

**Variation in the Order Diptera in Vernal Ponds as a Result of Differing Pond  
Characteristics**

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## **Abstract**

Vernal ponds are an important habitat for many insect orders, especially Diptera. The colonization of dipteran larvae depends greatly on pH and dissolved oxygen concentration, and this experiment tested the significance of these factors on diversity and richness of Diptera and other orders. 10 vernal ponds in the Upper Peninsula of Michigan were chosen for differing abiotic factors and were sampled for aquatic insects using a dip-net during three weeks in summer 2005. After insects were identified to Genus, statistical tests were performed to determine significance with abiotic factors. Though no abiotic factors showed significance among the dipterans, several other insect orders were very evidently affected by the differing abiotic factors.

## **Introduction**

Vernal ponds, as the name suggests, are pools of water which form and exist almost exclusively in the spring as a result of melting winter snow (Zedler 2003). Because they are fed by this seasonal source of water, these ponds often dry up as summer progresses. Though they are only available for a short time, vernal ponds become homes for many organisms within their few months of existence, including microcrustaceans, mites, and several orders of insects (Williams 1987, Brooks 2000). To those unaware of their significance, vernal ponds may appear to be an unimportant temporary habitat for a few organisms.

However, a 2003 study showed that many organisms use vernal ponds as their principal habitats, as they are a predictable location year after year (Zedler 2003).

The chemical diversity of vernal ponds (pH, dissolved oxygen concentrations, color, etc.) allows for diversity of organisms inhabiting each pond. Biotic and abiotic environmental influences create the unique makeup of each vernal pond, and this content can vary from location to location (Gerhardt and Collinge 2003). The diverse range of insects allows many species to inhabit this variety of vernal ponds that occur, including myriad Dipteran types, which will be the focus of this study.

Previous studies have shown that in vernal ponds with acidic conditions (pH<4.5), the number of organisms is generally lower than the number collected in a pond with circumneutral pH conditions, and that levels of dissolved oxygen in the water can change the insect demographic in each pond (Resh and Rosenberg 1984). I expect this data to be consistent with the findings from my experiment with Dipterans, and the other collected insects here at UNDERC, causing vernal ponds with lower pH to be found with fewer individuals, and ponds with differing levels of dissolved oxygen will contain differing insect populations .

## **Methods**

In this experiment, ten vernal ponds, ponds 5, 6, 7, 9, 10, J, K, N, P, and Wood Duck, were sampled for aquatic invertebrates. These ponds were selected for their diverse properties and characteristics, as well as their convenience to be used in a comparison to the results of a similar experiment done on the some of the same ponds in summer 2004. These ponds differed in size, water chemistry, and habitat this year, and as shown by measurements from previous years. Each of the study sites was sampled three times (unless ponds were completely desiccated before third sampling could occur); sampling took place during the weeks of May 23 -28, June 20-26, and July 11-17, 2005.

Each study site was sampled between 1000 h and 1600 h. A dip net was swept through vegetation, along the bottom, and throughout the water column to collect the invertebrates. Collection times ranged from 10 - 120 min, depending on pond size and depth at time of sampling. The invertebrates were removed from the dip nets with forceps at the sampling site and stored in jars of ethanol until they could be analyzed, identified, and more permanently stored in the lab. All invertebrates will be identified to Order; Dipterans will be identified to genus. This project was completed in conjunction with Joshua Vogel, who was responsible for identifying Coleopterans to genus.

The physical and chemical properties (pH, conductivity, dissolved oxygen concentration, water temperature, maximum depth, and area) of each vernal pond were characterized in the same week as the first sampling. Total water area and

maximum depth were measured using a tape measure and meter stick. Water color was estimated on a spectrophotometer using a water sample from the pool. To measure pH, dissolved oxygen, conductivity, and temperature, we sampled five spots throughout each pond so that an average measurement was estimated.

When identification of the specimens was completed, data was entered and graphs of differing abiotic factors and linear regressions were plotted on Microsoft Excel. SYSTAT 11 ran a one-way ANOVA to determine patterns among the ponds and the abiotic factors. A series of linear regressions tested for significance between the abiotic factors and any of the biotic factors. A Shannon-Weiner diversity index was calculated to test for genus diversity.

## **Results**

The one-way ANOVA used to test for significance among the abiotic factors revealed significance, so a Post Hoc test for each individual factor was performed ( $df=9$ ). The Post Hoc test revealed that there was significance in pH (Figure 1), dissolved oxygen (Figure 2), water temperature (Figure 3), and conductivity (Figure 4).

