

Survey of Invertebrate Species in Vernal Ponds at UNDERC

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## **Abstract**

Vernal ponds are an important environment for many species, with invertebrates being the dominant inhabitants. Previous studies have shown that vernal pond destruction can cause significant damage to the surrounding ecosystem. This study examines the biota of vernal ponds at UNDERC and attempts to find a relationship between the invertebrates and the unique characteristics of each pond. While there are a few correlations to be found, most conclusions show that invertebrate and insect abundance and diversity, is the consequence of a widespread and indiscriminate dispersion of invertebrates and invertebrate larvae.

## **Introduction**

Vernal ponds are temporary bodies of water which exist for only a few months following snow melt, during the spring and early summer. Still, this transitory habitat is home for many species of plants and animals. Although many engineers regard vernal ponds as obstructions to building projects, it is known that these areas provide temporary, albeit important habitats to many species of invertebrates, reptiles, amphibians, birds, and even some species of fish. Without these habitats the extinction rates of many organisms in and around the ponds may increase dramatically (Simon et al. 2000, Zedler 2003). The biota which exist at each pond are often site-specific and have been shown to vary widely due to both biotic and abiotic factors (Gerhardt & Collinge 2003). Because of the

great number of invertebrates that compose more than half of the world's biota and their ability to exist in nearly any habitat, it is no surprise that invertebrate species dominate these seasonal ponds (Brooks 2000). Because they are the dominant group of species in the vernal ponds, it is necessary to know the presence and life cycles of invertebrates in order to understand the effect that vernal ponds have on the local and broader ecosystem. Previous studies of vernal ponds have shown that larger, longer-lasting ponds correlate to an increased number of species (Simon et al. 2000). This study will look to expand upon this idea, first verifying that this is indeed true in the vernal ponds of UNDERC, and then see if there is any way of estimating biota from abiotic factors and vice versa.

There are five major hypotheses to be tested which will collectively help to answer the above question: Some abiotic factors of vernal ponds change continuously in one direction until dried up. Invertebrate abundance and diversity as a whole will change with time. Certain invertebrates will be more abundant in ponds with specific abiotic characteristics. The percentage of insects divided by the total number of invertebrates will correlate with the number of niches in a vernal pond, determined primarily by its size and depth. And ponds which dry up the latest will have certain characteristics that differentiate them from ponds which dry up sooner, most noticeably size and depth.

## Materials & Methods

All of the vernal ponds which were surveyed were located at the University of Notre Dame Environmental Research Center (UNDERC) near the border of Wisconsin and the Upper Peninsula of Michigan. Ten ponds were surveyed over three separate periods: June 1-3, 23-24, and July 15. The following vernal ponds were used: 5, 6, 7, 9, 10, J, K, M, N, and P (see figure 1). These ponds were chosen to vary in size, depth, and many other abiotic factors.

Surveying each pond included determining the pond's maximum length, width, and depth, the area canopy cover, and measuring the water for dissolved oxygen content, temperature, conductivity, and pH. The process of capturing invertebrates was accomplished using a sweep net, and based on the size and depth of the vernal pond, either 30, 45, or 60 minutes was spent sweeping along all sides and depths and then collecting specimens from the net. Invertebrates were preserved in a 70% ethanol mixture and were later identified using a microscope and several identification guides (see reference section for complete list). Each organism was identified to at least order, and whenever possible to family and genus.

Invertebrate diversity at each site was calculated using the Shannon-Weiner index of diversity (MacArthur and MacArthur 1961):

$$H = -\sum p_i (\ln p_i)$$

Where  $p_i$  is the proportion of individuals that belong to the  $i^{\text{th}}$  species. This index provides a statistical quantity which can be used to compare diversity between sites.

After all invertebrate species had been identified, data was entered onto a spreadsheet and correlations were sought relevant to the hypothesis previously presented. Graphs were prepared using Microsoft Excel to show invertebrate diversity at different sites and to identify characteristics which could be significant in determining the biota of each vernal pond. Statistical analysis was performed using SYSTAT v. 10.

