Abiotic and Biotic Influence (pH, Total Nitrogen, Insect Biomass, and Light Intensity) on Northern Pitcher Plant (*Sarracenia purpera*) Distribution at UMBS Sites

Catherine Doktycz, Rachel Hillabrand, Alex Yragui, Jerry Ilar

Abstract.

In contrast to the current knowledge about physiology and morphology of the northern pitcher plant, *Sarracenia purpurea,* very little is known about its habitat. In this study, we attempt to fill this knowledge gap by exploring the possibility of a significant relationship between *S. purpurea* fitness and abiotic and biotic factors including pH, light intensity, nitrogen content of groundwater and area-specific insect biomass. Measurements of the variables being studied were taken from two sites, Mud Lake Bog and Grass Bay Preserve, each located within 30 miles of UMBS. At each site, four arbitrary plots were chosen for a total of eight plots containing *S. purpurea,* at which we found a positive correlation between number of pitchers and diameter through a linear regression. This allowed us to use the diameter of each *S. purpurea* plant as a model for plant fitness. The mean number of pitchers for each site was determined to be significantly different by an independent t-test, but comparison of the dependent variable, diameter, with the independent variables, light intensity, pH, total nitrogen in groundwater, and area-specific insect biomass was insignificant. This study therefore accepts the null hypothesis; that there is not significant relationship between *S. purpurea* fitness and these abiotic and biotic factors.

Introduction

There are different types of wetlands, each consisting of a unique composition of organisms. Interdunal swales and bogs are two wetlands found in northern Michigan. Swales are moist, marshy, low tracts of land with rank vegetation (NH Division of Forests and Lands). Interdunal swales occur between sand dunes along a coast and they are very prevalent along the shorelines of all of the Great Lakes. They are shallow, usually freshwater, and contain a thin surface of organic soil on top of wet sandy soil (Ibid). Interdunal swales can be unvegetated or can contain low shrub communities. Swales are usually basic (around 7); however they still do not contain an extreme diversity of species due to the succession of the area creating a lack of nutrients in the newly formed sections of the swale (Ibid).

Bogs, unlike swales, are acidic wetlands, having a pH of less than 5 (Bräuer et al, 2006).These are commonly found in the northern hemisphere and are usually found in basin-like areas where water has very little inflow or outflow. Bogs are largely comprised of mosses and acidic peat (Johnson, 1985). The bog environment is regulated by sphagnum moss, the dominant species that thrives there. Sphagnum moss removes mineral nutrient cations from the water, such as nitrogen, giving off hydrogen ions in exchange, which results in a significantly lower pH (Givnish, 1989). Additionally, oxygen and nitrogen are limiting resources in a bog (Hemond, 1983). Therefore, it is difficult for other organisms to live in this type of environment, not only because of the low pH, but also because of the lack of both oxygen and nitrogen.

Swales and bogs are both challenging environments for plant establishment. Suprisingly, one unique plant is found in both habitats, this being the northern pitcher plant (*Sarracenia purpurea*). It is able to tolerate low oxygen, acidity, and low nutrients. *S. purpurea* is a carnivorous plant that relies on insects to obtain the necessary nutrients that its environment lacks. We also found that most of this plant's growth relies on the nitrogen and phosphorous from the soil, other than the nitrogen from insects (Chapin & Pastor, 1995). Insects account for less than 10 percent of the plant's nitrogen intake (Gotelli & Ellison, 2002). Additionally, the optimum pH for a pitcher plant is around 5.5 (Jett, 2005). Furthermore, the more acidic the leaf fluid, the older the pitcher plant tends to be. This may be a result of control by the plant through its secretions or a result of insect tissue degradation (Fish & Hall, 1978). The pitcher plant is comprised of a large flower (usually purplish-red), leafless stalk, curved leaves, and vestigial roots. The flowers of the plant are around 5cm wide and the leaves range from 10-30cm (Ellison & Gotelli, 2002). It feeds on flies, crickets, grasshoppers, other insects, and larvae (Bartelemy, 2008). Furthermore, the pitchers produce enzymes to start the decomposition of prey as well as downward hairs to prevent insects from escaping.

