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PURPOSE

The purpose of the JOURNAL OF ECOLOGICAL RESEARCH is to print original ecological research reports written by undergraduate students enrolled in ecology and environmental science courses at Juniata College. Some of the research included here has been carried out at the Raystown Field Station. Through participation in this journal, it is hoped that students will begin to gain an appreciation of the publication process in science. In addition, the journal serves as an archives of past student research projects that current students can potentially use as background for their own projects. Topics on all aspects of the science of ecology are welcome.



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ANT BIODIVERSITY SURVEY OF HUNTINGDON, PENNSYLVANIA

Peter W. DeMuth, Alexander B. Weimer, Krista M. Leibensperger and Evan L. Beatty

ABSTRACT

Ants are an omnipresent facet of most non-arctic/tundra ecosystems, yet studies of species richness tend to focus on more charismatic fauna. This study aimed to investigate the diversity and relative abundance of ant species in Huntingdon, Pennsylvania while testing various ant sampling methods. Ants were captured at four unique locations in the Huntingdon area during early April using bait prepared with tuna or cracker crumbs and in addition to pitfall traps. Pitfall traps on the Juniata College campus captured two species not found at the other locales, yet no unique species were found elsewhere (Table 1). Results of bait trap counts found that *Tetramorium caespitum* was by far the most abundant species, and the highest abundances were found in the downtown Huntingdon area (Table 2). An analysis of variance on ant foraging preferences found no significant difference between bait types ($F_{(0.05,1)}=0.15$, $p=0.704$), temperature ($F_{(0.05,2)}=2.36$, $p=0.137$), or trap placement ($F_{(0.05,2)}=2.83$, $p=0.099$) in downtown Huntingdon. Further analysis of ant species at three other locations was not possible due to small sample sizes.

Keywords: Formicidae, distribution, Huntingdon county, bait trap, pitfall trap

INTRODUCTION

In 1866, Ernst Haeckel invented the word “ecology” (or Ökologie as it is in the original German) to describe a budding branch of biology which sought to integrate concepts and theories from geology, organismal biology, and natural history. Today, this young science has grown into a rigorous discipline uniquely suited to describe and assess the stunning biodiversity seen throughout Earth’s biosphere. Of principle importance to ecologists is the reporting of species abundance, distribution, and diversity. Together, these characteristics are tantamount to the understanding of the communal structure and health of local ecosystems. The global human population is exploding and habitats are being destroyed at unprecedented rates without much foresight in an effort to satiate humanity’s growing appetite (Wilson 1988). Because biological diversity itself holds intrinsic value—in addition to its known and potential economic, medical, and even recreational benefits—it is ever more imperative to have a finger on the pulse of the biosphere.

Traditional biodiversity studies have focused primarily on vertebrates, flowering species, and

charismatic invertebrates (e.g. lepidopterans). However, no single taxon can be representative of the diversity of an area. As such, the addition of other groups in biodiversity surveys is imperative to best characterize regions which are of the utmost importance to protect. Due to their high diversity and vast numbers of individuals, ants make for excellent organisms of focus in biodiversity studies (Agosti, Majer, Alonso, & Schultz 2000). By far earth’s most abundant animals, individual ants easily outnumber humans by a factor of over one million. They inhabit nearly every habitat on earth, outside of Antarctica, and typically dominate all of the other insect fauna both through sheer numbers and the versatility and supremacy of their colonial lifestyle (Moffett 2010).

The ubiquitous nature of ants makes them easily identified by even laypeople (as a group, though not at the species or genus level). To an ecologist, they should be an obvious group to include in biodiversity studies since they are easily collected, but also not so diverse that identification becomes tedious and overwhelming (Agosti et al. 2010). But still, when sampling ants, the net must be cast wide. Behavioral diversity in ants is astounding—colonies can range from half a dozen individuals (*Taumatomyrmex*) to

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billions (*Linepithema humile*); they eat everything from seeds and nectar, captured prey, honeydew from aphids, and farmed fungus (Moffett 2010, Bolton 1994). Thus, in order to obtain a true picture of the diversity of ants present in an ecosystem, a variety of sampling methods must be employed. The present study aims to assess the efficacy of two simple, common sampling methods: baiting and trapping in pitfalls. Sampling will employ different types of bait and will be carried out over different times of day to capture as many individuals of different species as possible. Additionally, this study will utilize said techniques to characterize the ant biodiversity in a range of habitats in Huntingdon, Pennsylvania, USA.

METHODS AND MATERIALS

In this study, ants were captured from the four locations of the Juniata College campus, peace chapel, cliffs, and Huntingdon downtown. Capturing was conducted in morning and evening sessions, lasting approximately thirty minutes for bait traps and all day for pitfall traps. At each location sampled, temperature and field characteristics were recorded (Appendix A). For bait trapping, tuna was placed in two small, plastic vials and crackers were placed in two identical, small plastic vials. The vials were opened in locations near each other and placed sideways on the ground. After sampling, the vials were closed and the number of ants inside was counted. For pitfall trapping, two, three inch deep plastic cups were buried even with the ground and left for 24 - 48 hours

with the lid remaining on so that local fauna would become accustomed to the disturbance (Agosti et al. 2000). After this time, isopropyl alcohol was poured into the container and the lid was removed for the duration of sampling (~8-10 hours). Ants were taken back to the lab and examined for distinguishing characteristics under a dissecting microscope.

RESULTS

Species richness was determined at each of the four locations in the study using both pitfall and bait traps. Only the pitfall traps located on the Juniata College campus collected any individuals; however, three species were recorded (Table I). The common pavement ant *Tetramorium caespitum* was present in the bait traps downtown and on campus. Though rare (i.e. one individual), the carpenter ant *Camponotus pennsylvanicus* was collected in the bait traps as the Huntingdon Cliffs and downtown (Table II). *T. caespitum* was by far the most numerous ant species. (A summary of raw, bait trap data is available; Appendix B).

Analysis of differences in ant capture between bait types and temperatures could only be conducted for the downtown site due to small sample sizes at the other sites. An Analysis of Variance found no significant difference between bait types ($F_{(0.05, 1)}=0.15$, $p=0.704$), temperature ($(F_{(0.05, 2)}=2.36$, $p=0.137$), or trap placement ($(F_{(0.05, 2)}=2.83$, $p=0.099$). Residuals were normally distributed and heteroscedasticity was not apparent.

	Campus	Downtown	Cliffs	Peace Chapel
<i>Camponotus castaneus</i>	2	0	0	0
<i>Camponotus pennsylvanicus</i>	0	0	0	0
<i>Formica fusca</i>	1	0	0	0
<i>Tetramorium caespitum</i>	0	0	0	0

Table I: Individual species counts in pitfall traps at four locations (Juniata College campus, peace chapel, cliffs, and downtown Huntingdon)

	Campus	Downtown	Cliffs	Peace Chapel
<i>Camponotus castaneus</i>	0	0	0	0
<i>Camponotus pennsylvanicus</i>	0	1	1	0
<i>Formica fusca</i>	0	0	0	0
<i>Tetramorium caespitum</i>	19	285	0	0

Table II: Individual counts in bait traps at four locations (Juniata College campus, peace chapel, cliffs, and downtown Huntingdon)

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DISCUSSION

Of the ants present, *Tetramorium caespitum* was by far the most abundant species found in the bait traps (Table II). Based on these data, *T. caespitum* was significantly more abundant than other ant species in the downtown and campus area. However, one alternative explanation is that *T. caespitum* ants were quicker to respond to food stimuli in their habitat. Since bait traps were only left exposed for thirty minutes, it is possible that other ant species may have been captured if the time was extended. This is supported by the pitfall trap results of the campus location (Table I). In pitfall sampling of the Juniata College campus—which occurred on the same day as bait trapping—there were two species captured, none of which were *T. caespitum* (Table I). This suggests that the *T. caespitum* ants were in some way more perceptive to the bait traps, since the pitfall results show that other species exist in the area (Table I). Additionally, this study affirmed the notion of *T. caespitum* as a pavement ant species. This species is known for residing in residential environments, choosing locations near buildings and pavement (Tarkington 2001). Not surprisingly, this species was found in great abundances in an urban location.

In analyzing the data, there was no significant preference for either of the two food types, tuna or cracker. This suggests that ant species captured from bait traps were opportunists, which is consistent with *T. caespitum*'s observed diet (Tarkington 2001). However, due to the small sample size of *Camponotus pennsylvanicus*, it is difficult to confirm any food preferences from the bait trap data. While ants were predominantly captured in the afternoon when the temperatures were warmer than the morning, temperature was not found to be a significant factor. This is most likely attributable to the study's small sample size, as other studies suggest that during the springtime, when mornings are typically cold, ants prefer to forage in the afternoon (Sanford and Tschinkel 1993).

Between the four locations sampled, there was a marked difference in ant abundance. Most noticeably, there was a near total absence of ants captured at the peace chapel and cliff locations. One explanation could be the amount of shade at these locations. Temperatures were recorded from the National Weather Service for Huntingdon, PA; the temperatures at the cliffs and peace chapel were most likely lower the area average due to being removed from the urban heat island. This could produce a suboptimal temperature for ant activity and could partially account for the lack of ants captured. It has further been shown that within forest stands, altitude,

slope steepness, and direction the slope faces can all impact the abundance of ant mounds (Kilpeläinen 2008).

The present sampling data showed that different ant species were collected in bait traps than were found in pitfall traps. These results imply that the employment of multiple sampling methods can dramatically improve the effectiveness of ant biodiversity surveys. Additionally, the overwhelming presence of *Tetramorium caespitum* at the two sampling sites where ants were captured could evidence their dominance as an ecologically invasive species. Pitfall traps are more random in their sampling and control for competitive exclusion. Future research should recreate this experiment during the summer, when temperature differences between local environments are unlikely to contribute to discrepancies in ant activity. Additionally, just as different bait types were used in this experiment, future studies should include different types of pitfall agents, as some may differentially attract or repel ants.

ACKNOWLEDGEMENTS

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APPENDIX A

Ant Survey Data Sheet

Sample Information

Observer/Collector: _____

Survey Type: Bait Aspirator Pitfall Trap

Sample Code: _____

Location: _____ Date/Time of Day: _____

Collection Started: _____ Collection Finished: _____

Ambient Temp: _____ Soil Temp: _____ Weather Condition: _____

Trap Size/Bait Type/Sample Area: _____

Percentage Ground Cover

Bare Soil: _____ Litter: _____ Stone: _____ Plant: _____ Other: _____

Dominant vegetation: _____ Soil Type: _____

Percent Canopy Cover: _____

Notes/Observations:

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APPENDIX B

Code	Time (Start)	Time (End)	Locale	Bait Type	Temp (°F)	Total # Ants
AW_Bait_0 1	10:20	10:50	Downtown- sidewalk	tuna	46	0
AW_Bait_0 2	10:20	10:50	Downtown- sidewalk	cracker	46	0
AW_Bait_0 3	10:20	10:50	Downtown-grass	tuna	46	0
AW_Bait_0 4	10:20	10:50	Downtown-grass	cracker	46	0
AW_Bait_0 5	10:20	10:50	Downtown-under tree	tuna	46	0
AW_Bait_0 6	10:20	10:50	Downtown-under tree	cracker	46	0
AW_Bait_0 7	2:35	3:10	Downtown- sidewalk	tuna	57	0
AW_Bait_0 8	2:35	3:10	Downtown- sidewalk	cracker	57	18
AW_Bait_0 9	2:35	3:10	Downtown-grass	tuna	57	2
AW_Bait_1 0	2:35	3:10	Downtown-grass	cracker	57	1
AW_Bait_1 1	2:35	3:10	Downtown-under tree	cracker	57	62
AW_Bait_1 2	2:35	3:10	Downtown-under tree	tuna	57	117
AW_Bait_1 3	5:40	6:10	Downtown- sidewalk	cracker	60	19
AW_Bait_1 4	5:40	6:10	Downtown- sidewalk	tuna	60	0
AW_Bait_1 5	5:40	6:10	Downtown-under tree	tuna	60	0
AW_Bait_1 6	5:40	6:10	Downtown-under tree	cracker	60	43
AW_Bait_1 7	5:40	6:10	Downtown-grass	cracker	60	22

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AW_Bait_1 8	5:40	6:10	Downtown-grass	tuna	60	2
EB_Bait_1	7:00	7:20	Peace Chapel	cracker	54	0
EB_Bait_2	7:00	7:20	Peace Chapel	cracker	54	0
EB_Bait_3	7:00	7:20	Peace Chapel	tuna	54	0
EB_Bait_4	3:00	3:30	Peace Chapel	cracker	60	0
EB_Bait_5	3:00	3:30	Peace Chapel	tuna	60	0
EB_Bait_6	3:00	3:30	Peace Chapel	cracker	60	0
PD Bait 01	9:05	9:40	Observatory	cracker	55	4
PD_Bait_02	9:05	9:40	Observatory	cracker	55	0
PD_Bait_03	9:05	9:40	Observatory	tuna	58	0
PD_Bait_04	9:05	9:40	Observatory	tuna	58	0
PD_Bait_05	3:38	4:08	Observatory	cracker	70	0
PD_Bait_06	3:38	4:08	Observatory	cracker	70	0
PD_Bait_07	3:38	4:08	Observatory	tuna	70	0
PD_Bait_08	3:38	4:08	Observatory	tuna	70	0
PD_Bait_09	4:10	4:40	Observatory	cracker	69	0
PD_Bait_10	4:10	4:40	Observatory	cracker	69	0
PD_Bait_11	4:10	4:40	Observatory	tuna	69	0
PD_Bait_12	4:10	4:40	Observatory	tuna	69	15
KL_Bait_1	8:00	8:30	Cliffs-open	cracker	36	0
KL_Bait_2	8:00	8:30	Cliffs-open	tuna	36	0
KL_Bait_3	8:00	8:30	Cliffs-trees	cracker	36	0
KL_Bait_4	8:00	8:30	Cliffs-trees	tuna	36	0
KL_Bait_5	5:00	5:30	Cliffs-open	cracker	60	0
KL_Bait_6	5:00	5:30	Cliffs-open	tuna	60	1
KL_Bait_7	5:00	5:30	Cliffs-trees	cracker	60	0
KL_Bait_8	5:00	5:30	Cliffs-trees	tuna	60	0

Table 3: Raw measurements of bait trap results at all locations.

EFFECTS OF LIQUID MIRACLE-GRO ON *GAMMARUS MINUS* SURVIVORSHIP

Erick Wilson, Kristin Michalak and Marissa Trone

ABSTRACT

The purpose of this experiment was to investigate the survivorship of *Gammarus minus* based on large (>5mm) and small (<5mm) body size when treated with the household fertilizer, Miracle-Gro. We hypothesized that large *Gammarus minus* would survive longer than small *Gammarus minus*. We collected amphipods from Petersburg Spring and brought them back to the lab where Miracle-Gro was added. Survivorship of individuals was observed for five days and pH was recorded. We found that size was a significant factor in survivorship of *Gammarus minus* that were treated with Miracle-Gro. By the end of the experiment all small *Gammarus minus* that were treated with Miracle-Gro had died but 48.3% of the large group was still alive. Using Two-Way Chi-Square analysis, a significant difference in survivorship was found between these groups with a P-value of 0.000. There was also a significant difference in pH between the large and small groups based on a One-Way ANOVA statistical test with a P-value of 0.00. Our results show that when treated with Miracle-Gro there is a direct relationship between *Gammarus minus* body size and survivorship.

Key words: Gammarus minus, Miracle-Gro, household fertilizer, survivorship

INTRODUCTION

A number of sources state that running waters are one of the most obstructed ecosystems in the world, due to the amount of factors that could enter, harming the waterways and the organisms living in that environment (Hart et al 2004). The most harmful components include ecosystem destruction, water chemistry alteration, and the removal or addition of a species to an area (Obire et al., 2008). However, many times people unknowingly harm the waterways without thinking of the long-term effects of a substance. Over time runoff increases the amount of harmful substances that enter the waterway, causing the chemical balance of the water to be altered (Winter et al., 2002); which in turn can harm the macroinvertebrates, since they are the most vulnerable organism in that specific environment (Beasley et al., 2002).

Miracle-Gro is a common household fertilizer that is readily available to the average citizen. While its purpose is to enhance the growth of household plants, flowers, vegetables, etc. it also may have effects on local species within the area of application. A particularly affected area is where the Miracle-Gro is applied near a stream where run-off contamination from the Miracle-Gro can easily enter the stream and affect the organisms living in it. In one study conducted by Winter et al., fertilizer runoff from golf courses had a significant negative impact on macroinvertebrates (2008). Because of this we chose to study these effects upon the indicator species *Gammarus minus* and how those effects would change due to body size and concentration of Miracle-Gro. We hypothesized that the concentration chosen will affect juvenile *Gammarus minus* (<5mm) such that they exhibit a higher death rate than adult *Gammarus minus* (>5mm).

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FIELD SITE

The field site chosen was Petersburg spring located in Huntingdon, Pennsylvania. *Gammarus minus* were collected from here but all manipulations took place in the lab. The pH of this spring is 6.63. The substrate within the spring contains about 10% boulders, 20% cobbles, 60% gravel, 8% sand, and 2% silt. The macrophytic coverage is about 75% moss and vines. Along the spring were many shrubs, bushes, and ivies.

MATERIALS AND METHODS

We went to Petersburg spring to collect the *Gammarus minus* and used hand nets to gather them. We then placed the *Gammarus minus* into a bucket filled with water from the spring and brought them back to the lab. The amphipods were then separated by body size by using a ruler to distinguish between large (>5mm) and small (<5mm) organisms. After separation, they were individually placed into small cups with 50mL of Petersburg spring water in each. For this experiment 30 small and 30 large amphipods were tested. Using a micropipette, we placed 10 μ L of the liquid Miracle-Gro into each cup. As a control, 15 large and 15 small additional amphipods were set in individual cups but were not treated with the liquid Miracle-Gro. After the initial set up of the experiment, the pH was measured for each cup to determine the initial pH when all amphipods were alive. For the next two days the amphipods were observed once per day to record the death count and pH of the water. After pH was collected on Day 2 20 μ L of liquid Miracle-Gro was added to the 60 experimental cups. The amphipods were then observed and pH and death counts were taken for three more days.

RESULTS

Over the course of six days, survivorship of *Gammarus minus* was observed in the control, small (<5mm), and large (>5mm) groups. For the first three days, survivorship was high for all groups. After the third day, when more Miracle-Gro was added, survivorship began to decrease among the small and large groups. For the large group on the fourth day survivorship declined to 89.7% while the survivorship of the small group declined to 41.7%. By the fifth day, survivorship of the large group dropped to 62% while survivorship of the small group dropped to 25%. On day six all small *Gammarus minus* were dead while the large group retained 48.3% survivorship. From the fourth to fifth days, survivorship of the control group decreased to 96% due to one death (Fig. 1).

We used the One-Way ANOVA statistical test to compare the pH of the large, small, and control groups at the completion of the experiment. When comparing all groups together, the F value was found to be 739.06 and the P-value was 0.00. When comparing the small and large treated groups, the F value was found to be 24.260 and the P-value was 0.00 (Table 1). Please refer to Appendix 1 for raw pH data.