Overall, 1343 individuals (not including those in the Order Trichoptera—see Discussion) were collected during the three sampling weeks, including 273 Dipterans. The dipteran genus found most frequently, and in the

greatest number was the Phantom Midge genus Chaoboridae *Chaoborus* (Table 1). This Dipteran was found in the greatest magnitude in the first sampling week in vernal ponds 7 and Wood Duck.

Total invertebrate genus diversity, as measured by the Shannon-Weiner index, among all the sampling periods varied from 0.312 in vernal pond 10 to 2.153 in vernal pond 6. A one-way ANOVA test showed that genus diversity in vernal pond 10 was significantly lower than vernal ponds 6, Duck, and P ( $p=0.012$ ,  $0.024$ , and  $0.023$ , respectively). Vernal pond J also showed to be marginally different than vernal pond 6 ( $p=0.078$ ) with genus diversity measuring lower in vernal pond J

A multiple regression was performed to test for significance between biotic and abiotic factors in the experiment. Significance was found between the total number of individual insects collected per minute and the dissolved oxygen concentration ( $p=0.006$ , positive interaction). The number of Heteropterans collected per minute showed significance against dissolved oxygen concentration (positive interaction,  $p=0.003$ ) as well as pH (negative interaction,  $p=0.003$ ). Total Odonata collected per minute exhibited significance against conductivity (negative interaction,  $p=0.002$ ), temperature (positive interaction,  $p=0.002$ ), and maximum depth (negative interaction,  $p=0.002$ ).

## **Discussion**

Diptera did not exhibit significance with any of the abiotic factors because of their ephemeral presence in the ponds. The Diptera found were all mainly found in the first sampling period, and after this point. The extremely low numbers in the following sampling weeks could mean that they emerged into adulthood and were no longer present in the water. This is very possible, as this has been a hot, dry summer and some experiments suggest this can boost growth schedules (Bradshaw 1972).

Another factor affecting the lack of significance in the Diptera was the lack of true replication in this experiment. Multiple sampling periods took place, but over time, therefore they were not true replicates. To solve this problem, sampling should have taken place in several shorter periods per pond than one long sampling period. This lack of replication is also why an ANOVA analysis could not be performed as proposed, and a multiple regression had to take its place.

Dissolved oxygen content, combined with pond depth, is a major factor in the aquatic invertebrate populations in vernal ponds. In previous studies, it has been discovered that the optimum depth for most insect populations is about 10 cm, and at 80 cm very few individuals are present, and this inability to exist in great numbers at these depths is greatly affected by the dissolved oxygen concentration (Resh and Rosenberg 1984). The data collected showed significance in the relationship between dissolved oxygen concentration and the

total number of invertebrates collected per minute (Figure 1). This significance was expected because the greater the concentration of dissolved oxygen in the water, the more conducive the environment is to invertebrate colonization.

Significance was also found between pH and the number of Heteropterans collected per minute. This should mean that the Heteropterans collected have fairly impervious exoskeletons to keep the acidic water out of their systems, and that their body fluids are buffered to resist quick changes in chemistry (Resh and Rosenberg 1984). Significance was also found between dissolved oxygen content and the number of Heteropterans collected per minute (Figure 3). This was expected because invertebrate life is more likely to exist at high oxygen concentrations.

The order Odonata presented three areas of significance: conductivity, maximum depth, and water temperature. Conductivity is dependent upon the ion concentration in the water, and the Odonata seemed to be present more often in areas of low ion concentration.

Odonata collected per minute was highest in areas with a max depth of about 10 cm. This is expected because 10 cm is the optimum level for most insect life, due to dissolved oxygen levels, and other factors as well (Resh and Rosenberg 1984). They were also found more often high temperature areas than in low temperature areas. This could mean that the higher temperatures are more conducive to their living conditions, or it could mean that as the summer

progresses (and water temperatures rise) more larva are emerged from the eggs and present in the water.

Individuals from the order Trichoptera were also collected in high numbers, but not used in data analysis. One reason for this was instruction to count, rather than collect, the caddisfly cases that were unusually abundant in vernal pond 10 (651 cases were found in vernal pond 10 alone). Though this gave a one-time estimate for about how many caddisflies were in each pond, there was no way of assuring that cases would not be counted more than once in the three sampling periods.

The caddisfly cases most likely seemed so abundant in vernal pond 10 because of its very shallow depth. In the first sampling period vernal pond 10 had a maximum depth of only 17 cm, and by the second sampling period the pond had dried up completely. At this depth, the floating caddisfly cases were easy targets for the dip-netting technique, even if unintentionally. This sampling bias would have provided this experiment with very inaccurate statistical data.

