Effect of Allochthonous Inputs on the Inquiline Community in *Saracenia purpurea* (purple pitcher plant)

BIOS 569: Practicum in Field Biology

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Abstract:

The purple pitcher plant (*Saracenia purpurea)* forms a rosette of cup like leaves each season which catch water from rainfall events. Living in the water is an inquiline community that is reliant on insects drowning in the water to provide resources for consumption. This experiment was designed to determine what is the effect of various resource additions to the richness and diversity of the protozoa, rotifer, and mite inquilines. Five treatments were used (control (no resources added), unground midges, ground midges, unground June beetle, and ground June beetle) to test whether small particle size ground inputs support a more diverse community than whole insect inputs. Whole June beetles were found to foster statistically richer populations than ground midge, and overall unground insect inputs had statistically richer populations than ground insects. However, the control had more diversity than pitchers with either ground or unground insects. These findings suggest that the pitchers may have been resource overloaded resulting in rapid population growth of a few inquiline species which reduced the diversity of species living in the treated pitchers.

Introduction:

Saracenia purpurea (purple pitcher plant) is a carnivorous species common to wetlands, and bogs along the east coast of the United States and Canada stretching west through the Great Lakes region and even further west in Canada (Bradshaw et al. 2000; Chapin and Pastor 1995). Of special use to this species are the pitcher shaped leaves that collect up to fifty milliliters of water from rainfall events. This

water becomes the final resting place for insects that fly into the pitcher. The water drowns small insects, which primarily compose the allochthonous inputs to the food web that lives within the pitcher plants (Miller and Kneitel 2005; Hoekman 2007).

Within each of the leaves is an intricate inquiline food web (Figure 1) which is made up of a bacterial assemblage, rotifers, protozoa, and processing detritivores including midge larvae, flesh fly larvae and water mites (Hoekman 2007; Heard 1994 a, b). The processing detritivores feed on the insects and release small insect particles to the water which are fed on by bacteria. The bacteria are consumed by protists and rotifers which are eaten by mosquito larvae (Cochran-Stafira and von Ende 1998). The allochthonous inputs are important for maintenance of the interdependent inquiline community.

Experiments that manipulate the allochthonous inputs look at the bottom up effects on the system. If more nutrients are available a denser and usually more diverse community develops within the pitcher plant (Hoekman 2007). Processing detritivores increase insect surface area allowing for rapid breakdown of leaf inputs leading to larger populations of processing detrivores and other species found lower in the food web (Heard 1994; Hoekman 2007).

In this study I hypothesize that resource addition will increase the diversity, richness, and abundance of protozoa, rotifers and mites living within the leaves. Increased surface area of insect inputs (addition of ground up midges or June beetles) will lead to further increases in diversity and population. A study by

Kneitel and Miller (2003) in Florida manipulated the allochothonous inputs by adding autoclaved fire ants to the pitchers. As resources were added to the pitchers the local diversity increased (Kneitel and Miller 2002; Hoekman 2007). I believe that a similar effect will be seen in northern Michigan pitcher plants.

Methods:

***Study Site*:** For this project, I manipulated pitcher plants located at the University of Notre Dame Environmental Research Center (UNDERC, 46'13" N by 89' 32"W) in the upper peninsula of Michigan. My site was Forest Service Bog where pitcher plants are common. All pitchers manipulated were found at least four meters from the forest edge and within a five by ten meter plot in this sphagnum dominated bog.

Project Description: I set up the experiment on June 22, 2007, by finding and marking with flags, 35 pitcher plant leaves that had opened this season and were large enough to hold at least 20 mL of fluid. I collected fluid from each flagged leaf, and then filtered the liquid to remove large particles. I stored this combined fluid in the fridge for 24 hours until the experiment began on June 23, 2007 (day 0).

The purpose of the experiment was to alter the allochthonous inputs to the pitcher leaves. I collected local insect species including June beetles *Phyllophaga congrua* which I captured individually and then froze. For small inputs, I ran a New Jersey light trap from 9 pm to 8 am for several consecutive nights to collect small dipterans, especially midges which were also placed in a freezer. I cut the

beetles in half (between the hardened forewings) to reduce the insect weight added to the pitchers. I recorded the average wet weight of the half June beetles to be 150 mg, so I weighed out 150 mg of midges for each of the treatments requiring smaller particle inputs. The treatments I used for this experiment are: **1)** No resource addition (control). **2)** 150 mg (unground) midges. **3)** 150 mg of ground up midges. **4)** Half a (unground) June beetle (~150 mg.). **5)** Ground up June beetles (~150 mg). These five treatments were replicated seven times for a total of 35 pitchers in the field.