Two wetlands near the University of Michigan Biological Station (UMBS) that contain the pitcher plants are Mud Lake Bog and Grass Bay Preserve. Grass Bay is a forest-swale ecotone, while Mud Lake is a more acidic bog complex. Both differ greatly in pH and composition of organisms. We chose to study these two different types of wetlands in order to understand how *S. purpurea* is able to grow in both. At both sites, the pitcher plants were more abundant near the forest-wetland boundary. The plants were much smaller in size and number of pitchers further out in the wetland. In previous research, Baker (1996) found the optimal environment for the pitcher plant is in full sunlight.

The majority of research previously done on *S. purpurea* has focused largely on its carnivorous lifestyle and its morphological traits (Baker, 1996), while the plant's life history and distribution has been overlooked. We selected this topic because it has not been studied extensively. Due to the limited prior knowledge about pitcher plants, we decided to explore the relationship between *S. purpurea* and its environment throughout two different habitats. Based on our own observations, we predict that across the bog and swale study sites, the *S. purpurea* plants with the most pitcher leaves and largest diameter will grow in less sunlight and in acidic environments with less nitrogen (Baker, 1996). With this experiment, we hope to fill a knowledge gap about the species by studying the distribution and preferred conditions of the species within and between bogs.

Methods/Materials

At two sites, Mud Lake Bog and Grass Bay Preserve, we took measurements and samples to compare the abiotic and biotic factors affecting the growth of *Sarracenia purpurea*. Mud Lake Bog is just east of Douglas Lake, and is an acidic area and has relatively low species diversity (Breeman, 1995). Here, the pitcher plants grow on a layer of sphagnum that covers the ground. Grass Bay Preserve is a swale on the shore of Lake Huron. Its pH is more basic, and there are large areas of standing water, bordered by forest. Also, the older areas have more organic matter and therefore more nutrients and a greater diversity of species (Breeman, 1995).

Four 2 m x 4 m sampling plots were arbitrarily chosen, measured, and marked at each of the two locations (Grass Bay and Mud Lake Bog). At Grass Bay, plot 1 was marked in a dense, shrubby region; this area was located at the edge of the marsh area and was shaded by the surrounding tree line. Plots 2, 3, and 4 were located in the wetlands area that was completely exposed to sunlight. The soil here was comprised of an orange, clay-like surface layer, and wet, black soil underneath. At Mud Lake Bog, plots 1 and 4 were located on the perimeter of the sphagnum moss lawn. This area was shrubby, slightly shaded, and bordered by the tree line.

Plots 3 and 4 were in the middle of the sphagnum moss lawn, completely exposed to sunlight. In total 77 pitcher plant clumps were studied; 54 at Grass Bay and 23 at Mud Lake Bog.

Within each plot, the following measurements of the pitcher plants were recorded: the number of clumps, the number of pitcher-leaves in each clump (dead leaves were omitted, juvenile, unopened leaves were included), the diameter, the height of the tallest pitcher leaf, and the number of flower buds (dead buds were included). Next, aspects of the physical and biotic environments within each of the plots were quantified. Using a Li-Cor Quantum/Radiometer/Photometer Model LI-189, the light-intensity at the height of the pitcher plant-leaves was taken at each clump in each plot. Three water pH measurements in each plot were taken with a Fisher Scientific Accumet Portable AP10 pH/mV. Also, at either end of each plot, a length of sticky flypaper was left for three days to accumulate a sample of the insects that inhabit the area of each plot. The total biomass of the insects on each strand of fly paper was then measured, by taking the mass of a plain fly paper roll, and subtracting that from the mass of the fly paper with insects (Carver, Kryscynski, Lindow, & Roty, 1996). Finally, 1 water sample (about 100 mL) was drawn from each plot, and analyzed for total nitrogen content. To obtain one sample, a turkey baster was used to draw water from 3 arbitrary places in the plot, to fill the 125 mL container. Additionally, the data we collected is going to be analyzed through linear regressions and t-tests to look for significant correlations.

