

Abstract

Though it has been well-documented that northern pitcher plant (*Sarracenia purpurea*) individuals vary in physical, chemical, and environmental characteristics and inquiline community composition, little research has focused on variation among plants growing in different bogs in the same region. A survey of 100 pitcher plants from 5 different bogs was performed on the Upper Peninsula of Michigan to investigate bog-based variance. The variables surveyed include soil pH, pitcher fluid pH, leaf depth, leaf circumference, potential pitcher volume, actual pitcher volume, larval midge density, larval mosquito density, protozoa diversity, rotifer density, and prey density. Significant variance was found in protozoa diversity and prey density but no correlations between the two variables were evident. This suggests that inquiline community composition may be affected by the specific bog environment but further investigation and variable manipulation is necessary.

Introduction

The nutrient poor conditions of a bog create a distinct niche for a number of plant species that have adapted to this environment. One major growth-limiting factor is a lack of readily available minerals and nutrients in the soil or sphagnum. The slow rate of decomposition of organic matter limits the supply of necessary minerals, including calcium and magnesium. Bog plants compensate for this poor soil quality by absorbing cations such as Ca^+ and Mg^{2+} from rain water. The plants then release H^+ into the water, creating an acidic environment which ranges from 3.2 to 4.2. This high hydronium ion content further retards the decomposition process and thus reduces the nutrients available

to plants. Some plants have altered their nutritional strategy such that invertebrates, rather than rain water, serve as a major source of minerals and nutrients (Crowl 2003). These carnivorous plants, or their symbionts, breakdown insect carcasses and derive the necessary ions from the remains (Judd 1959).

The leaves of the northern pitcher plant (*Sarracenia purpurea*) are morphologically adapted such that they both capture invertebrates and absorb nutrients from their carcasses (Heard 1998). The characteristic pitcher-shaped leaves, with the hollow central cavity, act as pitfall traps for various flying and crawling insects (see Appendix A). As the carcasses break down, the plant is then able to absorb nutrients through digestive glands (Owen and Lennon 1999). In this manner, the northern pitcher plant is able to take in nitrogen, sulfur, and phosphorous (Bradshaw and Creelman 1984).

Other than supplementing nutrient uptake, the pitcher plant leaves also function to house an entire inquiline community ranging from bacteria and protozoa to larval invertebrates, primarily larval mosquitoes and midges (Cochran-Strafira and von Ende 1998). This community subsists on a number of symbiotic relationships between the pitcher plant and the inquiline community as well as within the inquiline community itself. The primary trophic level of the inquiline food web consists of trapped invertebrate prey, including ants, beetles, and flies as well as other insects (Judd 1959). While the pitcher plant needs to absorb the nutrients from these carcasses, the rate of decomposition is too slow to allow for sufficient nutrient uptake. Therefore, it is necessary that the carcasses be reduced to smaller, easily degraded pieces. The pitcher plant flesh fly *Blaesoxipha fletcheri* fulfills this role of reducer, as does the pitcher plant midge *Metriocnemus knabi*; the flesh fly feeds on suspended carcasses whereas the midge

reduces those that sink to the bottom of the pitcher. The various bacteria and protozoa present in the pitcher then feed on the particulate carcasses, and they in turn serve as food for rotifers and the pitcher plant mosquito *Wyeomyia smithii* (see Appendix B) (Bradshaw and Creelman 1984, Kneitel and Miller 2002). The pitcher plant benefits from the presence of these organisms, particularly the flesh fly and midge larvae as they increase the rate of decomposition of carcasses and thus increase the plant's rate of nutrient uptake.

The larvae, as symbionts, also benefit from their relationship with the pitcher plants. The cavity of the leaf provides the environment necessary for the completion of the life cycles of the pitcher plant midge, mosquito, and flesh fly (Nastase et al. 1995, Heard 1994). The eggs of these insects are usually laid in new pitcher plant leaves in late July and early August. The following year, pupation and the emergence of adults occurs in mid- to late July (Heard 1994). These dates are latitude dependent but according to the calculations of Bradshaw and Lounibos (1976), there will not be a significant change in seasonal pupation and emergence. Thus, Heard's (1994) estimated times for egg laying, pupation, and emergence are sufficient for the UNDERC locale.

While these insects and the relationships with the pitcher plant have been the focus of many studies, there is a lack of research concerning variation among pitcher plants and their inquiline communities between different bogs in the same region. It is the aim of this study to determine if pitcher plants from different bogs in the same region vary in physical, chemical, or environmental characteristics and in inquiline community composition. Harvey and Miller (1996) researched spatial variance in inquiline community composition; they found that despite the variance in individual pitchers,

inquiline communities do not significantly vary among subpopulations and populations of pitcher plants. From this, it is anticipated that there will be no significant variation among the physical, chemical, or environmental characteristics or in inquiline community composition of pitcher plants in different bogs but even though bog will have a characteristic pH.