We used the Two-Way Chi-Square statistical test to compare the expected and observed survivorship of the small, large, and control groups. When comparing all of the groups together, the Chi² value was 49.034 with a P-value of 0.000. When comparing the small treated and small control groups, the Chi² value was 38.000 with a P-value of 0.000. When comparing the large treated and large control groups, the Chi² value was 9.1030 with a P-value of 0.003 (Table 2). Raw data of amphipod survivorship can be consulted in Appendix 2.

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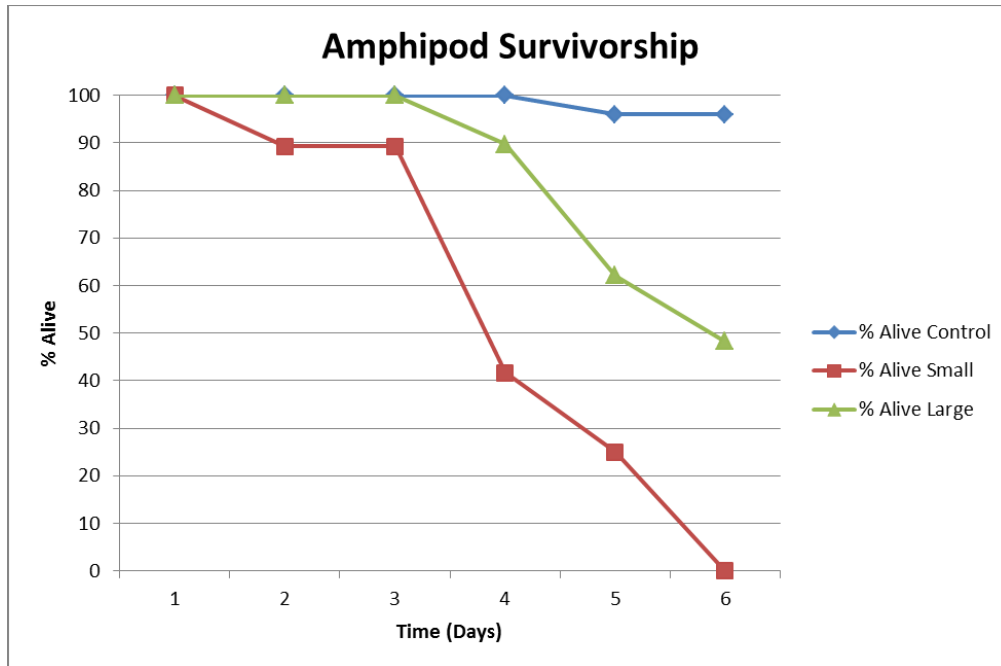


Figure 1. Amphipod (*Gammarus minus*) survivorship of control, small, and large groups based on percent alive per day.

Table 1. One-Way ANOVA Test to compare large, small, and controlled group pH after final treatment.

One-Way ANOVA			
Values	F	P	DF
Comparing Large/Small/Control	739.06	0	2
Comparing Large/Small	24.26	0	1

Table 2. Chi-Square Test comparing all control and treatment survivorship, control small and treated small survivorship, and control large and treated large survivorship of *Gammarus minus*.

Chi-Square Test				
	Control Small	Control Large	Treatment Small	Treatment Large
Alive Observed	14	11	0	14
Dead Observed	0	0	24	15
Alive Expected	7	5.5	12	14.5
Dead Expected	7	5.5	12	14.5
	Chi Value	P-Value	DF	
Comparing All	49.034	0.000	3	
Comparing CS&TS	38.000	0.000	1	
Comparing CL&TL	9.1030	0.003	1	

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DISCUSSION

We hypothesized that there would be a significant difference in *Gammarus minus* survivorship based on body size. Our Chi-Square test supports our hypothesis by showing a significant difference in survivorship between the small and large treated groups as well as the control group (Table 2). As displayed in Figure 1, the survivorship of both small and large treated groups declined. However, the small group declined more rapidly than the large group. By the end of the experiment all of the individuals in the small group had died while 48.3% of the large group remained alive. This shows that the adult *Gammarus minus* have a significant advantage over the juvenile *Gammarus minus* when treated with Miracle-Gro.

Using the One-Way ANOVA test we determined that the pH was significantly different at the end of the experiment across the three groups. This result could be one factor that contributed to death of the *Gammarus minus* over the period of experimentation. However, this may not be the only reason behind their death and further research should investigate other possible causes of death.

One source of error in our experiment could be due to the loss of *Gammarus minus* throughout the experiment. At times, when testing the pH amphipods swam into the crevices of the pH meter. Unfortunately, we did not realize this until the next day when we found them in the deionized water of the pH meter kit. Because of this our sample size was reduced. For the small group, five amphipods went missing and were classified as "MIA." For the large group, one amphipod was MIA and for the control group five were MIA. This could have affected our data because a decrease in sample size could reduce the accuracy of our results.

Ultimately, contrary to the Miracle-Gro MSDS, we determined that liquid Miracle-Gro does have an adverse effect upon the survivorship of *Gammarus minus*. We also found a direct relationship between *Gammarus minus* body size and survivorship when treated with Miracle-Gro such that adult *Gammarus minus* exhibited a higher survival

rate than juveniles. Further research could determine the exact cause of death as well as what element of the liquid Miracle-Gro is lethal to *Gammarus minus*.

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APPENDICIES

Appendix 1. Raw data of pH taken each day for small, large, and control groups of amphipods.

Container#	pH																	
	Large (>5mm)						Small (<5mm)						Control (1-15 large, 16-30 small)					
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
1	6.72	7.39	6.96	7.41	7.60	7.71	6.87	7.14	7.03	7.08	7.44	7.64	5.86	7.01	7.31	6.24	6.63	6.84
2	6.71	7.26	7.06	7.49	7.61	7.66	6.85	7.04	6.90	7.07	7.53	7.65	6.02	7.04	7.08	6.89	6.94	7.03
3	6.70	7.40	7.08	7.39	7.51	7.62	7.12	7.21	6.99	7.17	7.61	7.62	6.11	7.09	7.15	6.95	6.85	7.03
4	6.68	7.39	7.09	7.34	7.48	7.64	6.84	7.20	7.03	7.02	7.50	7.60	6.15	7.06	7.14	7.03	6.84	6.93
5	6.68	7.33	7.09	7.35	7.54	7.64	6.86	7.16	7.02	7.06	7.48	7.56	6.13	6.98	7.09	7.19	6.93	7.00
6	6.70	7.32	7.05	7.40	7.55	7.66	6.84	0.24	6.87	7.03	7.55	7.62	6.19	7.03	7.17	7.05	6.94	7.02
7	6.71	7.38	7.07	7.39	7.56	7.71	6.87	7.17	6.97	7.01	7.47	7.63	6.24	7.01	7.09	6.95	6.98	7.07
8	6.70	7.33	6.95	7.43	7.59	7.71	6.83	7.11	6.89	6.96	7.43	7.62	6.29	7.03	6.99	7.10	6.90	7.02
9	6.69	7.31	7.16	7.31	7.50	7.63	6.87	7.15	7.02	7.03	7.50	7.66	6.28	7.03	6.98	7.19	7.01	7.11
10	6.71	7.27	7.06	7.27	7.47	7.58	6.86	7.15	7.01	7.02	7.47	7.58	6.19	6.97	7.06	7.18	6.97	6.99
11	6.69	7.36	7.11	7.43	7.63	7.61	6.87	7.18	7.25	7.04	7.43	7.56	6.28	7.00	7.15	7.21	7.10	7.11
12	6.72	7.36	7.10	7.37	7.54	7.61	6.84	7.20	7.04	7.24	7.60	7.56	6.32	6.97	7.08	7.07	7.00	7.09
13	6.72	7.32	6.89	7.40	7.57	7.64	6.83	7.19	7.09	7.16	7.50	7.52	6.33	7.02	7.10	7.13	6.88	7.07
14	6.72	7.34	7.02	7.39	7.51	7.62	6.86	7.13	7.07	7.06	7.49	7.54	6.34	6.99	7.24	7.28	6.97	7.05
15	6.71	7.33	7.01	7.33	7.50	7.60	6.84	7.19	7.22	7.09	7.51	7.53	6.32	7.09	6.98	7.08	7.05	7.16
16	6.74	7.28	6.93	7.08	7.49	7.64	6.85	7.18	7.09	7.10	7.46	7.68	6.35	6.98	7.10	7.23	7.07	7.18
17	6.73	7.33	7.01	7.37	7.57	7.61	6.87	7.17	7.14	7.03	7.49	7.59	6.32	7.02	7.08	7.06	7.10	7.14
18	6.75	7.27	6.95	7.41	7.60	7.60	6.84	7.20	7.02	7.07	7.53	7.59	6.38	6.99	7.02	7.00	7.04	7.11
19	6.72	7.29	6.98	7.08	7.48	7.57	6.88	7.16	6.95	6.97	7.52	7.63	6.34	7.01	7.06	7.12	6.93	7.12
20	6.73	7.30	6.92	7.34	7.59	7.62	6.85	7.22	7.17	7.23	7.55	7.54	6.37	6.99	7.13	7.23	7.01	7.16
21	6.76	7.31	6.92	7.36	7.55	7.65	6.81	7.27	7.04	7.03	7.48	7.54	6.40	6.99	7.21	7.23	6.97	7.17
22	6.71	7.40	6.88	7.43	7.59	7.63	6.76	7.21	7.05	6.98	7.47	7.60	6.39	7.00	7.11	7.26	6.95	7.15
23	6.76	7.30	6.85	7.43	7.60	7.69	6.78	7.22	7.06	6.97	7.51	7.59	6.43	6.99	7.06	7.30	7.01	7.11
24	6.76	7.29	6.86	7.14	7.49	7.65	6.91	7.16	7.00	7.06	7.50	7.58	6.45	6.98	7.18	7.32	7.02	7.21
25	6.75	7.31	6.97	7.14	7.46	7.59	6.88	7.22	7.15	6.96	7.46	7.57	6.40	6.98	7.06	7.12	7.03	7.23
26	6.76	7.23	6.89	7.20	7.49	7.66	6.78	7.12	7.08	6.97	7.52	7.58	6.36	6.96	7.09	7.16	7.06	7.15
27	6.75	7.36	6.87	7.06	7.44	7.65	6.76	7.17	7.12	6.98	7.54	7.59	6.39	6.96	7.20	7.31	6.99	7.16
28	6.76	7.30	6.94	7.40	7.59	7.60	6.75	7.13	7.10	7.07	7.46	7.50	6.40	6.97	7.13	7.29	7.00	7.13
29	6.77	7.13	6.97	7.39	7.58	7.65	6.74	7.21	7.09	6.97	7.50	7.57	6.38	6.90	7.08	7.21	7.02	7.17
30	6.75	7.32	6.85	7.45	7.63	7.67	6.87	7.14	7.12	6.98	7.49	7.54	6.41	6.88	7.05	7.12	7.06	7.20
Average	6.73	7.32	6.98	7.33	7.54	7.64	6.85	6.94	7.05	7.05	7.50	7.59	6.29	7.00	7.11	7.12	6.98	7.10

Appendix 2. Raw data of amphipod survivorship. "MIA" indicates amphipods that were lost because they unknowingly became stuck to the pH meter during pH testing.

Survivorship									
Day	Large (>5mm)			Small (<5mm)			Control (1-15 large, 16-30 small)		
	Alive	Dead	MIA	Alive	Dead	MIA	Alive	Dead	MIA
0	30	0	0	30	0	0	30	0	0
1	30	0	0	25	2	3	29	0	1
2	30	0	0	25	2	3	26	0	4
3	26	3	1	10	14	6	25	0	5
4	18	11	1	6	18	6	24	1	5
5	14	15	1	0	24	6	24	1	5

TEMPERATURE TOLERANCE OF AMPHIPODS FROM LOCAL PETERSBURG SPRING

Cory Kutruff, Molly Snyder, Alex Belonis, Arie Pollard

ABSTRACT

Gammarus minus is an ectothermic amphipod where we tested if temperature was dependent on their survival. We also wanted to see if this followed Shelford's Law of Tolerance. This experiment consisted of collecting amphipods from Petersburg Spring and placing them in a laboratory setting into different temperatures. The temperatures chosen were 4 and 20, but used 10 degrees at a control since that is their natural temperature. Our results indicated that they do follow Shelford's law of tolerance because outside of their natural temperature, we had significant deaths. Most amphipods died in the 4 and 20 degree temperatures when we ran the experiment twice, while most survived both times in the 10 degrees Celsius.

Keywords: *Gammarus minus*, Temperature, Petersburg, Tolerance, Spring

INTRODUCTION

Gammarus minus is an ectothermic amphipod, whose metabolism is increased in warmer climates. Warmer water temperatures, correlate with its growth and reproduction (Blockwell & Taylor 1995). *G. minus* is an amphipod that is found in springs and streams where limestone is prevalent (Haley). Especially in hard-water springs (Glazier 1992). After a recent transplant study conducted earlier this semester, it was determined that *G. minus* cannot sustainably live in acidic conditions. The study consisted of transplanting the amphipods from a basic stream to an acidic stream and checking on

them after a week. Results showed that after a week all the amphipods were dead. The reason for this is the acidic water, prevents or slows regenerations of the exoskeleton (Glazier 1992).

This paper continues this study but looking at temperature tolerance with respect to Shelford's law of tolerance. It states that populations are negatively affected when removed from the optimal point of growth which produces a bell shaped curve (see Figure 1). By exposing *G. minus* at different temperatures, we hypothesize that we will see a reduction in population over time at temperatures outside of the control.

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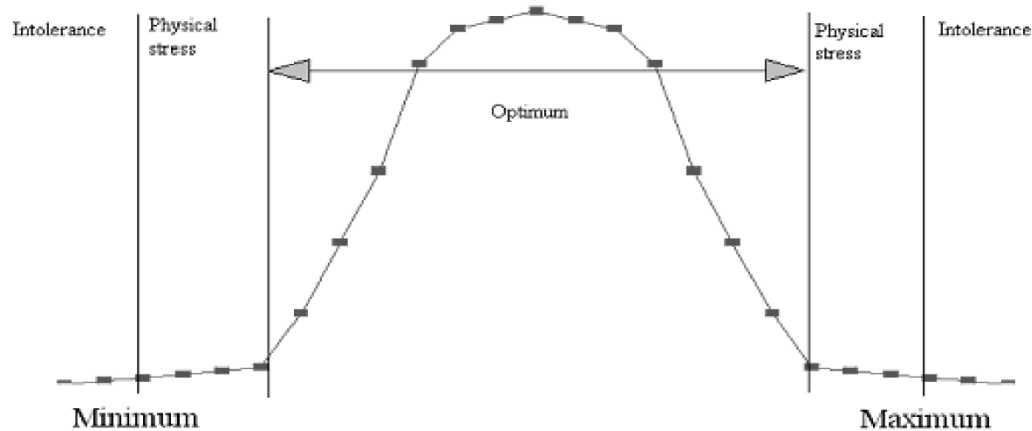


Figure 1 Shelford's Law of Tolerance Diagram (Landrey 2002)

FIELD SITE

The amphipods (*Gammarus minus*) were collected in Petersburg Spring. The spring is located in Petersburg PA (central PA) at the coordinates of 40.539708 longitude and -78.030978 latitude. The area is a brushy area with a lot of vegetative life surrounding the stream.

Amphipods were collected on Tuesday March 20th 2012. Weather conditions were good with an air temperature of 21.1 degrees Celsius (70 degrees Fahrenheit). A second collection was done on April 10th, 2012. Air temperature was colder at 11.6 degrees Celsius (53 degrees Fahrenheit) and it was cloudy. For spring conditions, see table 1.

For experimental detail see Materials and Methods section.

Table 1 Characteristics of Petersburg water spring conditions

Characteristic	March 20 th 2012	April 10 th 2012
Temperature	10.3 degrees Celsius	10.0 degrees Celsius
pH	6.63	6.60
Conductivity	106.4 (microsiemens)	105.2 (microsiemens)
Dissolved Oxygen	0.1 (ppt)	0.1(ppt)
Flow Rate	0.94 (m/s)	0.94(m/s)
Depth (average)	10.76 (cm)	10.76 (cm)
Width (average)	2.18 (m)	2.18 (m)

METHODS AND MATERIALS

Amphipods were collected randomly at random sites within the stream. They were collected by kicking up some dirt with a net, and then swiping the net through that area. If amphipods were on the net, they were then placed into a plastic container that contained water from Petersburg spring. To acquire enough amphipods we did this with three plastic containers. A spare container contained about five gallons of stream water from Petersburg, which will be used as one of the experimental conditions. Also, detritus was collected at the site to be used as a food source for the amphipods.

Amphipods were sorted back in the lab and were chosen only based on their size. For this study, we chose large amphipods but neglected gender as a factor. They were placed into a pod pad. A piece of filtered cloth was placed between both caps to allow water to enter in and out, but prevent the amphipods or their food source from escaping. This method was chosen so the amphipods would remain isolated from each other as well as give us the capability to control their food source. A nickel size piece of detritus (dead leaves) was given to each amphipod. The food source was taken directly from the field site. A complete container consisted of an initial live amphipod and a food source.

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From here the completed container was placed into one of three rectangular plastic bins that were filled with Petersburg spring water. The three bins each initially contained ten completed containers (so 30 amphipods total). The three bins were subject to temperatures of 4, 10, and 20 degrees Celsius. Temperatures were regulated precisely. The 4 degrees Celsius bin was placed in a refrigerator that was regulated to that temperature. The 10 degrees Celsius also the control (see Petersburg stream Temperature above in FIELD SITE) was regulated by a walk in cooler. The 20 degrees Celsius was kept in a warm bath (need to find out the name of it).

After getting some results, we decided to run the experiment again to obtain more data. We went to out to Petersburg stream and collected a plethora of amphipods. Everything remained the same from the first run, except the sample size was larger. Twenty five amphipods were placed in each temperature (75 total amphipods). Each bin would contain 12 to 13 completed containers, so there would be two bins in each temperature.

After experimental set up, the amphipods were checked twice a day, every day. They were checked once in the morning and once in the evening. Checking amphipods consisted of taking each completed container out of the rectangular bin and see that they were alive. If a container was taken out and there was no initial movement, the container was then re-submerged in that water bin and/or tapped to see if the amphipod(s) were sleeping. From here if there was no movement, and the amphipod had a yellow color, they were considered dead. If the amphipod was dead, their container was removed from the rectangular bin, and disposed of appropriately. Living amphipods were place back into their bin and there metabolic activity was commented on. When water supply became low due to evaporation in the 20 degree bin, it was replenished to rule out the death of amphipods from lack of water.

RESULTS

The experiment for the amphipods collected on March 20th ran until April 5th. Reasons for stopping on this day were because; we had significant number of deaths in the 4 and 20 degrees containers. Also, it was noted that the amphipods food source was depleted. Stopping the experiment would ensure that the amphipods cause of death was solely dependent on temperature and was not from malnutrition. We looked at how the amphipods died over time to see if they all died at once. If this

occurred, it would tell us that it probably associated with something other than temperature. Tables 2, 3, & 4 document the data recorded each day for amphipods and has the times they were checked. At first, there were two amphipods that died in the ten degrees C. We do not believe this is temperature dependent, but rather their death was caused by injury during transfer to a completed container. This is speculated because there were no deaths within the 10 degrees Celsius for the rest of the experiment. This is also another reason for why the experiment was rerun with more data. For the data collected however, noted on how many were alive and how many were dead at the end of the experiment (Table 5) and made a percent alive vs temperature graph and compared it to Shelfords Law of Tolerance (Figure 2).

