebrates (number of taxa)	Moderately tolerant macroinvertebrates (number of taxa)	Pollution tolerant macroinvertebrates (number of taxa)	Stream Classification
Site 1	1	3	7	Moderate pollution
Site 2	1	4	8	Moderate pollution
Site 3	0	4	4	Moderate pollution
Site 4	1	4	4	Moderate pollution

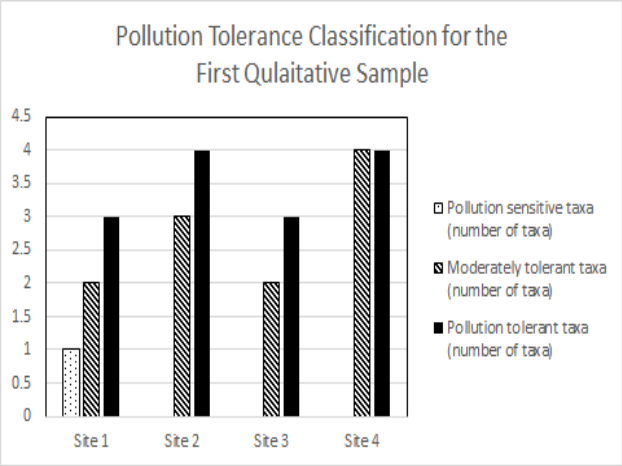

Figure 6. Number of macroinvertebrate taxa for each pollution tolerance classification for the first qualitative sample (Sharp, Kimmel, and Buda).

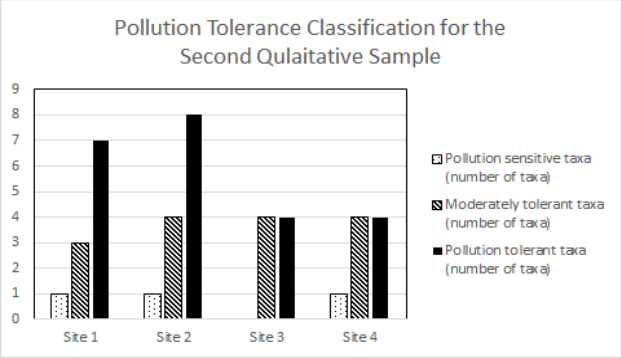

Figure 7. Number of macroinvertebrate taxa for each pollution tolerance classification for the second qualitative sample (Sharp, Kimmel, and Buda).

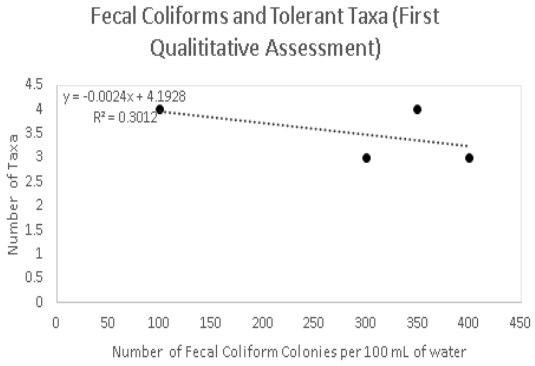

Figure 8. Correlation analysis between number of pollution tolerant macroinvertebrates (identified to class or order) and number of fecal coliform colonies for the first qualitative assessment. ***p = 0.45****.*

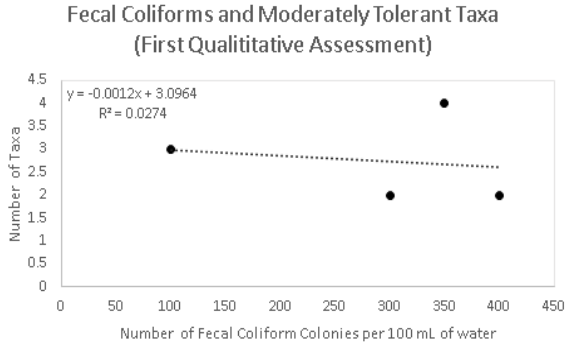

Figure 9. Correlation analysis between number of moderately tolerant macroinvertebrates (identified to class or order) and number of fecal coliform colonies for the first qualitative assessment. ***p = 0.83****.*

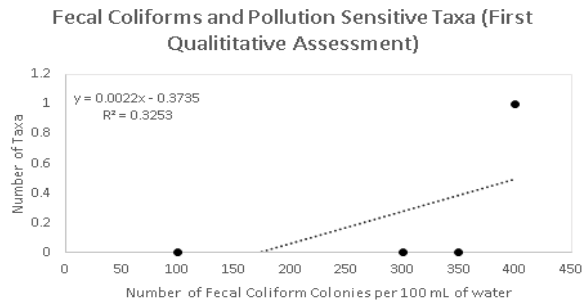

Figure 10. Correlation analysis between number of pollution sensitive macroinvertebrates (identified to class or order) and number of fecal coliform colonies for the first qualitative assessment. ***p = 0.43****.*

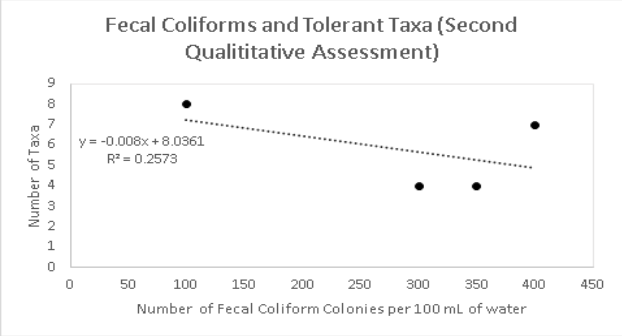

Figure 11. Correlation analysis between number of pollution tolerant macroinvertebrates (identified to class or order) and number of fecal coliform colonies for the second qualitative assessment. ***p = 0.49****.*

