Nutrient Gradient within Mud Lake Bog and How that Affects the Pigment Patterns of *Sarracenia*

Abstract:

Bogs are one of the most unique wetlands on the planet and provide multiple ecosystem services. The central question we focus on in this study is to find out whether Mud Lake Bog, located near Pellston, Michigan has a nutrient gradient between Mud Lake and the surrounding forest. We then related these nutrient findings to see how the nutrient gradient affects the pigmentation of *Sarracenia* within the bog. In order to complete this task we sampled for nitrate, ammonium, phosphate, dissolved oxygen, and pH level in order to identify a nutrient gradient and calculated red pixel proportions of pitchers to find the effects the nutrients have on pigmentation. The results showed a nitrate gradient starting low at the lake and raising as we got closer to the surrounding forest. There was no statistical significance related to the other nutrients' gradients, or a relationship between *Sarracenia* redness and nutrient availability. This shows that nitrate is affected by some factor at the bog, potentially the makeup of tree communities on either side of our transect. These results also show that other factors besides the visible light spectrum, measured as redness, such as ultraviolet radiation, microhabitat existence, or nectar production may be a more determinant factor related to nutrient availability. Understanding these distinctive habitats can garner information used to implement protection strategies for conservation of these valuable wetlands.

Introduction:

Wetlands are one of the planet's most important natural ecosystem because they are sinks for large amounts of greenhouse gases (Weltzin et al. 2000). Wetlands provide other services in addition to containing large amounts of these gases including providing a flood barrier, filtering out pollutants from entering water sources, and providing habitat for wildlife (Mitsch and

Gosselink 2000). These wetlands are of importance as we move forward in our understanding of climate change and the dynamic Earth as the human population exponentially increases. Gibbs (2000) concluded that there is a clear reduction in wetland density as human density increases and determined action towards conservation needs to be taken in order to maintain minimal habitat required for continued wetland species biodiversity.

Bogs are one of the most unique wetlands. They contain species and species interactions that can only be found in a limited number of areas. What makes the bog so unique, are the distinctive conditions associated with the habitat. These environments are composed of poor nutrient availability and low pH levels. One condition of this is a slow rate of decay and a large buildup of organic matter. Damman (1986) found that these nutrient poor conditions primarily are the result of how water cycles within the bog. He found that most of the water and nutrients cycling within the bog comes from precipitation rather than a combination of precipitation and groundwater. Typically habitats have nutrients flowing in and out of them through a series of groundwater flows. Precipitation alone is not capable of transporting the required nutrients to support more common wetland species. Though this cycle of nutrients has been researched, there have been further studies showing a more complex nutrient path. Damman (1978) discovered that nutrients in the deep organic layer are more mobile that once thought. Nutrients are actually capable of shifting towards the surface layer of the bog after they are leached from the slowly decomposing materials. Overall, this leads to a collection of species who found their ecological niche within bog systems.

Common vegetation that have found this niche within a bog include *Larix laricina*, *Sphagnum* species, *Sarracenia* species, *Picea mariana*, and *Drosera* species. These species have evolved the ability to tolerate these low nutrient environments. *Sarracenia* and *Drosera* are two species who have adapted to survive these conditions through carnivory. Captured insects provide for the lack of available nutrients. Other species within the bog are able to physically alter the harsh conditions. Andrus (1986) focused pieces of his research on the ability of *Sphagnum* species to lower the pH levels in the water surrounding them. This technique eliminates competition from plants who are unable to tolerate strong acidic conditions. *Sphagnum* is capable of then dominating its habitat leading to *sphagnum* bogs where large mats of the moss form.

The purpose of this study was to examine these limiting nutrients within Mud Lake Bog, a *Sphagnum* Bog near Pellston, Michigan, and to see how this deficiency affects species of plants that exist in the bog, specifically *Sarracenia*. In order to investigate these questions, we mapped out a nutrient gradient within Mud Lake Bog. Surrounding the bog is Mud Lake consisting of a thin shoreline of woody plants and trees on one side with a forested area on the other side. The surroundings of the lake and forest are related to the formation of the bog. The bog begins forming over an open body of water and over time early parts of the bog form more solid surfaces while newer parts still float out over the lake (Gates 1942). Our hypothesis for the study was that there exists a nutrient gradient of high concentrations of nutrients near Mud Lake, lower concentrations near the middle, and higher concentrations towards the forest opposite the lake because the lake and forest will act as a source of nutrients for the sphagnum mat.

The second hypothesis of the study is that *Sarracenia* will be redder in areas of low nutrients because they will allocate their available resources towards developing red pigmentation in order to catch more insects rather than towards developing photosynthetic pigmentation. As *Sarracenia* are carnivorous, they need to be capable of attracting prey. Multiple studies have found relationships between *Sarracenia* and their process of nutrient absorption. At a more basic level, Iversen, Bridgham, and Kellogg (2010) asserted that the success of plants is directly related by their ability to uptake nitrate, ammonium, and phosphate. Cresswell (1993) found that the increase in red pigmentation in *Sarracenia* attracts more prey for the *Sarracenia* and thus additional nutrients. Ellison and Gotelli (2001) found that added nutrients from insectivory increases a plants ability to uptake nitrogen in the soil and also (2002) found that *Sarracenia* experience phenotypic plasticity and have different phenotypes based off of the amount of insect and soil nutrients available. We predicted that as nutrients are lower towards the center of the bog, there would be an increase in the red pigmentation among the *Sarracenia* while *Sarracenia* closer to the higher-nutrient bog boundaries have more green pigmentation. The insects are able to provide more limiting nutrients to the attractive red *Sarracenia* while the green *Sarracenia* can focus more on executing photosynthesis because of the higher nutrient content in the soil.

Methods:

We began by choosing an area of 70 meters long by 40 meters wide. The area was chosen because the dimensions coincided with the boundaries between the lake and the forested area and it was a sample representation of the bog gradient in question. Within this plot we split the area into 5 transects taking a sample every 10 meters at the center of the quadrat. This process totaled to 40 samples taken (Fig 1). A meter-squared quadrat was used to mark the area where the samples would be taken from. GPS coordinates were recorded at each of the sample sites in order to mark quadrat locations within ArcGIS (Environmental Systems Research Institute Incorporated, Redlands, California) programming.