Materials and Methods

The survey of pitcher plants, their contents, and the bogs in which they grow involved several quantitative measurements taken in the field. For this study, 20 pitchers each in 5 different bogs were sampled for a total of 100 pitchers. The field sites were Cranberry Bog, Forest Service Bog, Tender Bog, Ed's Bog, and North Gate Bog, all of which are located on the UNDERC property. These sites were chosen for their abundance of pitcher plants as well as for geographic dispersion around the UNDERC property (see Appendix C). Paths were walked around the perimeter of the open water in each bog and every visible pitcher plant flagged within approximately five meters of the water's edge. These flags were then numbered and, using Microsoft[®] Excel, 20 random numbers were chosen for each bog to correspond with the flagged plants. Plants which were submerged by walking on the sphagnum mat nearby were thrown out of the valid list of plants as the inquiline community was most likely flushed out of the pitchers; new random numbers were then drawn. One pitcher was chosen from each plant for sampling. The pitcher chosen was of average size for that plant, had no holes (if all pitchers on a given plant had a hole, then the pitcher with the smallest hole closest to the lip of the leaf was chosen), and had the least evidence of grazing (i.e. had the most intact

hood). Red flagging was then tied loosely around the stalk of the pitcher and labeled with the bog letter and sample number written in permanent marker. Sample number ranged from 1 to 20 for each bog, and the bog letters were as follows: Cranberry Bog—C, Ed's Bog—E, Forest Service Bog—F, North Gate Bog—N, and Tender Bog—T.

The first data taken were the temperature of the pitcher fluid prior to any disturbances caused by sampling. A standard alcohol thermometer was gently rested inside the sample pitcher and remained there while other measurements were made. The pH of the soil/sphagnum was taken approximately one inch below the surface by parting the sphagnum and inserting the pH meter to the appropriate depth. The depth of the pitcher plant leaf was measured from the lip of the pitcher to the point on the stalk where the hollow cavity ended in solid stem. Circumference was measured at the widest, enclosed portion of the leaf (see Appendix B). The thermometer was then read, having had sufficient time to adjust to the temperature of the pitcher fluid.

Next, the contents of the leaf were pipetted into a graduated cylinder and the volume of fluid in the pitcher recorded as the actual volume. The pitcher fluid was then poured into one or more Petri dishes, as the volume required, and midge, mosquito, and flesh fly larvae were counted. The number of prey carcasses present were also counted and classified as flying, non-flying, ants, beetles, spiders, or unidentified carcass. The pH of the pitcher plant fluid was taken by holding the pH meter in the Petri dish in a region with minimal larvae in order to avoid damaging them. While the pitcher fluid was out of the pitcher, the potential volume was measured by filling the pitcher to the point of overflowing with distilled water from a graduated cylinder. This water was then pipetted

out of the pitcher and the inquiline community and pitcher fluid returned to the pitcher plant. This sampling lasted from June 2 to June 7.

From July 12 to July 17, the pitchers were revisited and samples were for protozoa analysis. A 0.75 mL sample of the fluid was pipetted out, stored in plastic transfer vials, and taken back to the lab for protozoa analysis. Using Palmer counting cells, the diversity of the protozoa in a 0.1 mL sample of the pitcher fluid was measured. For each sample, one half of the Palmer cell was scanned in columns, continuously when the density permitted it, such that the entirety of that half of the cell was viewed. Each species of protozoa was notated by letters, though not identified to specific species. A running tally was kept for each lettered species in each slide. Any slide containing more than 50 individuals of a given lettered species was recorded as “>50” rather than making a precise count as species diversity rather than abundance was of interest. The number of rotifers was also noted for each Palmer cell to determine rotifer abundance.

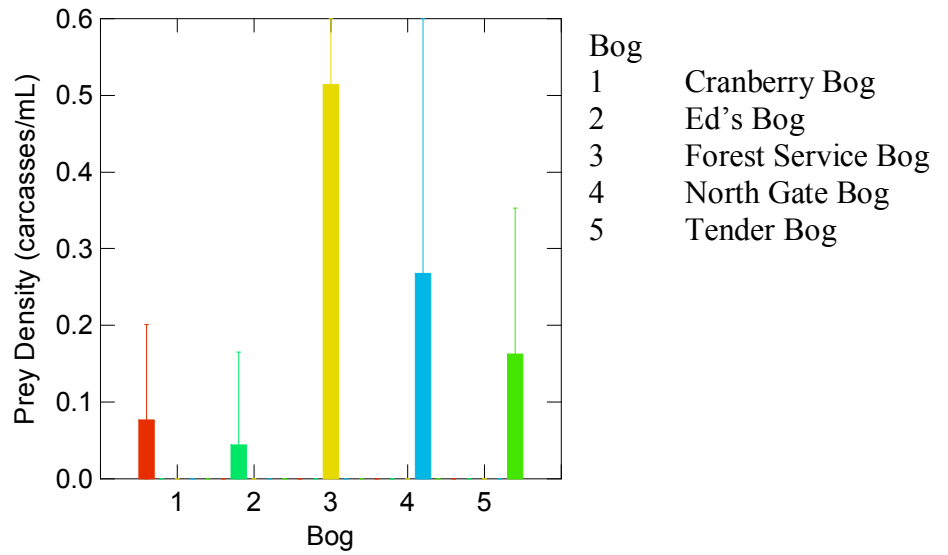
Due to the anticipated pupation and emergence of adult midges, mosquitoes, and flesh flies in mid- to late July, it was necessary to sample in June and early July and over a short period of time so as to avoid dramatic changes in the inquiline community. As the inquiline community was likely to change in composition over the course of the summer as larvae died or pupated and emerged, the gap between the first and second samplings prevent correlating protozoa diversity and rotifer abundance with the inquiline data taken during the first sampling. Should sampling of the same variables in different bogs have been drawn out into the period of pupation and emergence, the data would show reduced larval counts in the later samplings, skewing data for those bogs.