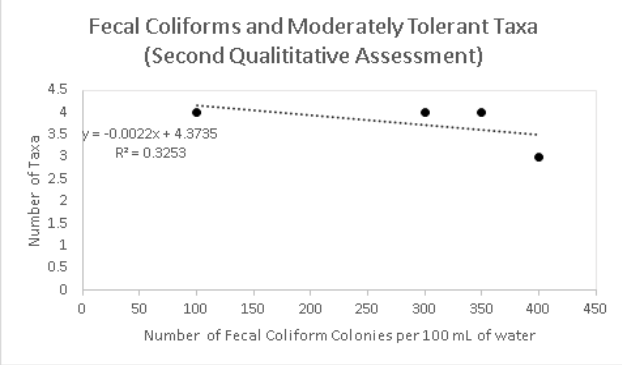

Figure 12. Correlation analysis between number of moderately tolerant macroinvertebrates (identified to class or order) and number of fecal coliform colonies for the second qualitative assessment. ***p = 0.43****.*

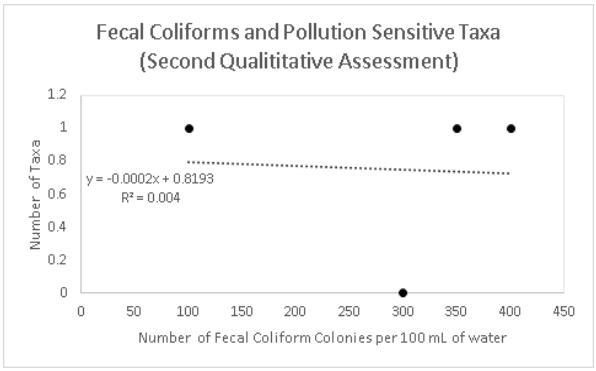

Figure 13. Correlation analysis between number of pollution sensitive macroinvertebrates (identified to class or order) and number of fecal coliform colonies for the second qualitative assessment. ***p = 0.93****.*

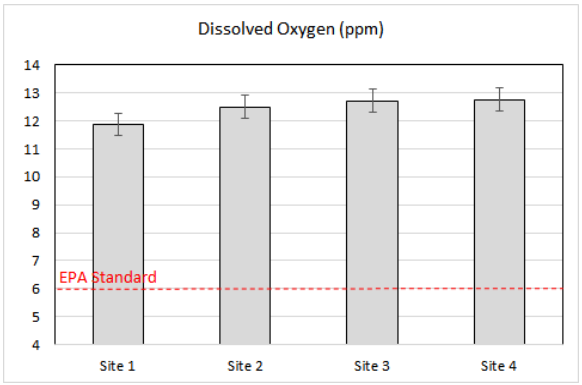

Figure 14. The dissolved oxygen measurements in parts per million for each of the four sites of Muddy Run compared to the EPA's Water quality standard for dissolved oxygen according to Chapter 93 of the Pennsylvania Clean Streams Law (EPA 1971).

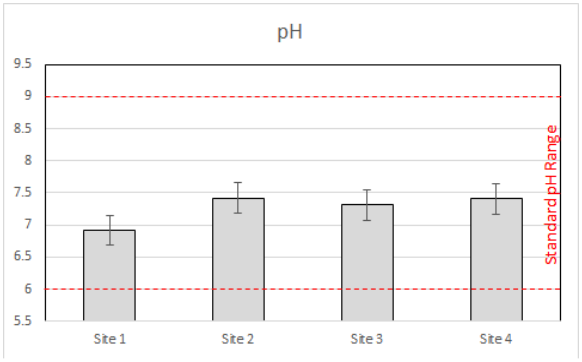

Figure 15. The pH measurements for each of the four sites of Muddy Run. The red dotted lines represent the EPA's Water quality standard range of pH according to Chapter 93 of the Pennsylvania Clean Streams Law (EPA 1971).

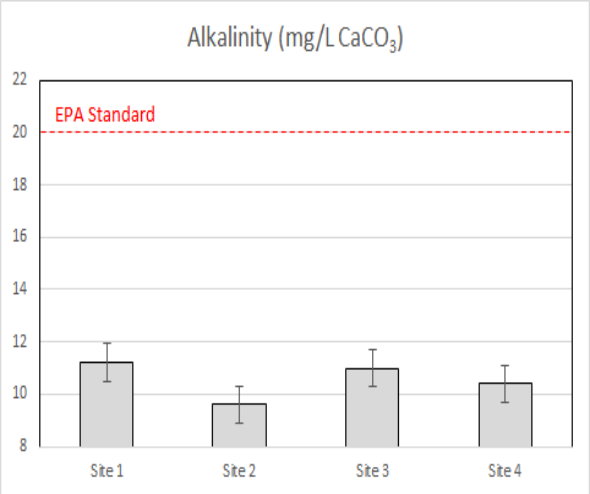

Figure 16. The alkalinity measurements in mg/L of calcium carbonate for each of the four sites of Muddy Run compared to the EPA's Water quality standard for alkalinity according to Chapter 93 of the Pennsylvania Clean Streams Law (EPA 1971).