Within each quadrat we tested for pH, dissolved oxygen, nitrate, phosphate, and ammonium. To test for pH we used a pH meter and to test for dissolved oxygen we used a DO meter. The pH meter and DO meters were placed in the water available at the surface of the bog. Water samples were then sent to the chemistry lab in order to test for the remaining nutrients. These samples were collected be using a siphon pump. The pump was inserted underground until the researcher hit water that was available for the plants to uptake into their roots. Organic matter was removed using a fine particle filter and a funnel in order to provide a consistent water sample for analysis. The nutrient level result were then mapped out individually using ArcGIS.

Each individual *Sarracenia* pitcher within each quadrat was photographed totaling 137 *Sarracenia* pitchers. The pictures were taken in shade created either by the clouds or by blocking out the sun. This procedure reduced the effects of lighting and glare. Adobe Photoshop CS6 (Adobe Systems Incorporated, San Jose, California) allowed us to calculate the amount of red pixels compared to total pixels of each pitcher which gave us the proportion of red pigmentation of each *Sarracenia*. Regression analysis was then used to compare the nutrient availability as a function of distance from the lake and pigmentation patterns as a function of nutrient availability across the plot. In addition to running regressions, we were able to run ANOVA tests by separating the quadrats into zones ranging from the lake to the forest. Zone 1 included the quadrats 0-10 meters away from the lake, Zone 2 was 20-30 meters away from the lake, Zone 3 was 40-50 meters away from the lake and Zone 4 was 60-70 meters away from the lake.

Results:

Results for pH showed that the pH values were relatively stable ranging from values of 3.21-4.27. The regression analysis of dissolved oxygen as a function of distance showed no statistical significance (Fig. 2, R^2=.026, p=.322). Phosphate gradient results (Fig. 3) showed no statistical significance as a function of distance from the lake after being calculated using regression analysis (Fig. 4, R^2=.0097, p=.544), though the overlain ArcGIS map of phosphate (Fig. 3) shows small hotspots near tree stands. Ammonium nutrient gradient (Fig. 5) data analysis yielded similar results as the regression analysis was once again not significant (Fig. 6, R^2 = .0136, p=.474). There is a visible nitrate gradient on the overlain ArcGIS map (Fig. 7) stretching from low values at the lake to high values near the forest and the regression analysis comparing Nitrate level against the distance from Mud Lake illustrates a statistically significant result (Fig. 8, R^2=.8416, p=.000). ANOVA analysis comparing nitrate levels between zones revealed that there was a statistically significant difference in nitrate levels between zones 1 and 3, zones 1 and 4, zones 2 and 3, zones 2 and 4, and zones 3 and 4 (Fig. 9, $F=22.304_{df=28}$, p=.000).

The gradient of the proportion of redness of the *Sarracenia* is shown using the ArcGIS map (Fig. 10). When compared to our significant nitrate gradient there was no correlation between percent redness and nitrate levels (Fig. 11). Compared to all other nutrient values the percent redness did not correlate to Ammonium (R^2=.003, p=.781), Phosphate (R^2=.013, p=.553), or DO (R^2=.001, p=.847).

Discussion:

Although there was no relationship between the distance from the Mud Lake and phosphate levels there were apparent hotspots when the data was shown using ArcGIS. When observing the data as a transparent gradient the hotspots appeared to be where there were tree stands scattered throughout the bog. This would imply that the presence of tree communities correlates to an increase in phosphate levels in bog communities. Tyrell and Boerner (1987) found that as *Picea* leaves fall there is a small amount of phosphate that leaches into the soil. *Picea* was a common tree found within the bog community and could explain the increase in phosphate levels around tree covered quadrats. Further surveying of the trees in the bog and our transect specifically could help solidify this information.

Additionally Kellogg and Bridgahm (2002) found that as trees grew in the bog, their root biomass doubled as they expanded deeper into the soil. As the root biomass increases into the soil there is more decomposable matter available for microorganisms to breakdown. These microorganisms then have the ability to mineralize the phosphorus stored in the roots of the tree into phosphate that is found in the soil. Tree stands would produce this increase in root biomass, thus increasing the microorganism activity creating phosphate the hotspots.

The findings of the nitrate gradient could be a relationship towards the composition of the tree communities at either end of the lake. Williams and Crawford (1983) found that a variety of ammonifying, denitrifying, and nitrogen fixing bacteria live in peat lands. Dijkstra et al. (2009) found that when *Larix laricina* is growing as a monoculture, the sites have a high nitrogen mineralization rate, but when the *Larix laricina* is growing together with *Picea mariana* the sites have low nitrogen mineralization rates. As both species of trees were present all across the bog, the nitrate gradient could potentially be explained if there was a high proportion of *Larix laricina* found on the forest end of the transect where high nitrate values were found and a mixed proportion of both species near the lake where the nitrate levels were low. Further research could be done examining the composition of trees at both the lake and forest ends of the transect.

As our data does not imply any significant relationships between the pigmentation of *Sarracenia* and nutrient values we examined a few other possibilities as to what may affect the pitcher pigments. Joel, Juniper, and Dafni (1985) suggested that like some other species of vegetation the *Sarracenia* may attract insects by using ultraviolet radiation rather than the visible light spectrum. This would imply that the visible color of the *Sarracenia* pitchers does not relate to nutrients because they compensate for low soil nutrient availability using methods other than visible light. Bennett and Ellison (2009) also advocated that *Sarracenia* attracts insect prey through their nectar production. This would produce the same conclusion that because the visible light spectrum is not involved in attracting prey the visible light spectrum is not related to nutrient availability.