We assumed that *S. purpurea* clumps included multiple flowers/buds, pitcher clumps were accurately counted and measured, and that the tools of measurement (light intensity and pH probes) were taking accurate measurements. Another assumption was that nitrogen uptaken by the soil-water environment is the primary nutrient used by the plant. Lastly, we assumed that the plots were representative of their respective study sites.

Results

A positive correlation between the diameter (cm) and the number of individual pitcher leaves of *S. purpurea* was found between the two dependent variables (R^2= .849 , p= .001, Figure 4) . We therefore selected just one, diameter, to compare to light intensity, pH, total amount of nitrogen in the water, and the biomass of insects in the air. We found no other significant correlations within dependent or independent variables.

We used the linear regression to compare the diameter of *S. purpurea* with each independent variable. First we conducted regressions at the sample level of the diameter of pitcher clumps by light intensity, and pH across both sites (n=77). Then we conducted regressions at the plot level across both sites for total nitrogen of the water and biomass of insects (n= 8). There were no significant relationships between diameter and any of the independent variables at the sample or plot level across both sites (Figure 5).

An independent t-test confirmed the mean number of pitchers at both sites were significantly different (p= .029). We then stratified our data between the two sites, Mud Lake Bog and Grass Bay Preserve, and performed the linear regressions within sites. We regressed diameter on pH within each site and this was a significantly negative relationship between pH and diameter in the Grass Bay site (t=-.092, df=1, p=0.001). Our collection of biomass of insects provided observational data of Grass Bay Preserve having a more abundant amount insect species, while Mud Lake Bog had smaller and less insect species.

pH appeared to be significant (R^2= .182 , P= .001) at the Grass Bay Preserve. The linear regression contained a negative correlation between the diameter of S. purpurea and the pH (Figure 6). All other independent variables were insignificant compared with the diameter when regress within each site.

We then created a histogram to visualize the distribution of *S. purpurea* plants by pH (Figure 7). The pH of plants found in Mud Lake Bog ranged from 3-4, while the pH of plants found in Grass Bay Preserve ranged from 5-8. In comparing the mean pH's of the plots in Grass Bay Preserve, we discovered that plot 1 proved to be an outlier (Figure 6) with a much lower pH than any of the other plots in the site (and much lower than when taken 2 days prior) (Figure 8).

Consequently, we performed the linear regression again, stratified between the two sites without the data of plot 1 from Grass Bay Preserve due to it being an outlier. The regression between the diameter of *S. purpurea* and pH became insignificant in both plots and slopes near zero (Figure 9a & 9b). All of our independent variables compared to the dependent variable, diameter, resulted in insignificant relationship.

Discussion

Our results revealed significant correlation between pitcher plant diameter and number of pitcher leaves. Since we assumed a greater number of pitcher leaves corresponded to greater plant fitness, this meant we could correlate greater diameter with greater plant fitness, and use diameter as a dependent variable in our multiple linear regression. In addition, a significant difference in the mean number of pitchers between Mud Lake Bog and Grass Bay Preserve suggested that we might better detect differences in pitcher diameter by examining pitcher diameter across sites instead of just within a single one. Unfortunately, the variables we selected do not seem to significantly relate to pitcher diameter.

As stated in the results, pH between plots within Grass Bay Preserve was noted as significant, but was then proven insignificant after the outlying pH data for plot 1 was removed. Plot one readings may have been so drastically different over such a short period of time, because plot 1 was located in both an older part of the swale and drier soil. The plot age is relevant because an older area is more likely to contain older pitcher plants. And, older plants have been shown to create a more acidic immediate environment from more acidic leaf fluid, due to control through secretions/insect degradation (Fish and Hall, 1978). The uncharacteristically acidic pH readings of plot 1 suggest that plot 1 is very different from other areas in Grass Bay, because pH is a significant factor in how available soil nutrients is made to plants. Macronutrients tend to be less available in soils with low pH; micronutrients tend to be less available in soils with high pH (Plant Nutrients).