## **Results**

Through two weeks, all 10 vernal ponds were present and invertebrates collected. During the final sampling period in mid-July, only three of the vernal ponds had yet to dry up: ponds K, M, and P. The total number of invertebrates collected and identified from these 23 replicates was 1082. Of these, 8 were leeches, 17 were water mites, 52 were gastropods (which were of the genus *Aplexa* and *Bithynia*), 68 were aquatic earthworms, 282 were bivalves and 655 were hexapods, or true insects. The hexapods identified belonged to one of five orders: Odonata, Diptera, Coleoptera, Heteroptera, and Trichoptera. A complete list of p values for statistical tests is shown on pages 14-16.

The tests which compared time to abiotic factors consisted only of the data from the first two weeks, because there were not enough replicates from the third

week to find significant results. Linear regression tests were run comparing time to maximum volume (found by multiplying length, width, and depth), dissolved oxygen content, canopy cover, conductivity, temperature, and pH. Linear regression showed that volume decreases with time ( $p= 0.001$ ) and canopy cover increases ( $p= 0.001$ ).

Tests which related abundance and diversity of invertebrates included those which tested both of these factors against each other, against time. The same tests were then run with insect abundance and diversity. Of these six tests, the only one which had a significant negative linear relationship was insect abundance versus time of year ( $p= 0.006$ ). Several species specific tests were also run, showing whether the abundance of three of the most common insects in the vernal pools, the Diptera larvae Chironomidae and Chaoboridae and the Coleoptera larvae *Acilius*, increases or decreases with time of year. The only significant correlation found was that the abundance of Chaoboridae larvae decreased in later weeks ( $p= 0.014$ ).

Further tests studied the abundance of specific insects against particular characteristics of the vernal ponds. Two of the most prevalent species of insects in the vernal ponds were the larvae of Chaoboridae and Chironomidae. Neither family's abundance directly correlated with any of the vernal pond's abiotic characteristics. However, tests were run to test whether either of these families' dominance (which was determined by being the most abundant species in a vernal

pond) correlated with ponds which were large or deep. A significant relationship was found for both families, with Chaoboridae dominance correlating with increased depth ( $p= 0.011$ ) and Chironomidae dominance correlating with decreased volume ( $p= 0.039$ ).

One of the most prevalent predators in the vernal ponds was the water beetle larvae, genus *Acilius*. Although they were never the dominant species in any of the ponds, they were found in 9 of the 10 ponds and 21 of the 23 replicates. Testing their abundance against all of the recorded factors, no single factor was found to be significant in determining *Acilius* abundance.

Another factor tested was the percentage of insects out of all invertebrates. This was tested against many abiotic factors as well as the volume and depth of the lake. The only significant correlation found through these tests was the percentage of insect abundance increases with depth ( $p= 0.006$ ).

Finally, tests were run to determine whether there were correlations between those ponds which lasted the longest and many different biotic and abiotic factors. The three ponds that had standing water in them the third week, were categorized as long lasting. Using data from the first week, the characteristics between these two groups of ponds were compared to see if there was a correlation between pond longevity and the following factors: initial volume, depth, invertebrate abundance and diversity, insect abundance and

diversity, or the percentage of insect abundance. None of these tests showed a significant statistical relationship.

### **Discussion**

The first hypothesis posed was that some abiotic factors will consistently change throughout all of the ponds. The two factors which were found to have a significant correlation with time were volume ( $p= 0.001$ ) and canopy cover ( $p= 0.001$ ). Both of these results were anticipated. The volume of a vernal pond must decrease and eventually dry out, by definition. And canopy cover also blocks out more sunlight as the trees mature from winter to summer. Dissolved oxygen showed almost no correlation ( $p= 0.662$ ), but temperature ( $p= 0.145$ ), conductivity ( $p= 0.131$ ), and pH ( $p= 0.066$ ) all showed an insignificant relationship. To further test these unexpected findings, t-tests were run for all three of these factors, with temperature ( $p< 0.001$ ) and conductivity ( $p= 0.011$ ) having a certifiable change from weeks 1 to 2. However, the graph of temperature (figure X), clearly shows that this trend is reversed in week 3. However, because vernal ponds are small bodies of water, it is likely that the change in temperature is due to the change in air temperature between the days that the survey was completed. The conductivity (figure X) also appears to reverse trends in week 3 and it is possible that these two factors are related to each other. This study does not have enough replicates throughout the season to validate this.