Chapin and Pastor (1994) researched the possibility of simple competition interaction between the *Sarricinia* and other species and microbes surrounding them, asserting that the pitchers may not absorb the available nitrate in the soil. Without absorbing the nitrates in the soil the idea that a nutrient gradient would affect the pigment would be completely null. The concept of phenotypic plasticity brought up by Ellison and Gotelli (2002) can be applied, saying that similar genotypes may change their pigment based on the nutrient availability in their specific environment. What determines the phenotype would still be in question as there are multiple ideas as to how soil nutrients are absorbed and distributed and what component of the *Sarricinia* attract prey.

One of the biggest possibilities that could explain our lack of correlation between *Sarracenia* redness and our nutrient gradient is the existence of microhabitats within Mud Lake Bog. We took each water and nutrient sample at the center of each quadrat. There exists the possibility that there were microhabitats within each quadrat where the concentration of nutrients under each individual pitcher. If the difference in nutrients changed within small spaces of tenths, or hundredths of meters then we could have missed the exact value under the pitchers. For future research, taking 137 water samples under each pitcher plant in order to control for the possibility of microhabitats we could get a more accurate representation of the nutrients absorbed by each pitcher. Belyea (1996) found that the position in the water table, litter type, and breaking down of peat all can have an effect on creating microhabitats within a bog. Taking account for microhabitats could bring a significant relationship between *Sarracenia* pigmentation and the nutrient gradient.

Future studies could further explore the bog tree communities and bog microhabitats. Overall, we found that there existed a nitrate gradient with increasing nitrate as one moved from the Mud Lake towards the surrounding forest. This relationship of this nitrate gradient, or the relationship between any other nutrients, provided no significant data in an explanation of *Sarracenia* pigmentation. The further understanding of bog communities will help ensure workable protection for this unique wetland as populations expand and wetlands are put at risk.