With plot 1 removed as an outlier, the insignificant correlation between pH and pitcher plant diameter tells us that the natural history of *Sarracenia purpurea* does not prefer a particular pH. Although we would expect pH (and simultaneously nutrient availability) to be a determinant of plant fitness, our negative results seem plausible based on prior research. A study titled "Nitrogen availability alters the expression of carnivory in the northern pitcher plant, Sarracenia purpurea", concluded that the production of carnivorous organs in *S. purpurea* is extremely phenotypically plastic. Plants in the genus *Sarracenia* produce both carnivorous pitcher leaves and noncarnivorous but photosynthetically functional phyllodia, and when nitrogen is overly abundant in the environment, more photosynthetic phyllodia and fewer carnivorous pitchers are produced. The results favored the cost-benefit model for the evolution of carnivory, where carnivory can be expressed on different levels based on plant needs. However, in this experiment the phenotypic plasticity was observed during ecological, not evolutionary time. The pitcher plant's ability to readily adapt to environments with different nutrient levels supports the conclusion that pH is not a determining factor for plant diameter (Ellison and Gotelli, 2002).

Contrary to what we hypothesized, light intensity did not have a significant effect on pitcher plant diameter. One explanation for this is that the photometer we used was extremely sensitive, which may have given us readings that were overly variable. We could not take every reading within each plot simultaneously; even from one minute to the next, if a cloud were to go in front of the sun for a moment, that reading would be significantly lower than the rest for that plot.

Similarly, our method of measuring insect biomass in each plot area was inaccurate. We collected the fly papers on 2 different days, and on the second it rained all day. Since the papers were bagged, the samples were still wet when weighed, so a significant amount of weight recorded was due to water. We also noticed that the rain had washed off adhesive and some insects on the fly papers that were left to dry. In addition, because the fly paper was Wal Mart brand, not all unused papers were equal in weight, so our method of subtracting the mass of an unused one from a used one was flawed. For these reasons, we omitted the fly paper mass data.

The average total nitrogen content in water samples was about the same at each site, so this variable was also concluded insignificant. One error in our collection methods here could be that in some cases water was drawn from the nearest standing pool, instead of from the ground of the actual plot. Also, it rained just before the samples were collected, so we could have taken groundwater mostly diluted with rainwater.

Despite the pitcher plant's adaptability, we were still surprised that none of the variables tested proved to have a significant effect on the fitness of the species. Assuming some factor must influence the plant's growth, one such variable could be intraspecific competition. A study done in Switzerland regarding newly introduced populations of *Sarracenia purpurea* deduced that the species grew quickly and aggressively, outcompeting rare native peat bog species (Parisod, Trippi, and Galland, 2004). This growth strategy suggests that a limiting factor for *S.*

purpurea may be competition with members of its own population. This is a growth aspect that we would like to further investigate.

According to the statistical tests we ran and their corresponding p-values, we must accept the null hypothesis: there is no relationship between pitcher plant (*Sarracenia purpurea)* diameter and light intensity, pH, nitrogen availability in water, and insect biomass, in UMBS wetlands. Since the experiment was limited to a period of four weeks, we believe that further testing of the same variables over a more extended length of time would be a necessary continuation of this experiment. Specifically, the measurements of pH should be taken over a longer time period (not just one day), because of variances in pH due to rainwater. Futher testing might also include a test for Phosphorous because it too is an important nutrient for *S. purpurea* (Chapin & Pastor, 1995). For a comparative study, variables in bogs and other wetlands with different ranges of abundance of *S. purpurea* (including an area with no pitcher plant growth) should be tested. Regarding insect biomass, future researchers should find a more efficient way to take the mass of the insects in the area. Insect diversity should be calculated using the Shannon-Weiner diversity index ($H' = -\sum(p_i \text{ x } \log p_i)$) for a more accurate analysis of the insect population.

Literature Cited

Baker, F. 1996. Observations of The Pitcher Plant, Sarracenia purpurea.

Bartelemy, C. 2008. Sarracenia Purpurea: Effects of Pitcher Leaf Size, Density, and Coloration on Species Richness and Mass of Organic Material Found Inside.

