The role of dipteran larvae in controlling Euglena concentrations in the pitchers of *Sarracenia purpurea L.*

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Abstract- Field studies suggest that in phytotelm communities of *Sarracenia purpurea,* the absence of pitcher-plant specific larvae of the mosquito *Wyeomyia smithii* and the chironomid midge *Metriocnemus knabi* resulted in higher concentrations of *Euglena.* Conversely, a pitcher inquiline in which these larvae were present had lower concentrations of *Euglena* and algae, in general. *In vitro* experiments demonstrated that the *Euglena*-concentration with the absence of larvae, rose from near zero to 10^5 cells/mL after 14 day and by day-28, it exceeded 3 x 10^5 cells/mL. When only *M. knabi* larvae were present, the *Euglena*-concentration rose to 7 x 10^4 cells/mL by day-14 and to 2 x 10^5 cells/mL by day-28. When the larvae of the mosquito *W. smithii* were present, either by themselves or commensally with *M. knabi*, the *Euglena*-concentration remained below 10^4 cells/mL. Microscopic inspection of both *M. knabi* and *W. smithii* larvae revealed that, in the presence of *Euglena*, their guts turned green. We concluded that predation by *W. smithii* and *M. knabi* larvae suppress the growth of *Euglena.* These larvae prevent algae from overwhelming the pitcher-plant phytotelm and thus may play a critical role in maintaining the integrity of *S. purpurea*'s inquiline community.

Index Terms- *Sarracenia purpurea, Wyeomyia smithii, Metriocnemus knabi, Euglena,* phytotelm community

I. INTRODUCTION

There are few reports of algae in the phytotelm of the purple pitcher plant *Sarracenia purpurea* L. (Maguire 1971; Schnell, 1976; Dudley, 1984**),** in spite of the fact that the nutrient-rich liquid of *S. purpurea* pitchers (Heard, 1994 a) coupled with the plant's open and sunny bog-habitat (Schnell, 1976), would seem ideal for supporting algal growth. Dudley (1984) determined that the leaves of *S. purpurea* are capable of supporting a diverse community of algae. Of the 56 pitchers sampled 40 contained a total of 23 genera of algae. No algae were detected in the remaining 16 samples. Of the genera identified 10 were from the Chlorophyta (green algae), seven from the Chrysophyta (diatoms), four from the Cyanophyta (cyanobacteria) and one genus from the Euglenophyta (euglenoids). No other organisms identified nor quantitative assessments given.

A preliminary screening of a number of *S. purpurea* populations by our research group revealed that algae were absent or infrequently encountered in the pitcher-liquid when larvae of the pitcher-plant specific dipterans of the mosquito *Wyeomyia smithii* and those of the chironomid midge *Metriocnemus knabi* were present. Conversely, algae were abundant in populations in which these mosquito and midge larvae were absent (Douglas and Petersen, 2005). Based on these observations we decided to investigate through a combined field and laboratory study if these dipteran larvae were preventing algal blooms from forming in the pitchers of *S. purpurea.*

The larvae of most Culcididae are filter feeders (Clements, 1963), including those of the mosquito *Wyeomyia smithii* (Addicott, 1974) and planktonic algae are included as a food of filter-feeding mosquito larvae (Clements, 1963). The larvae of most, if not all, Chironomidae are opportunistic omnivores [Oliver, 1971; Berg, 1995), though a significant subset show a propensity for carnivory (Berg, 1995). This subset includes members of the genus *Metriocnemus* which have well-developed mandibles and other mouthparts for grasping and shredding food (Berg, 1995; Petersen, Faust, Thomas, and Vilmenay, 1995). Larvae of the pitcher plant midge *Metriocnemus knabi* have been described both as detritus feeders (Fish and Hall, 1978) and as voracious predators (Petersen et al., 1995**)**.

Considering the differences in feeding of *W. smithii* (filtration) and *M. knabi* (active ingestion) we hypothesized that the larvae of *W. smithii* utilizing planktonic algae as food suppress algal growth in the *S. purpurea* leaf communities. This hypothesis was suggested by the findings of Cochran-Stafira and von Ende (1998) and Miller, Kneital, and Burns (2002) have determined that *W. smithii* larvae control bacterial and protozoan growth and composition in the pitcher-plant phytotelm community. In addition to what was reported, *M. knabi* are detritus feeders with grasping and chewing mouth parts have a lesser role in algal suppression.

II. METHODOLOGY

Field Studies

Leaves from two *Sarracenia purpurea* populations were surveyed for algae. In one population, Suitland Bog, Prince Georges County, Maryland, U.S.A, the larvae of the pitcher plant species-specific mosquito *Wyeomyia smithii* and midge *Metriocnemus knabi* were present in the pitchers. In the other population, the National Botanic Garden greenhouse - Plant Growing Facility, Washington, D.C., U.S.A., these larvae were absence.