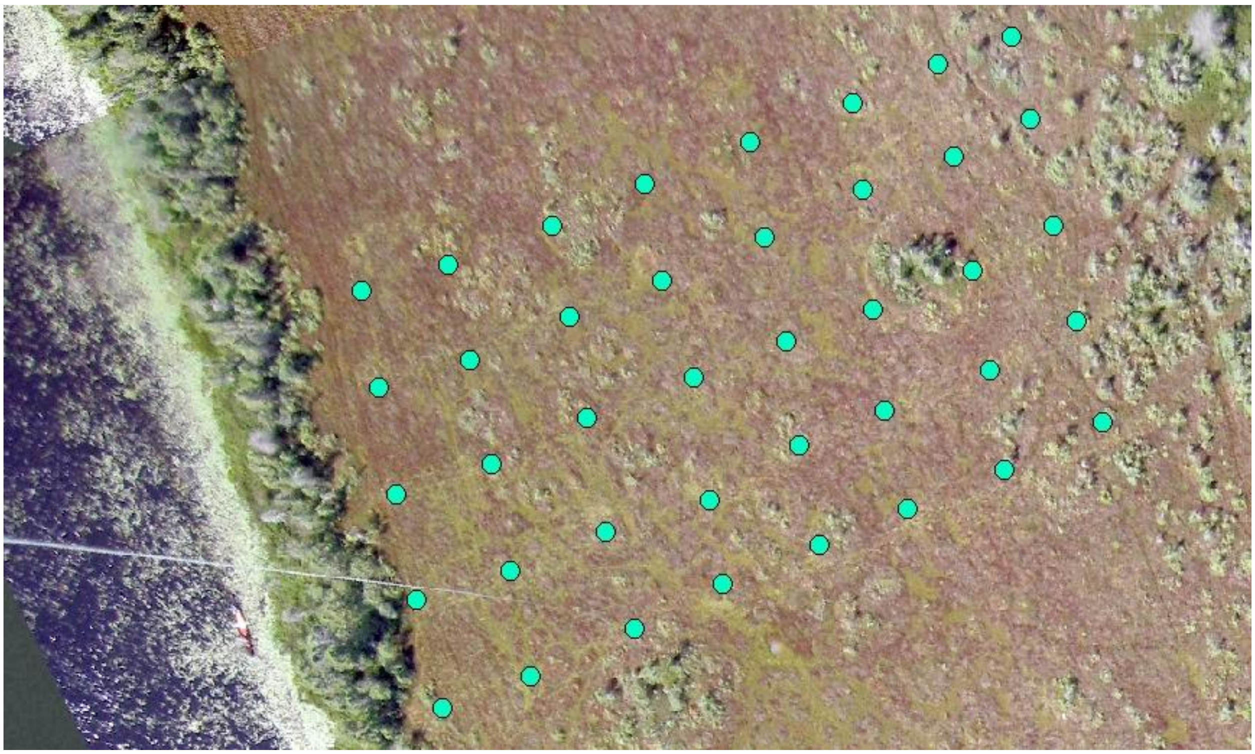

Figure 1 - Overview of Mud Lake Bog Transect Locations

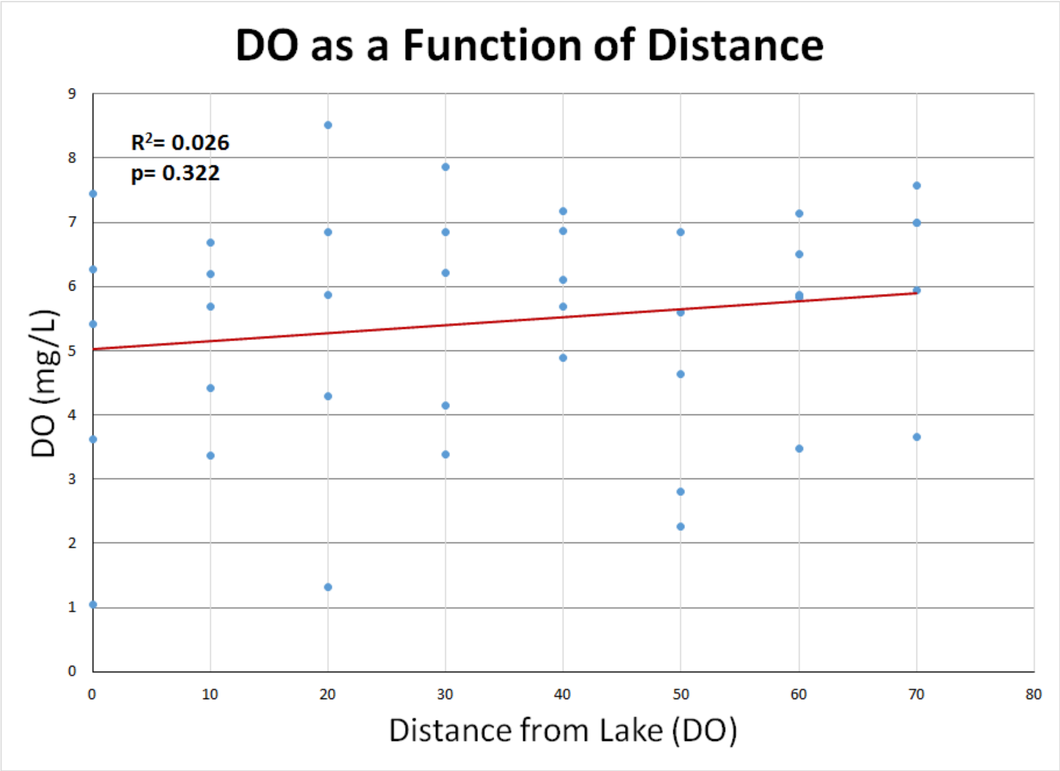

Figure 2 - Regression analysis of DO versus Distance from Mud Lake

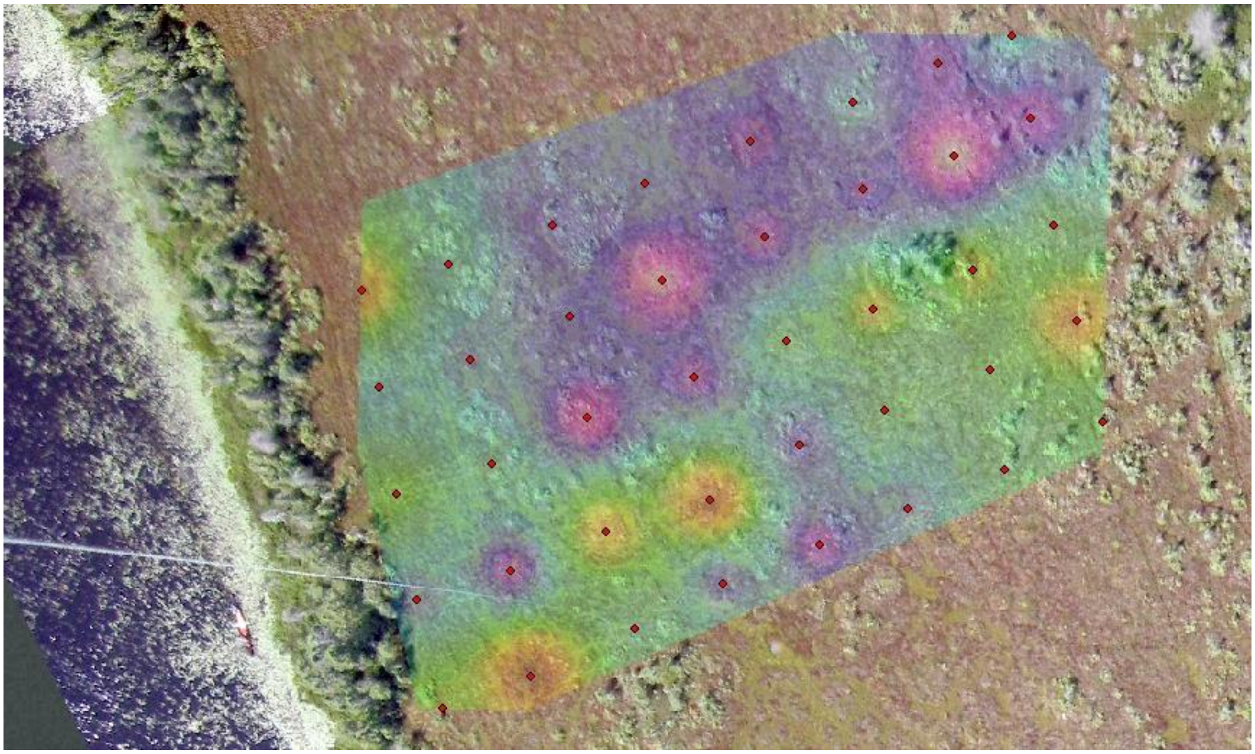

Figure 3 – Phosphate Gradient laid over transects using ArcGIS

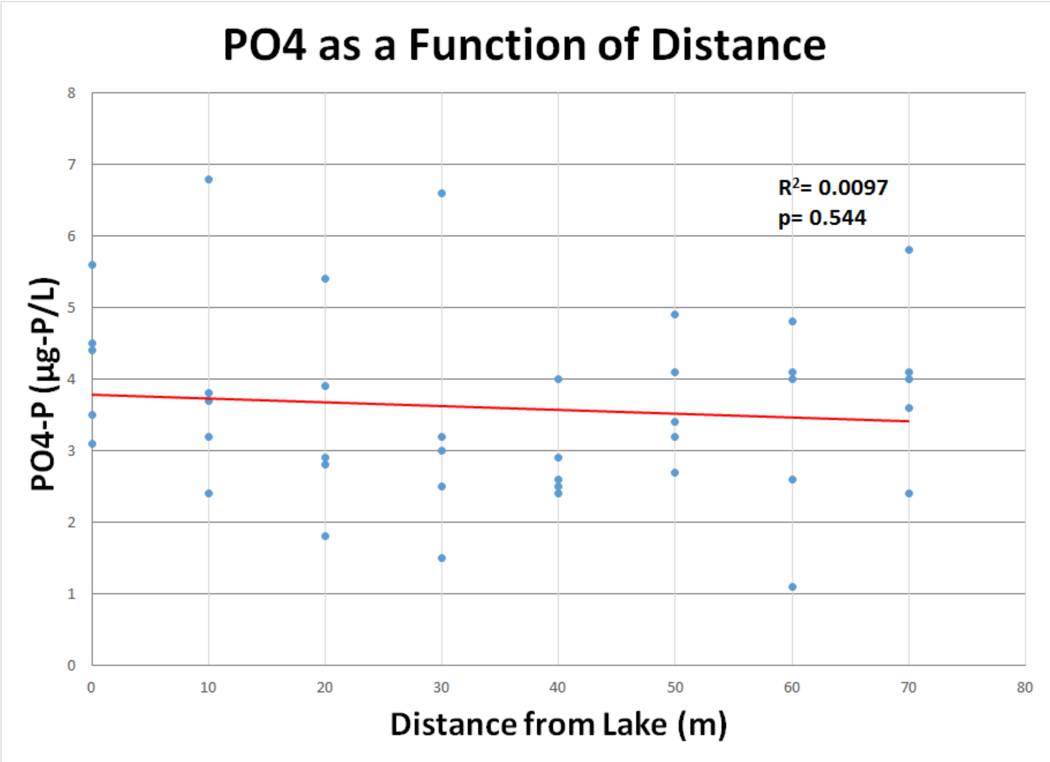

Figure 4 – Regression analysis of PO4 versus distance from Mud Lake

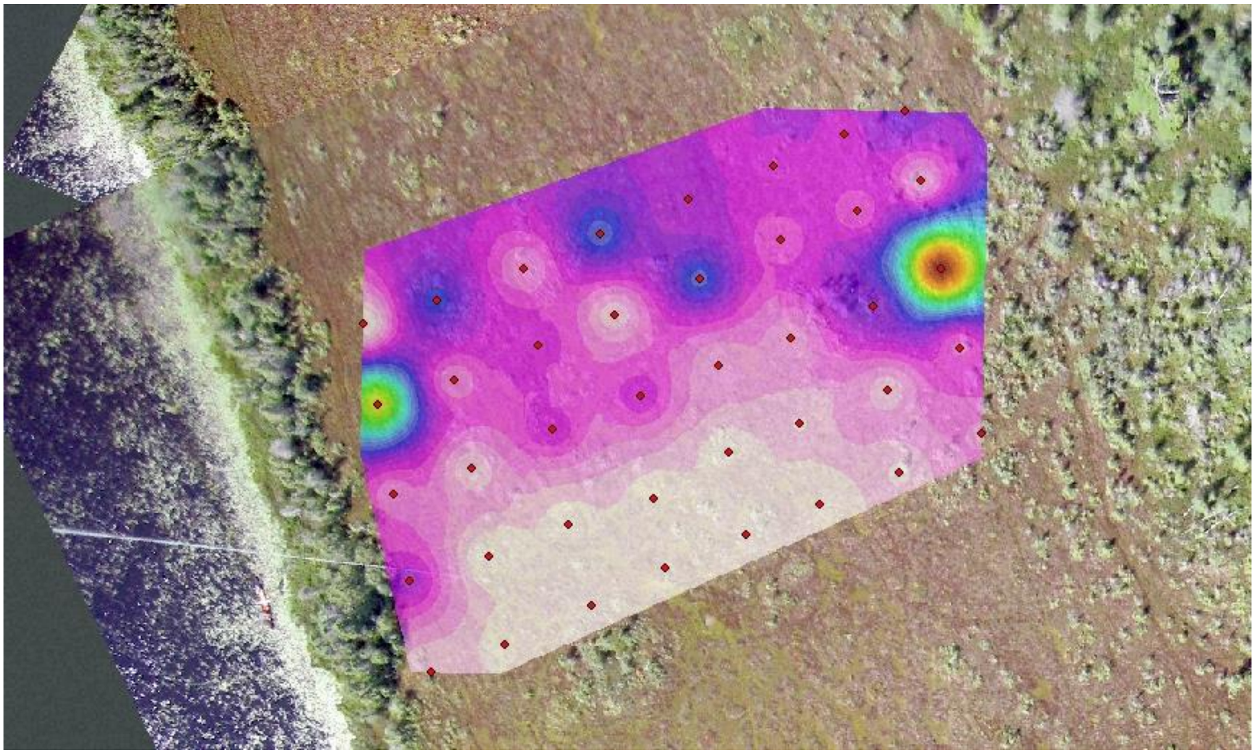

Figure 5 – Ammonium gradient laid over transects using ArcGIS

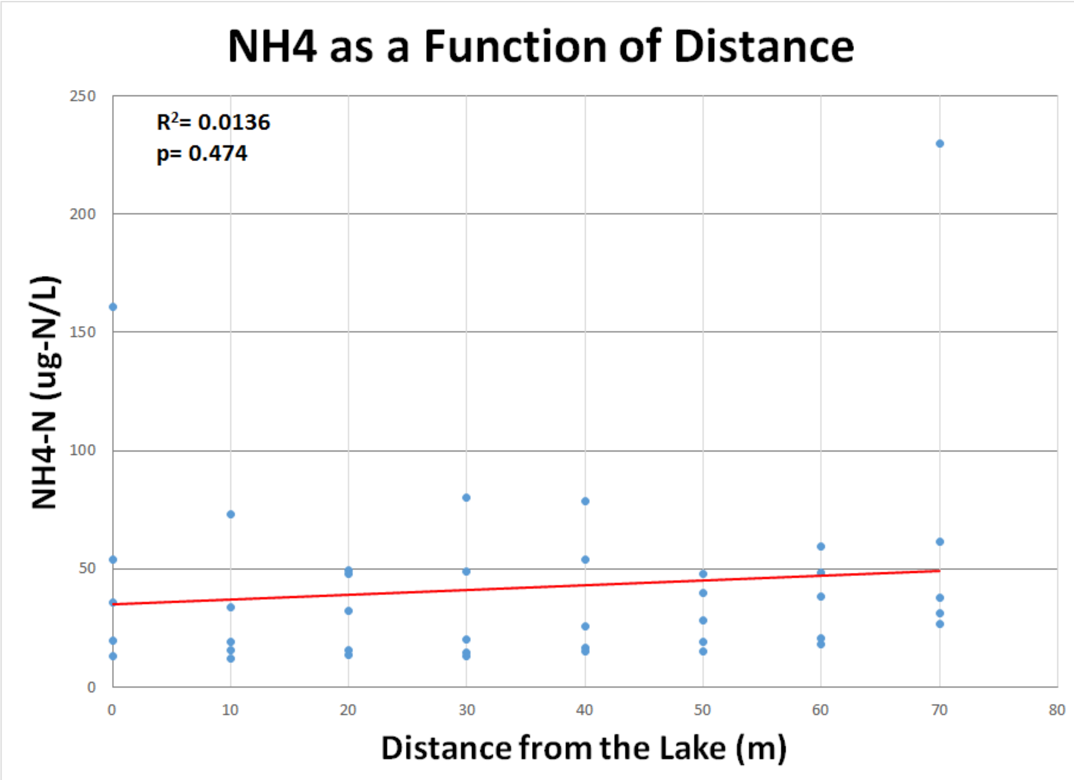

Figure 6 – Regression analysis of NH4 as a function of distance from Mud La

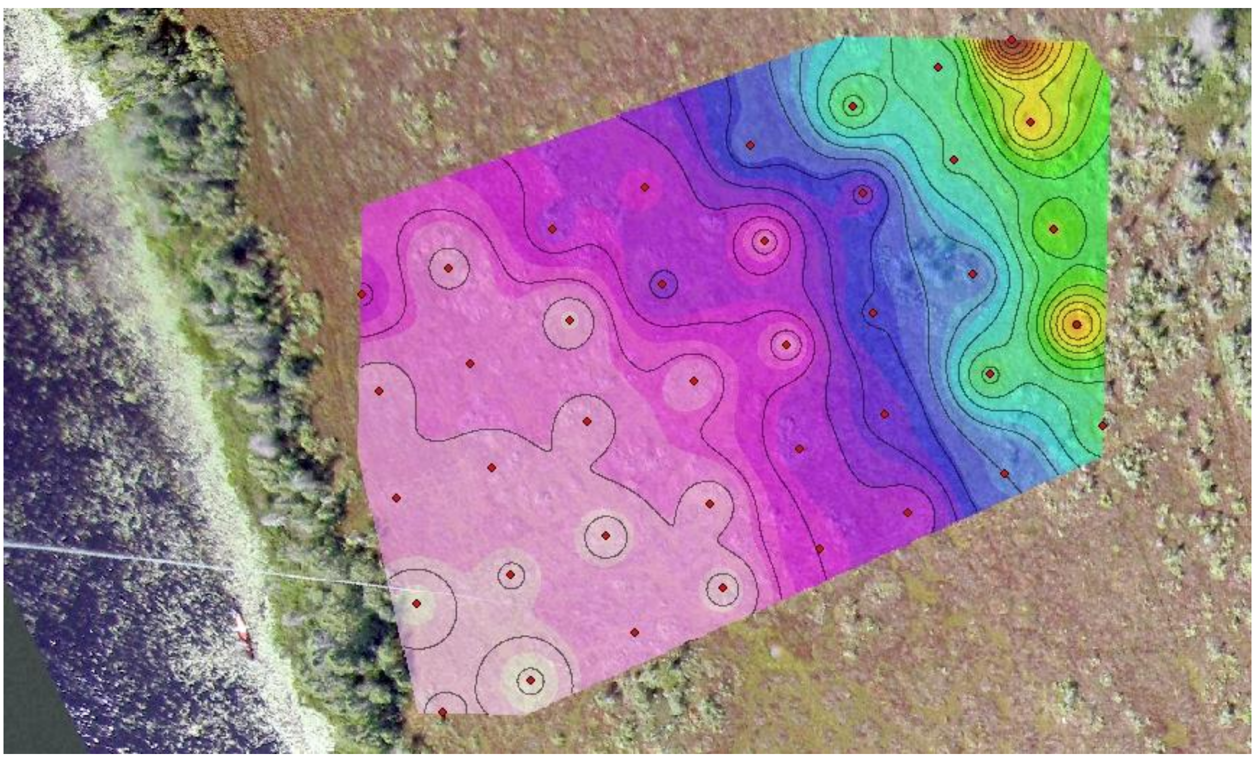

Figure 7 – Nitrate gradient laid over transects using ArcGIS

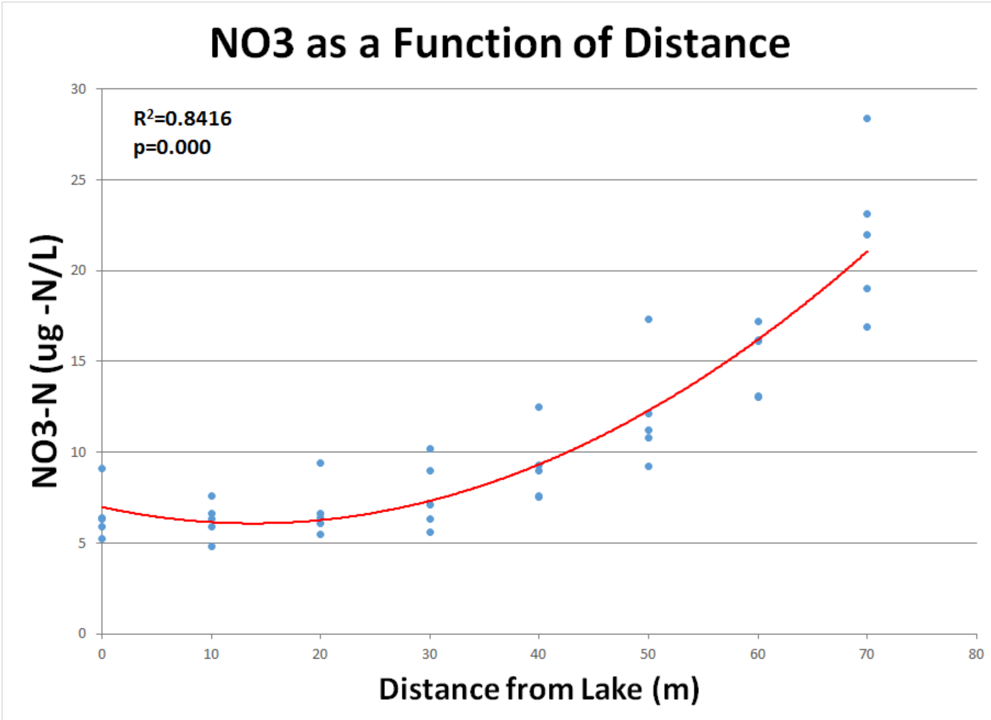