Bräuer, S. L., Cadillo-Quiroz, H., Yashiro, E., Yavitt, J. B., Zinder, S. H., 2006. Isolation of a novel acidiphilic methanogen from an acidic peat bog. Nature 442, 192-194

Breeman, N., 1995. How Sphagnum bogs down other plants. Trends in Ecology & Evolution Vol 10, 7: 270-275

Carver, A., J. Kryscynski, E. Lindow, & B. Roty. 1996. Marsh Marigolds (Caltha Palustris): The Influence of Abiotic Factors on Distribution.

Chapin, C.T., Pastor, J., 1995. Nutrient limitations in the northern pitcher plant *Sarracenia purpurea*. Canadian Journal of Botany Vol. 73, 728-724

Ellison, A. M., N. J. Gotelli. 2002. Nitrogen availability alters the expression of carnivory in the northern pitcher plant, Sarracenia Purpurea. The National Academy of Science. Vol. 99, 7: 4409–4412

Fish, D., Hall, D.W. 1978. Succession and Stratification of Aquatic Insects Inhabiting the Leaves of the Insectivorous Pitcher Plant, Sarracenia purpurea. 172-183

Givnish, T. J. 1989. Ecology and Evolution of Carnivorous Plants. Plant-Animal Interactions. 243-290.

Hemond, H. F., 1983. The Nitrogen Budget of Thoreau's Bog. Ecological Society of America. Ecology 64, 1: 999-109.

Johnson, CW. 1985. Bogs of the Northeast. University Press of New England Hanover, NH, p. 15-19

Parisod, C., Trippi, C., Galland, N. 2004. Genetic Variability and Founder Effect of the Pitcher Plant Sarracenia purpurea in Populations Introduced into Switzerland: from Inbreeding to Invasion. 277-286.

NH Division of Forests and Land [Internet] (US); [cited 2011 June 18]. Available from: http://www.nhdfl.org/about-forests-and-lands/bureaus/natural-heritage-bureau/photo-index/coastal-interdunal-marsh-swale.aspx

Plant Nutrients [Internet] North Carolina Department of Agriculture (US); [cited 2011 June 18]. Available from: http://www.ncagr.gov/cyber/kidswrld/plant/nutrient.htm#top