Composite samples were prepared by taking 10-mL phytotelm water sample from each pitcher plant. Samples were placed in plastic test tubes and microscopically examined using an Olympus B071 research microscope for the presence of dipteran larvae and algae.

In vitro-controlled Experiment

Larvae of the pitcher plant mosquito (*Wyeomyia smithii*) and midge (*Metriocnemus knabi*) were obtained from plants at the Suitland Bog site. A culture of *Euglena* was established form an isolate obtained from pitcher-plant water sampled from the National Botanic Garden population. This culture was maintained on a bacterial infusion medium (see, below for details). Inoculates from this culture of *Euglena* were used in all experiments.

Six mL of a bacterial infusion, grown for five days on a 2 gm suspension of Purina Catfish Chow© (Petersen et al., 1995) in 500 mL of 1/5 strength Hoagland's Solution (Sigma Corporation), was placed in each well of 6-well Falcon Brand© Tissue Culture Plates. One drop of a suspension of *Euglena* sp. (3×10^4 *Euglena* / drop) was added to each well.

The experiment consisted of four treatments, Treatment-1 – No Dipteran larvae – the control; Treatment-2 –six *W. smithii* mosquito larvae/well; Treatment-3 – six *M. knabi* midge larvae/well; and Treatment-4 – mosquito and midge larvae three each/well. The numbers of samples were chosen based on the six well cell culture plate. There were 24 wells for each of the four treatments (n=24). Plates were maintained under controlled conditions of light (fluorescent light at 300 µmol m^2 s^2 on a 12-hour diurnal cycle) and temperature (20-24^o C) for 28 days (more larvae was added as needed).

Daily estimates of *Euglena*-concentration for each well were made over a 28-day period. Aliquots of 0.25 mL were taken from each well and placed on a hemacytometer slide (Delta Environmental). To facilitate counting the *Euglena* 5µL of Lugol's iodine was added after filling the hemacytometer to this sample. The iodine solution both kill and stain the *Euglena*. Concentration-estimates of *Euglena* were expressed as number of *Euglena*/mL.

Statistical Analysis

Statistical analysis consisted of descriptive statistics, linear regression of days vs Euglena growth, and one-way ANOVA showing the variation among groups of larvae. Data were $\log_{10}$ transformed to meet normality and homogeneity-of-variance assumptions of ANOVA. Significant ANOVA results at $p < 0.05$ were further examined with Tukey least significant difference (LSD) comparisons (SAS Institute, 1996).

Direct Microscopic Observations

Wyeoyia smithii and *Metriocnemus knabi* larvae were placed in tissue culture plates. A suspension of *Euglena* cells after 24 hours were inspected microscopically using a Nikon SMZ-10 stereomicroscope for the presence or absence of green-coloration in their guts.

Field Survey

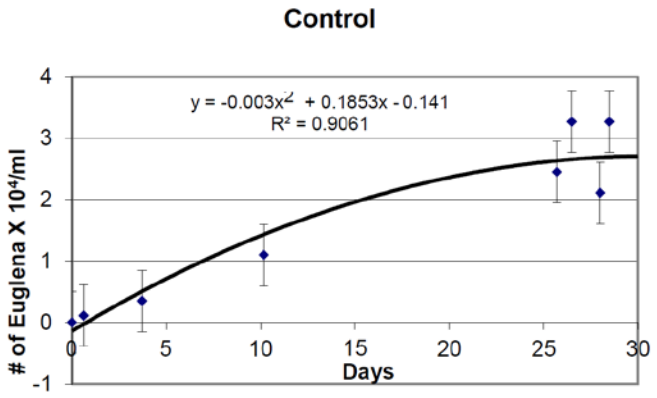
Algae were not observed in pitcher-water samples taken from the *S. purpurea* population (Suitland Bog) containing *W. smithii* mosquito larvae and *M. knabi* midge larvae. Conversely, in the population in which these diptera larvae were absence, the botanical garden site, algae were abundant with a unicellular non-motile Chlorella-like green alga and a species of *Euglena*, which predominated.

III. RESULTS AND DISCUSSION

In-Vitro Control Experiment

Linear regression lines of average concentrations of *Euglena* as a function of the presence or absence of larvae, including the control (no larvae) and three experimental treatments (only mosquito larvae presence; only midge larvae presence, and both mosquito and midge larvae presence to replicate the normal conditions) are presented in Figures 1 A-D. Regression lines were generated for each treatment at specified intervals to show differences within leaf activity.

The greatest increase in *Euglena*-density occurred when dipteran larvae were absent –the control– treatment (Fig. 1A). The next greatest was when only *M. knabi* midge larvae were present (Fig. 1B). There were little no significant increases in *Eulgena* in the remaining two treatments in which *W. smithii* mosquito larvae were present alone or commensally with *M. knabi* (Figs. 1 C and D). Analysis of variance revealed a significant difference ($p < 0.00001$) among the four treatments.