I dried the insects in a drying oven at 60°C for 24 hours to make the grinding easier. I ground the insects using a mortar and pestle until they were of a small particle size. After each half June beetle or equal weight of midges was ground, I rinsed the mortar and pestle with deionized water. This slurry was put into centrifuge tubes. I prepared centrifuge tubes for all treatments, each tube had its treatment of 150 mg of insect portions (except control), one mL of pitcher fluid, and then the total volume was brought up to 20 mL with deionized water.

In the field I removed all pitcher fluid from my 35 pitchers, and then rinsed each flagged leaf several times to remove as much of the inquiline community as possible from the leaf. Next I randomly added the contents of each centrifuge tube (treatments 1-5) into a flagged pitcher, and then labeled the flags with the treatment and replicate. I covered each pitcher hood with fine mesh held in place by a rubber band to prevent the addition of other insects.

I began collecting samples from the pitchers on the second day of the experiment. I randomly divided the pitchers into two groups so half the pitchers were sampled each day. During sampling I first stirred up the pitcher by taking fluid into the pipette and then sending it out rapidly. Next, I would remove .3 mL of fluid and put it into a labeled microcentrifuge tube (.6 mL). After the samples were taken back to the lab, I pipetted 100 µL of pitcher fluid onto a Palmer counting cell to count and identify protozoa, rotifers, and mites under a compound microscope. The next sampling took place on the fourth day of the experiment. Each day I recorded the richness and abundance of species found in each sample which was then used to calculate Simpson's index of diversity. The last sampling session was on the sixth day of the experiment, and all 35 pitchers were sampled on that day.

***Statistical tests*:** The data I collected was analyzed for statistical significance using SYSTAT 12. No transformations of the data were required and the data met all assumptions of the tests. I ran two way ANOVA and Tukey post hoc tests to look for statistically significant differences between the various treatments. I ran tests with the independent variables of the five treatments against the dependent variables richness, abundance and diversity. Then I ran the independent variable of ground/unground against the three dependent variables richness, abundance and diversity.

Results:

On the second sampling session I found flesh fly larvae in several of the pitchers. I promptly removed them from the pitchers to reduce any effect they may have had on the inquiline community. Flesh flies feed on allochthonous inputs thereby increasing the surface area of insect carcasses. They also eat rotifers and protozoa, so for the purposes of my experiment it was important that I removed them as soon as they were found. I also found two pitchers that had a thick slime in the leaf. This slime had a strong foul odor and these pitchers had reduced inquiline community numbers, so the slime may have altered the inquiline microhabitat to some extent.

The size of insects added as prey influenced species richness in pitcher plants (overall ANOVA P=0.01, $F_{4,30}$=3.8). Treatments with allochthonous inputs of June beetles (unground) had significantly greater inquiline community richness than pitchers that had allochthonous inputs of ground midge (Tukey test P=0.009, mean error= 2.57) (Figure 2). Also, by the end of the experiment the controls (no prey added) had significantly greater diversity than the ground midge treatments (Tukey test P=0.02, mean error=0.68) (Figure 3).

Inquiline community diversity was affected by grinding up insect prey (overall ANOVA P=0.002, $F_{2,32}$= 5.48). The unground insects had significantly greater richness than ground insects (Tukey test P=0.015, mean error= 1.71) (Figure 4). In this test, the control did not have significantly greater richness than the ground treatment. The control however, had significantly more diversity than

either ground or unground insects (Tukey tests $p=0.02$ and $p<0.001$ respectively, mean error=-0.377031200 and -0.636048909 respectively) (Figure 5).

Discussion:

Both the surface area and the type of insect added to pitcher plant leaves had effects on the richness and diversity of the inquiline community found within the pitchers. I hypothesized that ground allochthonous inputs would make nutrients more immediately available, so a richer inquiline community could exist. Instead, unground treatments had significantly greater richness than the ground treatments (Figure 4) which is inconsistent with my hypothesis. Unground June beetles treatments had statistically greater richness than the ground midge treatments (Figure 2). It seems that unground June beetles or midges required the inquiline community to work from the outside towards the center of the insect(s). This allows inquiline species to specialize on different parts of the prey. In contrast, the ground treatments are of a more homogenous mixture when placed in the leaves, so there are fewer opportunities for specialist species to colonize specific regions of the prey carcasses. Ground treatments allow for fewer species to dominate because there are fewer niches available for colonization. This results in a less rich inquiline community.