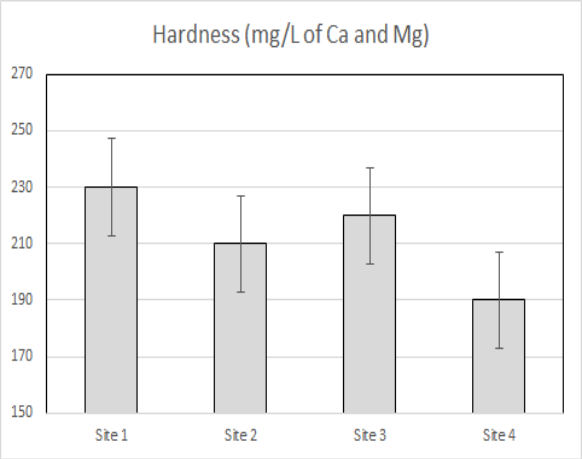

Figure 17. The hardness measurements in mg/L of calcium and magnesium for each of the four sites of Muddy Run.

DISCUSSION

As we analyzed our data, we realized that some factors may have affected our results. One major factor could be the dates and times that we collected our data. For our initial macroinvertebrate collections on March 25 and 29 we also collected water samples for the fecal coliforms on the same days. However, weeks later on April 12, we did not determine the number of fecal coliforms that correlated to the second sample of macroinvertebrates collected that day. With the first and second macroinvertebrate collections being half a month apart, it is possible that the water composition of Muddy Run changed as we moved further into spring. Perhaps, if we had collected abiotic water quality measurements for both macroinvertebrate collections we would have had more data to analyze.

Another factor that could have given us a better sense of the water quality of Muddy Run is our macroinvertebrate collection method. We collected macroinvertebrates qualitatively, in which we wrote down the types of taxa we found at each site of the stream. Qualitative macroinvertebrate collection provides us only with the types of taxa in Muddy Run, and does not describe which taxa are most abundant. If there were a higher abundance of pollution sensitive taxa in comparison to the moderately and pollution tolerant taxa at site 1, then we would have a better picture of the water quality. Using a quantitative macroinvertebrate collection method would have provided us with the abundances of taxa within the different pollution sensitivities, which could potentially give us different conclusions.

As shown in the Results section, we did not find any significant correlation between the number of fecal coliform colonies and the types of macroinvertebrates found in a stream. Based on our findings, it does not seem like the concentration of fecal coliforms in the water of Muddy Run had a significant influence on the types of macroinvertebrates living there. However, these results might be due to the fact that we did not take enough samples in the stream, as well as use enough sample for the Coliscan Easygel kit, and our sampling sites did not have a large range of water qualities. Another study that used both fecal coliforms and macroinvertebrates; Mcnett, Hunt, and Osbourne found a negative correlation between fecal coliforms and water quality based on the macroinvertebrates for one of their three stream classifications (2010). That group had the greatest range in stream water qualities, giving evidence that a lack of sufficient variation among our sample sites may have affected our results.

Our results for fecal coliform colonies were interesting, as they showed Muddy Run should not be used for drinking or recreation. For recreational waters, the mean number of fecal coliform colonies should not exceed 200 colonies per 100 mL of water, for five samples over a thirty-day period (EPA 1986a). Except for site 2, which had 100 colonies per 100 mL of water, all of the other sites had more than 300 colonies per 100 mL of water; therefore, we can tell that Muddy Run does not have a good concentration of fecal coliforms for humans, especially for drinking. According to Swistock, Clemens, and Sharpe, it is unsafe to drink water with any detectable amount of fecal coliforms (2017).

The qualitative assessment indicated that Muddy Run has decent water quality since several

species appear to be surviving and thriving. Interestingly, this finding contrasts with the results of a previous study by Juniata students that concluded Muddy Run was polluted based on the absence of some pollution intolerant taxa and the abundance of chironomid fly larvae, a pollution tolerant taxa (Chilcote et al. 1998). Therefore, our qualitative assessments give some evidence that Muddy Run's water quality is better now than it was previously. The mayfly we found is especially notable since it is a pollution sensitive taxon (Sharpe, Kimmel, and Buda) that was not previously found in Muddy Run (Chilcote et al. 1998).

Our abiotic measurements also indicated that Muddy Run is a fairly healthy stream. All our pH values are around 7 so we can consider that the water of our stream is neutral, which is a very good pH to sustain life in a stream, as the pH of streams is normally between 6.5 and 8.5 (Oram 2014b). Therefore, a pH outside of that range could be evidence of pollution. The alkalinity we found for Muddy Run is between 9.6 and 11.2 mg/L of $CaCO_3$; it is smaller than the standard minimum alkalinity of the streams in PA, which is 20 mg/L. Consequently, even though the water of Muddy Run has a neutral pH, the water is not very resistant to changes in pH, so the pH could change rapidly. The effect of changes of pH could be dramatic for aquatic organisms, especially for those that have calcium exoskeletons.

Temperature also affects aquatic ecosystems. With temperatures remaining constant throughout the examined sections of Muddy Run, we inferred that temperature did not play a large part in the differing characteristics of the stream sections, including the stream classifications in Table 3 and 5. Similarly, the dissolved oxygen values we found were all around 12 mg/L; however, it is noticeable that the concentrations increased heading downstream. During our data collection, we observed that the flow of Muddy Run also increased as we moved further downstream. With faster waters being able to diffuse more atmospheric oxygen into water than slower waters, it explains why the dissolved oxygen concentrations increased going downstream when temperature is relatively constant. The minimum level of dissolved oxygen needed is between 4 and 7 mg/L for most aquatic organisms (Cary Institute of Ecosystem Studies 2016). Therefore, Muddy Run has a suitable level of DO to sustain life.