Figure 8 – Comparison between nitrate levels and distance from the lake

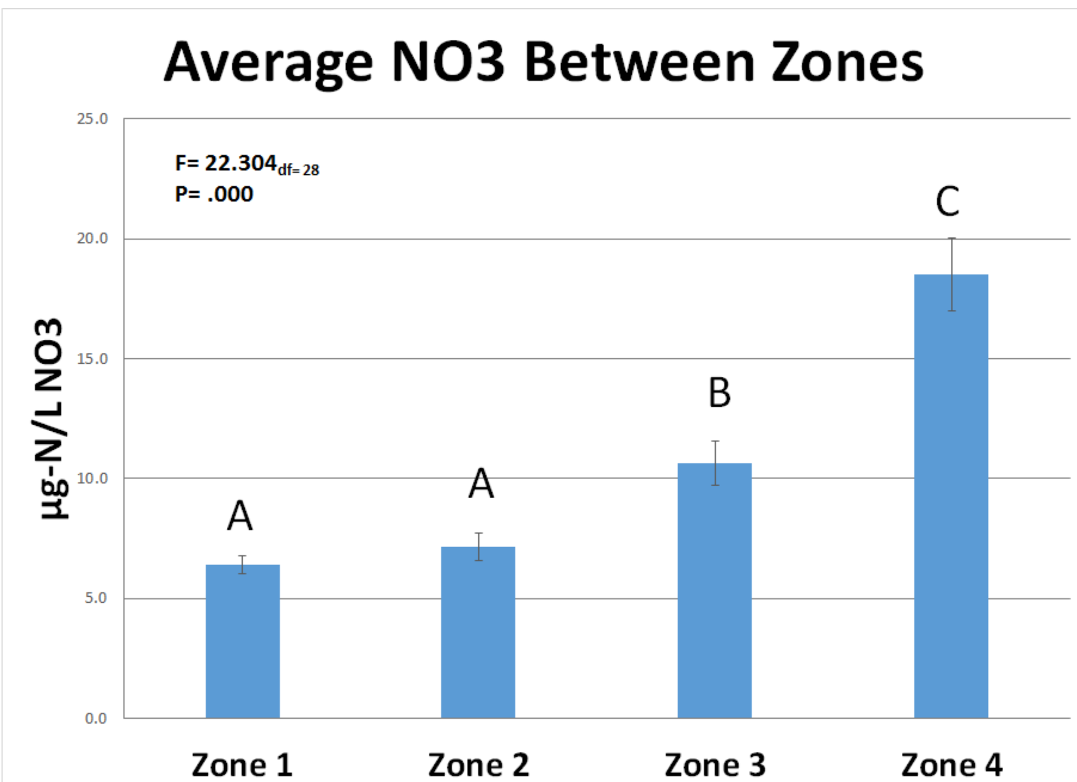

Figure 9 – ANOVA showing nitrate differences between zones

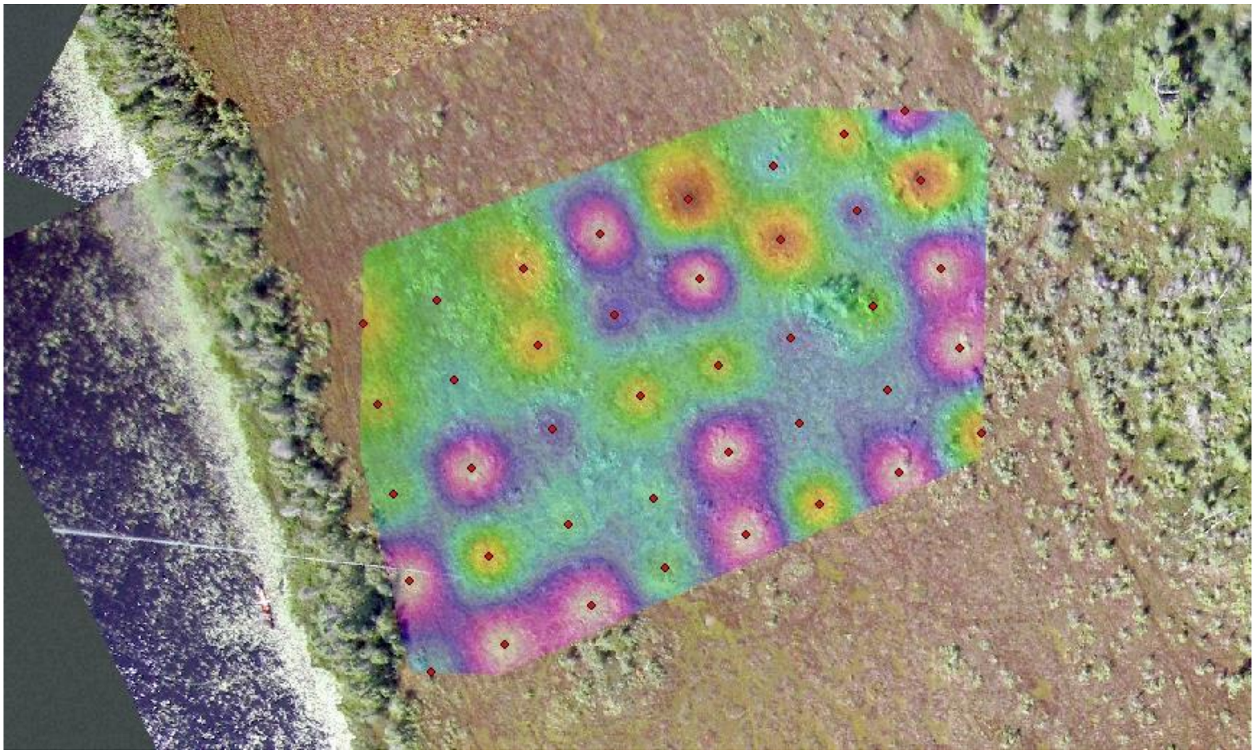

Figure 10 - *Sarracenia* redness percentage shown using ArcGIS

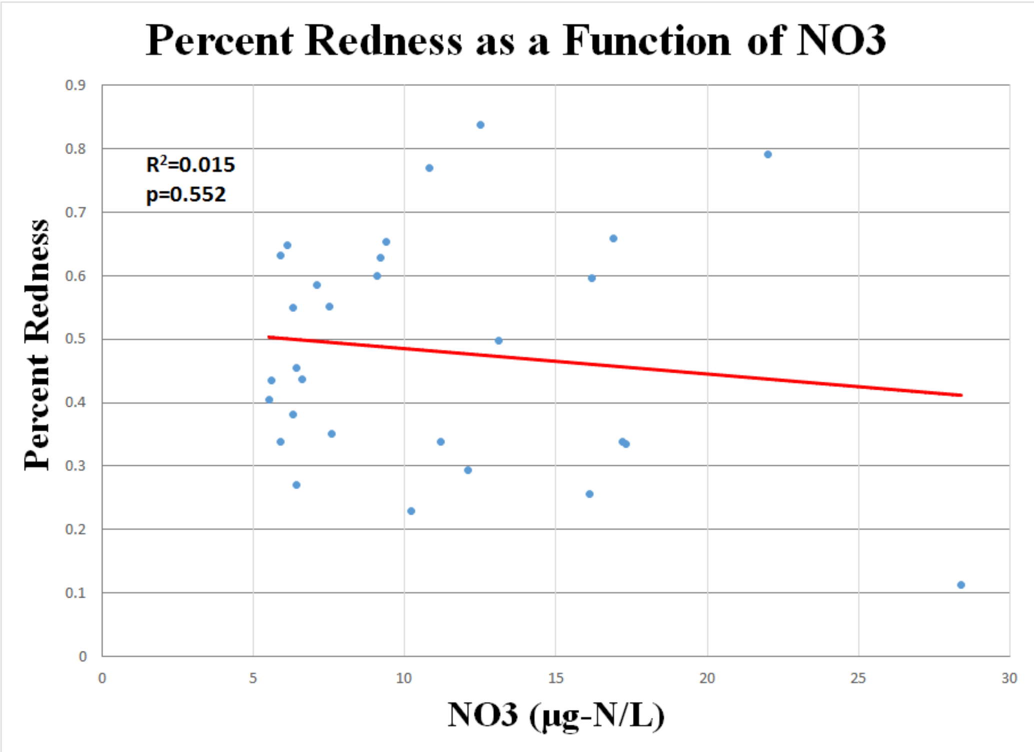

Figure 11- Comparison between Percent redness and Nitrate levels

Citations:

Cresswell, J. E. 1993. The Morphological Correlates of Prey Capture and Resource Parasitism in Pitchers of the Carnivorous Plant Sarracenia purpurea 129(1): 35-41.

Andrus, R. E. 1986. Some aspects of Sphagnum ecology 64: 416-426.

Damman, A. W. H. 1986. Hydrology, development, and biogeochemistry of ombrogenous peat bogs with special reference to nutrient relocation in a western Newfoundland bog 64: 384-394.