Control treatments (no prey added) were found to have significantly higher diversity than unground or ground treatments (Figure 5) which did not support my hypothesis. The control treatments also had statistically greater diversity than the ground midge treatments (Figure 3). An explanation for this

may be that the allochthonous inputs made the inquiline communities very productive which allowed quickly reproducing species to take advantage of the high resource levels and achieve high densities. The control treatment in my experiment would not have experienced a population explosion because resources were not added. This maintained greater inquiline diversity for the control treatments because no species could become super abundant and exclude other species.

Other studies have been done which show a similar relationship between the productivity of a resource and the diversity of species that can live in/on it. In Interlandi and Kilham's (2001) paper on phytoplankton diversity in lakes, they found that the more productive lakes had less diverse communities, while the less productive lakes could support a more diverse community of phytoplankton species. This reaction to increased resources was first called the paradox of enrichment in 1971 by Rosenzweig. I think a similar pattern was visible in the pitcher plants. The enriched pitchers were more productive which limited the diversity of species, while the control (not enriched) had a more diverse inquiline community.

Sources of Error: When conducting this experiment I wanted both large and small bodied insects used as treatments. June bugs were common on the property, but they are rather large, so I decided to put half June bugs into the pitchers. By providing an already opened carcass to the inquiline community I was giving it a head start of sorts and provided more regions for initial colonization. In

retrospect, using a smaller insect that could have been added to the leaves whole may have been more representative of what actually may happen in nature.

Despite using half June beetles (~150 mg) and the same weight in midges for my other treatments I may have provided the leaves with more nutrients than necessary. Looking at a previous experiment with northern Michigan pitcher plants, the maximum input used was 10 ants. Predators were also present in that experiment by Hoekman (2007) which helped break down the inputs and slow the growth rates of the inquiline community (Knietel and Miller 2002). In my study, I may have overloaded the leaves with resources which then caused the rapid population growth of a few dominant species.

This experiment ran for a total of seven days with only three sampling sessions. This short amount of time yielded some statistically significant results, but it is also possible that with more sampling some trends may have become significant. Also, this experiment was replicated at only one bog. Other bogs would probably react similarly which would add replicates and possibly show other significant trends within the results.

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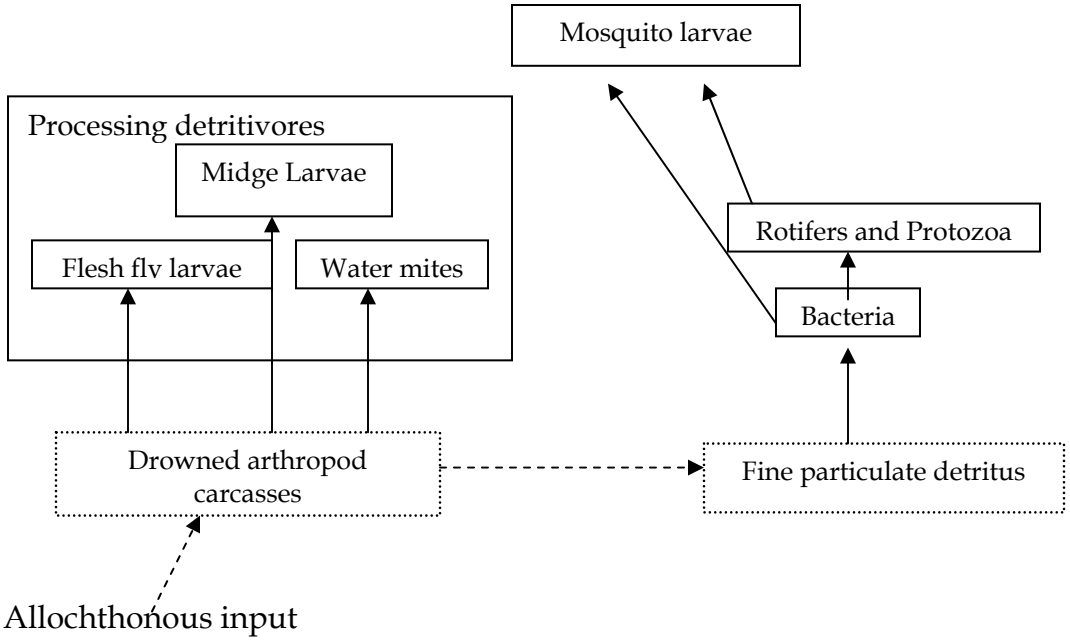

Figure 1. Food web found within the purple pitcher plant. Solid arrows designate feeding relationships. Dashed arrows designate basal resource pathways (Hoekman 2007).