Appendix

Figure 1

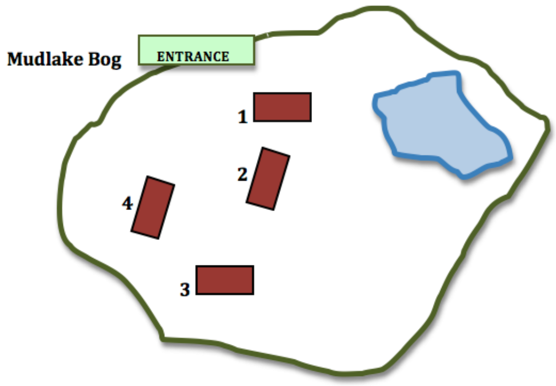

Figure 2

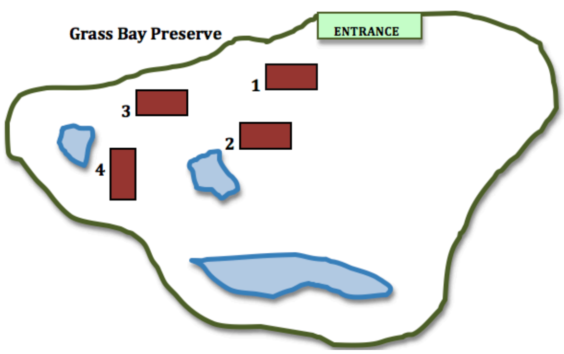

Figure 3a

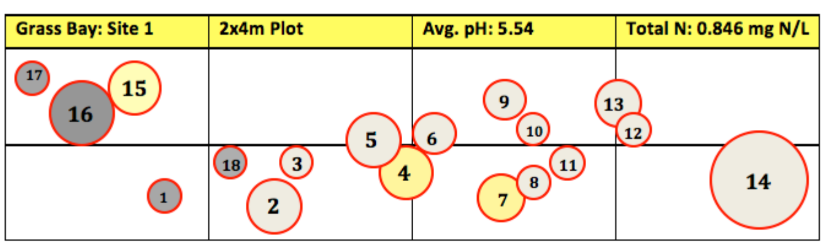

Figure 3b

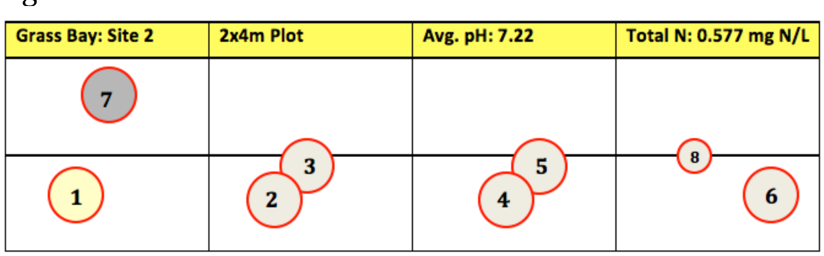

Figure 3c

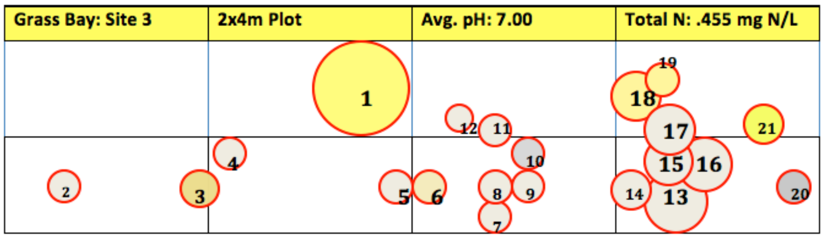

Figure 3d

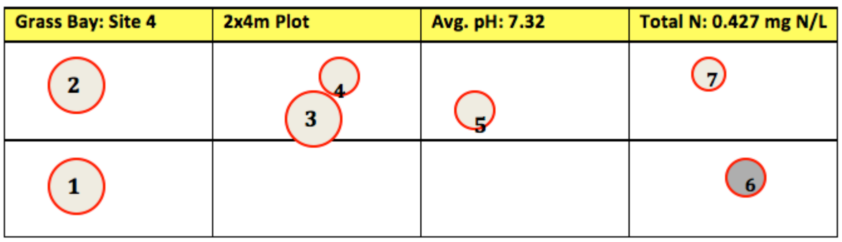

Figure 3e

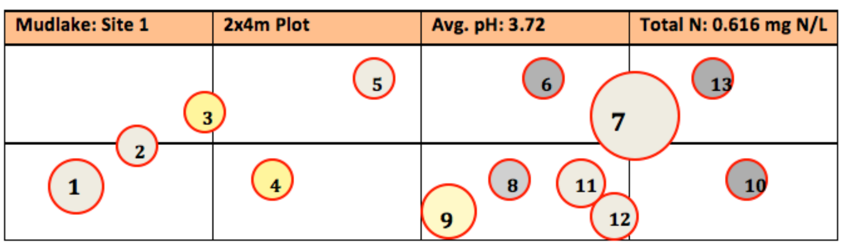

Figure 3f

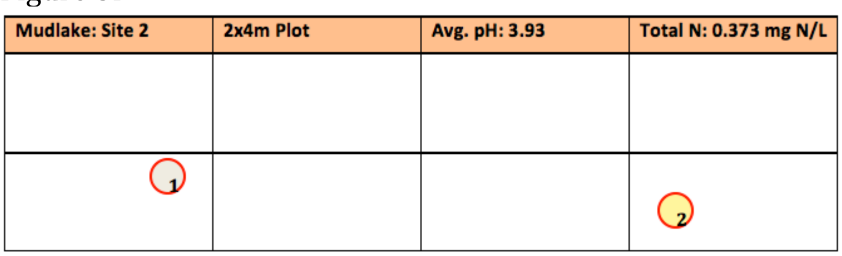