Though no significance was found in any factor of the initial experiment involving dipterans, this experiment exhibited several key characteristics of vernal ponds. First, dissolved oxygen content (along with maximum depth) plays a major role in diversity and richness. Secondly, pH, a factor that usually produces very predictable results, can sometimes correctly produce a negatively correlated response. Last, areas where significance was expected (i.e. total number vs. area)

did not necessarily produce significance. Had true replication been adhered to, more findings and more significance may have surfaced. Though in some ways the data collected in this experiment was quite limited produced by this experiment, the importance of these vernal ponds to the insect population is very evident.

### **Acknowledgements**

I thank Dr. Ronald Hellenthal for his guidance in invertebrate identification and sampling methods, and Dr. Karen Franel for her sampling advice and writing recommendations. I would also like to thank Dr. Gary Belovsky for his help in statistics and data analysis. I am also very grateful for the identification and sampling assistance provided by Joshua Vogel, without whom this project would have seemed very overwhelming. The Hank family is also to be greatly acknowledged for their benevolent donations to the University of Notre Dame Environmental Research Center, making research facilities and materials available for this and many other research projects.

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## Tables

Table 1. Diptera Genus Distribution (from all sites)

Family	Genus	Amount
Chironomini	<i>Chironomus</i>	1
Chaoboridae	<i>Chaoborus</i>	268
Ceratopogonidae	<i>Alluaudomyia</i>	3
Psychodidae	<i>Psychoda</i>	1

Table 2. Distribution of Diptera Occurrence.

Vernal Pond	Total Number of Diptera Individuals		
	1	2	3
5	39	2	0
6	13	1	0
7	60	4	1
9	37	0	n/a
10	39	n/a	n/a
J	3	0	n/a
K	19	0	0
N	0	0	n/a
P	10	0	0
Wood Duck	59	0	1

Table 3. Analysis of Variance Table for Significant Biotic Factors.

Dependent variables of Significance	Degrees of Freedom	F-ratio	p-value
Total Number of Individuals Collected (per minute)			
Regression	1	9.258	0.006
Residual	23	<del>          </del>	<del>          </del>
Total Number of Heteropterans Collected (per minute)			
Regression	2	7.472	0.003
Residual	22	<del>          </del>	<del>          </del>
Total Number of Odonates Collected (per minute)			
Regression	3	7.197	0.002
Residual	21	<del>          </del>	<del>          </del>

Table 4. Abiotic factor Average Readings

	pH	D.O. (mg/L)	Conductivity (µS/L)	Temperature (°C)
VP 5	6.34	0.882	57.86	15.04
VP 6	4.58	2.818	43.62	18.1
VP 7	5.32	2.35	35.26	17.12
VP 9	5.4	1.214	61.3	19
VP 10	5.28	1.362	63.66	19.18
VP Wood Duck	5.38	3.254	37.498	21.02
VP J	6.1	0.87	68.4	17.18
VP K	5.84	0.962	102.58	20.04
VP N	5.96	0.798	63.7	15.94
VP P	6.06	4.11166667	33.48	16.22