The hardness we measured in Muddy Run is around 190 and 230 mg/L, which is within the acceptable range for hardness in fish cultures, 63-250 mg/L (Wurts 2014). The EPA does not have an acceptable range for hardness, but it notes that a higher hardness can reduce the effects of toxic heavy metals in an aquatic ecosystem (EPA 1986b).

Overall, the abiotic parameters of Muddy Run are pretty good and adequate for the survival and thriving of many aquatic organisms. The main issue is that the alkalinity is pretty low and that the pH could change rapidly, for instance, the pH could dramatically decrease if acidic rain were to occur. Our qualitative index provides good evidence that Muddy Run's water quality has improved, showing the value of re-examining an area after several years, instead of just assuming quality remained unchanged. Additionally, the conflicting indications given by the qualitative assessment and the fecal coliform results illustrate that a variety of measurements and factors must be considered in order to evaluate water quality accurately, contributing to the wealth of studies that have previously been done on water quality.

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We would like to thank Dr. Glazier and our teaching assistants, Laura and Jessica, for aiding us through the experimental design process as well as supplying us with the necessary technology and equipment. We would also like to thank Ms. Shannon Wray for providing us with the kits for the alkalinity, hardness, and fecal coliform tests. Lastly, we would like to thank the Ecology Department for providing us with waders, even though Alanna suffered the consequence of three pairs of leaky waders.

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ASSESSING THE RELATIONSHIP BETWEEN INVASIVE BERRY PRODUCING PLANTS AND BIRD COMMUNITIES IN WETLANDS

Luke Fultz and Karl Suttmann

ABSTRACT

Invasive plants have been shown to have a profound effect on their ecosystem. Wetland ecosystems are easily impacted by these invasive plants which then may shape the wildlife community in the wetland. Research was conducted at four constructed wetland sites of similar composition, in Central Pennsylvania, to determine whether there is a relationship between the density of invasive berry producing plants and bird communities in wetland environments. Berry scores, based on abundance and density, were calculated to rank each of the four wetlands from least to greatest availability of invasive berry producing plants. We found no significant correlation between frugivorous bird density and invasive berry density (r= -0.322, p= 0.678). Conservation scores were assigned to each species. We found no significant correlation between the density of frugivorous birds of conservation concern and berry density (r= -0.453, p= 0.547). An analysis of species richness for birds by site also produced statistically insignificant results (r= 0.239, p= 0.761). Analysis of migratory vs. residential species richness (r= 0.742, p=0.258) and proportion (r= 0.829, p=0.171) compared to invasive berry density also exhibited little to no significant correlation. We also compared the density (r= -0.114, p= 0.886) and species richness (r= 0.895, p= 0.105) of insectivorous birds to the density of invasive berry producing plants and found no significant correlation. Overall, the study was unable to confirm that there is a relationship between the density of invasive berry producing plants and the density of frugivorous bird species in wetland environments.

Key words: Birds, wetlands, invasive berries, avian community structure, species density

INTRODUCTION

Many bird species use wetland sites due to the abundance of resources (Stewart, 2016). Food availability in wetland habitats supports a wide distribution of different bird species with varying dietary restrictions and many bird species find wetlands especially attractive due to the abundance of herbaceous plants (Weller, 1999). Some birds use wetlands exclusively. 138 of the roughly 1900 North American bird species are wetland dependent (Stewart, 2016). Wetlands provide key habitat with necessary resources, meeting a multitude of needs for bird species. The unique environment allows birds to stay in a small area, protected from predators and weather (Stewart, 2016).

Arguably one of the most important habitats types to conserve presently is wetlands. Wetlands have a history of being destroyed and reclaimed for mankind's own use, causing wetlands to become some of the most degraded habitats, resulting in habitat loss for many species (Amezaga et. all, 2002). Due to the loss of habitat and their importance as stopover, wetlands became the first major ecosystem to be protected by an international treaty. The treaty was known as the "Convention on Wetlands of International Importance Especially as Waterfowl Habitat" and it designated 1108 sites as wetlands of international importance for migrating birds (Amezaga et. all, 2002).

With wetlands in many areas being protected and new wetlands being created, wetland habitats are slowly coming back to the landscape in the United States. However, there are still many threats to wetland habitat. Rich soils, ample water, abundant nutrients, and extensive canopy gaps, cause wetlands to be especially vulnerable to invasive species (Kercher 2010). When invasive species take over a

wetland they alter the habitat, outcompeting native species that would normally be prevalent, forming monotypes, ultimately lowering the overall biodiversity of the wetlands (Kercher 2010).

During the autumn months, berries and fleshy fruits become an important food source for birds as the availability and presence of insect decline (Baird, 1980). Birds that consume berries are attracted to wetlands by the many different berry producing plants that thrive in these environments such as Pokeweed (*Phytolacca americana*), Greenbrier (*Smilax rotundifolia*), Autumn olive (*Elaeagnus umbellata*), Multiflora rose (*Rosa multiflora*), Barberry (*Berberis thunbergii*), and Honeysuckle (*Lonicera*). However, many of these plants are invasive and reproduce with very little competition (Weller, 1999). Wetlands are especially susceptible to the spread of invasive plants and while studies have shown the availability berries increases the abundance of frugivorous bird species, less is known whether birds have a food preference with respect to invasive berries, or if berries from invasive plants result in increased bird density or species richness in wetlands (Stewart 2016).