Statistical data for March 20th data was performed using a chi-squared test of independence (Table 6). However, because of limited data, the null hypothesis is excepted. Figure 2 shows that our data fits Shelfords law of Tolerance, so we suspected that our data was limited and that we needed to rerun the experiment (see Materials & Methods). Looking at the data as a whole, shows that it does follow the trend that temperature does affect the amphipods.

Data that was collected on April 10th, was ran until April 29th because of time constraints on the lab report. This run was longer and had a larger sample to give more statistically significant results. The experiment was ran with the same procedure as the March 20th data. It should be noted that in experiment, water levels in the 20 degrees Celsius was low and had to be refilled twice. Also, there was an accumulation of ice forming in the 4 degrees container 2/3rds of the way into the experiment. Tables 7, 8, & 9 show how the amphipods died over time. Looking at this you can speculate that they will follow Shelfords law of tolerance. Table 10 shows the percent of amphipods alive after the conclusion of the experiment and is graphed in Figure 3 which can be compared to Shelfords law of tolerance and Figure 2.

We performed a chi-square test of independence (Table 11). The chi-square test of independence ($\chi^2 = 29.806$, $p < 0.001$) indicates that you are rejecting the null hypothesis that there is no relationship between temperature and status (alive or dead); thus, there is a significant relationship between the temperature and whether or not amphipods were alive or dead. The Cramer's V-square value indicates that 39.7% of shared variance exists between temperature and status.

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Comparing the results from March 20th and April 10th, show similar out comes. However, more amphipods did die in the 20 degrees Celsius from the April 10th data, from which time may be the factor

(see discussion section). The control shows that amphipod survival is at 10 degrees Celsius, and outside of that range makes it difficult for the species to survive.

Table 2 Amphipod data collected on March 20th at 4 degrees Celsius

Petersburg	Morning		Evening	
4 Degrees	Alive	Dead	Alive	Dead
21-Mar	10	0	9	1
22-Mar	9	1	9	1
23-Mar	9	1	9	1
24-Mar	9	1	9	1
25-Mar	9	1	9	1
26-Mar	9	1	5	4
27-Mar	5	4	5	4
28-Mar	5	4	5	4
29-Mar	5	4	5	4
30-Mar	5	4	4	5
31-Mar	4	5	4	5
1-Apr	4	5	4	5
2-Apr	4	5	4	5
3-Apr	4	5	4	5
4-Apr	4	5	4	5
5-Apr	4	5	4	5

Table 3 Amphipod Data collected on March 20th at 10 degrees Celsius

Petersburg	Morning		Evening	
10 Degrees	Alive	Dead	Alive	Dead
21-Mar	10	0	10	0
22-Mar	10	0	10	0
23-Mar	9	1	9	1
24-Mar	9	1	9	1
25-Mar	9	1	8	2
26-Mar	8	2	8	2
27-Mar	8	2	8	2
28-Mar	8	2	8	2
29-Mar	8	2	8	2
30-Mar	8	2	8	2
31-Mar	8	2	8	2
1-Apr	8	2	8	2
2-Apr	8	2	8	2
3-Apr	8	2	8	2
4-Apr	8	2	8	2
5-Apr	8	2	8	2

Table 4 Amphipod Data collected March 20th at 20 degrees Celsius

Petersburg	Morning		Evening	
20 Degrees	Alive	Dead	Alive	Dead
21-Mar	10	0	10	0
22-Mar	9	1	9	1
23-Mar	9	1	9	1
24-Mar	8	2	8	2
25-Mar	8	2	8	2
26-Mar	8	2	8	2
27-Mar	8	2	8	2
28-Mar	8	2	8	2
29-Mar	8	2	8	2
30-Mar	7	3	7	3
31-Mar	7	3	7	3
1-Apr	6	4	6	4
2-Apr	6	4	6	4
3-Apr	6	4	6	4
4-Apr	6	4	6	4
5-Apr	6	4	6	4

Table 5 Shows the percent of amphipods

alive after completion of April 10th experiment

Temperature	% alive
4	0.4
10	0.8
20	0.6

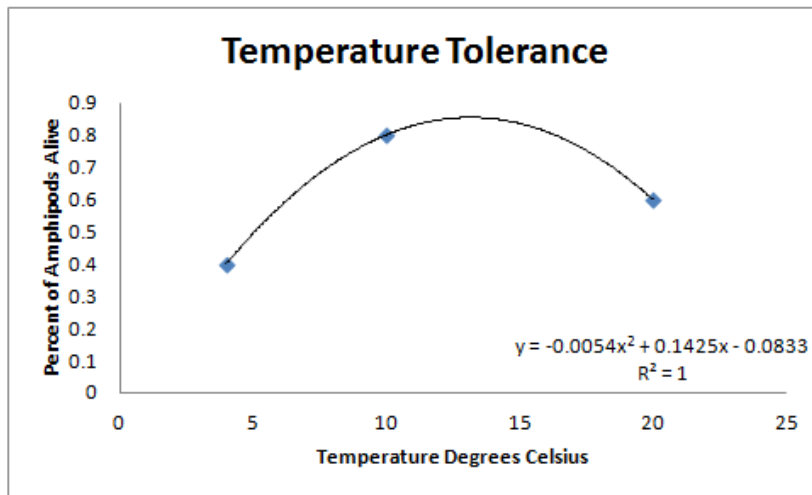


Figure 2 Graph of Percent of Amphipods alive on April 5th vs Temperature.

Shows a close comparison to Shelford's Law of Tolerance.

Table 6 Statistical chi-squared test of independence for March 20th data. Not enough data to complete the test

Tabulated statistics: Temp B, Status B			
Rows: Temp B Columns: Status B			
	Alive	Dead	All
4	4	6	10
	6	4	10
	-0.8165	1.0000	*
10	8	2	10
	6	4	10
	0.8165	-1.0000	*
20	6	4	10
	6	4	10
	0.0000	0.0000	*
All	18	12	30
	18	12	30
	*	*	*
Cell Contents: Count			
Expected count			
Standardized residual			

Pearson Chi-Square = 3.333, DF = 2, P-Value = 0.189

Likelihood Ratio Chi-Square = 3.452, DF = 2, P-Value = 0.178

* NOTE * 3 cells with expected counts less than 5

Cramer's V-square 0.111111

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Table 7 Amphipod data collected April 10th

at 4 degrees Celsius

Petersburg	Morning		Evening	
4 degrees	Alive	Dead	Alive	Dead
11-Apr	25	0	25	0
12-Apr	24	1	24	1
13-Apr	24	1	24	1
14-Apr	24	1	24	1
15-Apr	22	3	22	3
16-Apr	21	4	21	4
17-Apr	21	4	21	4
18-Apr	21	4	20	5
19-Apr	20	5	20	5
20-Apr	18	7	18	7
21-Apr	15	10	15	10
22-Apr	15	10	15	10
23-Apr	15	10	15	10
24-Apr	15	10	15	10
25-Apr	15	10	14	11
26-Apr	12	13	12	13
27-Apr	12	13	12	13
28-Apr	12	13	12	13
29-Apr	12	13	12	13

Table 8 Amphipod data collected April 10th

at 10 degrees Celsius.

Petersburg	Morning		Evening	
10 degrees	Alive	Dead	Alive	Dead
11-Apr	25	0	25	0
12-Apr	25	0	25	0
13-Apr	25	0	25	0
14-Apr	25	0	25	0
15-Apr	25	0	25	0
16-Apr	25	0	25	0
17-Apr	25	0	25	0
18-Apr	25	0	25	0
19-Apr	25	0	25	0
20-Apr	25	0	25	0
21-Apr	25	0	25	0
22-Apr	25	0	25	0
23-Apr	25	0	25	0
24-Apr	25	0	25	0
25-Apr	25	0	25	0
26-Apr	25	0	25	0
27-Apr	24	1	24	1
28-Apr	24	1	24	1
29-Apr	24	1	24	1

Table 10 Percentage of Amphipods alive

from the April 10th data on April 29th

at each Temperature.

Temperature	% alive
4	0.48
10	0.96
20	0.2

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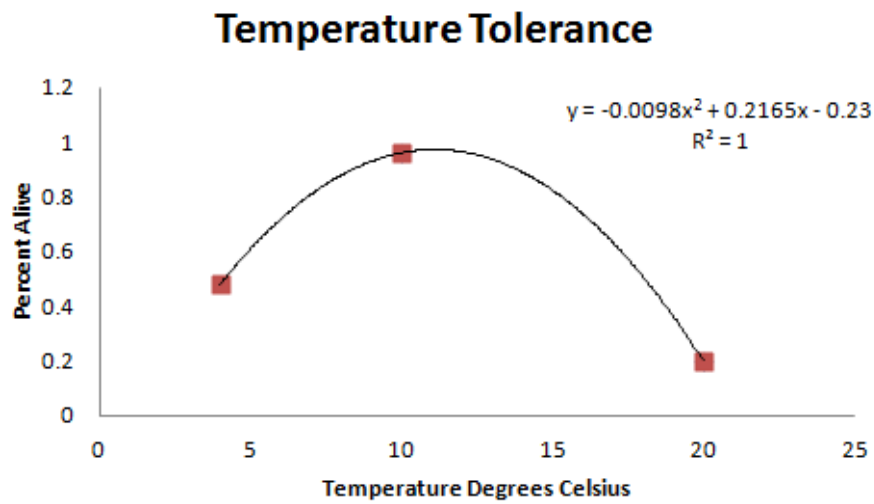


Figure 3 Graphical representation of percent of amphipods alive vs. temperature they were stored in. Data is based off of April 10th collection and the percentages are from the end of the experiment.

Table 9 Amphipod data collected on April 10th

at 20 degrees Celsius

Petersburg	Morning		Evening	
20 degrees	Alive	Dead	Alive	Dead
11-Apr	25	0	25	0
12-Apr	25	0	25	0
13-Apr	25	0	25	0
14-Apr	24	1	23	2
15-Apr	23	2	23	2
16-Apr	23	2	23	2
17-Apr	23	2	23	2
18-Apr	23	2	23	2
19-Apr	19	6	19	6
20-Apr	17	8	16	9
21-Apr	15	10	15	10
22-Apr	15	10	15	10
23-Apr	10	15	10	15
24-Apr	8	17	7	18
25-Apr	7	18	7	18
26-Apr	5	20	5	20
27-Apr	5	20	5	20
28-Apr	5	20	5	20
29-Apr	5	20	5	20

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Table 11 Statistical analysis of April 10th data, Chi Squared test.

Tabulated statistics: Temp, Status			
Rows: Temp Columns: Status			
	Alive	Dead	All
4	5	20	25
	13.67	11.33	25.00
	-2.344	2.574	*
10	24	1	25
	13.67	11.33	25.00
	2.795	-3.069	*
20	12	13	25
	13.67	11.33	25.00
	-0.451	0.495	*
All	41	34	75
	41.00	34.00	75.00
	*	*	*
Cell Contents: Count			
Expected count			
Standardized residual			
Pearson Chi-Square = 29.806, DF = 2, P-Value = 0.000			
Likelihood Ratio Chi-Square = 35.283, DF = 2, P-Value = 0.000			
Cramer's V-square 0.397418			

DISCUSSION

After conducting the experiment twice, we obtained similar results therefore holding our hypothesis true. Both sets of data followed a similar pattern as well as Shelford's Law of Tolerance. The results for 10 degrees Celsius, the temperature at which Petersburg spring is showed that amphipods prefer to live at this temperature. When put outside this temperature range the amphipods were at risk of death, which we saw from our data. The reason for their deaths outside their range is thought to be caused differently.

For the 20 degrees Celsius, amphipod metabolism was increased in the beginning. Increased metabolism was interpreted by movement and food consumption. The detritus was consumed faster in this container as opposed to any other bin. We interpreted their death as to hunger starvation as a

result of their increased metabolism. To explain this, their increased metabolism caused them to eat more. Increased hunger caused them to deplete their food source more rapidly, which was not replenished. Their death was then caused by starvation. Also, this correlates with the data because you do not see a lot of amphipods dying at the beginning of the experiment but more towards the end; at a point which their food source was significantly depleted.

As for the 4 degrees Celsius, the temperature was low enough that probably did not allow sufficient metabolism for survival. So the water temperature directly affected their survival because they did not maintain a metabolism that was high enough to sustain life.

We observed that in the containers, especially the 4 degrees container that the amphipods would hide behind their food source. This may be a technique used to compensate for the cold.

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temperatures by using the leaf as insulation. However, that is outside the context of this paper.

Our study tested whether temperature was dependent on *G. minus*. and followed Shelford Law of Tolerance. Our hypothesis held true and was consistent with Shelfords Law of Tolerance. As soon as amphipods move outside their optimal temperature, their population becomes at risk for extinction.

ACKNOWLEDGMENTS

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CORRELATION BETWEEN BODY SIZE AND HABITAT SELECTION OF AQUATIC SALAMANDER SPECIES

Kathleen LaForce, Emily Layman, Trey Shope and Tim Moore

ABSTRACT

Throughout our study we found a correlation between body length of aquatic salamanders and their choice in habitat selection. The larger and stronger salamanders generally lived under rocks and on substrate that was mostly gravel, while the smaller salamanders were mostly found occupying areas of sandy substrate with little or no rock cover.

Keywords: *Desmognathus fuscus*, *Gyrinophilus porphyriticus*, *habitat selection*, *body length*

INTRODUCTION

Central Pennsylvania hosts many different types of aquatic salamander species that play an important role as environmental indicators. Our experiment involved analysis of *Gyrinophilus porphyriticus* and *Desmognathus fuscus*, two species of salamanders present in Warm Spring. *Gyrinophilus porphyriticus* was the larger of the two species and has an average adult body length of twelve to nineteen centimeters. *Desmognathus fuscus* was the more plentiful species at our field site and ranges in adult size between six and eleven centimeters. We believe that both of these species exhibited a paedomorphic phenotype, in which the species is sexually mature but lives in an aquatic habitat and exhibits larval features (Jackson and Semlitsch, 1993). This explains why we found all of our subjects in water as opposed to land. This paedomorphic phenotype also indicates that Warm Spring is likely to be a habitat with few predators, because this phenotype is most common in ponds without predators (Jackson and Semlitsch, 1993).

We were interested to see if these species selected habitat based on their size. This would be a relevant question because finding a correlation between these two factors in a stream with few or no predators would accurately display competition between species for optimal habitat. We believe that

larger salamanders will be more likely to inhabit areas with gravel substrate and live under boulders, while smaller salamanders will be forced to live in areas with finer substrate and out in the open. A positive correlation between body size and habitat selection would indicate significant interspecific and intraspecific competition within the stream.

FIELD SITE

We evaluated Warm Spring in Huntingdon County. At the study location there was a pH of 7.11, temperature of 17 degrees Celsius, and a depth ranging from 1 to eight inches. A substrate of sand, gravel, cobbles, and boulders, with dense clumps of aquatic vegetation interspersed with open areas of faster water flow in between. Macrofauna observed at the location-included crayfish, turtles, minnow type fishes (3-8 centimeters) and water snakes.

METHODS AND MATERIALS

Samples were collected on two separate days over the span of a week. At our study location, we designated 15 meters, from the mouth of the spring onward, as our sampling site. pH and conductivity were measured near the source using a Markson pH meter and a YSI conductivity meter. We decided to divide the stream up by the stream bottom

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substrate and rock cover, and divided the substrate into 6 different groups: vegetation, sand, gravel/sand, sand/vegetation and gravel/vegetation (As seen below in the results section). We evaluated as many areas of substrate as possible in our location. Salamanders were located by sight, and we removed areas of vegetation and rocks to find the submerged animals. We used fine mesh dip nets to collect specimens. We quickly learned the most efficient method of capture was to place the wire frame of the net firmly against the streambed, and gently pushed the salamander into the net.

Once captured, the salamanders were measured using waterproof metric rulers. We then placed them into a five-gallon bucket, which was partially submerged in the stream to maintain correct water temperature. This was done to ensure that we

were not re-capturing the same animals twice. We also made sure to refresh the water in the bucket every few minutes to avoid low oxygen levels and any buildup of ammonia and other waste products. We also photographed any unusual markings and several individuals from each species to ensure correct identification.

When our fifty specimens were captured and all of the data was collected we could then begin combining our results and perform our tests. We chose to do a Kruskal-Wallis Test to compare median values of all fifty salamanders present in each habitat code, as well as determine the P-Value. A graph was created to compare the salamander size by habitat type and an ANOVA one-way statistic test was completed to display mean value and confidence intervals of salamander length in each habitat code.