The second hypothesis tested was that the quantity and diversity of invertebrates and insects would change along with the season. This was found to be the case for insects, as an almost perfect linear relationship was found between time of year and insect abundance ( $p= 0.006$ ). Invertebrate abundance did not show the same result, which is strange because more than half of the invertebrates in this study were classified as insects. However, of the non-insect invertebrates, many of them were solely aquatic organisms, while all of the insects changed or could change between aquatic and terrestrial or aerial habitats.

Because many insects have a peak seasonal emergence, it was interesting to see the effect that time of season would have on individual species. Three of the most abundant insects were used for this analysis, Chaoboridae and Chironomidae, both Diptera larvae, and the Coleoptera larvae, genus *Acilius*. Comparing their abundance to the time of year using the statistical test ANOVA, only Chaoboridae had a significant change in abundance. One possible explanation for this is that Chaoboridae larvae do not continually reproduce like many other families of insects. Instead have just one or a few mating season per year and as the season moves along, either less and less of the larvae are found in the ponds, or have a few select periods where their larvae population explodes, which happened to coincide with only the first week of surveying, but not the next two. Another explanation is that adult female Chaoboridae would be more likely to find and lay eggs in a larger, more permanent home later on in the summer.

The next hypothesis to be tested was that the above mentioned insects had populations which would change linearly with varying abiotic factors. Neither of the Diptera larvae families' abundance levels gave a significant result when compared to depth, volume, dissolved oxygen content, canopy cover, temperature, conductivity, or pH. However, both of these larvae were the dominant species in at least three of the ponds. By categorizing ponds as those where Chaoboridae was the dominant species and those where they were not, and running them against the same seven factors, it was found that Chaoboridae larvae were statistically more likely to be the dominant species in deep ponds ( $p= 0.011$ ). Doing the same thing with Chironomidae, a correlation was found between those ponds dominated by Chironomidae and low volume ponds ( $p= 0.039$ ). The same test was also run with *Acilius* but none of the seven factors gave a significant relationship. These tests show that either the ecological niches or the seasonal emergence of Chaoboridae and Chironomidae larvae are probably very different. The dominance of Chaoboridae in the deep pools could be because they mature early in the season when all vernal ponds are deeper. But because they dominated only in three of the deepest ponds, specifically, ponds 7, 10, and M, it seems more likely that they thrive in ponds which are deep. This may be because of their predators' inability to hunt them in these locations, or possibly because of its penchant to metamorphosis into an adult before the vernal ponds become too shallow or dry up. The lack of correlation for the very common larvae of *Acilius*

may show that they are very resilient insects which are able to thrive in numerous conditions.

The fourth hypothesis tested was that the percentage of insects versus the total number of invertebrates will change with some of the abiotic factors of the vernal ponds. Of all the factors tested, only depth showed a direct positive correlation with an increase in percentage of insects ( $p= 0.006$ ). While pond volume was not found to be statistically significant, this makes sense that pond depth would be a better indicator of this insect dominance, if one considers that there was a much greater diversity of insects in the vernal ponds than of non-insect invertebrates. With greater diversity, there is usually a broader spectrum of ecological habitats that the diverse class can occupy. Ponds which are wide but shallow do not have as many niches for invertebrates as a pond which is smaller but deep. The difference in habitat in a meter of depth is much more drastic than in a meter of width.

The final hypothesis presented is that it could be possible to determine which ponds would be the longest lasting, by characteristics of the pond measured early on. For this test, the ponds which were present in mid-July were classified as long lasting. The long lasting ponds were then compared to all other ponds in the following categories: length, width, depth, volume, dissolved oxygen content, canopy cover, temperature, conductivity, pH, invertebrate abundance and diversity, and insect abundance and diversity. None of these tests had a p value of

less than  $p=0.200$ . This shows that not one single factor that was measured, related absolutely with those ponds that dried out latest. In retrospect, a better factor to help determine this would probably be elevation. Most of the ponds that seemed to last until the final survey were those that were located at major declines along the road. Although this makes sense it also is odd that the deepest ponds in the beginning were not necessarily the ponds that were at these declines.