The data collected were compiled with the bog being the independent variable and all other data being dependent variables. The mean of each variable was taken for each bog and graphed in order to determine a rough estimate of possible significance. The mosquito number, midge number, and prey number were all converted to density values by dividing the count by the actual fluid volume of the pitcher. The rotifer counts were originally rotifers/0.05 mL and were converted to rotifers/mL. A one-way MANOVA was run for the following variables: pH of the soil, pH of the fluid, percentage of pitcher filled with fluid, midge density, mosquito density, prey density, rotifer density, and protozoa diversity. Separate one-way ANOVAs were run for pitcher depth, pitcher circumference, and potential volume of the pitcher as these three variables are intrinsically correlated. All variables showing a significant difference, as defined by a P value less than or equal to 0.05, were plotted against one another in order to determine what, if any, correlation. A post hoc test was also run on these variables. The temperatures of the fluid in the pitchers fluctuated throughout the day in accord with the air temperature, and thus were not statistically evaluated. The flesh fly larval counts were also disregarded for statistical analysis as there were none found in any pitcher plant in any bog, most likely due to the early sampling date.

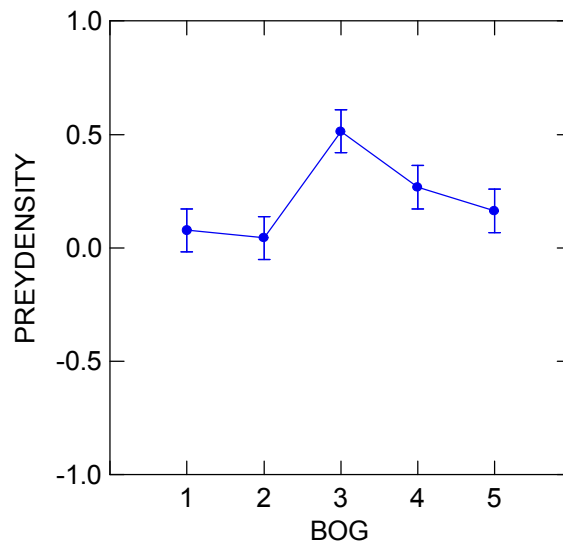
Results

Prey density $df=4$, $F=2.865$, $P=0.028$

Prey Density by Bog



Least Squares Means

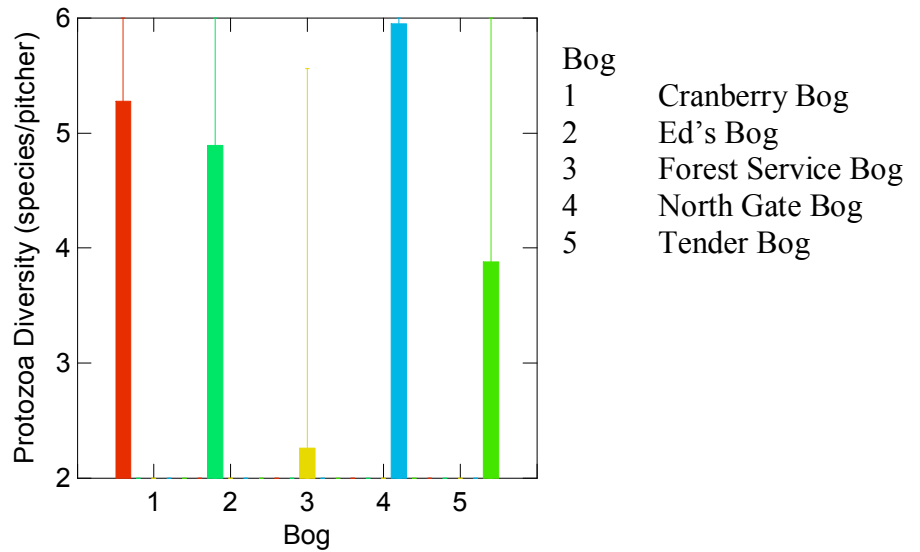


Prey Density Post Hoc Test
 Tukey HSD Multiple Comparisons.