RESULTS

Table 1. *Habitat Codes: Categories of habitat type are based on six combinations of substrate found in Warm Spring*

1	Vegetation
2	Sand
3	Gravel
4	Gravel/Sand
5	Sand/Vegetation
6	Gravel/Vegetation

Table 2. *Kruskal-Wallis Test: Compares median values of all fifty salamanders present in each habitat code and provides P-values showing significance of our data.*

Kruskal-Wallis Test on C1				
C2	N	Median	Ave Rank	Z
1	8	2.500	11.6	-2.94
2	19	4.500	26.1	0.21
3	7	5.500	37.1	2.26
4	7	4.200	18.2	-1.43
5	5	4.100	24.6	-0.15
6	4	6.100	44.3	2.68
Overall	50		25.5	
H = 20.07 DF = 5 P = 0.001				
H = 20.12 DF = 5 P = 0.001 (adjusted for ties)				

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Table 3. *Kruskal-Wallis Test: Compares median values of the forty-six Desmognathus fuscus salamanders present in each habitat code and provides P-values showing significance of our data. Does not include the four Gyrinophilus porphyriticus salamanders.*

Kruskal-Wallis Test on C1

C2	N	Median	Ave Rank	Z
1	8	2.500	11.6	-2.75
2	18	4.350	25.1	0.64
3	5	5.100	31.8	1.46
4	7	4.200	18.2	-1.13
5	5	4.100	24.6	0.19
6	3	5.500	42.3	2.51
Overall	46		23.5	

H = 15.45 DF = 5 P = 0.009

H = 15.49 DF = 5 P = 0.008 (adjusted for ties)

Specimen Number	Habitat Code	Length (cm)	Rock Cover Type
26	2	2.3	cobble
27	2	4	cobble
28	3	5.1	-
29	3	8.7	boulder
30	3	4	-
31	3	5.8	-
32	3	3.8	-
33	3	10.5	boulder
34	3	5.5	-
35	4	4.5	-
36	4	5	-
37	4	2.2	-
38	4	4.2	-
39	4	2.25	-
40	4	4.3	cobble
41	4	2	cobble
42	5	3.4	-
43	5	4	boulder
44	5	5.1	boulder
45	5	4.3	cobble
46	5	4.1	cobble
47	6	6.7	boulder
48	6	5.5	-
49	6	5.5	-
50	6	8	-

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Specimen Number	Habitat Code	Length (cm)	Rock Cover Type
1	1	3.1	-
2	1	2	-
3	1	3.2	-
4	1	2.1	-
5	1	2.7	-
6	1	4.5	-
7	1	2	-
8	1	2.3	-
9	2	2.4	-
10	2	4.2	boulder
11	2	2.7	-
12	2	2.6	-
13	2	3	-
14	2	4.5	-
15	2	2.2	boulder
16	2	6.5	boulder
17	2	2.3	-
18	2	5.5	-
19	2	4.5	boulder
20	2	5.5	-
21	2	5.25	cobble
22	2	5	cobble
23	2	5.2	cobble
24	2	5.2	-
25	2	5.75	cobble

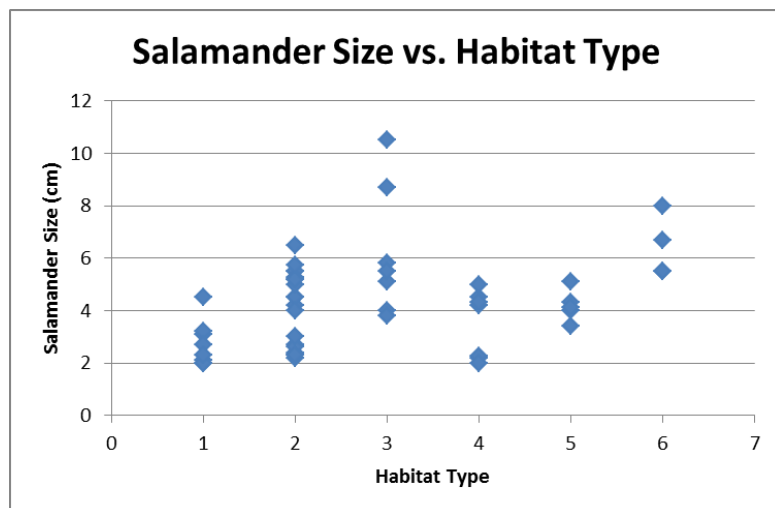


Figure 1. *Salamander Size vs. Habitat Type: Each of the fifty salamanders is plotted based on habitat code in correlation with individual size.*

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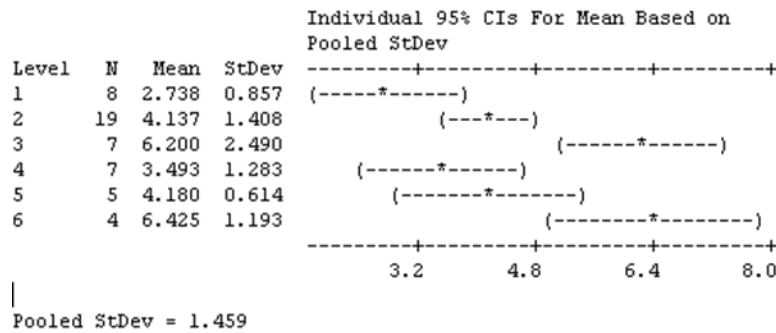


Figure 2. ANOVA one-way statistic test: Displays mean value and confidence intervals of salamander length in each habitat code. Includes all fifty salamanders.

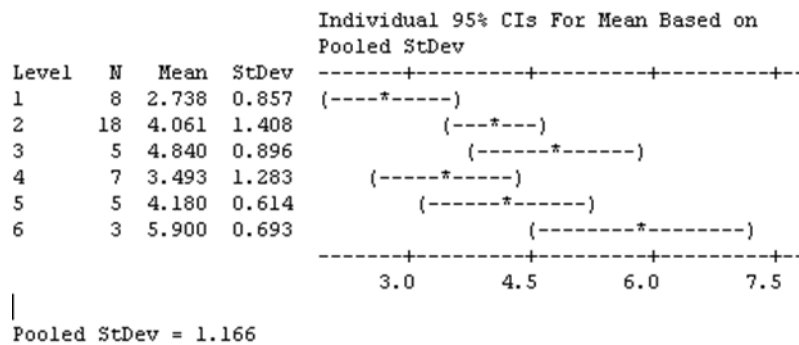


Figure 3. ANOVA one-way statistic test: Displays mean value and confidence intervals of salamander length in each habitat code but does not include four *Gyrinophilus porphyriticus* salamanders.

DISCUSSION

Table 2 confirms that the data collected on all fifty salamanders, including the Dusky salamander (*Desmognathus fuscus*) and Spring salamander (*Gyrinophilus porphyriticus*) is significant, and therefore the correlation between the length of the salamanders and the habitat in which we found them is significant. The results of our study show a definite habitat preference in the salamanders we sampled.

Although our data contains information regarding two different salamander species, we have chosen to look at the salamander species as a whole in our study. Since *Desmognathus fuscus* and *Gyrinophilus porphyriticus* have the same general body morphology, we are looking at the dynamics of the entire niche that salamanders occupy in this stream environment. It should be noted that *Gyrinophilus porphyriticus* is generally larger than *Desmognathus fuscus* and may appear as outliers in our data. However, when we removed all data points

relating to *Gyrinophilus porphyriticus* as seen in Figure 3, the same trends are observed in size vs. habitat type. Gravel (code 3) showed a large amount of change in relative mean and median values, but still remains the second highest mean value. This indicates that our data can be applied across the salamander species in our study site.

Figure 2 indicates that the smallest salamanders collected seemed to have a preference for vegetation. During the collection, these larvae were found living within large, dense clumps of *Riccia fluitans* and waterweed. This environment may have been selected by the smaller salamanders because of its greater protection from predators, such as fish, crayfish, and aquatic insects. These clumps of vegetation also provided a calm area without significant current to the smaller individuals, which may not yet be large enough to battle the stream flow. The aquatic vegetation hosts many small insects and a number of amphipods, which would be an ample food source to the smaller larvae.

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Interestingly, Figure 2 also shows the largest salamanders found were also located around vegetation, but were typically located under the clumps on a gravel substrate. This habitat preference may be due to a number of factors. As previously stated, there is a wealth of prey located within the vegetation. Salamanders sitting just below the main plant mass on gravel substrate could be foraging for amphipods, young fish, insects or possibly even smaller salamander larvae as they get washed out. The larger salamanders would have trouble moving through the dense vegetation, which is most likely why they were not found within it. Because of their size, these salamanders may be at increased risk of predation from large predators, such as herons, turtles, or snakes. Therefore, it would be in their best interest to remain concealed under the mats of plant material. We feel that the vegetation mats are the optimum habitat patches in the stream, so it is also a possibility that these large individuals, able to out compete smaller salamanders, could have and dominated these areas. Other Studies have indicated that both interspecific and intraspecific competitions are strong factors in habitat selection by salamanders (Keen, 1982) (Southerland, 1986).

In addition to our analysis of plants and substrate, we recorded which salamanders were collected under rocks. In total, 19 individuals were found under cobbles or boulders. The average size of salamanders found under rocks of any size was 4.98 cm. This was slightly larger than the average size of all fifty salamanders, which was 4.30 cm. This data becomes even more interesting when sizes of rocks are taken into account. Under cobbles, salamander length averaged 4.22 cm, slightly smaller than the overall average. However, salamanders collected under boulders averaged 5.82 cm in length. Once again, this indicates that the larger salamanders are choosing cover over being in the open. The increase in average length of the salamanders under boulders is most likely because the larger rock size allows them more range of movement while still being concealed. A study conducted by W. H. Keen on habitat selection and competition of salamanders in aquatic and terrestrial environments concluded: “*D. fuscus* was significantly influenced by the presence of the other, larger, salamander species. The largest rocks were strongly selected as cover by both species but smaller rocks were utilized more frequently by *D. fuscus*. Selecting large rocks for cover provides greater protection to adult salamanders from predators and maintain a more consistent and moist habitat (Keen, 1982).” Although this is discussing terrestrial salamanders habits, *Desmognathus fuscus* is the species that made up most of our study group.

It is possible that the same habitat preferences apply for terrestrial and aquatic environments.

Large portions of the 50 sampled individuals were found with no overhead cover in the form of rocks or vegetation. These particular individuals, 14 in total, averaged 4.00 cm in length, which is just below the average for all 50. Their individual lengths ranged from 2.2 to 5.8 cm. It is possible that these salamanders were moving between patches of vegetation or rocks, or they could have been pushed out by larger, more dominant individuals occupying the habitat patches. Another possibility is that these salamanders do not need to take cover from most predators, and therefore feel no need to spend all their time under cover. Since they fell into the mid-range of body lengths, they are too large to live within vegetation, and not large enough to be a major target of large, non-aquatic predators. We did not observe, nor did we believe, the conditions of the study site could support any fish larger than approximately 8 centimeters. We feel that fish this size would not pose a threat to a larval salamander of over 2.5 cm, and therefore the majority of these salamanders would be relatively safe without concealment.

The majority of salamanders sampled were found on a sand substrate. These individuals were also the second smallest in overall length. The portions of substrate with the most sand were located in the shallowest waters throughout the stream, and were usually without vegetation cover. A similar study stated “The largest salamander species was found in the deepest parts of stream beds while smallest salamander species was found in shallowest parts. Interspecific competition was the critical process in spacing (Keen, 1982).” We believe that this competition may be the reason so many salamanders were collected in the sandy shallows in our study as well. There were many larger salamanders, which could out compete the smaller individuals for the best habitats in this stream.

On average, the second largest salamanders were collected on a gravel substrate. The coloration of most of the salamanders collected would indicate that they are best suited for camouflage against a gravel background. The current was faster flowing over this substrate, which could be an advantage to respiration. In addition, there were generally more prey species associated with gravel than the sand substrate. The study previously mentioned also found these trends in *Desmognathus fuscus* and *Desmognathus monticola*: “A clear preference was shown for coarser substrate. *D. fuscus* (*dusky*)

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generally showed a more diverse substrate texture pattern than *D. monticola*. *D. fuscus* also selected a more diverse array of microhabitats. However, salamanders may compete for microhabitats with coarse substrate because it is a refuge from predators (Keen, 1982).” Since this is an optimum habitat within the stream, we again believe that the larger salamanders are selecting this habitat and pushing the smaller individuals out into less desirable habitats.

ACKNOWLEDGEMENTS

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THE APPLICABILITY OF OPTIMAL FORAGING THEORY TO SALAMANDERS PREDATION AND THE DEPENDENCE OF GAPE SIZE IN OPTIMAL PREDATION

Wilbert Beachy, Nathan Higgins, Kelsey Kohrs, Bradley Medellin, and Alex Raymond

ABSTRACT

This study tested the prey selection of salamanders from Warm Spring in the controlled lab environment. Based on optimal foraging theory we predicted that salamanders of all sizes should select larger *Gammarus minus* (amphipods) as prey. Results showed that in this particular lab setting optimal foraging theory was not applicable. Small salamanders were constrained to eating only small amphipods due to gape size of these salamanders. Larger salamanders showed no preference of large or small amphipods in laboratory cultures most likely due to an increased environmental stress on these salamanders.

Keywords: *Desmognathus ochrophaeus*, *Pseudotriton ruber ruber*, *Gyrinophilus porphyriticus*, *Gammarus minus*, *Optimal Foraging Theory*, *Natural Selection*

INTRODUCTION

Optimal foraging theory has been a tremendously successful theory in describing size selective predation demonstrated in many ecological communities. Central to this theory is that predators optimize their caloric intake by feeding on the largest thus most energy rich foods available while using the smallest amount of energy and time to intake this food. Natural selection therefore works on the population of prey in systems where optimal foraging theory is demonstrated because often the larger more visibly appealing prey are selected for predation. Applying this theory two local Huntington County springs, Petersburg Spring and Warm Spring, we found a prevalent macro invertebrate in these streams, *Gammarus minus*, appears to demonstrate this selective pressure. *G. minus* (amphipods) in Warm spring where salamanders, small fishes, and crayfish are abundant show a largely reduced size as compared to Petersburg spring where predators are rarely found and *G. minus* is the dominant species. Since optimal foraging theory appeared to be at work in these populations we decided to set up a predation study *in vitro* with a common predator of *G. minus*, the salamander. The salamander being a generalist

predator would be an adequate species to test the applicability of optimal foraging theory (Wilbur and Morin 1983). Several sizes of salamanders should show that optimal foraging theory holds true for all salamander species in these population in that they will actively seek out and eat the larger prey in the lab setting. As long as the salamanders have a gape size width enough to eat the large amphipods all salamanders should selectively pick the most calorically dense food. However nature may not be capable of being mirrored in the laboratory setting.

MATERIALS AND METHODS

Salamander cultures were kept in plastic containers in the cold storage control room. Lids of the cultures were kept cracked for proper gas exchange to occur. A feeding event occurred when amphipods disappeared. Dead amphipods were noted but not counted as a feeding event. Forceps, pipettes, and a separation pan were used daily to count the number of live amphipods in each culture. Size category of salamanders was determined by amount of salamanders available to conduct the study. The study used 14 salamanders and a 1 cm length gap between 3.5cm and 4.5cm separated the salamanders into

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categories. 8 salamanders were put into the less than 3.5cm category, or small salamander category. 6 salamanders were put into the greater than 4.5cm category, or large salamander category.

Field Work

The two sites chosen for data collection were Petersburg and Warm springs. Both sites had more alkaline water pHs. *G. minus* were collected at Petersburg Spring. Salamanders *Desmognathus ochrophaeus*, *Pseudotriton ruber ruber*, and *Gyrinophilus porphyriticus* were collected at Warm Spring by riffing upstream about a foot above where nets were placed. Water and vegetation were collected from both sites.

Laboratory Work

Post field work, male amphipods were separated out to reduce amphipod reproduction within the running cultures. Large and small amphipods were determined by visual inspection. Salamander size was measured from snout to tip of the tail. Cultures including vegetation, one salamander, and ten amphipods (five large and five small) were made. Cultures were replenished with fresh water every two days. Data was collected daily, missing and dead amphipods were replaced. Dead salamanders were also replaced with new size recorded.

RESULTS

Table 1. Shows a two way Chi-Square table comparing the feeding of large salamanders ranging from sizes of 4.5cm to 9cm to the feeding of small salamanders ranging in size from 2cm to 3.5 cm. Data for the large salamanders was taken from N=6 salamanders falling into the large classification. Data for small salamanders was taken from N=8 salamanders falling into the small salamander classification. Chi-square shows the difference in feeding activity of the two size ranges of salamanders on either large or small amphipods. The numbers in the rows with the salamander category are the observed feeding counts on either large or small amphipods. Numbers below the observed counts represent contribution of each value to the Chi-square value. Chi-square value is 10.935 from 1 degree of freedom and the p-value for the counts observed is 0.001.

	Large Amphipod	Small Amphipod	Total
Small Salamander	0	12	12
	4.85	7.15	
	4.851	3.292	
Large Salamander	19	16	35
	14.15	20.85	
	1.663	1.129	
Total	19	28	47

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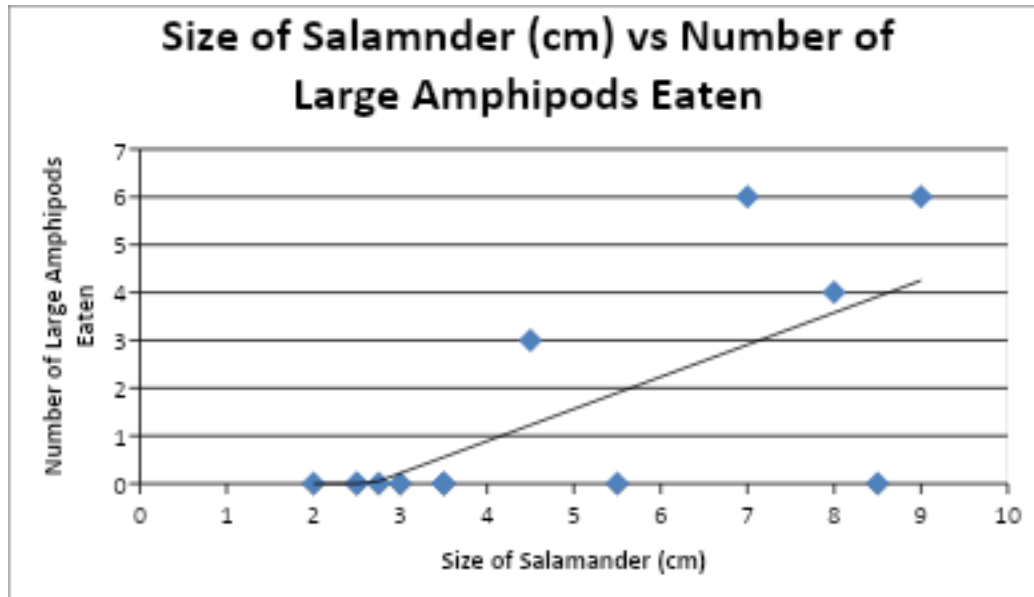


Figure 1. The graph shows the number of the large sized amphipods versus the size of each salamander. The body size of each salamander is recorded in centimeters. The data points represent the total number of small amphipods eaten during the study. The graph shows a positive correlation with more amphipods of the large size being eaten by the larger salamanders. The R squared value shows the goodness of fit for the line considering the points. The p -value of the regression is 0.107.

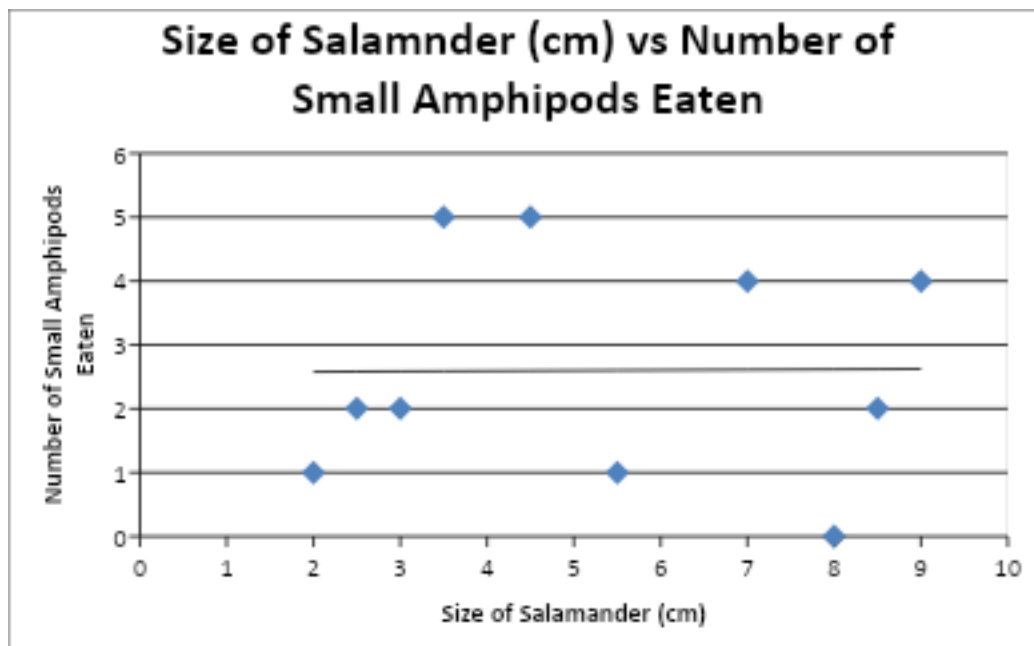


Figure 2. The graph shows the number of the small sized amphipods versus the size of each salamander. The body size of each salamander is recorded in centimeters. The data points represent the total number of small amphipods eaten during the study. The graph shows no correlation with small amphipods compared to size of salamanders. The R squared value is close to zero so points are essentially random. The p -value of the regression is 0.399.