Weltzin, J.F. J. Pastor, C. Harth, S.D. Brigham, K. Updegraff, and C.T. Chapin. 2000. Response of bog and fen plant communities to warming and water-table manipulations 81: 3464–3478.

Gates, F. C. 1942. The Bogs of Northern Lower Michigan 12(3): 213-254.

Mitsch, W.J. and J.G. Gosselink. 2000. The value of wetlands: importance of scale and landscape setting 35: 25-33.

Gibbs, J.P. 2000. Wetland Loss and Biodiversity Conservation 14(1): 314-317.

Damman, A. 1978. Distribution and movement of elements in ombrotrophic peat bogs. Oikos 30:480–495.

Iversen, C., S. Bridgham, and L. Kellogg. 2010. Scaling plant nitrogen use and uptake efficiencies in response to nutrient addition in peatlands. Ecological Society of America 91:693–707.

Ellison, A., and N. Gotelli. 2001. Evolutionary ecology of carnivorous plants. Trends in ecology & evolution 16:623–629.

Ellison, A., and N. Gotelli. 2002. Nitrogen availability alters the expression of carnivory in the northern *Sarracenia*, Sarracenia purpurea. Proceedings of the National Academy of Sciences of the United States of America 99:4409–12.

Tyrrell, L., and R. Boerner. 1987. Larix laricina and Picea mariana: relationships among leaf life-span, foliar nutrient patterns, nutrient conservation, and growth efficiency. Canadian Journal of Botany.

Kellogg, L., and S. Bridgham. 2002. Phosphorus retention and movement across an ombrotrophic-minerotrophic peatland gradient. Biogeochemistry:299–315.

Dijkstra, F., J. West, S. Hobbie, and P. Reich. 2009. Antagonistic effects of species on C respiration and net N mineralization in soils from mixed coniferous plantations. Forest Ecology and Management 257:1112–1118.

Williams, R., and R. Crawford. 1983. Microbial diversity of Minnesota peatlands. Microbial ecology 9:201–14.

Joel, D., B. Juniper, and A. Dafni. 1985. Ultraviolet patterns in the traps of carnivorous plants. New Phytologist 101:585–593.

Bennett, K., and A. Ellison. 2009. Nectar, not colour, may lure insects to their death. Biology Letters 5:469–72.

Chapin, C., and J. Pastor. 1995. Nutrient limitations in the northern pitcher plant Sarracenia purpurea. Canadian Journal of Botany 734:728–734.

Belyea, L. 1996. Separating the effects of litter quality and microenvironment on decomposition rates in a patterned peatland. Oikos 77:529–539.