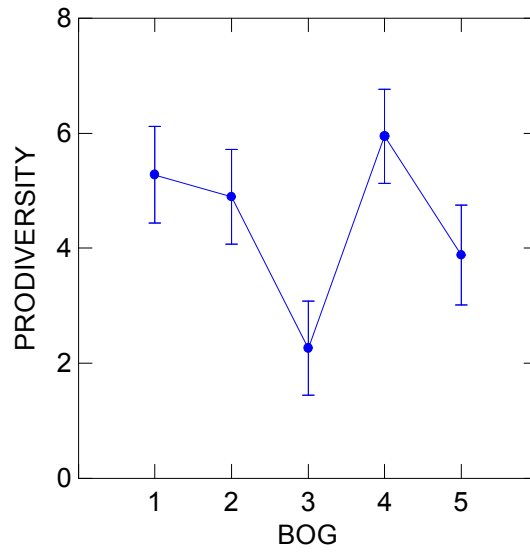
	1	2	3	4	5
1	1.000				
2	0.999	1.000			
3	0.014	0.006	1.000		
4	0.617	0.459	0.362	1.000	
5	0.969	0.903	0.076	0.935	1.000

Protozoa diversity $df=4$, $F=2.652$, $P=0.039$

Protozoa Diversity by Bog



Least Squares Means

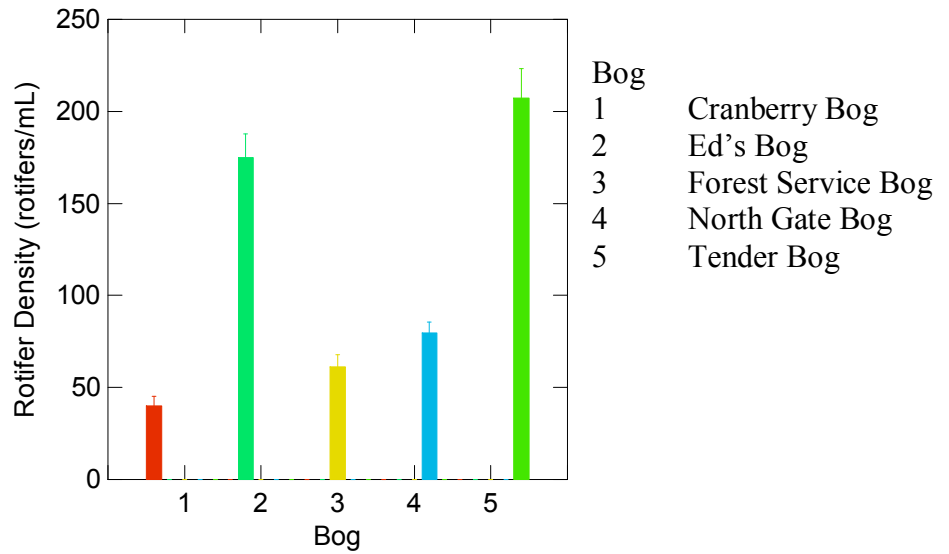


Protozoa Diversity Post Hoc Test
Tukey HSD Multiple Comparisons.

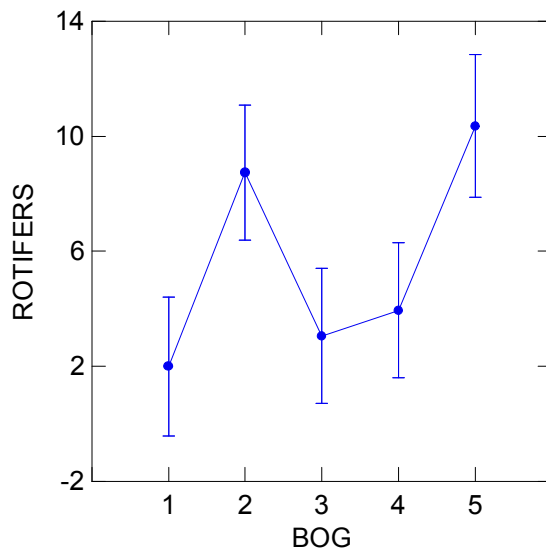
Bog	1	2	3	4	5
1	1.000				
2	0.998	1.000			
3	0.086	0.164	1.000		
4	0.979	0.893	0.017	1.000	
5	0.777	0.914	0.656	0.420	1.000

Rotifer abundance $df=4$, $F=2.459$, $P=0.052$

Rotifer Density by Bog



Least Squares Means



Rotifer Density Post Hoc Test
 Tukey HSD Multiple Comparisons

	1	2	3	4	5
1	1.000				
2	0.274	1.000			
3	0.998	0.432	1.000		
4	0.978	0.602	0.999	1.000	
5	0.121	0.990	0.214	0.338	1.000

The MANOVA showed no significant difference between bogs for any of the following variables: pH of the soil (df=4, F=0.926, P=0.433), pH of the fluid (df=4, F=2.319, P=0.064), adjusted pitcher volume (df=4, F=1.727, P=0.152), mosquito density (df=4, F=2.089, P=0.089), and midge density (df=4, F=0.661, P=0.621) (see Table 1).

Table 1: Mean Values of Pitcher Plant Environmental and Inquiline Characteristics

	Soil pH	Fluid pH	Transformed Pitcher Volume (mL)	Mosquito Density (larvae/mL)	Midge Density (larvae/mL)
Cranberry Bog	4.225	4.847	0.867	0.240	0.146
Ed's Bog	3.975	4.637	0.895	0.373	0.308
Forest Service Bog	4.085	5.058	0.747	0.403	0.264
North Gate Bog	3.965	4.537	0.913	0.227	0.187
Tender Bog	4.270	5.211	0.839	0.771	0.901

Similarly, the individual ANOVAs run on leaf depth ($df=4$, $F=0.523$, $P=0.719$), leaf circumference ($df=4$, $F=0.624$, $P=0.647$), and potential volume ($df=4$, $F=1.453$, $P=0.223$) showed no significant differences between bogs; the measurements were as varied in each bog as they were between bogs (see Table 2).