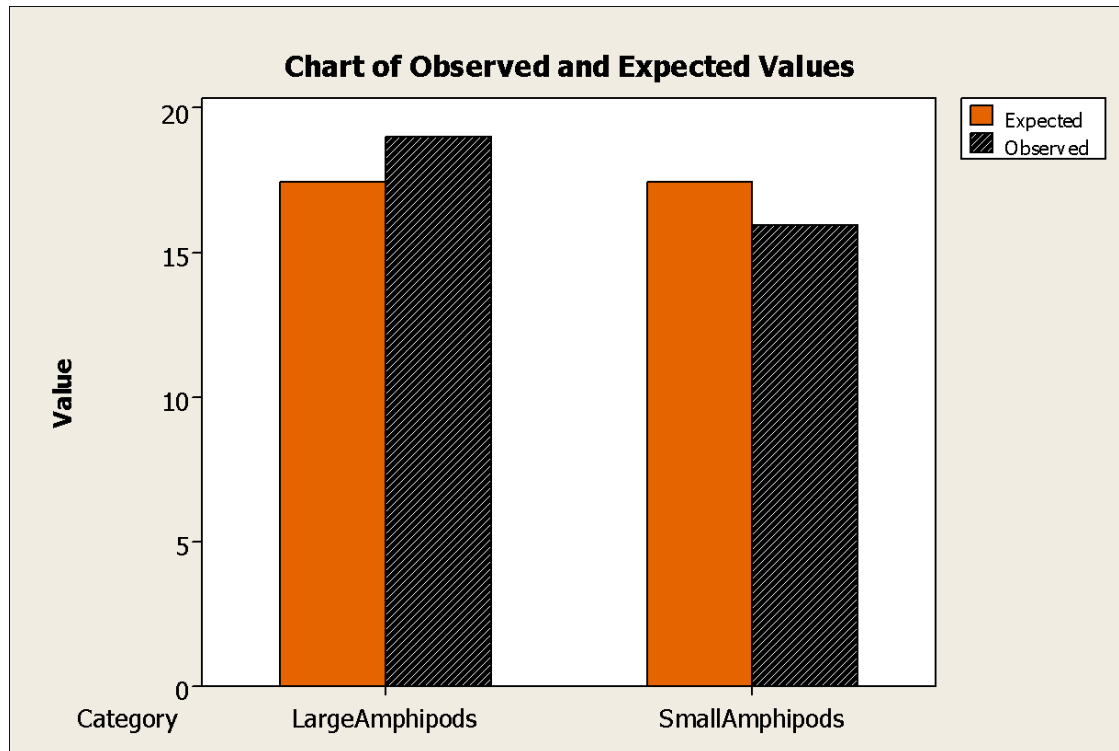


Figure 3. The Chi-square goodness of fit test shows the difference between the large and small amphipods eaten by salamanders of the large size classification. Solid orange bars represent the expected value of the null hypothesis that no difference in the size of amphipods eaten will be observed and both sizes will be eaten at equal proportions by large sized salamanders. The striped blue bar show the observed counts of large amphipods eaten $N=19$ and small amphipods eaten $N=16$ for the large sized salamanders. P -value of 0.612 shows no significant difference between the observed and expected counts.

The Chi-square test in Table 1 showed with a p -value of 0.001 that the feeding events observed with small salamanders significantly differed from the feeding events observed with large salamanders. The table shows the result that no salamanders in the small classification ate any of the large amphipods whereas the salamanders in the large classification had a relatively even spread of large and small amphipods taken.

The regression between the number of large amphipods eaten and size of salamanders shows a positive correlation with more amphipods being eaten by the larger salamanders. The p -value of 0.107 is suggestive of a correlation; however it is not significant and thus the positive correlation may be due to randomness.

The regression between the number of small amphipods eaten and the size of salamanders shows no correlation and an almost completely flat line. The r squared value of this graph is minimal at 0.00009 showing that the large variation between the points and the line associated with them. Being so close to

zero the effects of salamander size on the predation of small amphipods appears to be random. With a p -value of 0.399 the correlation shows no significant and is assumed to be due to randomness.

Since no observed counts of small salamanders eating larger amphipods were present we deemed it unnecessary to conduct a Chi-square goodness of fit test on the difference between the two types of amphipods eaten for the small salamander group. However the Chi-square goodness of fit test for the difference between the sizes of amphipods eaten by the large classification of salamander is shown above. The p -value of 0.612 shows no significant difference between the large and small amphipods eaten in the cultures. This suggests that the null hypothesis that the feeding behavior of the salamander is random is confirmed.

DISCUSSION

The findings previously displayed produce counterintuitive results as to how our experiment was designed. The study produced results which appeared

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to be trending toward the selection of small amphipods in the laboratory study of salamander predation. This does not match the hypothesis that all sizes of salamanders would select the bigger amphipods due to optimal foraging theory's prediction that predators will select prey with a larger caloric value in order to maximize their resource intake. The hypothesis was ground on the assumption that in culture the prey would be selected by salamanders visually selecting the preferable prey. We account for this difference first, by consulting studies done on the biological limitation of gape size in small animals (Schmitt and Holbrook 1984).

Salamanders in the small size category did not eat a single large amphipod for the entirety of the study this must be due to a biological limitation of the salamanders gape width at length sizes below 3.5 cm. The salamanders in the small size classification were limited to prey that the salamanders could physically open their mouths wide enough to eat (Schmitt and Holbrook 1984). As the size of the salamanders became larger more of the larger amphipods were eaten. Salamanders at larger sizes showed that amphipods could be potential food when they were encountered. This biological limitation of salamander is a reason for the suggestive correlation in Figure 2. Gape size therefore has a large contribution to the chi-square values observed in the differences in feeding habits of large and small salamanders on different sized amphipods. The significant difference observed in Table 1 between the sizes of salamanders thus is partially due to the gape size limitation.

We then expected a negative correlation with Figure 2 for smaller amphipods eaten versus the size of salamander. We based this idea on the fact that the small salamanders were only capable of eating smaller amphipods, while larger salamanders should theoretically select larger amphipods, based on the biological ability of these salamanders and optimal foraging theory (Wilbur and Morin 1983). However, we saw no correlation between the size of salamander and how many small amphipods these salamanders ate. This suggests that the larger salamanders were eating smaller amphipods just as much as the smaller salamanders.

Although the large salamanders did eat large amphipods they did not appear to have a selective preference between the two. Figure 3 showed that the large salamanders contrary to optimal foraging theory were not selecting an optimal diet. In other words in our lab setting optimal foraging theory was apparently not at work, though in the actual springs the large size of *G. minus* in the spring without predators suggested

that selective predation was occurring. The difference between the predation behaviors of salamanders *in vitro* versus predation stresses on *G. minus* in its natural habitat raised the issue of how effective our lab culture was at emulating the salamanders' natural environment.

We expect that this is due to the environmental stress that the salamanders were under in culture. Environmental stress has been shown to reduce the predation selectivity in a generalist predator like the aquatic salamander (Maiorana 1976). Although the lids of all cultures were cracked for gas exchange the cultures were not aerated and thus didn't have a normal amount of oxygen to sustain normal spring qualities. Lower oxygen levels decrease the metabolic rate of salamanders and increases the amount of environmental stress that these salamanders experience. Along with this lack of oxygen, were not exposed to sunlight, though the water temperatures were kept relatively consistent and new water was added to the cultures regularly. Environmental stress of the culture in the later days of the study was apparent when several cultures began to die. Either outside contamination or the death within the cultures caused two of our laboratory cultures to completely die during the last two days of data taking. The total deaths of all large and small amphipods as well as the salamanders in these cultures are indicative of the severe environmental change these organisms experienced between the lab and the natural spring habitat.

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EFFECTS OF WATER TEMPERATURE ON EGG DEVELOPMENT IN WOOD FROGS (*RANA SYLVATICA*) AND SPRING PEEPERS (*PSEUDACRIS CRUCIFER*)

Zachary Bordner, Bridget Wade, and Hannah Long

ABSTRACT

Temperature is an extremely important factor affecting the rate of metabolism, particularly in ectotherms. The objective of this experiment was to assess the effects of temperature on the larval development of the spring peeper, *Pseudacris crucifer*, and the wood frog, *Rana sylvatica*. Establishing the relationship between temperature and amphibian developmental growth is an integral component in understanding the influence climate change could potentially have on our environment. The developmental stages were recorded at 24 hour intervals for 32 days at three temperatures 4°C, 10°C, and 20°C. The recorded data established a rate of development which confirmed our hypothesis that the 20°C group would develop most rapidly followed by the 10°C group.

Key words: Wood Frog, temperature, eggs, development, *Rana sylvatica*, *Pseudacris crucifer*, spring peeper

INTRODUCTION

The beginning of a Pennsylvania spring is audibly established by the deafening sound of spring peepers in early March. As soon as night temperatures consistently reach 4.4°C (40°F) and the winter ice thaws, the peepers emerge from hibernation and migrate to vernal ponds where they proceed to find mates. While spring peepers, *P. crucifer*, are the most noticeably abundant at this time due to their outstanding noise, *R. sylvatica*, the wood frog, also comes out of hibernation in early spring in order to mate.

Although adult *R. sylvatica* are on average at least 25% larger than *P. crucifer*, and their tadpoles are significantly larger as well, fertilized eggs of both species are roughly the same size; clear differentiation only occurs about two week after the tadpoles have hatched. The biggest distinction between *R. sylvatica* and *P. crucifer* tadpoles is size and prevalence of gold mottling.

Since both *R. sylvatica* and *P. crucifer* are ectotherms, temperature plays an important role in determining rate of development since, more than in endotherms, it has a greater effect on metabolic

processes. Metabolic rate is generally exponential and a 10°C increase in temperature approximately doubles metabolic rate (Porter, 1972). Tadpole tissue is known to be unresponsive to thyroid hormone at temperatures lower than 10°C (Porter, 1972). Additionally, 3,5-3'-triiodo-L-thyronine has significantly reduced effects on tail tissue at temperatures less than 20°C and no effect below 5°C (Porter, 1972). From fertilization to hatching, temperature exclusively affects development. Upon hatching, larval development is influenced by population density and food availability as well as temperature (Herreid & Kinney 1967)

FIELD SITE

Egg masses were collected at the Fouses Crossing Wetland Area (40°21'48.62 N 78°10'29.22W), located adjacent to Raystown Lake. The 3,000 acre area is managed by the Pennsylvania Game Commission as part of a Wildlife Mitigation Project. We chose this location for its suitable habitat for amphibian species.

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METHODS AND MATERIALS

Six egg masses (3 *R. sylvetica* and 3 *P. crucifer*) were collected from Fouses Crossing Wetland Area. All egg masses were then kept in 10°C (50°F) for 24 hours. Each mass was then divided into thirds, which were then placed under varying climatic conditions. Using environmental control chambers set to 4, 10, and 20 degrees Celcius, six samples were exposed to each temperature for 32 days. Eggs were contained in plastic lidded vessels and once hatched, tadpoles were provided with a constant food supply (boiled lettuce). Tanks were cleaned and filled with clean water as needed. Multiple randomly selected individuals from each sub-group were examined daily and assigned a stage of development based on various physical and functional milestones outlined in a 1972 publication by Porter. Both microscope and naked eye observations were utilized.

RESULTS

The rates of growth demonstrated by each temperature group proved to have significantly different slopes (Figures 1-3). The 4°C group exhibited a mean value of 15, with the 10°C group only slightly higher at 17. The 20°C degree groups had a significantly higher mean stage value at 27 (Figure 5). A one-way ANOVA test yielded a p-value of $<2e-16$. Figure 4 shows significantly varied progressions among the three temperature groups. The mean starting stage for all temperature groups was 13.83. The 4°C cultures progressed an average of 2.67 stages over the 32 day study. The 10°C cultures progressed an average of 6.47 stages and the 20°C groups advanced a total average of 17.5 stages.

Cultures at 4°C

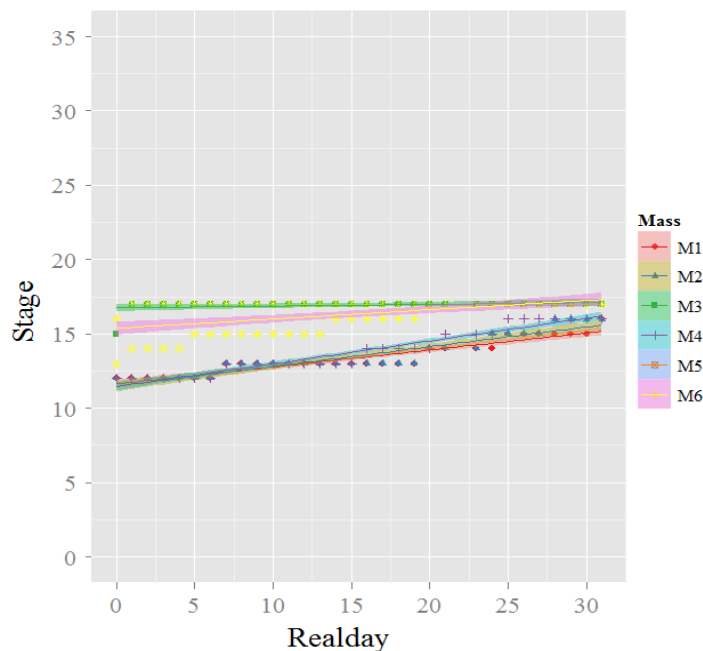


Figure 1. Growth rate for six egg masses cultured at 4°C. A linear regression model yielded a p value of $2.06e-10$ indicating that data is significant. RESET and Shapiro-Wilk tests show that the regression model has both a linear and normal distribution.

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Cultures at 10°C

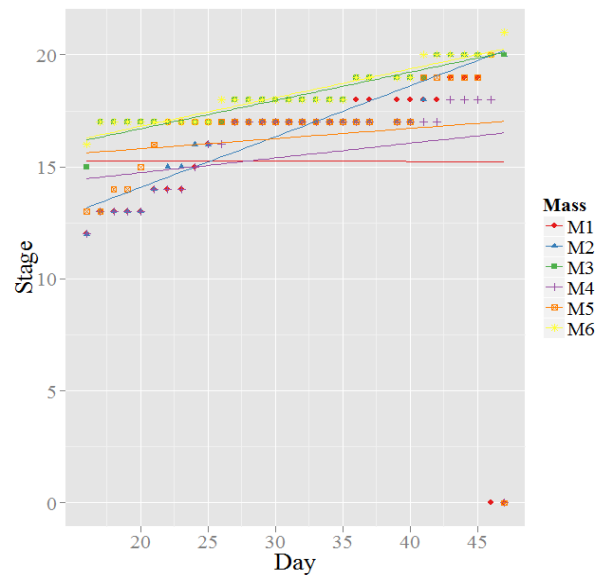


Figure 2. Growth rate for six egg masses cultured at 10°C. A linear regression model yielded a p value of $5.38e-05$ indicating that data is significant. RESET and Shapiro-Wilk tests show that the regression model is non-linear and normal in distribution.

Cultures at 20°C

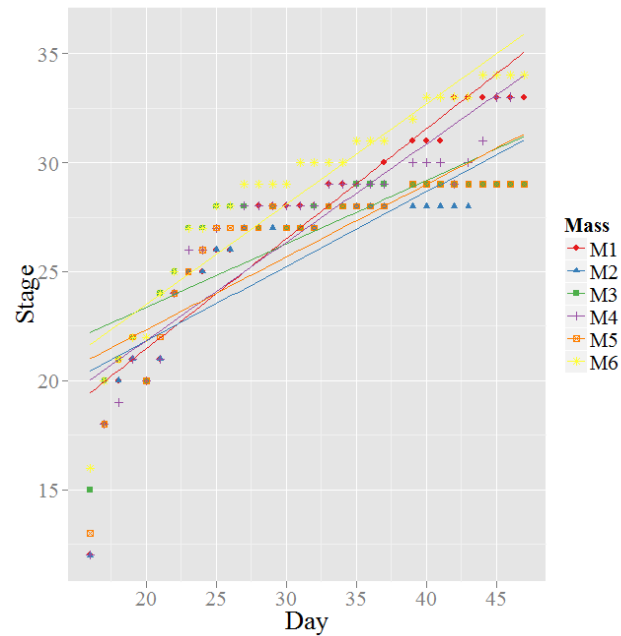


Figure 3. Growth rate for six egg masses cultured at 20°C. A linear regression model yielded a p value of $<2e-16$ indicating that data is significant. RESET and Shapiro-Wilk tests show that the regression model is non-linear and normal in distribution.

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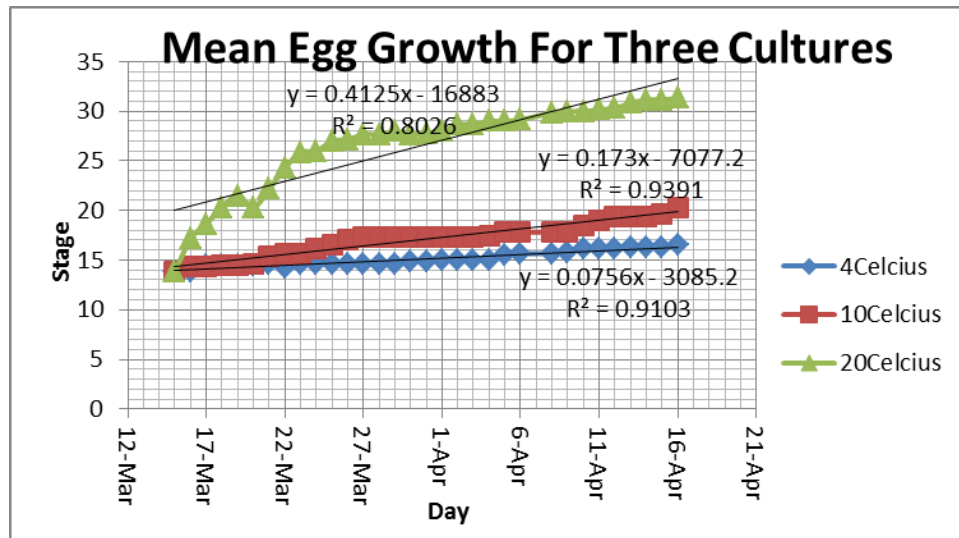


Figure 4. Mean daily larval growth for 4°C, 10°C, and 20°C cultures. All R^2 values approach 1 indicating that the lines fit the original data points well.