Figure 3g

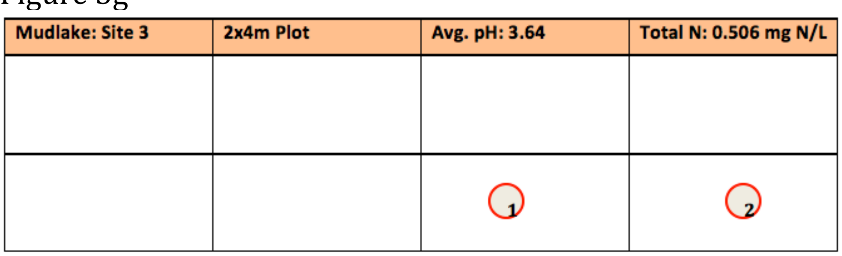

Figure 3h

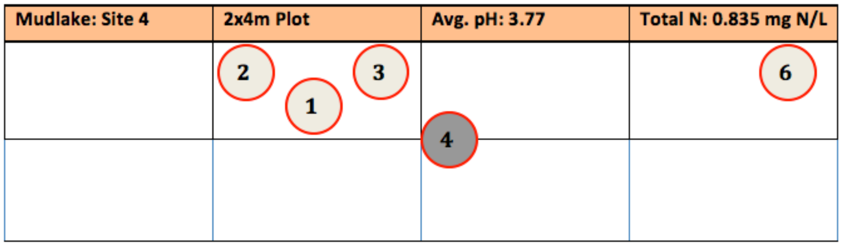

Figure 4

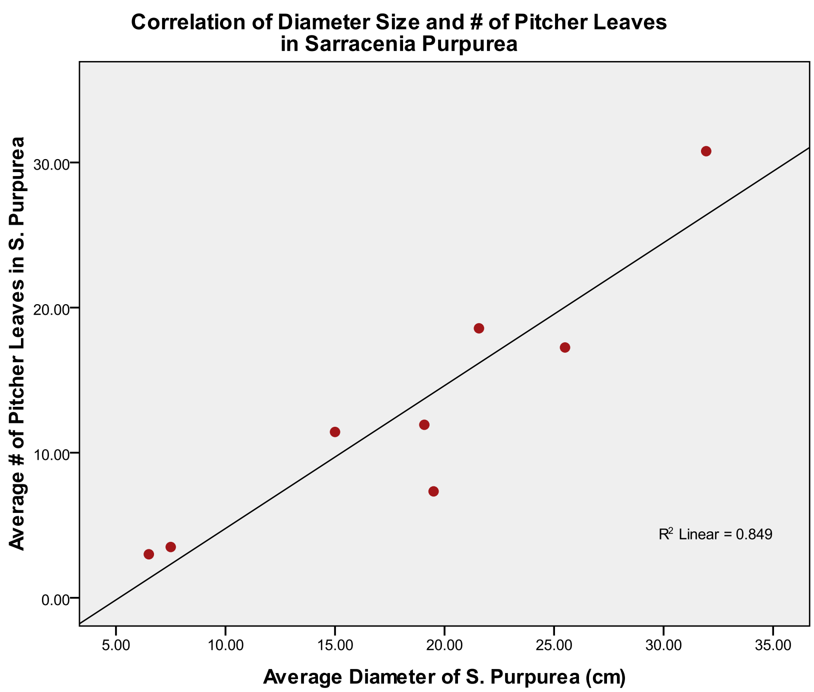

Figure 5

Linear Regression of Diameter of *S. purpurea* to Each Independent Variable Across Sites		
Independent Variables	R^2	P-Value
Light Intensity	.000	.957
pH	.015	.285
Total Nitrogen in the Water	.155	.334
Biomass of Insects	.116	.408