Table 2: Mean Physical Properties of Pitcher Plant Leaves

	Leaf Depth (cm)	Leaf Circumference (cm)	Potential volume (mL)
Cranberry Bog	9.474	10.340	40.230
Ed's Bog	8.947	9.845	29.400
Forest Service Bog	8.525	10.585	35.425
North Gate Bog	9.144	10.575	37.150
Tender Bog	8.932	10.385	34.850

Discussion

The lack of statistically significant variation of soil pH between bogs was unexpected as pH generally varies in accordance with bog age and level of decomposition. It is possible that the bogs at UNDERC are quite similar chemically and formed at very similar times and under similar conditions. On the other hand, this discrepancy could be the result of measuring soil pH as opposed to water pH. The surface sphagnum most likely excretes similar quantities of hydronium ion, regardless of bog, as it takes in its necessary cations. Furthermore, rain water, rather than bog water, is most likely to be in the upper portion of the sphagnum mat, standardizing pH across a given region. As a result, there would be a similar pH across every sphagnum mat, regardless of regional variation in locale. In order to account for this in future

experimentation, it would be best to measure the pH of the bog water from which the pitcher plants imbibe.

On the other hand, the lack of significant variation of the physical and chemical characteristics of the pitcher plant leaf and its contained fluid is in accordance with the proposed hypothesis. The physical attributes of the leaf, including depth, circumference, and potential volume, varied as much between bogs as within each bog itself. This may be due to the lack of variation in the chemical character of the bogs as the pitcher plants do not appear to be experiencing variable growing conditions in different bogs. It is evident, however, that the species *Sarracenia purpurea* has a general size distribution for this region. Similarly, the consistent inquiline chemical conditions were expected as the pitchers are most often filled by rain water which would have a constant pH over a given region.

Just as the physical and chemical attributes of the pitchers in all five bogs were similar, the density of mosquito larvae and midge larvae did not vary significantly between bogs. This indicates that either the breeding adult populations of *Wyeomyia smithii* and *Metriocnemus knabi* are evenly distributed across the UNDERC property or that the pitcher leaves preferred for oviposition are evenly distributed among the various bogs. Furthermore, the density of flesh fly larvae did not vary, but their complete absence suggests that this was an issue of timing and sampling too early in the season, rather than an issue of similarity between bogs and even population distribution. Future experimentation should take this into account, and if flesh fly larvae are to be counted, then the sampling period should be pushed later into the summer. However, given the

lack of variation in the midge and mosquito larvae density, it is unlikely that there would be significant differences in flesh fly larvae density.

While these top predators had only insignificant variation between bogs, there were statistically significant differences among the lower trophic level organisms, specifically the density of captured prey and protozoa diversity. Prey density significantly varied between Forest Service Bog and both Cranberry Bog and Ed's Bog. The different levels of pitcher plant prey density between bogs suggests that there are different abundances of prey in different bogs or that plants in some bogs are more efficient than their counterparts in other bogs. Given the consistency of other inquiline community variables and the physical characteristics of the pitchers, it is unlikely that the plants themselves are varying; it is more probable that it is indeed the prey availability that is varying. This could be determined in the future by capturing insects outside of pitcher plants and doing a density study of the external insect population as well as examining the prey capture of the pitcher plants themselves, looking for a correlation between external prey density and captured prey density. Furthermore, it would be worthwhile to investigate the pitcher plant density in various bogs as plant density may have an effect on prey capture rate and thus prey density.

Protozoa diversity also varied significantly by bog, but there was no general correlation between protozoa diversity and prey density. The significant variation of protozoa diversity was found between Forest Service Bog and all four other sample sites. Unfortunately, the low protozoa diversity observed in Forest Service Bog is currently unexplainable as the origin of pitcher plant protozoa is unknown.

The predator of the protozoa, the rotifer, showed marginally significant variation of density between pitchers of different bogs. The rotifer density had a P value of 0.052 which is only slightly higher than the significant value of 0.05. Though the post hoc test showed no significant variation between any two bogs, the graphic display indicates that there are two groups between which there is significant variation. Ed's Bog and Tender Bog showed high rotifer density while Cranberry Bog, Forest Service Bog, and North Gate Bog showed significantly lower rotifer density. This marginal significance is likely due to differences in protozoa populations. The general diversity of the protozoa showed no correlation with the rotifer density, but there may be a correlation between dominant protozoa species or abundance. This could be determined from a more extensive evaluation of the protozoa communities, including species identification and precise enumeration.

Conclusions

The data obtained is not wholly consistent with the proposed hypothesis which anticipated that there would be no significant differences in physical, chemical, or environmental characteristics and in inquiline community composition. Based on the general survey of *Sarracenia purpurea* performed on the UNDERC property, there is no significant variance of physical or chemical properties of pitcher plants growing in different bogs. However, there are some differences in inquiline community composition based upon the bog in which the plants are located. Protozoa diversity and prey density had significant variation between bogs while rotifer density was marginally significant. Also, variability in the age of pitchers was not evaluated; other studies suggest that there is a correlation between pitcher age and an inquiline community composition (Nastase et

al. 1995). It is necessary to further examine the inquiline communities for variation before concluding possible bog influence on inquiline communities.