As a whole, this data suggests that there are some trends to be found involving invertebrate abundance and diversity. However, most of these tests did not find significant relationships, suggesting that many, if not most, of the behaviors of invertebrates are determined by more than one factor. Some of this data seems to imply that the success of insects is due primarily to their abundance and their ability to survive in a wide variety of habitats. This is not surprising, because insects are the most abundant macroinvertebrate and can be found in nearly every ecological niche known to man.

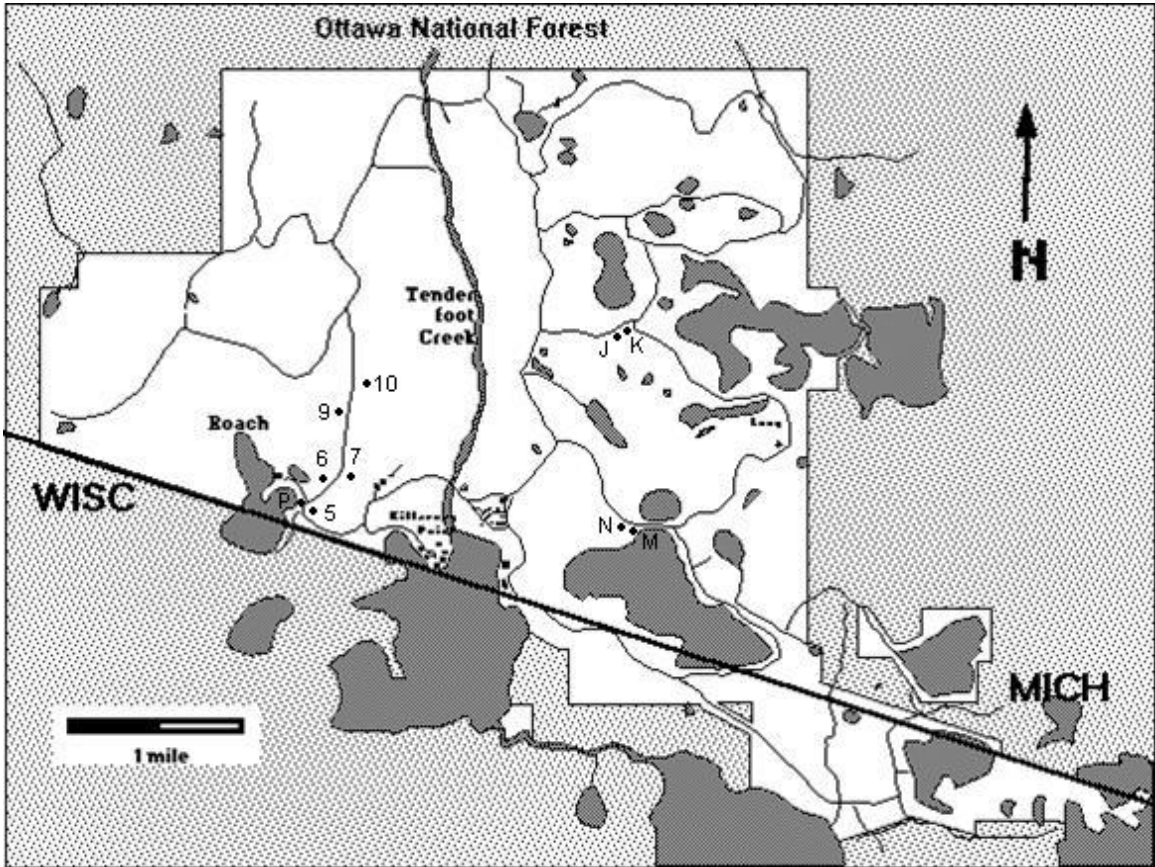


Figure 1: Map of UNDERC and location of vernal ponds studied.