## Figures

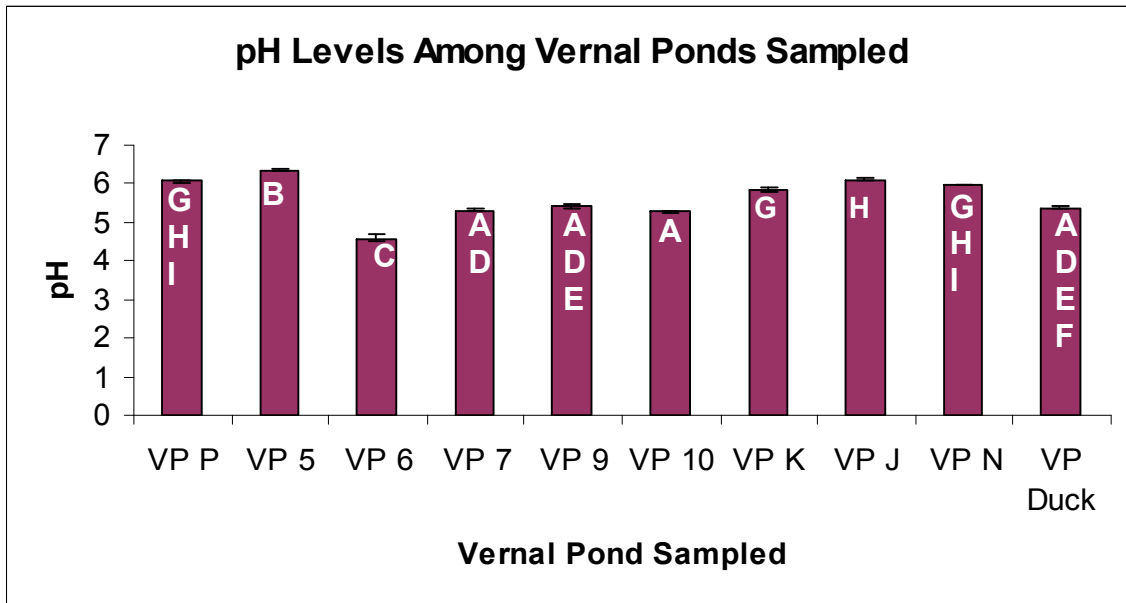


Figure 1. pH Significance among all ten vernal ponds, as a result of one-way ANOVA.

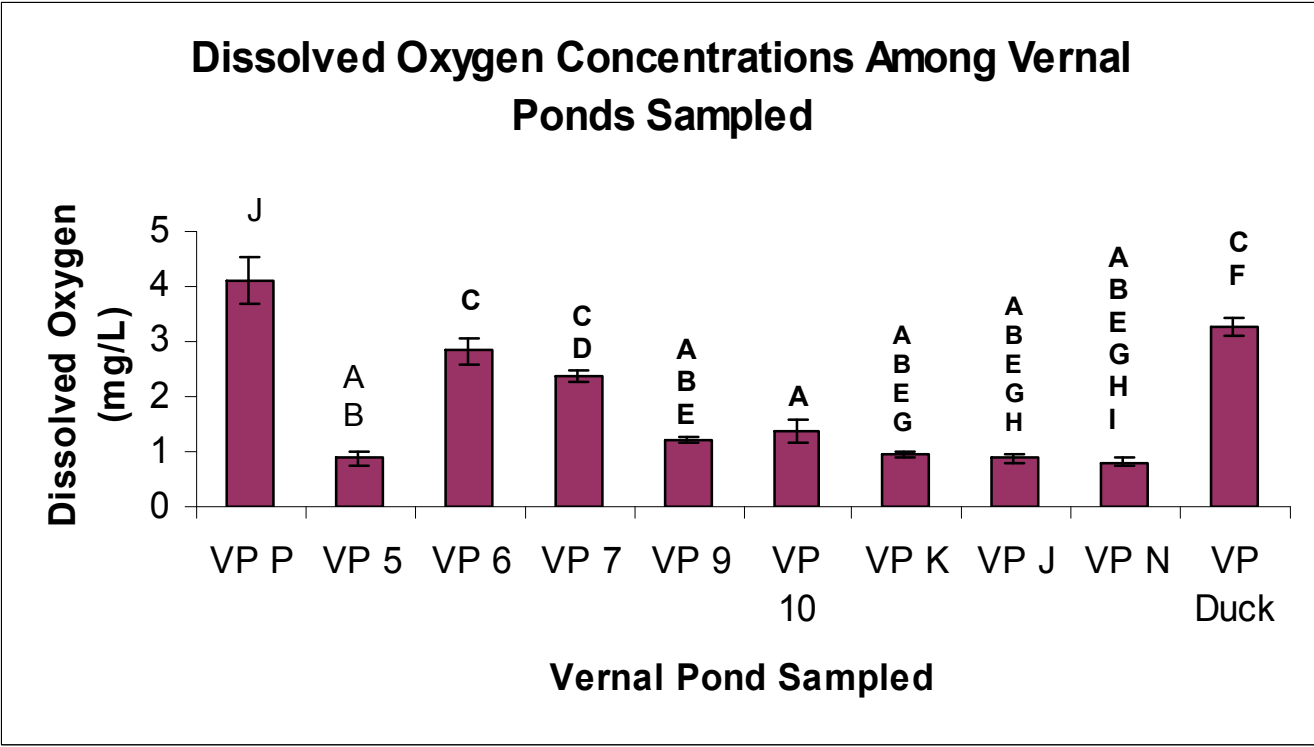


Figure 2. Dissolved Oxygen Concentration Significance among all ten vernal ponds, as a result of one-way ANOVA.