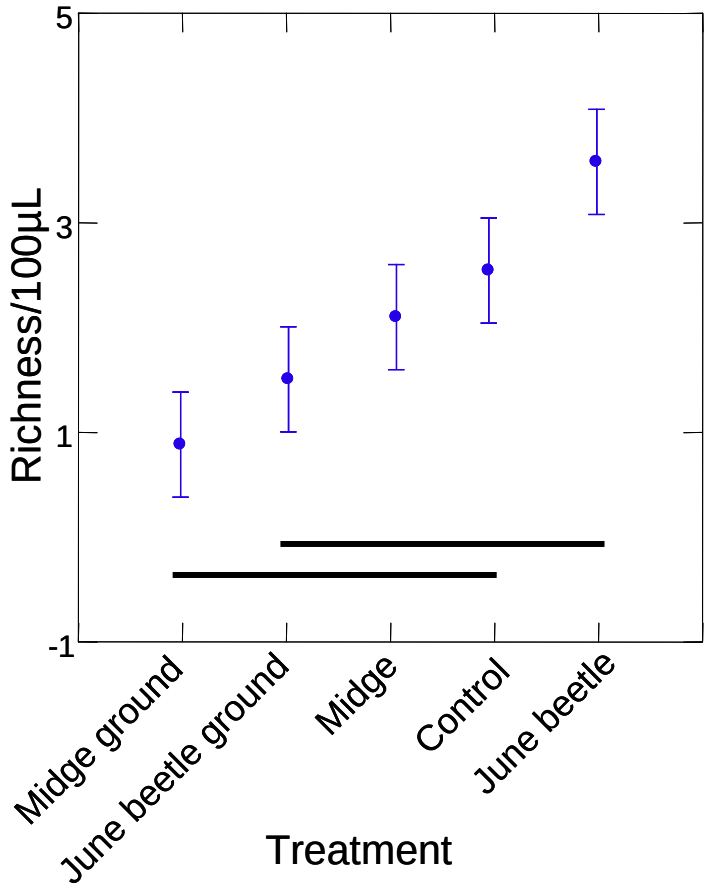

Figure 2. June beetle treatment leaves have statistically greater richness than ground midge treatments (Tukey test P=0.00869) four days after start. Lines connect points that are not statistically significant (P>0.05). Error bars represent one standard error about the mean.

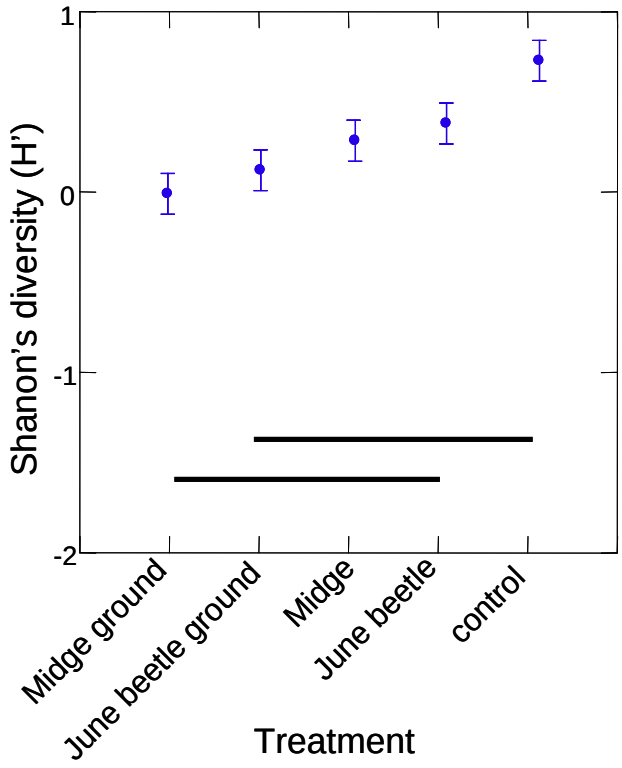

Figure 3. Control treatments had significantly greater diversity (Shannon index) than the ground midge treatments (Tukey test P=0.0016) six days after start. Lines connect points that are not statistically significant (P>0.05). Error bars represent one standard error about the mean.