Acknowledgements

I would like to thank David Hoekman for his guidance throughout the entire design, experimentation, and writing process as well as for his assistance in the field. A special thanks to Rachel Clavers for her extensive field and protozoa work and to Dave Choate for his unending patience with numerous miscellaneous questions and Systat[®] conundrums. Thank you to Karen Francl for her ability to locate Ed's Bog and to Gary Belovsky for his Systat[®] assistance. Also, thank you to those who helped count protozoa at all hours of the day and night: Andy Borden, Lynne DeFilippo, Christine Mingione, Jennifer Jeffers, Elisabeth Solchik, and Tony Hollowell. This research has been funded by the University of Notre Dame Environmental Research Center.

Literature Cited

- Bradshaw, W. E. and R. A. Creelman. 1984. Mutualism between the Carnivorous Purple Pitcher Plant and Its Inhabitants. *American Midland Naturalist* 112 (2):294-304.
- Bradshaw, W. E. and L. P. Lounibos. 1976. Evolution of Dormancy and Its Photoperiodic Control in Pitcher-Plant Mosquitoes. *Ecology* 31 (3):546-567.
- Cochran-Stafira, D. L. and C. N. von Ende. 1998. Integrating Bacteria into Food Webs: Studies with *Sarracenia Purpurea* Inquilines. *Ecology* 79 (3):880-898.
- Crowl, Todd. "Bogs." University of Notre Dame Environmental Research Center. Land O' Lakes, WI, 10 June 2003.
- Harvey, E. and T. E. Miller. 1996. Variance in composition of inquiline communities in leaves of *Sarracenia purpurea* L on multiple spatial scales. *Oecologia* 108 (3):562-566.

- Heard, S. B. 1994. Pitcher-Plant Midges and Mosquitoes: Processing Chain Commensalism. *Ecology* 75 (6):1647-1660.
- Heard, S. B. 1998. Capture Rates of Invertebrate Prey by the Pitcher Plant, *Sarracenia purpurea* L. *The American Midland Naturalist* 139:79-89.
- Judd, W. W. 1959. Studies of the Byron Bog in Southwestern Ontario X. Inquilines and Victims of the Pitcher-Plant, *Sarracenia purpurea* L. *The Canadian Entomologist* XCI March:171-180.
- Kneitel, J. M. and T. E. Miller. 2002. Resource and Top-Predator Regulation in the Pitcher Plant (*Sarracenia purpurea*) Inquiline Community. *Ecology* 83 (3):680-688.
- Nastase, A. J., C. De La Rosa, and S. J. Newell. 1995. Abundance of Pitcher-plant Mosquitoes, *Wyemyia smithii* (Coq.) (Diptera: Culicidae) and Midges, *Metriocnemus knabi* Coq. (Diptera: Chironomidae), in Relation to Pitcher Characteristics of *Sarracenia purpurea* L. *American Midland Naturalist* 133:44-51.
- Owen, T. P. and K. A. Lennon. 1999. Structure and development of the pitchers from the carnivorous plant *Nepenthes alata* (Nepenthaceae). *American Journal of Botany* 86 (10):1382-1390

Photos Used

- Ellison, A. M. 2002. Food for thought: a review of recent research on pitcher-plant bogs in New England. *Conservation Perspectives*.
www.massscb.org/epublications/summer2002/images/ellison/fig2_web.gif
- Gross, Michael. 2003. Plants of the New Jersey Pinelands.
www.mercy.georgian.edu/pinebarrens/bi_p_spu.htm