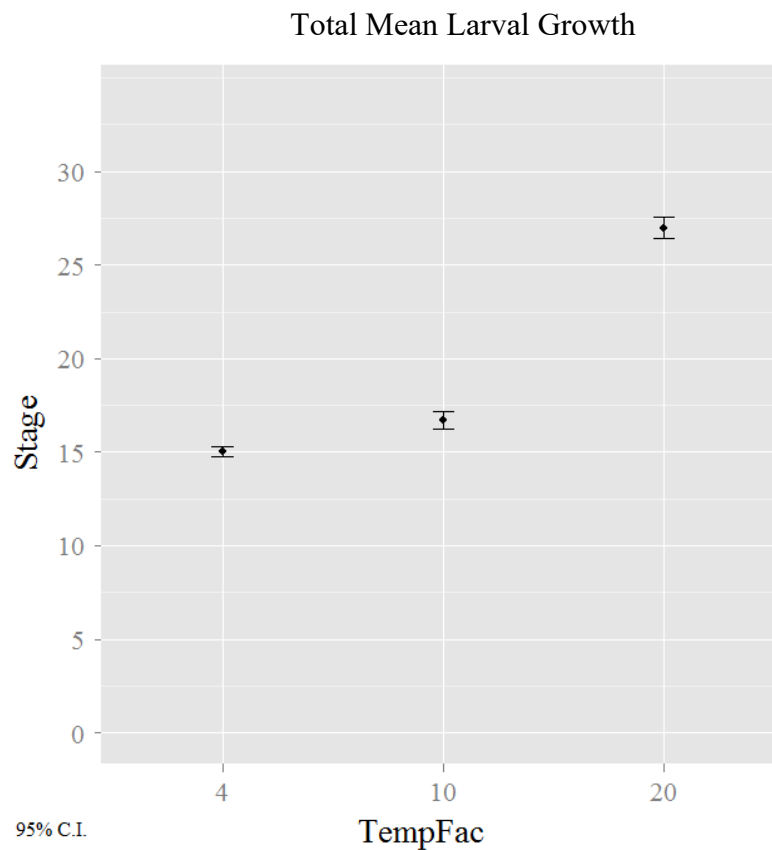


Figure 5. Total mean developmental stages plotted for each temperature. A one-way ANOVA test yielded a p value of $<2e-16$ indicating that the data is significant.

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DISCUSSION

The data collected from this study indicates that temperature significantly influences the developmental growth in *R. sylvatica* and *P. crucifer*. A comparison of the data from each temperature group manifests obvious differences in development with the 4°C being the most inhibitory, suppressing all development beyond the formation of a tail bud, and 20°C encouraging the most development, allowing for the development of hind limb buds within 3 weeks.

The data also suggests that *R. sylvatica* develops at a faster rate than does *P. crucifer* with noticeable advanced development in all *R. sylvatica* subgroups within the 20°C group. However, this difference could be due to the lower density of tadpoles in the three containers with *R. sylvatica*. High densities and crowding are well documented in their inhibiting effects on tadpole development (Porter, 1972). The low density of *R. sylvatica* in these containers was due to large die-offs within the first week of hatching which reduced all subgroup populations of *R. sylvatica* within the 20°C group to fewer than 20 individuals. The subgroup populations of *P. crucifer* at 20°C remained around 100 individuals throughout the experiment. We concluded that these die-offs most likely occurred due to crowding and low dissolved oxygen caused by the decomposing feces in the containers. We assume that the main reason that *P. crucifer* was not affected by crowding is that it is able to live at higher densities and can survive in water with low dissolved oxygen whereas *R. sylvatica* is extremely sensitive to these factors. Because of this distinction, using larger containers could have improved the survival rate of our test subjects and potentially could have positively influenced the rate of development.

The disparity in population size between our two species also affected our daily sampling to determine developmental stage. While we consistently sampled multiple individuals each day, the reduced number of *R. sylvatica* in each container meant that we were likely sampling the same individuals more frequently. Because the die-off of *R. sylvatica* only occurred in the 20°C group, this problem did not alter our results for the 10°C and 4°C groups. The developmental staging of these lower temperature groups did, however, produce complications due to the fact that staging had to be

done with the whole egg mass for the majority of our experiment since no tadpoles hatched in any of 4°C and only a few tadpoles managed to hatch and survive in the 10°C group. The main complication that accompanied working with the entire mass was the difficulty in handling and clearly observing minute changes in individual eggs. Additionally, early stages of development, especially those occurring at 4°C, potentially went undetected due to their small size.

Our data is significant due to the implications it has on the life history of amphibious indicator species. While conducting our experiment at constant temperatures is unlike an environment that these amphibians would experience in wild, understanding these rates of change is important in modeling the effects that certain temperatures will have on the environment. This data can also be used to help predict how the dynamic between organisms within a certain environment will develop. With this data we can also more effectively monitor the status of wild local populations by applying the known rate of development, with consideration to natural daily temperature fluctuation, to wild populations in order to evaluate developmental health and potentially identify developmentally inhibiting anthropogenic factors.

ACKNOWLEDGEMENTS

We would like to thank Dr. Doug Glazier for his mentoring guidance which gave our research project direction. He provided the necessary materials and resources needed to complete our data collection and analysis. We would also like to acknowledge Dr. John Matter for providing knowledge and resources related to amphibian development. Dr. Neil Pelkey's statistical expertise was also invaluable to our research.

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BRYOPHYTE RICHNESS, DIVERSITY AND ABUNDANCE IN RELATION TO SOIL AND WATER PH

Gabrielle Cannon, Mollie Lewis, and Steph Macri

ABSTRACT

In stream ecology, bryophytes such as mosses and liverworts are commonly used as bioindicators due to their sensitivity to changes in pH levels and other environmental factors. A relative dearth of bryophytes could signal of a polluted stream, possibly indicating a danger to other species. In a comparative study, we collected bryophyte samples from four local freshwater streams in Huntingdon County, Pennsylvania. To achieve an even data collection, two of the chosen streams were known to be acidic, and the other two alkaline. Along with samples, bryophyte coverage was recorded in randomly selected plots along and in each of the four springs. Soil and water samples were also collected. We hypothesized bryophyte diversity and abundance would decrease with decreasing soil and water pH. Statistical analyses determined that soil pH is strongly negatively correlated with species richness ($P = 0.024$, Coefficient = -2.90 , $R\text{-sq} = 95.2\%$), but no other correlations were found. Further study with larger sample sizes and more spring locations is recommended.

Keywords: bryophytes, soil pH, water chemistry, Simpson index, freshwater springs, bioindicators

INTRODUCTION

Non-vascular land plants include mosses, liverworts and hornworts and are collectively referred to as bryophytes. Because they are not capable of effectively transporting water through their tissues, bryophytes must grow low to the ground and their distribution is mostly confined to moist areas. Lack of both vascular tissue and waxy cuticles make bryophytes useful bioindicators, especially with regard to air quality and mineral monitoring (Glime 2007). Bryophytes are important ecologically because they reduce soil erosion and provide shelter where macroinvertebrates are safe from desiccation (Australian National Herbarium 2012).

The environmental significance and usefulness of bryophytes warrants extensive research. Despite this, bryophytes have garnered relatively little scholarly interest (Munch 2006). However, if bryophytes are to be practical bioindicators, their ecology must be studied thoroughly. J.M. Glime (2007), Vellak *et al* (2003) and others have shown that bryophyte diversity and abundance can be affected by humidity, surrounding vegetation, stream current velocity and many other factors. Bryophytes are most

commonly studied in relation to pH levels, as it has been suggested that non-vascular plants have narrower pH amplitude than vascular plants (R. Virtanen 2000). The influence of these environmental factors must be accounted for when using bryophytes as bioindicators; in order to fully understand the effects of a variable of interest such as a pollutant, natural variation due to environmental conditions must be considered as well.

This study examined the effects of both soil and water pH on richness, diversity and abundance of bryophyte species at two acidic and two alkaline springs in central Pennsylvania. Because bryophytes absorb their water and nutrients directly from the soil or water they are in, we hypothesized that bryophyte diversity and richness would decrease with decreasing pH. We believed low pH would lead to a direct decrease in abundance and diversity by damaging tissues and indirectly through leaching necessary nutrients from the soil. The findings of this study are significant because they provide insight into bryophyte ecology and can be applied to their use as bioindicators.

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FIELD SITES

The study was conducted at four freshwater spring locations in central Pennsylvania: Cold Spring, Dubbel Spring, Petersburg Spring and Blue Spring.

All springs were shallow and at least partially surrounded by forest. Blue Spring was unique in that it was largely surrounded by grass. Table 1, below, summarizes chemical and physical data collected in February, 2012.

TABLE 1. Summary of chemical and physical parameters at four freshwater springs in Huntingdon County, Pennsylvania: Cold, Dubbel, Blue and Petersburg. Data collected in February of 2012.

		Acidic		Alkaline	
		Cold	Dubbel	Blue	Petersburg
Chemical	Temperature (°C)	10.4	9.6	9.9	10.3
	pH	5.04	4.75	7.43	6.63
	Conductivity (microsiemens)	37.2	32.5	354.6	106.4
	Dissolved oxygen (ppm)		9.62	1.02	0.1
Physical	Width (m)	1.52	1.98	2.48	2.18
	Depth (cm)	6.20	16.81	23	10.76
	Pond present	No	Yes	Yes	Yes
	Flow rate (m/sec)	0.236	.11	0.28	0.94
	Discharge (m ³ /sec)	0.40	3.63	2.1	1.176
	Substrate (%)	Boulders	30	5	10
		Cobbles	10	20	15
		Gravel	25	15	30
		Sand	5	40	50
		Silt	30	20	0
	Macrophytic coverage	Grass, moss	Ferns, moss	Grass, moss	Moss, vines
	Springside vegetation	Wooded area; heavy leaf cover, fallen logs	Wooded area	Grass and moss	Shrubs and bushes; Trees further out
	Macrofauna		Turkeys, salamanders	Slimy skulpin, rainbow trout	

METHODS AND MATERIALS

General observations such as date, time of day, temperature and surrounding flora & fauna were recorded at each spring location. A random number generator was used to determine ten length and width coordinate pairs within the study area. Meter quadrats were placed accordingly, using the coordinate pairs as center points. A pH meter was used to record the pH of three randomly generated locations within the spring study area.

Bryophyte Sampling & Identification

Individual bryophyte species coverage was estimated to the nearest 5% for each quadrat. If a species was present but extremely rare, it was recorded as 1%. Pinch-sized samples of each species were collected and stored in labeled plastic cups. All samples were stored in a dark refrigerator. Identification was determined to at least genus level using Susan Munch's *Outstanding Mosses & Liverworts of Pennsylvania & Nearby States* and A.J. Grout's *Mosses with a Hand-Lens*. All identification

was completed within two weeks of sample collection.

Soil Sampling & Evaluation

Small, consistent amounts (roughly 5 g) of soil from each quadrat were collected in a single plastic sample cup for each spring. Soil samples were stored in the refrigerator for short periods of time (< 24 hours) to avoid significant alteration of the chemical composition due to microbial/biological processes. Soil samples were stirred well to homogenize the mixture. 10 g of the stirred soil was mixed with 20 mL distilled water and sifted until only very fine particulate remained. The pH of the resulting liquid was measured using a standardized pH meter. This process was repeated twice more in order to obtain a representative mean soil pH for each spring.

Statistical Analysis

To link soil and water pH with species richness, diversity and abundance, various statistical techniques were used. Species richness was simply recorded at each location. Each spring's bryophyte diversity was determined using the inverse Simpson's

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Biodiversity Index. Mean overall abundance was determined by taking the average of each spring's ten quadrats' total percent bryophyte coverage

Regression analysis was used to determine overall correlation between the soil pH and water pH with species richness, diversity and abundance. Data from all four locations was included. Regression analysis was conducted using Minitab software. Richness, diversity and abundance were

independently analyzed as the response variables to both soil pH and water pH.

To correlate richness, diversity and abundance with spring location, Kruskal-Wallis tests were performed. Minitab software was used to plot each quadrat's richness, diversity and abundance against its location. This time, the normal Simpson's Index was used instead of its inverse.

RESULTS

Table 2, left, summarizes the results of bryophyte sampling and identification. Two samples were unable to be identified; all others have been identified to at least genus-level. 24 taxa were identified in total. Dubbel Spring was the most species rich and Blue Spring had the fewest species.

Taxon	Acidic		Alkaline	
	Cold	Dubbel	Petersburg	Blue
Brachythecium rivulare				X
Bryoandersonia illicebra		X		
Catherinea undulata		X		
Climacium				X
Fissidens	X			
Hedwigia 1	X			
Hedwigia 2			X	
Hedwigia 3	X			
Hedwigia 4				X
Hedwigia 5		X		X
Leucobryum		X		
Mnium		X		
Mnium cuspidatum		X		X
Nowellia curviflora			X	
Porella		X		
Scapania 1	X			
Scapania 2			X	
Sphagnum 1		X		X
Sphagnum 2		X		
Sphagnum 3		X		
Thuidium	X			
Thuidium delicatulum		X		
Unknown 1	X		X	
Unknown 2				X
Total species	6	11	4	7
Unique species	5	8	3	4

Table 3, right, summarizes basic environmental and statistical variables. Only the mean values are reported.

Variable	Acidic		Alkaline	
	Cold	Dubbel	Blue	Petersburg
Soil pH	6.52	4.86	7.15	6.61
Water pH	5.04	4.75	7.43	6.63
Species Richness	6	11	4	7
Inverse Simpson's Index	2.125	6.052	3.732	2.116
Equitability	0.354	0.55	0.466	0.529
Overall Abundance (% coverage)	21	24.44	67	12.3

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Regression analysis of between-site correlations of soil and water pH with diversity, richness and abundance determined that the only statistically significant correlation was soil pH with species richness. There was a strong negative correlation of soil pH with species richness ($P=0.024$, Coefficient=-2.90, $R\text{-sq}=95.2\%$). Its regression equation is:

$$\text{Richness} = 25.2 - 2.90 \times \text{Soil pH}$$

Kruskal-Wallis one-way analysis of spring location with species diversity, richness and abundance determined no significant correlation except for spring location with abundance ($P=0.001$). Mood's Median test of correlation between spring location and bryophyte abundance, however, did not conclude statistical significance ($P=0.083$). The results of these tests are shown below:

Kruskal-Wallis Test: Abundance versus Location					
Kruskal-Wallis Test on Abundance					
Location	N	Median	Ave Rank	Z	
1	10	20.000	21.4	0.30	
2	10	15.000	17.4	-0.95	
3	10	62.500	31.8	3.51	
4	10	8.500	11.4	-2.86	
Overall	40		20.5		
H = 16.13 DF = 3 P = 0.001					
H = 16.35 DF = 3 P = 0.001					
(adjusted for ties)					

FIGURE 1. Kruskal-Wallis Test on Bryophyte Abundance vs. Spring Location.
Location 1 = Cold Spring, 2 = Dubbel, 3 = Blue, 4 = Petersburg.
 $P = 0.001$ = Significant

Mood Median Test: Location versus Abundance					
Mood median test for Location					
Chi-Square = 12.57 DF = 7 P = 0.083					
Abundance	N<=	N>	Median	Q3-Q1	Individual 95.0% CIs
0	1	1	3.00	2.00	(-----*-----)
5	1	2	4.00	2.00	(-----*-----)
6	0	1	4.00	*	
7	0	1	4.00	*	
10	3	4	3.00	2.00	(-----*-----)
15	4	0	1.50	1.00	(----*----)
20	6	1	1.00	1.00	*-----)
30	3	1	1.00	2.25	*-----)
40	0	2	3.50	1.00	(----*----)
50	0	1	3.00	*	
55	1	0	2.00	*	
60	0	1	3.00	*	
65	0	1	3.00	*	
70	0	1	3.00	*	
85	1	0	2.00	*	
100	0	2	3.00	0.00	*
155	0	1	3.00	*	
Overall median = 2.50					
* NOTE * Levels with < 6 observations have confidence < 95.0%					

FIGURE 2. Mood Median Test on Bryophyte Abundance vs. Spring Location.
Location 1 = Cold Spring, 2 = Dubbel, 3 = Blue, 4 = Petersburg.
 $P = 0.083$ = Not Significant.

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DISCUSSION

Our Kruskal-Wallis findings indicate a negative correlation between pH and species richness; this suggests that as pH decreases, species richness of bryophytes should increase. This disproves both our initial hypothesis of a positive correlation and also our null hypothesis of no correlation. Our data may have been skewed by a disproportionately high number of species which prefer acidic environments, such as *Sphagnum* and *Mnium*. The correlation is likely to be reliable only within certain limitations; at some point the acidity will become less tolerable to bryophytes, and the relationship will break down.

Our Mood-Median test indicates from our results that there is no clear correlation between pH and abundance. Additional data would be needed to better analyze the potential for a relationship, as our sample sources were too few to provide a meaningful context.

Equipment malfunctions were a possible source of error: our pH meters sometimes gave inconsistent results during testing and had to be repeatedly recalibrated. We did not test for presence of contaminants in our springs, so that is another potential source of error. Mineral content was not taken into account, and may have contributed in ways we did not foresee.

Our findings are consistent with the recorded pH preferences of our moss samples where listed in *Outstanding Mosses & Liverworts of Pennsylvania and Nearby States*, but not with the findings of our sources (Vellak 2003, Virtanen 2000), which indicate a positive correlation between pH and species richness.

More sampling locations would greatly increase the accuracy of our results. We would suggest sampling from at least four additional springs, and if possible, from springs with higher pH levels. Another further topic for future research would be the comparison of preferences between mosses and liverworts, both of which are found within our sample springs.

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We thank Dr. Douglas Glazier for his guidance, assistance and editing and Professor Chris Grant for supplying us with Susan Munch's *Outstanding Mosses & Liverworts of Pennsylvania and Nearby States*. We also appreciate the General Ecology teaching assistants for their helpfulness with furnishing supplies and giving directions to our field sites.

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DIATOM SPECIES RICHNESS AND DIVERSITY IN ALKALINE AND ACIDIC SPRINGS

Breanne Hileman, Amanda Peretin, Aubrey Treese, and Melinda Wilk

ABSTRACT

This comparative study was performed to determine the difference in diatoms species richness and diversity between an acidic spring and an alkaline spring in Huntingdon, Pennsylvania. Our hypothesis that there will be an increased species richness and abundance of diatoms in Warm Spring (alkaline) compared to Dubbel Spring (acidic), due to increased H^+ concentrations in the water of the acidic spring, was rejected. However, the null hypothesis of no correlation between spring pH and species diversity was not accepted either. The hypothesis was rejected due to a p -value > 0.05 ($F_{1,42} = 0.41$, ns), calculated from the ANOVA statistical test performed on the raw data of the species diversity of Dubbel and Warm Springs. This indicates that the difference in species diversity between the two springs was not statistically significant. Species richness and diversity tests did show a slightly higher value for Warm Spring however, which corresponds to the results from other diatom studies, such as the one performed by Yangdong et al. The role of diatoms as biological indicators of environmental conditions emphasizes the importance of diatom studies.

Keywords: diatom, diversity, pH, spring, water quality

INTRODUCTION

Diatoms are microscopic algae and are a common type of phytoplankton. Freshwater systems as well as marine environments are composed of various species of diatoms. Silicon is the major limiting nutrient of diatoms because it is essential in the production of the cell wall or frustule. Diatoms are classified by the intricate siliceous structures in their cell walls and they are often grouped according to morphology (Hildebrand et. al 200). Diatoms are commonly used as biological indicators of environmental conditions such as stream health and many other complex environmental factors incorporated into an ecosystem. Diatoms are a useful indicator of stream health because they are very sensitive to changes in particular variables in an environment such as climate, geology, pH, nutrients, temperature, and toxic substances. Diatoms develop distinct physiological and morphological

characteristics that allow them to thrive in these diverse conditions. The special structure and morphology of diatoms adapted to these environments are the reason that there are over 10,000 species of diatoms living in streams, rivers, oceans, lakes, and wetlands (Stoermer, 1999). We decided to test the pH as a variable affecting the species richness and diversity of diatoms in Huntingdon, PA. The two springs observed in this study were Warm Spring, which is alkaline and Dubbel Spring, which is acidic. In a study performed by Yangdong et al, 49 water samples in the Mid-Atlantic Highlands Region of the United States were taken to observe any correlations between the diatom species and the difference in water chemistry parameters. The distribution of diatom species correlated with many conditions of the observed streams in this region. pH models in their experiment provided the highest predictability of all of the regression and calibration models. The study concluded that out of all the environmental characteristics, pH was the most important

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environmental factor that determined the species composition of diatom communities. We decided to further this study and determine whether or not there is a greater species richness and diversity in one spring over another. After previous experience with acidic and alkaline springs, we formed the hypothesis that there will be increased species richness and diversity of diatoms in Warm Spring (alkaline) compared to Dubbel Spring (acidic), due to increased H⁺ concentrations in the water of the acidic spring, which creates a harsher environment.