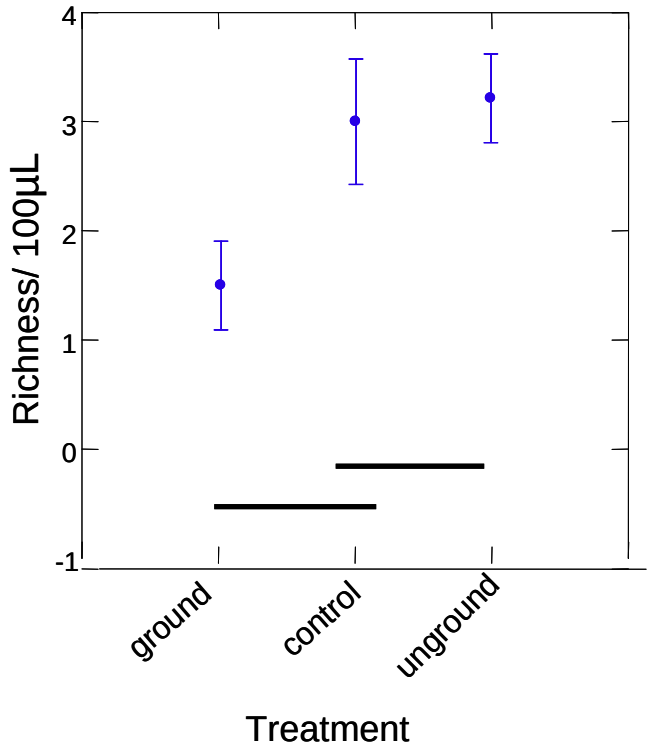

Figure 4. Unground treatments of June beetle and midges had significantly greater richness per 100 µL than the ground treatments (Tukey test P=0.0015) six days after start. Lines connect points that are not statistically significant (P>0.05). Error bars represent one standard error about the mean.

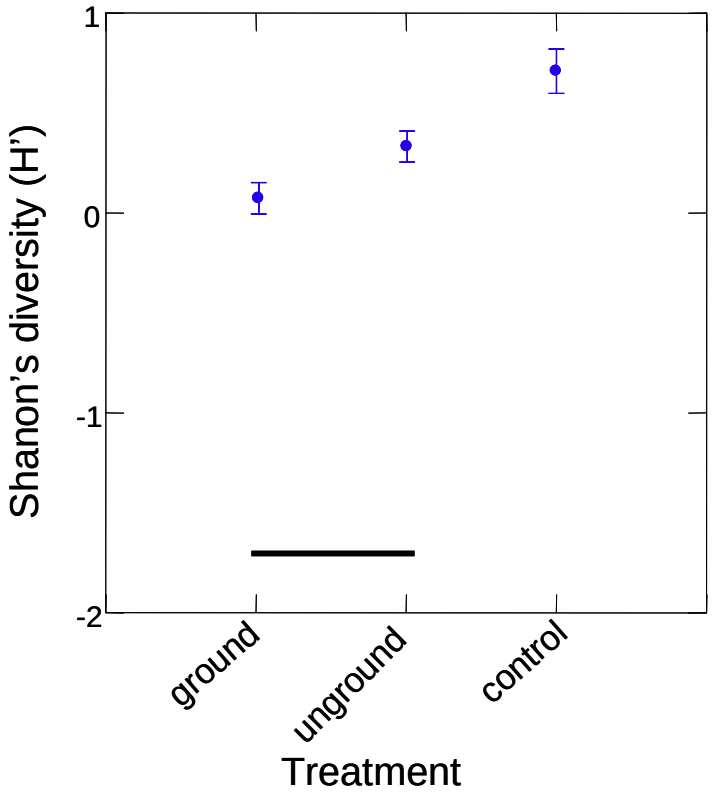

Figure 5. Control treatments had significantly more diversity than unground (Tukey test p=0.024) and ground (Tukey test p=0.00015) treatments of June beetles and midge six days after start. Lines connect points that are not statistically significant (P>0.05). Error bars represent one standard error about the mean.

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