Appendix A

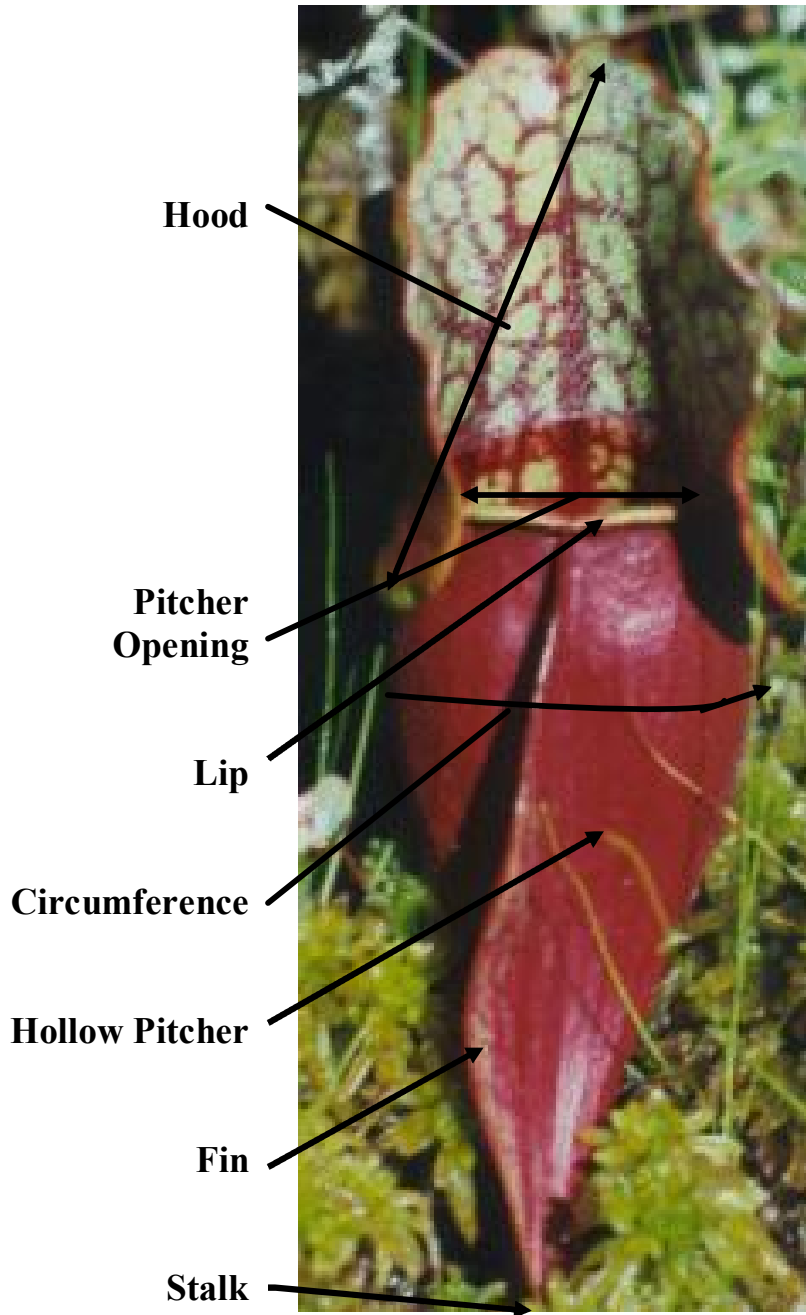
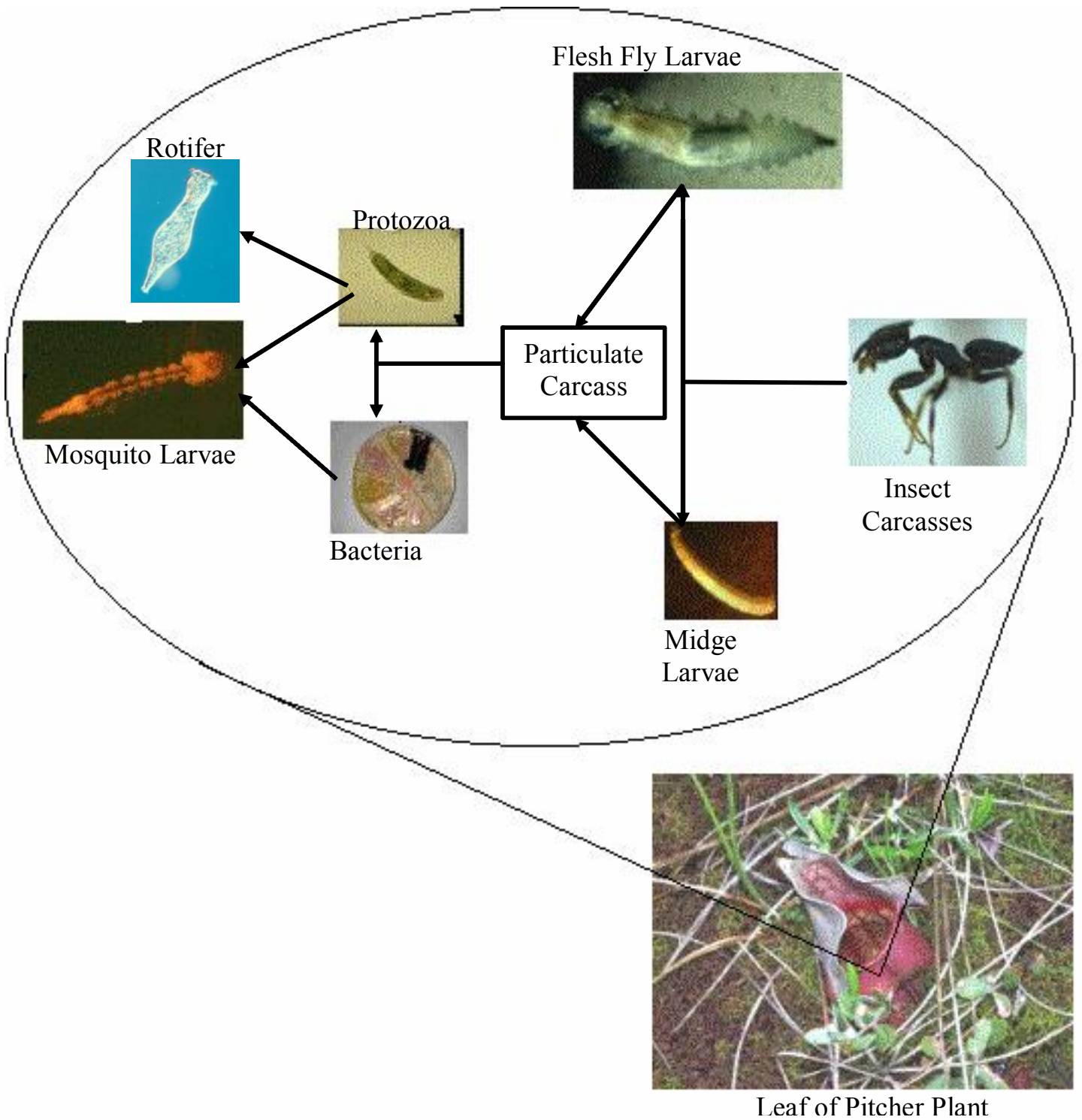