## Statistical Analysis Tests

### Hypothesis 1

factor 1	factor 2	p value	statistical test
time	volume	0.001	linear regression
time	dissolved oxygen	0.662	linear regression
time	canopy cover	0.001	linear regression
time	temperature	0.145	linear regression
time	conductivity	0.131	linear regression
time	pH	0.066	linear regression
time	temperature	< 0.001	t-tests
time	conductivity	0.011	t-tests
time	pH	0.954	t-tests

### Hypothesis 2

factor 1	factor 2	p value	statistical test
invertebrate total	invertebrate diversity	0.625	linear regression
time	invertebrate diversity	0.203	linear regression
time	invertebrate total	0.317	linear regression
insect total	insect diversity	0.820	linear regression
time	insect diversity	0.195	linear regression
time	insect total	0.006	linear regression
time	Chaoboridae	0.014	ANOVA
time	Chironomidae	0.629	ANOVA
time	<i>Acilius</i>	0.810	ANOVA

### Hypothesis 3

<b>factor 1</b>	<b>factor 2</b>	<b>p value</b>	<b>statistical test</b>
Chaoboridae total	depth	0.059	linear regression
Chaoboridae total	volume	0.855	linear regression
Chaoboridae total	DO	0.801	linear regression
Chaoboridae total	canopy cover	0.545	linear regression
Chaoboridae total	temperature	0.822	linear regression
Chaoboridae total	conductivity	0.163	linear regression
Chaoboridae total	pH	0.694	linear regression
Chironomidae total	depth	0.311	linear regression
Chironomidae total	volume	0.104	linear regression
Chironomidae total	DO	0.206	linear regression
Chironomidae total	canopy cover	0.146	linear regression
Chironomidae total	temperature	0.369	linear regression
Chironomidae total	conductivity	0.991	linear regression
Chironomidae total	pH	0.720	linear regression
Chaoboridae dominance	depth	0.011	linear regression
Chaoboridae dominance	volume	0.538	linear regression
Chaoboridae dominance	DO	0.901	linear regression
Chaoboridae dominance	canopy cover	0.387	linear regression
Chaoboridae dominance	temperature	0.916	linear regression
Chaoboridae dominance	conductivity	0.406	linear regression
Chaoboridae dominance	pH	0.633	linear regression
Chironomidae dominance	depth	0.090	linear regression
Chironomidae dominance	volume	0.039	linear regression
Chironomidae dominance	DO	0.115	linear regression
Chironomidae dominance	canopy cover	0.233	linear regression
Chironomidae dominance	temperature	0.509	linear regression
Chironomidae dominance	conductivity	0.892	linear regression
Chironomidae dominance	pH	0.647	linear regression
<i>Acilius</i> total	depth	0.129	linear regression
<i>Acilius</i> total	volume	0.414	linear regression
<i>Acilius</i> total	DO	0.587	linear regression
<i>Acilius</i> total	canopy cover	0.177	linear regression
<i>Acilius</i> total	temperature	0.498	linear regression
<i>Acilius</i> total	conductivity	0.603	linear regression
<i>Acilius</i> total	pH	0.535	linear regression

#### Hypothesis 4

<b>factor 1</b>	<b>factor 2</b>	<b>p value</b>	<b>statistical test</b>
% insects vs. invertebrates	depth	0.006	linear regression
% insects vs. invertebrates	volume	0.189	linear regression
% insects vs. invertebrates	DO	0.228	linear regression
% insects vs. invertebrates	canopy cover	0.908	linear regression
% insects vs. invertebrates	temperature	0.358	linear regression
% insects vs. invertebrates	conductivity	0.443	linear regression
% insects vs. invertebrates	pH	0.335	linear regression

#### Hypothesis 5

<b>factor 1</b>	<b>factor 2</b>	<b>p value</b>	<b>statistical test</b>
long lasting pool	length	0.203	linear regression
long lasting pool	width	0.722	linear regression
long lasting pool	depth	0.324	linear regression
long lasting pool	volume	0.619	linear regression
long lasting pool	DO	0.606	linear regression
long lasting pool	canopy cover	0.635	linear regression
long lasting pool	temperature	0.785	linear regression
long lasting pool	conductivity	0.687	linear regression
long lasting pool	pH	0.231	linear regression
long lasting pool	invertebrate abundance	0.489	linear regression
long lasting pool	invertebrate diversity	0.561	linear regression
long lasting pool	insect abundance	0.386	linear regression
long lasting pool	insect diversity	0.426	linear regression



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## References Cited