Figure 6

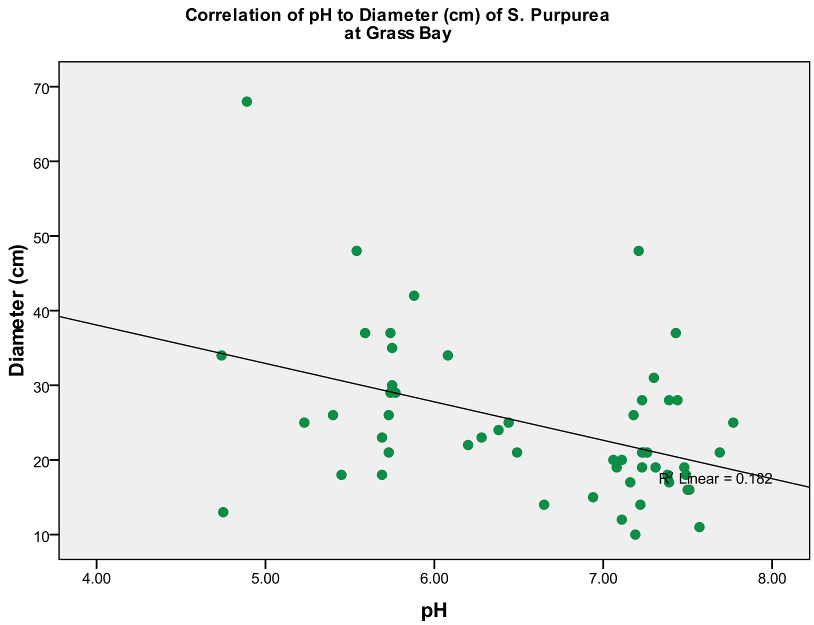

Figure 7

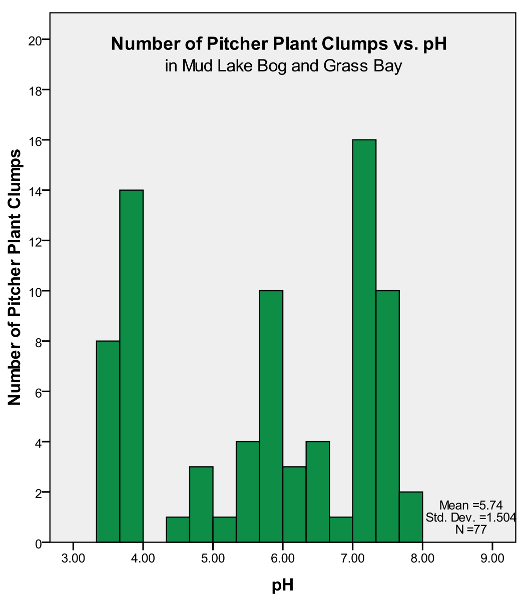

Figure 8

Mean pH of Plots at Grass Bay Preserve	
Plot Number	Mean pH
1	5.54
2	7.22
3	7.00

4	7.32

Figure 9a

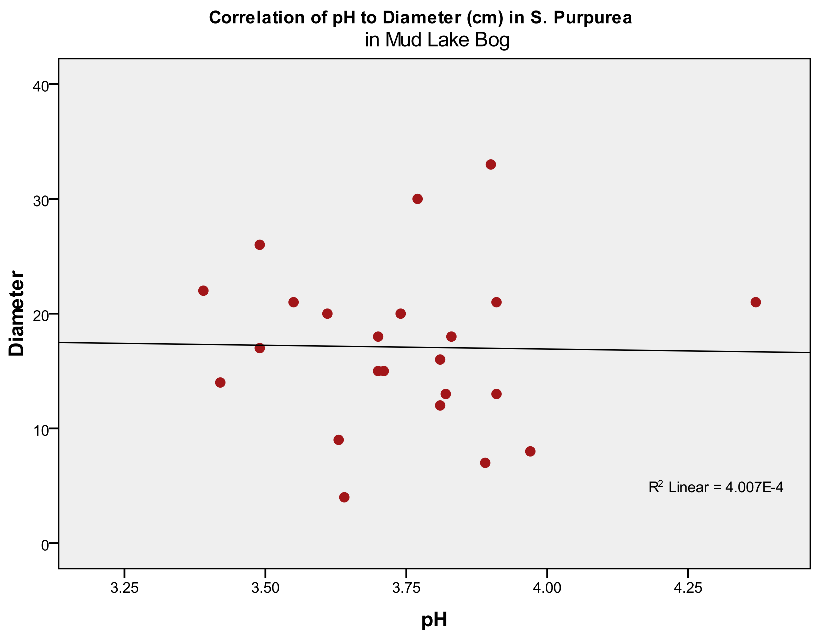

Figure 9b