Photo (www.georgian.edu/pinebarrens/bi_p_spu.htm)

Appendix B

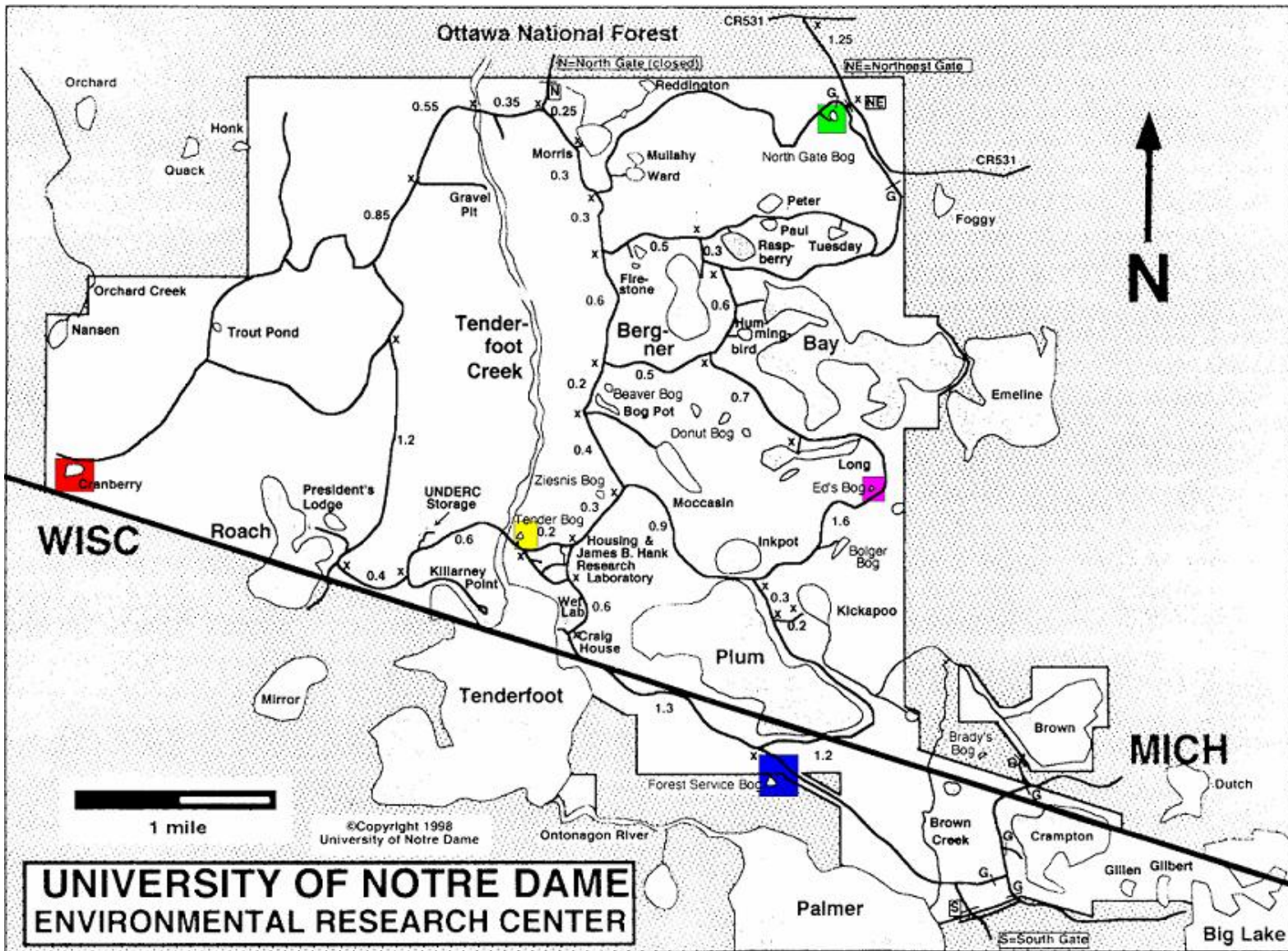
Sarracenia purpurea Inquiline Community Food Web



Photos and Design (Ellison 2002)

Appendix C

Geographic Distribution of Sample Sites on UNDERC Property



Northern Pitcher Plant and Inquiline Community Variation Relative to Bog Environment

A Research Paper for BIOS 569
University of Notre Dame Environmental Research Center

Heather Berry
Under the guidance of David Hoekman
July 22, 2003