While berries are an important food source in late fall, insects are still prevalent and important food sources during early fall migrations. Invasive plants have also been shown to impact the abundance and diversity of invertebrate populations in ecosystems as studies show that insects prefer native plants over invasive plants (Herrera and Dudley, 2003). This suggests that wetlands with higher invasive densities are likely to have less insects and support a lower diversity of insectivorous bird species. The goal of this study was to determine if there is a relationship between bird communities and the abundance of invasive berry producing plants in wetland habitats.

STUDY AREA

Four wetland sites in Huntingdon and Blair Counties, Central Pennsylvania, were studied during a six-week period in the fall of 2017. All Selected wetlands had similar habitat composition and varied in the amount of invasive berries present. Selected sites included: Old Crow Wetlands, the beaver ponds at Canoe Creek State Park, as well as Brumbaugh and Fouse's Crossings. All four of the selected wetland sites support a similar composition of herbaceous plants such as emergent cattails, grasses and sedges, as well as a variety of fruit bearing plant species. Each of the wetland sites also shared roughly the same amount of water cover and habitat type. Each of the wetlands exhibited varying amounts of invasive berry producing plant cover.

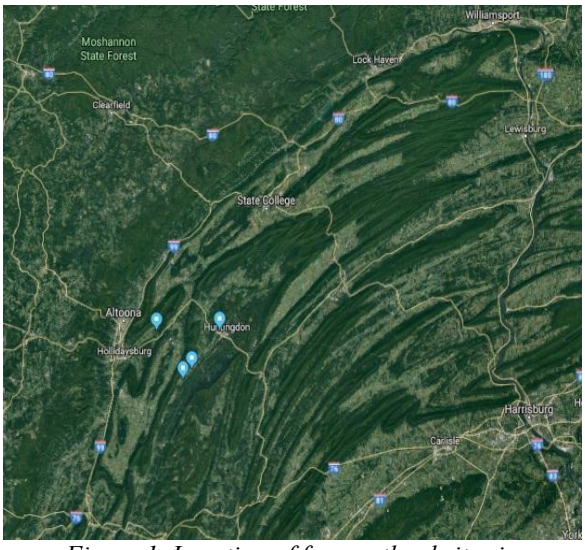

Figure 1. Location of four wetland sites in Huntingdon and Blair Counties, in Central Pennsylvania.

Old Crow Wetlands

Canoe Creek

Brumbaugh Crossing

Fouse's Crossing

Figure 2. Aerial photos of Four wetland sites, in Central Pennsylvania. Two transect lines in each wetland (marked in yellow) of 200 – 300m were used in this study during the fall of 2017.

Research conducted in the spring of 2018, studying the foraging behavior of insectivorous birds in the presence and absence of high invasive berry density at 2 of the wetlands that had been studied during the fall research period: Old Crow Wetlands and Brumbaugh Crossing.

METHODS

In each of the four wetlands, we established two lines of transects of 200 – 300 meters each, in which to determine invasive berry density as well as the density of frugivorous birds through visual and audible identification within 30 meters of the transect line (Figure 2). Each transect ran adjacent to the wetland area.

We quantified invasive berry abundance for each invasive berry producing plants identified using invasive berry density score cards on a scale of one to five, one representing little to no berries, and five representing a full load of berries (Figure 3). Each invasive berry producing plant observed was given a score based on the designated categories. The scores were then summed to produce an overall invasive berry score for each site. Each site was then categorized based on its overall invasive berry score. We calculated invasive berry scores for each of the four sites at the start of our 6-week research period and again at the close of our research period.

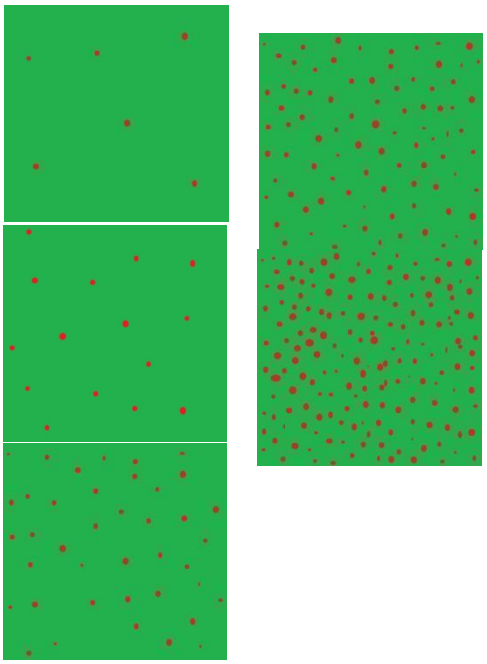

Figure 3. Example berry score system, ranking 1 – 5, in correspondence to the number of berries on each plant

Figure 4. Identifying invasive berry producing plant species and determining berry scores at research sites at the beginning of the research period, September 2017.

We surveyed each of the four wetland sites once every week for six weeks between the hours of 6 AM and 10 AM. Twenty - thirty minutes were spent identifying bird species seen or heard within 30 meters

of the two transect lines at each of the four sites (Figure 2). Birds observed outside of the designated transect area and outside of the time frame were not counted in the study. Only frugivorous birds, whose diet included berries and fleshy fruits, were counted in the survey. Surveys were not conducted on days exhibiting heavy rains and high winds, or days where weather limited bird activity. We assessed wind speeds using the Beaufort Wind Scale and weather was checked each day before going into the field.

We calculated the area of each of our transects into hectares and compared the area to the number of berry eating birds observed, species richness, and berry scores of each individual site to determine density. We used a Pearson's Correlation method to compare invasive berry densities to frugivorous bird densities and species richness densities (mintab, version 18).

Figure 5. Identifying bird species at Old Crow Wetlands during the fall of 2017.