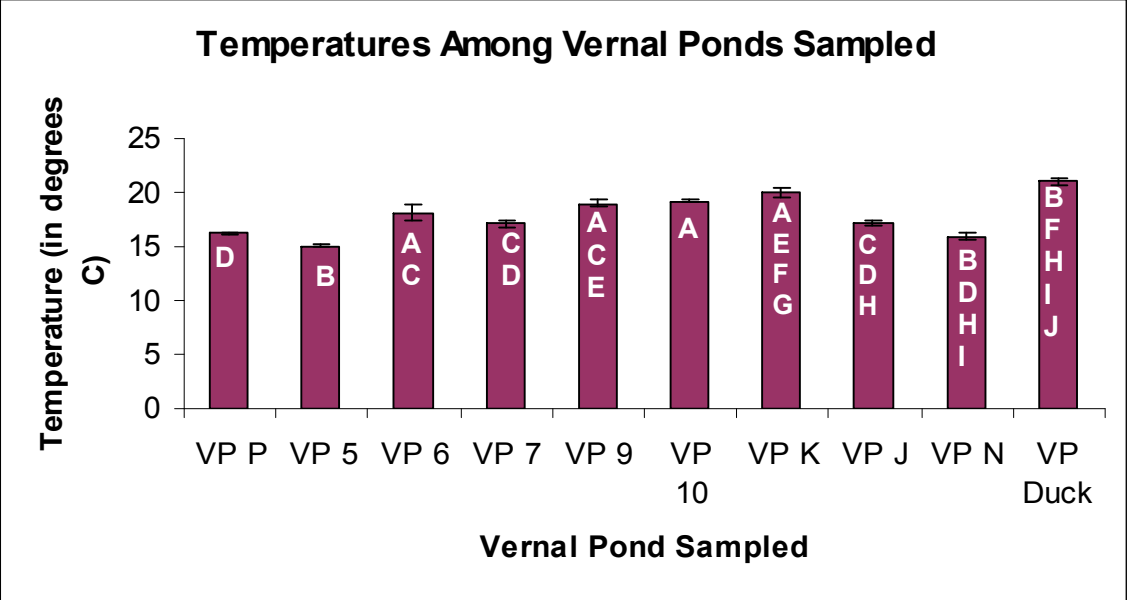


Figure 3. Water Temperature Significance among all ten vernal ponds, as a result of one-way ANOVA.

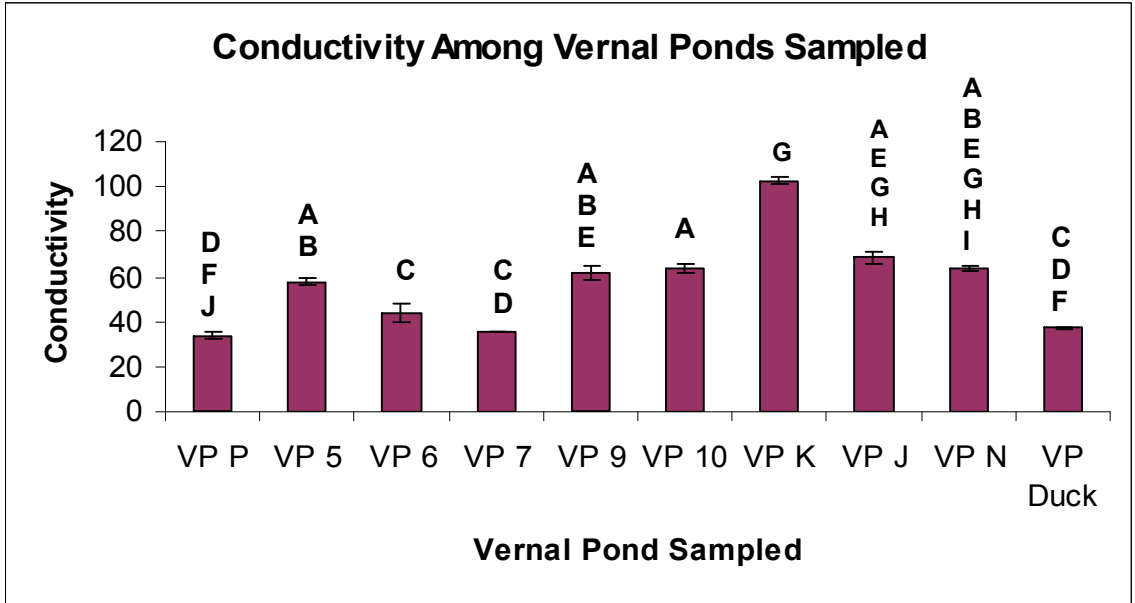


Figure 4. Conductivity Significance among all ten vernal ponds, as a result of one-way ANOVA.