FIELD SITE

The two springs used in this study were Dubbel Spring, an acidic spring, and Warm Spring, an alkaline spring, located in Huntingdon, Pennsylvania. These springs are located within a few miles of each other and are surrounded by a wooded area. Dubbel Spring has a soil streambed, while Warm Spring's streambed is composed of sand and rocks. Warm Spring has a more diverse marine life than Dubbel, including crayfish, salamanders, newts, and amphipods. Both springs are surrounded by and contain various forms of vegetation, such as moss, skunk cabbage plants, and mats of clover. Warm Spring is 7.6 to 12.7 cm deep, with the depth varying marginally throughout the entire length of the spring. Dubbel Spring has a mean depth of 16.81 cm, with depths ranging from 2 cm to 54 cm. The pH in Dubbel

Spring was approximately 5.87, while the pH in Warm Spring was 7.10 the day of sampling.

MATERIALS AND METHODS

Three microscope slides were placed on individual 7 x 7 inch clay plates. Four clay plates were placed in random locations in Dubbel and Warm Spring, and labeled 1-4 based on their locations in each spring (Figures 1 and 2). The location of the clay plates was determined using random sampling by dividing the springs into a grid scale and using numbers from a random numbers table. The slides were left on the clay plates to collect diatoms for five days. After the slides were collected, they had coverslips placed on them and were stored in closed Tupperware for one week before observations were made. We used a Scion microscope at 40x magnification to make the observations for each slide. This microscope allowed us to capture images of the diatoms observed on the slides. These pictures were later edited using ImageJ software. We performed three random counts for each slide by having one member arbitrarily move the slide around on the microscope stage using the knobs, while another member arbitrarily yelled stop. We would then look at the computer screen field of view to count the total number of diatoms present, indicating how many of each taxon was present. The field of view of the computer was approximately one-fourth the size of the microscope's field of view.

Dubbe Spring

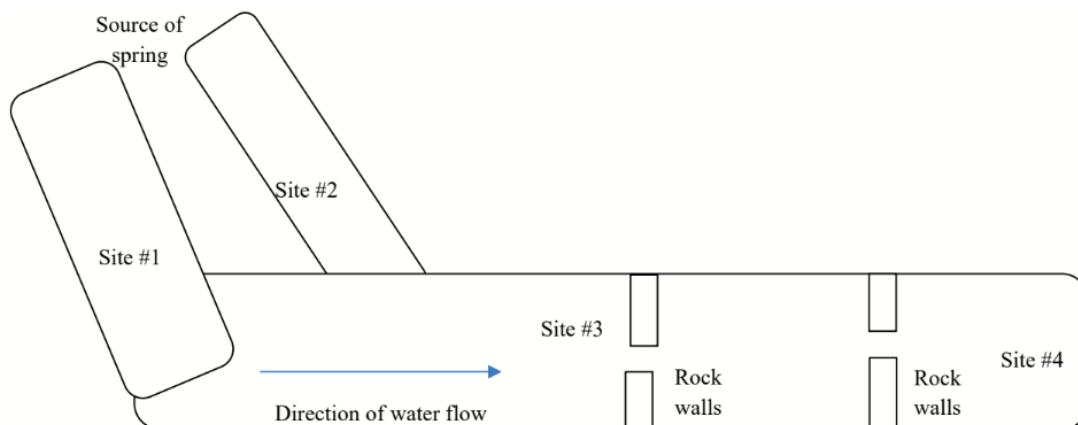


FIGURE 1. Location of random sampling. The streambed throughout Dubbel Spring was mostly soil and thick silt with rocks scattered throughout. Sites 3 and 4 were in water that was deeper than sites 1 and 2.

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Warm Spring:

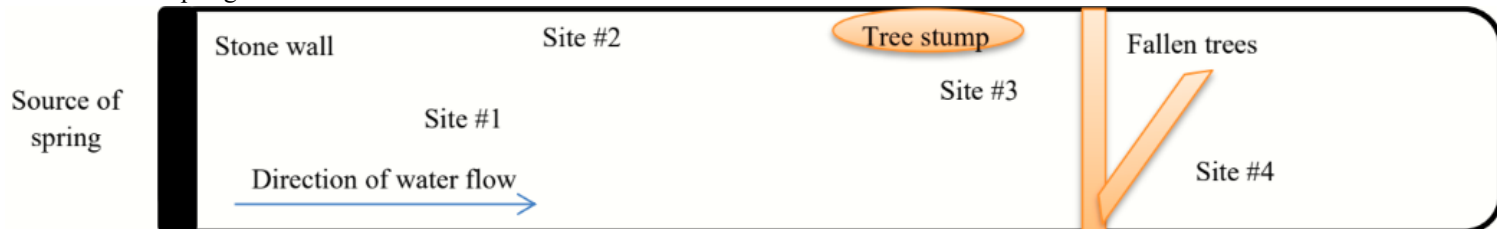


FIGURE 2. Location of random sampling. The streambed near sites 1 and 2 was mostly sand with a few scattered rocks and moss covering the rocks. The streambed near sites 3 and 4 was mostly rocks, with ferns, algae, and mats of clover floating on the surface of the spring.

RESULTS

Before analyzing our data we first had to assign each taxon a number that corresponded to the morphological codename we gave them (Table 1). Enlarged, detailed images of several of the taxa observed are depicted below; three taxa from Dubbel

Spring, one taxon found in both springs, and two taxa from Warm Spring (Figures 3-6). Table 2 lists a qualitative comparison between the two springs, indicating which taxa were found in each spring. There were 19 taxa specific to Warm Spring and 10 taxa specific to Dubbel Spring, with four taxa found in both springs.

Table 1: Taxonomic Key for Taxa of Dubbel and Warm Springs. The diatoms observed in this study were assigned morphological code names so that we could easily differentiate between them. The table below indicates the number that correlates with each code name. These numbers will be used throughout the results and discussion when referring to the taxon.

Taxonomic Key			
Taxon name	Number assigned to taxon	Taxon name	Number assigned to taxon
bowling pin	1	crocodile jaw	18
hairy nail	2	infinity symbol	19
raft	3	candy corn	20
hot dog bun	4	comb	21
ridge raft	5	lips	22
baby oval	6	machete	23
rod	7	ridged wedge	24
pickle	8	earthworm	25
slug	9	club line wedge	26
bamboo tube	10	needle	27
inoculation loop	11	flat lips	28
ridged pickle	12	fringed oval	29
mustache	13	dotted rod	30
oblong	14	beaded chain	31
oblong with teeth	15	nutshell	32
shark tooth wedge	16	asterisk	33
outlined oblong	17		

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FIGURE 3. The diatom shown above was found strictly in Dubbel Spring. It was assigned the morphological codename “mustache” (#13).



FIGURE 4. The diatom shown above was found strictly in Dubbel Spring. It was assigned the morphological codename “ridged pickle” (#12).

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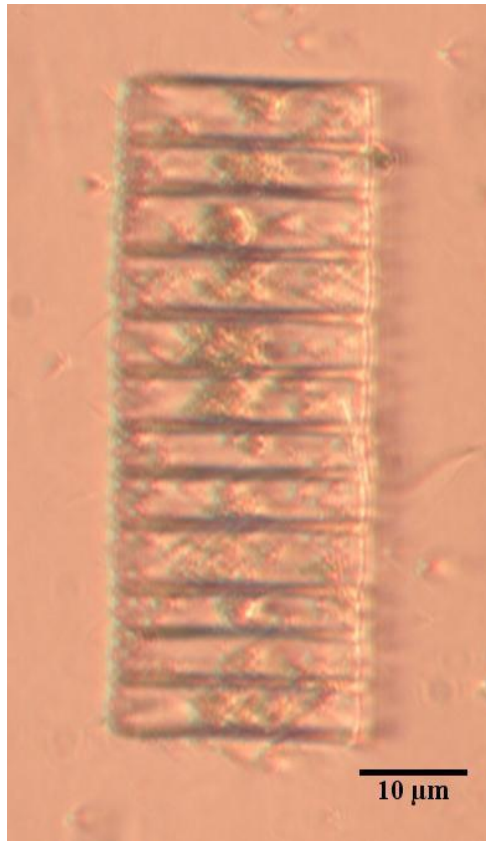


FIGURE 5. The diatom shown above was found in both Dubbel and Warm Spring. It was assigned the morphological codename “raft” (#3).



FIGURE 6. The diatoms shown above were found strictly in Warm Spring. The larger center diatom was assigned the morphological codename “needle” (#27), and the diatom above it is a “club line wedge” (#26).

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FIGURE 7. The diatoms shown above were also found strictly in Warm Spring. The larger center diatom is a “fringed oval” (#29), and the diatom underneath it is a “ridged wedge” (#26).

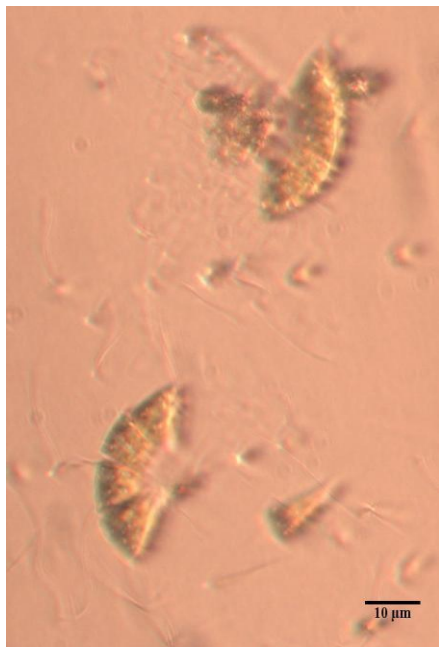


FIGURE 8. The diatoms shown above were also found strictly in Warm Spring. Diatoms commonly cluster together in colonies. There are two partial colonies shown above, made up of the “shark tooth wedge” (#16).

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Table 2. Taxon found in Dubbel and Warm Springs. An “X” indicates that a particular taxon was present at the associated spring. Four sites were randomly sampled at Dubbel and Warm Springs, with three slides placed on a clay plate at each site, so that a total of twelve slides were obtained from both springs. Only four taxa were found to overlap between the two springs.

Taxon	Dubbel	Warm
bowling pin	X	
hairy nail	X	
raft	X	X
hot dog bun	X	
ridge raft	X	X
baby oval	X	
rod	X	X
pickle	X	
slug	X	X
bamboo tube	X	
inoculation loop	X	
ridged pickle	X	
mustache	X	
oblong	X	
oblong with teeth		X
shark tooth wedge		X
outlined oblong		X
crocodile jaw		X
infinity symbol		X
candy corn		X
comb		X
lips		X
machete		X
ridged wedge		X
earthworm		X
club line wedge		X
needle		X
flat lips		X
fringed oval		X
dotted rod		X
beaded chain		X
nutshell		X
asterisk		X

To compare the differences in species richness we calculated the value of species richness per slide for each spring, and then calculated the mean and standard deviation. Dubbel Spring had a species richness of 1.667 ± 1.073 , while Warm Spring had a

species richness of 2.667 ± 2.146 (Tables 3 and 4). Both springs had large standard deviations. To compare the differences in species diversity we performed the Simpson’s diversity index, using the equation $d = (\Sigma P_i^2)^{-1}$, in which d is the diversity

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index. Dubbel Spring had a species diversity of 1.428 ± 0.993 , while Warm Spring had a species diversity of 1.783 ± 1.138 (Table 5). Again, both springs had large standard deviations. This raw data was then used in an ANOVA statistical test to determine the relationship between the species diversity of Dubbel and Warm Springs. The p-value was found to be 0.527 ($F_{1,42} = 0.41$, ns), indicating that the difference in diversity

between the springs was not statistically significant. To determine the rank abundance of each spring we calculated total counts for each taxon and assigned a rank number corresponding to the frequency of counts. The rank number was then graphed against the $\log(P_i * 1000)$ value for each taxon to determine the distribution curve of the taxon within their community (Figures 9 and 10).

Table 3. Species richness per slide sample from Dubbel Spring. The species richness for each corresponding slide sample is listed, along with the mean and standard deviation, 1.667 ± 1.073 .

Dubbel Spring	
Slide Number	Species Richness
1	0
2	1
3	2
4	1
5	2
6	0
7	2
8	3
9	3
10	1
11	2
12	3
Mean	1.667
Standard deviation	1.073

Table 4. Species richness per slide sample from Warm Spring. The species richness for each corresponding slide sample is listed, along with the mean and standard deviation, 2.667 ± 2.146 .

Warm Spring	
Slide Number	Species Richness
1	1
2	8
3	4
4	0
5	2
6	1
7	2
8	3
9	2
10	4
11	1
12	4
Mean	2.667
Standard deviation	2.146

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Table 5. Simpson's Diversity Index of Dubbel vs. Warm Spring. Simpson's diversity index quantifies the biodiversity of species in a community, allowing us to compare the differences in species diversity between Dubbel and Warm Spring. Dubbel Spring had a species diversity of 1.428 ± 0.993 , while Warm Spring had a species diversity of 1.783 ± 1.138 .

Simpson's Diversity Index		
	Dubbel Spring	Warm Spring
Slide number	Diversity index	Diversity index
1	0	1
2	1	3.982
3	1.324	3.103
4	1	0
5	2	1.180
6	0	1
7	1.142	1.385
8	1.241	2.778
9	2.882	1.800
10	2.882	1.394
11	1	1
12	2.667	2.778
Mean	1.428	1.783
Standard deviation	0.993	1.138

Table 6. One-way ANOVA statistical test comparing species diversity between Dubbel and Warm Springs. Below is a reproduction of the ANOVA table generated with Minitab15. There was no statistically significant relationship for species diversity between Dubbel and Warm Springs, $p\text{-value} > 0.05$, ($F_{1,42} = 0.41$, ns).

Source	DF	SS	MS	F	P
Species Diversity	1	6.6	6.6	0.41	0.527
Error	42	678.2	16.1		
Total	43	684.8			

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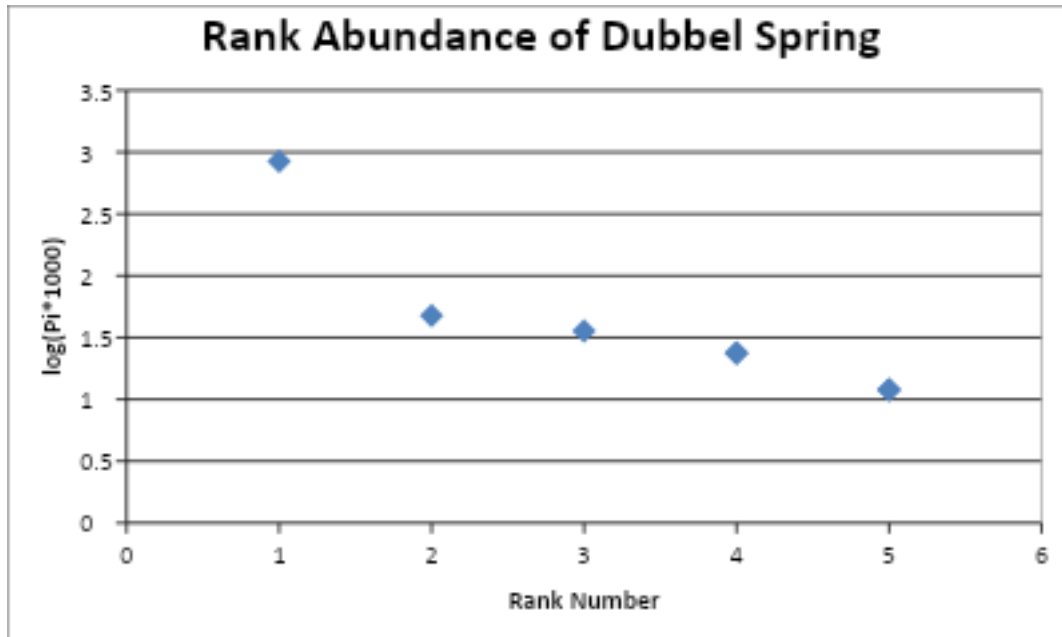


FIGURE 9. Rank abundance of Dubbel Spring taxa. Total counts for each taxon were recorded and assigned a rank number corresponding to the frequency of counts. The rank number was then graphed against the $\log(P_i \cdot 1000)$ value for each taxon to determine the distribution of the taxon within their community. Dubbel Spring taxa follow a distribution similar to the broken-stick curve.

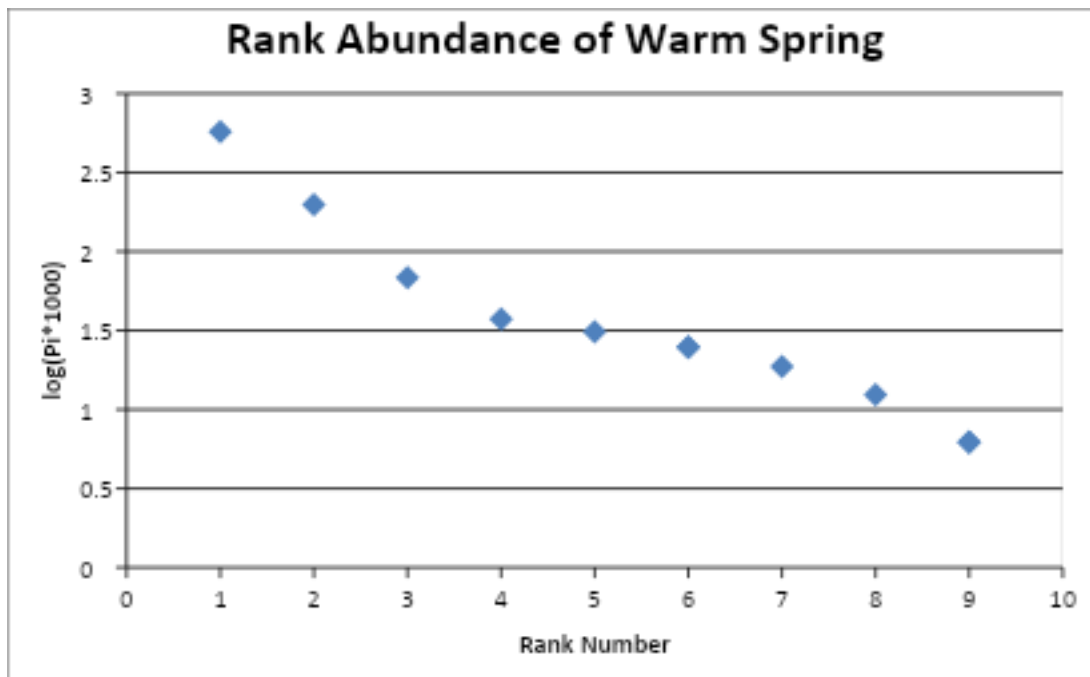


FIGURE 10. Rank abundance of Warm Spring taxa. Total counts for each taxon were recorded and assigned a rank number corresponding to the frequency of counts. The rank number was then graphed against the $\log(P_i \cdot 1000)$ value for each taxon to determine the distribution of the taxon within their community. Warm Spring taxa follow a lognormal distribution curve.