Further field research was conducted in the spring of 2018 at Old Crow Wetland and Brumbaugh Crossing. The goal of the spring research design was to determine the relationship between insectivorous birds and invasive berry producing plants in wetlands. We hypothesize that an increase in invasive plants would result in a decrease in insect abundance, and therefore a decrease in insectivorous bird species should be exhibited.

A feeding tray of meal worms was placed at each of the two wetland sites (Old Crow Wetlands and Brumbaugh Crossing) to record avian feeding events. Observers were positioned in blinds to observe feeding events for 1 hour at each of the two wetland sites between the hours of 5 pm and 8 pm, once a week for three weeks.

Figure x. Eastern Phoebe with mealworm at Old Crow Wetland, spring 2018.

During the spring of 2018, we also analyzed all our data from the six-week study period during the fall of 2017 to compare the density and species richness of migrant and resident bird species to the density of invasive plants at each of the four wetlands. Insectivorous bird density was also compared to invasive berry density at each of the four wetland sites. A Pearson's Correlation method was used for statistical analysis of the data compared. (minitab, version 18).

Figure x. Eastern Phoebe and feeding tray of mealworms at Old Crow Wetlands, spring 2018.

RESULTS

During our research, we observed a total of 1,588 birds that make up 63 different species. Of the 1,588 birds observed, 889 individuals in 29 species,

were frugivorous bird species. A wide variety of invasive berry producing plants were also identified at each of the four wetland sites. Although, no birds were actually observed consuming berries during our research period, we know that the berries were being eaten by the reduction of berry abundance and density between the start and conclusion of our research. Site ranking, based on berry score for each of the four wetland sites, did not change from the start of our research to the end.

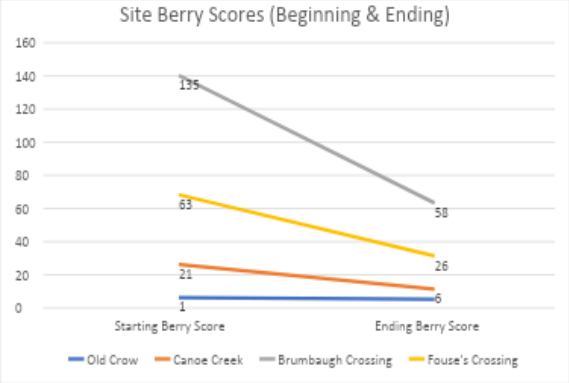

Figure 6. Invasive berry scores at four wetland sites in Central Pennsylvania were calculated in Fall of 2017 on the last week of September and the first week in November

Invasive berry score density did not affect the ranking of the four wetland sites (Table 1).

Table 1. Average Invasive Berry Score Per Hectare for Four Wetland Sites in Central PA, Fall 2017.

	AVERAGE BERRY SCORE	AREA (HA)	INVASIVE BERRY DENSITY
OLD CROW	0.5	2.76	0.181
CANOE CREEK	13.5	3.06	4.41
FOUSE'S CROSSING	44.5	3.33	13.4
BRUMBAUGH CROSSING	96.5	2.88	33.5

The total number of frugivorous birds recorded during our research as compared to the area of each of the four wetland sites shows the density of frugivorous birds per hectare (Table 2, Figure 7.)

Table 2. Frugivorous Bird Density Per Hectare for Four Wetland Sites in Central Pennsylvania, Fall 2017.

The density of frugivorous bird species and the density of invasive berry producing plants were not significantly correlated. (r= -0.322, p= 0.678)

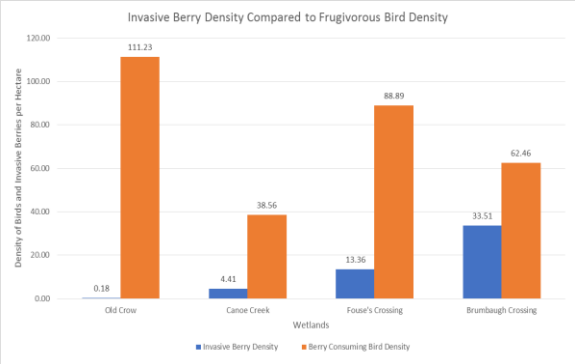

Figure 7. Comparison of invasive berry density and frugivorous bird density at four wetland sites in Central Pennsylvania during fall 2017.

The density of frugivorous birds of conservation concern and the density of invasive berry producing plants were not significantly correlated. (r= -0.453, p= 0.547)

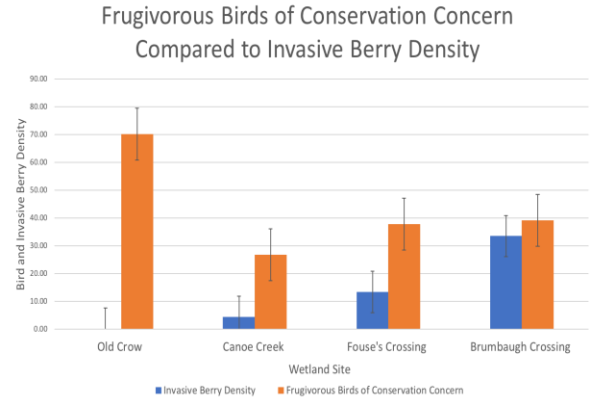

Figure 8. Frugivorous bird density compared to invasive berry density at four wetland sites in Central Pennsylvania during the fall of 2017, limited to birds of conservation score greater than 6.

Species Richness of frugivorous birds of conservation concern and invasive berry density was not significantly correlated. (r= -0.071, p= 0.929)

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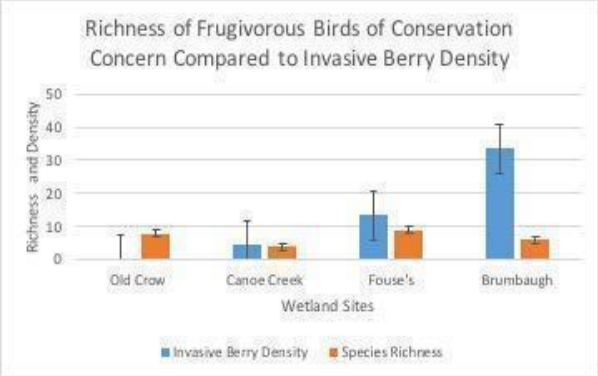

Figure 9. Species Richness of frugivorous birds compared to invasive berry density at four wetland sites in Central Pennsylvania during the fall of 2017, limited to birds of conservation score greater than 6.

Species richness and the density of invasive berry producing plants were not significantly correlated. (r= 0.239, p= 0.761)

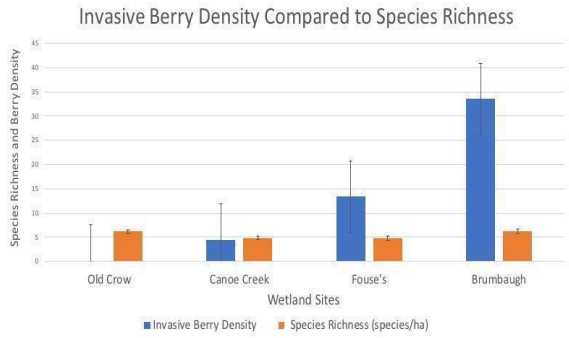

Figure 10. Comparison of invasive berry density and species richness at four wetland sites in Central Pennsylvania during the fall of 2017.

Species richness of migrants and residents was not significantly correlated to invasive berry density. (r= 0.742, p=0.258)

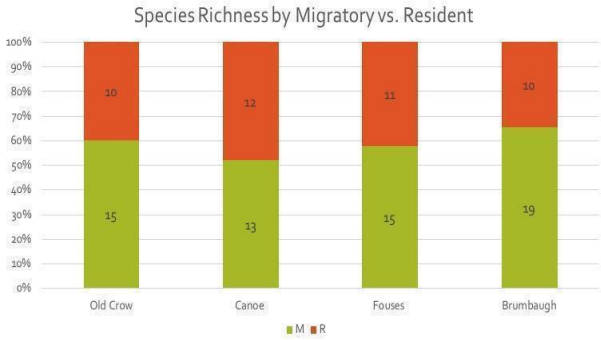

Figure 11. Migratory and resident species richness by invasive berry density at four wetlands in Central Pennsylvania during the fall of 2017.

Total number of individual migrants and residents were not significantly correlated to invasive berry density. (r= 0.829, p=0.171)

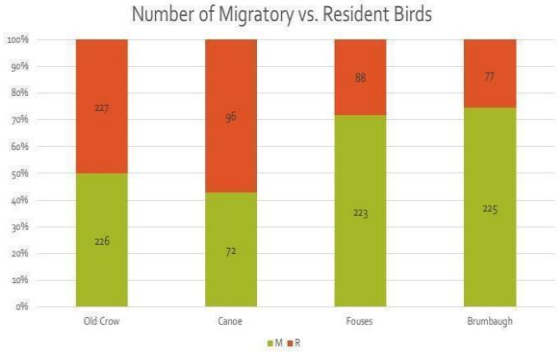

Figure 12. Total number of migratory and resident species by increasing invasive berry density at four wetlands in Central Pennsylvania during the fall of 2017.

Insectivorous bird density and invasive berry density was not significantly correlated. (r= -0.114, p= 0.886)

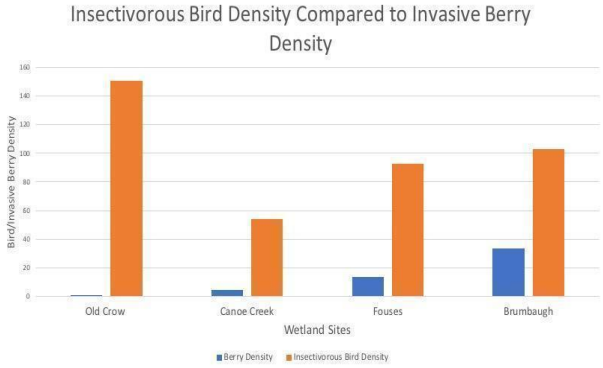

Figure 13. Insectivorous bird density compared to invasive berry density at four wetland sites in Central Pennsylvania during the fall of 2017.

Species richness of insectivorous birds and invasive berry density was not significantly correlated. (r= 0.895, p= 0.105)

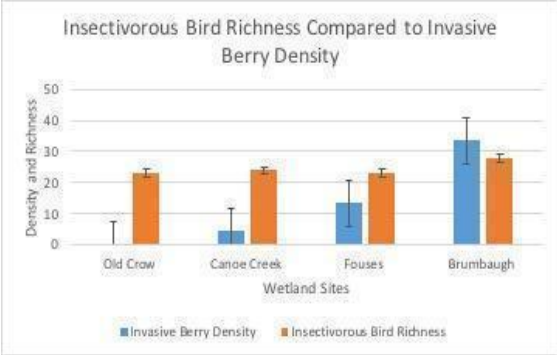

Figure 14. Species Richness of Insectivorous birds compared to invasive berry density at four wetland sites in Central Pennsylvania during the fall of 2017.