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DISCUSSION

Our hypothesis that there will be an increased species richness and diversity of diatoms in Warm Spring (alkaline) compared to Dubbel Spring (acidic), due to increased H^+ concentrations in the water of the acidic spring, was rejected. However, the null hypothesis of no correlation between spring pH and species diversity was not accepted either. The hypothesis was rejected due to a p-value of 0.527 ($F_{1,42} = 0.41$, ns) calculated from the ANOVA statistical test performed on the raw data of the species diversity of Dubbel and Warm Springs, which indicated that the differences in species diversity between the two springs was not statistically significant. Table 2 shows there is a greater number of different taxa in Warm Spring compared to Dubbel Spring (19 taxa vs. 10 taxa). There were four taxa that overlapped between the two springs. These include species #3, 5, 7, and 9. We believe this overlap is due to the small deviation (of 1.23) in pH between the springs; some diatoms may have a wider pH tolerance, allowing them to survive in both slightly acidic and slightly alkaline springs. If there was a larger difference in pH levels between the springs we would expect to see no taxa overlap. When comparing the species richness between the two springs we observed a value of 1.667 ± 1.073 for Dubbel Spring and 2.667 ± 2.146 for Warm Spring. The data used in the species richness calculations were from the random counts taken from each of the twenty-four slides. These counts did not accurately represent the species diversity of the slides, because many of the species found during the qualitative analysis were not viewed during the counts. This was largely due to the fact that the sample field was only that which was visible through the digital scope camera which only represented approximately one fourth of the microscopes normal field of view. The high frequency of zero species observed per count led to the large standard deviations. Although the standard deviations are large for both springs, there is a slightly higher species richness value for Warm Spring. This corresponds with the higher number of species observed for Warm Spring in the qualitative analysis, which occurred before the random counts were performed. According to Table 5, Dubbel Spring had a species diversity of 1.428 ± 0.993 , while Warm Spring had a species diversity of 1.783 ± 1.138 . Once again, Warm Spring had an overall greater diversity in comparison with Dubbel Spring. The standard deviations were again large due to the large variance in species diversity observed between each slide.

In order to determine ecological community interactions for each spring, we created rank

abundance graphs (Figures 9 and 10). The distribution curve for Dubbel Spring is similar to the broken-stick curve, which indicates that resource allocation is determined randomly, but competition still exists in the community. There is one dominant species indicated on the graph. It is common to have one dominant species in communities with low species richness, which our previous tests confirmed for Dubbel Spring. The distribution curve for Warm Spring follows a lognormal curve, where resources are split up fairly equally. This commonly occurs in communities with high species richness, because of an increase in the number of niches and the effects each has on the community structure. Therefore, the distribution curves in these figures indicate a more diverse and complex community is occurring in the alkaline spring. This increase in species diversity leads to an increase in the diversity of organisms in higher trophic levels, which adds to the complexity of the food webs.

Although the relationship between species diversity of Dubbel and Warm Spring was not statistically significant, our other tests indicate a correlation between spring pH and species diversity still exists. Our results are comparable to the study performed by Yangdong et al., which found significant correlations between pH and the location of specific diatom species. This data can help conservation biologists use diatoms as biological indicators. They can be used to aide conservation efforts for streams that are affected by acid mine drainage or other factors that can lower the pH of streams. Because diatoms base their morphology off of certain environmental factors such as pH, they can be used to assess the health and chemistry of stream ecosystems. The diatoms observed in our study could have been better classified if resources from diatom experts had been available for our use. If we had been able to properly classify each species of diatoms that were observed, we could have carried out our study further by investigating why certain species chose to live in an alkaline or acidic spring. Further studies could also determine the effect of other environmental factors on diatom species richness and diversity, such as conductivity or water temperature.

ACKNOWLEDGEMENTS

We would like to thank Mr. and Mrs. Dubbel and the owners of the Warm Spring property for the use of their property as field sites. We would also like to thank Dr. Doug Glazier for his direction and guidance and the Juniata College Biology Department for use of microscopes and other supplies.

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HABITAT SELECTION OF PEROMYSCUS IN CENTRAL PENNSYLVANIA

Skylar G. Kulbacki, Daniel S. Piper and James Y. Rixey

ABSTRACT

The species known as the white-footed mouse (*Peromyscus leucopus*) and the Deermouse (*Peromyscus maniculatus*) are widely abundant in the eastern parts of North America and are especially abundant in Pennsylvania. There have been a number of studies conducted concerning the habitat, distribution, and abundance of this species, *Peromyscus*, and it has been found that the optimal habitat for this species is deciduous woodland (Linzey et. al, 1997). We were interested in finding the preferred habitat selection of this species near the Juniata College Field Station, located in Raystown, Pennsylvania, and how the data compared to that of previous studies conducted on the habitat selection of *Peromyscus* and if they would indeed favor wooded areas compared to open fielded areas. Although difficulties arose concerning weather conditions and the ability to collect a sufficient amount of data, from what we had collected, we were able to conclude that *Peromyscus* in central Pennsylvania did indeed prefer wooded areas in comparison to open fielded areas.

Keywords: *Peromyscus leucopus*, *Peromyscus maniculatus*, *habitat selection*, *abundance*, *distribution*, *Pennsylvania*

INTRODUCTION

Specific characteristics of a habitat play a large role in the overall niche of a species. These characteristics influence the distribution and abundance of a particular species. The purpose of this study was to determine the preference of habitat selection for the *Peromyscus* species found in central Pennsylvania.

P. leucopus and *P. maniculatus* are rodents native to North America; coat color is variable but are must distinguishable by their white undersides and white feet; they also have an average weight range from 15-25g. These species are often most abundant in eastern woodlands. Geographically *P. leucopus* is native to and range from the eastern two-thirds of the United States and adjoining portions of southern Canada, and southward into southern Mexico. However, it does not reach down into the coastal plain areas located in the southeastern states of the United States and also does not reach into Florida (Fig. 1). *P. maniculatus* is the most widespread North American

rodent and is found throughout southern Canada, the United States, and north and central Mexico, similar to *P. leucopus* it also does not go down into the coastal plain areas of the southeast and Florida (Fig. 2) ("Peromyscus, 2012).

As previously mentioned this species prefer deciduous woodlands to inhabit, but will also inhabit a wide variety of other plant communities such as grasslands, brushy areas, and forests. *Peromyscus* create their nests underground, in brush piles, or in crevices among rocks; almost strictly nocturnal, this species does not leave their nest and go out into the open until the night time hours to feed and return to their nest in the early morning hours. The *Peromyscus* species has been determined to not be agricultural pests, and are considered an important part of the ecological community, mostly because they are the source of prey by several predator species such as owls, weasels, snakes, and many more. Omnivorous species, the *Peromyscus* have a diet that mainly consists of seeds, fruit, anthropods, leaves, and fungi. Eating habits vary throughout different seasons due to

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varying abundances of their food sources. The *Peromyscus* do not hibernate and continue breeding throughout all of the seasons. Population density is influenced by fluctuations in temperature and food availability. In the early part of their breeding season

(April-June), populations are at annual low densities; this may be due to a large mortality rate over the winter season because of the decline in food abundance and harsh weather (Wilder et. al, 2006).



Figure 1. Distribution of the white-footed mouse (*Peromyscus leucopus*).



Figure 2. Distribution of the deermouse (*Peromyscus maniculatus*)

Our study focused on the habitat selection of the *Peromyscus* species, more specifically *P. leucopus* and *P. maniculatus* in the central Pennsylvania area. Our trapping location was set up in Raystown, Pennsylvania, near the Juniata College Field Station. Reviewing past research (Linzey et. al, 1997) we expected that the mice would prefer wooded areas in comparison to open fielded areas. This is due to the amount of coverage and protection that wooded areas provide the mice from predators. Using 100 Sherman live traps, we tested this hypothesis by setting 50 of these traps in a wooded and open field area and utilizing the mark re-capture method we collected our data.

FIELD SITE

This study was conducted at Grove's Farm near the Juniata College Field Station in Raystown, Pennsylvania. We surveyed two different trap sites, one of which was in an open field adjacent to the farmhouse, and the other, the forested habitat, was within the sugar maple grove near a deer enclosure. Grids of fifty traps (arranged 5x10) were placed at each site.

METHODS AND MATERIALS

One hundred total Sherman live traps were laid out in a grid-like pattern at both trap sites, fifty at each location. Peanut butter was used as bait to attract *Peromyscus*, and cotton balls were placed in the traps to prevent hypothermia amongst sample individuals during cold nights. Data collection began on the sixth of April, 2010 and concluded on the sixteenth of April, 2012. Five total days were collected within this timespan due to inclement weather. Traps were opened at around 6:30pm on warmer evenings, and then samples were collected the next morning at around 7:00am. Sample collection was conducted early in the mornings to avoid over heating of individuals within the traps.

When an individual was caught, it was then weighed using a spring mass scale and a plastic bag to prevent the individual from escaping, tagged, the data was recorded, and then the individuals were released back into the environment. All traps were cleaned and re-baited halfway through the study. Whenever an individual was caught that trap was cleaned and re-baited before closing. If traps did not have individuals in them, they were closed to prevent access during the day for the reason stated above.

Due to the low sample size of trapped individuals the Lincoln index was used to estimate the population size of each area. The Lincoln index was defined as follows:

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$$\text{Predicted Total Population} = \frac{(\text{number individuals marked and released}) * (\text{number caught at next sample})}{(\text{number of marked individuals caught at next sample})}$$

RESULTS

Results indicated that *Peromyscus* shows a preference for forested areas over open fields. Six

unique mice were captured within the forested area and only four unique mice were caught within the open field area. There were two recaptures in the forested area and none in the open field.

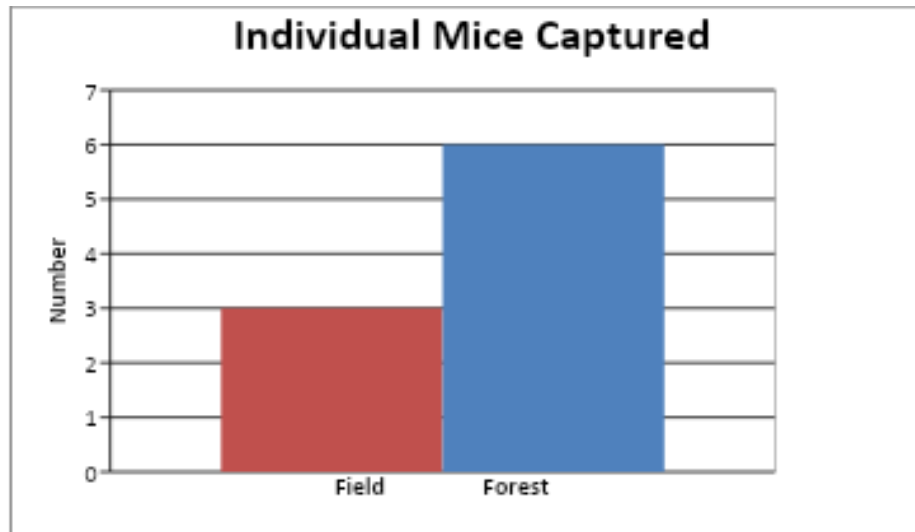


Figure 4. Number of unique *Peromyscus* captures within each area.

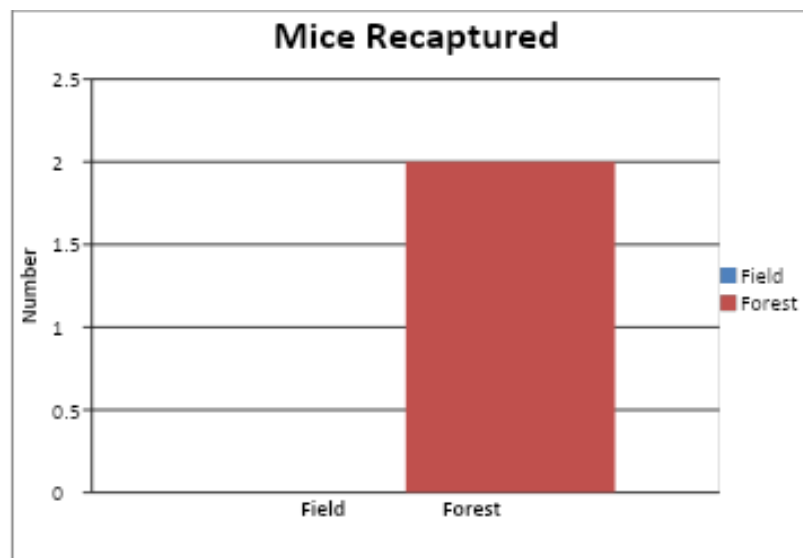


Figure 5. Number of recaptures within each area.

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Upon the capture of each individual its weight was measured and recorded. Individual weight varied greatly within the individuals captured in both the open field and forested areas (standard deviation =

9.38 and 5.16 respectively). Student's T-Test was performed on the data and the P-Value was found to be 0.72.

Field Area		Forested Area	
Individual	Weight	Individual	Weight
1	18	1	28
2	16	2	16
3	18	3	15
4	36	4	25
		5	18
		6	20
Average	22.00	Average	20.33
St. Dev	9.38	St. Dev	5.16

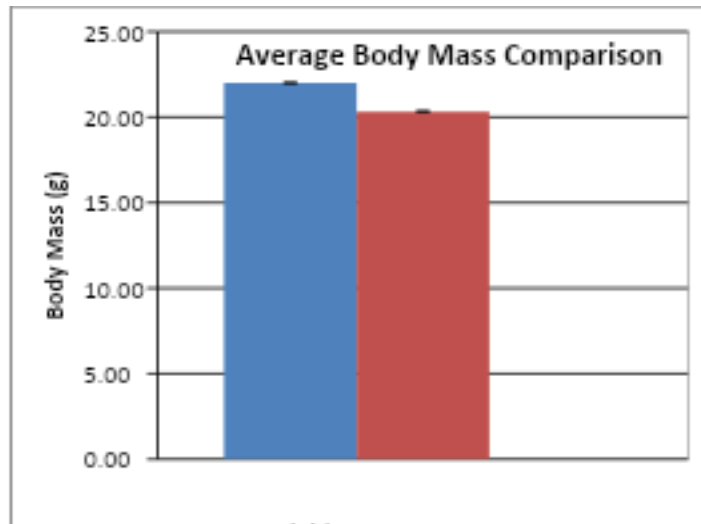


Figure 6. Comparison of the average weights of Peromyscus captured in each area.

Due to the small number of individuals captured over the course of the study a Lincoln index calculation was performed in order to attempt to estimate the number of individuals living in both the field and forested areas. The population size of the fielded area could not be estimated due to the lack of

recaptures. The population size of the forested area was estimated as low as two or four after the third and fourth days of capturing. Due to the lack of recapturing on the final day of the study, however, the Lincoln index indicates that the population could be significantly higher.

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Table 1. The Lincoln index for the captured mice in the field and forested areas.

Day	Field Mice captured	total # of Mice Recaptured	New Mice	Total Population <i>N</i>
1	0	0	0	~
2	0	0	0	~
3	1	0	0	~
4	1	0	1	Infinite
5	2	0	2	Infinite
Total s	4	0	-	

Day	Forest Mice captured	total # of Mice Recaptured	New Mice	Total Population <i>N</i>
1	0	0	0	~
2	1	0	1	~
3	2	1	1	2
4	2	1	1	4
5	3	0	3	Infinite
Total s	6	2	-	

DISCUSSION

Although a relatively small amount of data was collected, the *Peromyscus* appear to prefer a forest environment over a field environment. This is consistent with our hypothesis. We believe that this trend was observed because the forest area provided a greater amount of cover than the field area, allowing the species to hide from predators more effectively. It must be noted, however, that even though there were a greater number of individual mice in the forested area, the Lincoln index indicates that the population found in the field environment could potentially be much larger.

According to the data collected, we cannot determine the population size within the parameters of the Lincoln Index in the field area. This is because no individuals were recaptured. This, in effect, makes the population potentially infinite. Causes for this anomaly could include the population being so large that recaptures of a lone individual was statistically improbable. According to the Lincoln Index, before the final day of sampling, the forest population could be as low as two and as high as four. The results of the fifth day of testing in the forested area, however, when there were now recaptures suggest that the population size of *Peromyscus* could potentially be very large. With just one additional sample collection the potential results of the study were greatly shifted. This

demonstrates that statistical analysis of any data requires an adequate sample size, which is not obviously present here. To come up with a more accurate estimate of the true populations of the fielded and forested areas many more days of sampling would be required.

Possible sources of error are abundant. The foremost of these is the weather. During the two week period that we were conducting our study inclement weather would have been detrimental to the *Peromyscus* if they had been left for too long in the traps. Much of the time ambient temperatures dropped to below freezing, and wind chill factors would have made it even worse. On such inhospitable nights it was unlikely that the *Peromyscus* would leave their dwellings and become trapped. Further error could have been caused by faulty equipment. Some traps were prone to sticking and did not close without excessive stimulation. This issue was compounded by the frost we encountered on the first night of trapping. In the morning several of the traps had frozen over and would not close. There is a chance that the *Peromyscus* individuals entered that traps on the first night but, because of the weather, the traps did not close.

Another source of error is the location of the field traps. We were told to place the traps "on the hill" near Grove's Farm, and were pointed towards the flat lawn area behind the building. What our guide must

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have meant was to set them up on the slope behind the house and stretching above it. It is likely that we would have gotten more samples if we had set the traps up on the hill, but this cannot be confirmed. This sloped area did have much more brush area and tall grass for the *Peromyscus* to hide in, which could have been a factor. This potential source of error aside, the results of the study still indicate that mice are more abundant in more well-covered areas. It is possible that the mice are actually more sheltered and protected from predation in the tightly clustered shrub-laden field than in the forest. Repeating this study may produce results indicating a higher number of

A third source of error is our lack of test days. Due to scheduling conflicts between the three of us, we were unable to go to the trap sites every day. It would only take one person to open the traps in the evening, but we really needed two or more people at the site in the morning. Usually one person tagged and weighed the mice and the other recorded the data. It would have been too difficult for one person to make sure the mice did not get away. For this reason we did not force any one person to go by themselves to collect the data. Also, our survey time was cut short due to technical difficulties preventing us from getting to the trap site. On the fifth day a deer collision rendered our means of transportation unviable and we had to cease the study.

In the future the results could be made more reliable by avoiding the aforementioned sources of error. The largest factor in the credibility of the results of this study is the very small sample size. Data should be collected until many more individuals are trapped so that the Lincoln index can produce more reliable population size estimates for the different habitats.

ACKNOWLEDGEMENTS

We would like to thank Dr. Douglas Glazier for providing us with the equipment and knowledge necessary to complete this project, the Raystown Field Station facilities workers for putting up with our traps being scattered around Grove Farm for two-weeks, and Erie Auto Insurance for paying to repair one near-totaled Volvo.

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