DISCUSSION

The goal of our study was to examine four wetland locations in Central Pennsylvania to determine whether invasive berry producing plants are driving the community structure of avian species in wetlands. Based on our results, we found little correlation between invasive berry densities and bird densities (r=-0.322, p=0.678). When we restricted data analysis to species of high conservation value (r=-0.453, p=0.547) and using species richness (r=0.239, p=0.761), there was still little to no correlation found. Analysis of migratory and residential species richness (r= 0.742, p=0.258) and proportion (r= 0.829, p=0.171) compared to invasive berry density also exhibited little to no significant correlation. We also compared the density (r= -0.114, p= 0.886) and species richness (r= 0.895, p= 0.105) of insectivorous birds to the density of invasive berry producing plants and found no significant correlation. This indicates that invasive berry producing plants do not impact bird abundance.

This was the fourth year that this type of study has been conducted by Juniata College student researchers at the Raystown Field Station. Previous unpublished studies examined Old Crow Wetland, and Fouse's Crossing wetland, comparing berry production and bird species recorded. In one study conducted, student researchers Zachary Adams and Gordon Dimmig assessed the impacts of invasive berry producing plants on avian feeding guild structure. Adams and Dimmig found no significant difference in bird abundance between the two sites, nor did they find significant differences in density, richness, or species with high conservation values. This follows the same trend we observed in our own data, that there was no correlation in invasive berry producing plant density and bird density.

However, another unpublished study conducted by Juniata researchers in 2014 showed the opposite result. In 2014, there was a significant difference in abundance and richness, with Old Crow having higher abundances and richness than Fouse's Crossing. The differences in the results of 2014, 2015, 2016, and 2017 could be due to weather variations or other unforeseen variables. Once the data set continues to expand after multiple years of study, we may begin to see whether 2014 was an exception to the trend, or if invasive berries truly impact bird abundance.

Research conducted in the scientific community varies on the subject. Some studies have shown that birds do have a preference between choosing invasive berries and native berries (Lafleur et. all, 2006). Based on their studies, American Robins (*Turdus migratorius)* and European Starlings (*Sturnus vulgaris*) both preferred invasive berries over native in tests (Lafleur et. all, 2006). Their study would indicate that birds prefer invasive berries and we would expect to see higher bird densities in areas that have high densities of invasive berries. Other studies have also indicated that birds prefer invasive berries. One study recorded the visitations of birds at invasive plants versus native plants and counted the number of fruits consumed (Mokotjomela et. all 2012). Both of these studies contradict our study that showed no preference towards high density invasive berry sites. This also contradicts the previous 2014 study at Juniata College which indicated that there was a preference to sites with low invasive berry densities.

One study, conducted by Gleditsch and Carlo in 2011, suggest that some birds such as Gray Catbirds (*Dumetella carolinensis)* exhibit a preference for invasive berry producing plant species like honeysuckle (*Lonicera)*, forming mutualistic relationships. Research shows that catbirds are often absent from locations having honeysuckle levels <100 fruits per plot (Gleditsch and Carlo, 2011). Arguments have been raised that the abundance of invasive plant species limits the amount of non-invasive and native plant species that are present in location. This hypothesis has been tested by placing potted black-nightshade plants (*Solanum nigrum)* in a location where honeysuckle was present in both high and low abundances to determine a preference between native and non-native berries. Upon conclusion of the study, 96% of the black-nightshade berries had been consumed in the location of high abundance of honeysuckle, while only 67% of the black-nightshade berries were consumed in location of low abundance of honeysuckle. (Davis, 2011) These studies suggest a preference for invasive berry producing plant species such as honeysuckle,

while many others including our own do not show this relationship.

We hypothesized that other avian feeding guilds, such as insectivores, may be impacted by high densities of invasive berry producing plants in wetlands. In 2003, Herrera and Dudley, studied the impacts of exotic invasive plants on the abundance and diversity of riparian arthropod abundances by sampling aerial, ground-dwelling, and other terrestrial arthropods in the presence and absence of *Arundo donax*, and aggressive invader of riparian habitat. Results of the study show a 50% decrease in aerial invertebrates, while ground-dwelling arthropods did not exhibit differences as great as that of aerial invertebrates (Herrera and Dudley, 2003). These results suggest that vegetation cover is a significant driver of invertebrate abundance and diversity. Due to the significant impact that invasive plants pose on insects, we would expect to see similar impacts to insectivorous birds. However, throughout our study we saw little correlation between insectivorous bird densities and invasive berry densities. It is possible that the invasive berry producing plants at our site do not act in the same way as *Arundo donax*. The previous study only compared the abundances of insects to one invasive plant while through the course of our study, the wetlands had been occupied by multiple invasive berry producing plants. Different invasive plants could have different impacts on insects and could possibly positively or negatively impact insect abundance or even have no effect on abundance at all.

In conclusion, research suggests that we should see higher bird densities at wetland sites with higher invasive berry densities. However, we have noticed no correlation between invasive berry densities and bird densities and prior research from these sites has indicated the exact opposite, that birds prefer lower densities of invasive berries. The differences in our results could be caused by various uncontrollable variables impacting bird selection of wetland sites. It has been suggested that various amounts of canopy cover could impact birds using wetlands (Petersen & Westmark 2013). Our wetland sites, as similar as they were, still had differences such as tree canopy cover. Other differences at our sites could be impacting bird densities and skewing data. More data from our sites should be collected in order to see if the trends recorded in our study continue and determine the cause.

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