Figures 1 A-D. Regression line analysis of Euglena concentration as a function of time (days) and the presence or

absence of dipteran larvae. (± s.e.; n=24). Regression line equations and R2 – values. **A**. Control – no dipteran larvae presence.

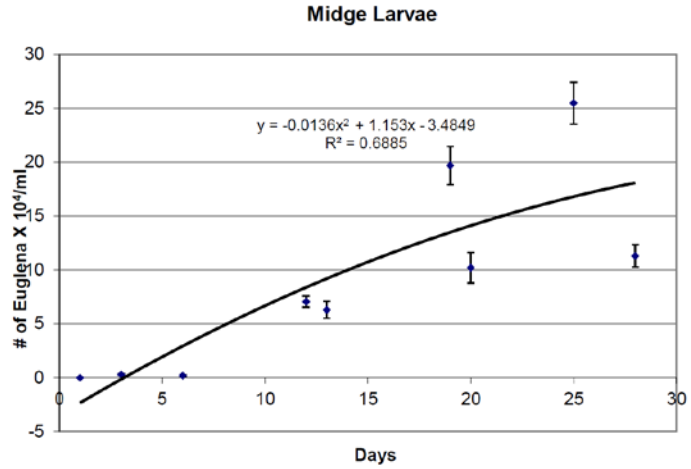


Figure 1 **B**. *Metriocnemus knabi* (midge) larvae presence.

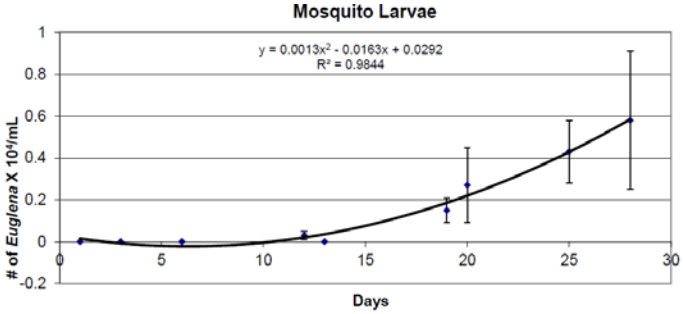


Figure 1 **C**. *Wyeomyia smithii* (mosquito) larvae presence.

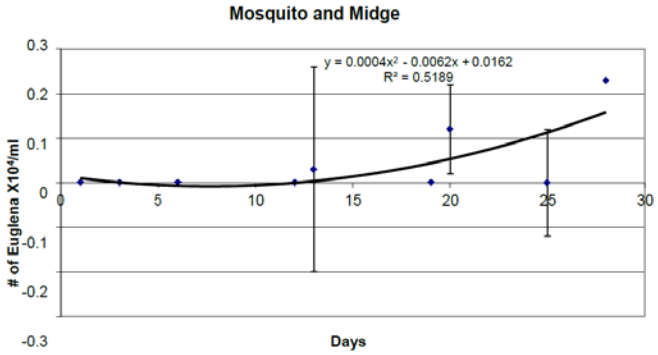


Figure 1 **D**. Both *Metriocnemus knabi* (midge) and *Wyeomyia smithii* (mosquito) larvae presence.

A summary of these findings, including Tukey LSD results, is presented as estimates of *Euglena*-densities for days 14 and 28 (Fig. 2). These estimates were derived from regression line equations for each of the four treatments. Regression line density-estimates for the control were 1 x 10^5 *Euglena*/mL by day-14 and nearing 3.3 x 10^5/mL by day-28. Corresponding estimates for the midge larvae only treatment were 7 x 10^4/mL (day-14) and 2 x 10^5 /mL by day-28 (Fig. 2). Though high, these midge-treatment densities represent a 30-40% decrease from those of the control-treatment. By comparison, in the remaining two treatments in which *W. smithii* mosquito larvae were presence, *Euglena*-densities are close to 0 ± s.e. (Fig. 2).

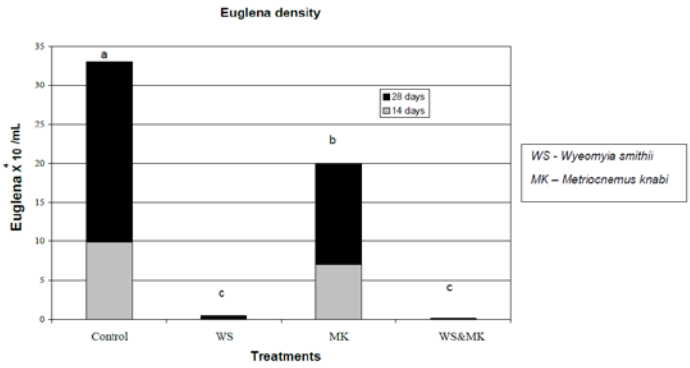


Figure 2. Summary of *Euglena* density. [Least significant difference (LSD) results indicated as a, b, and c.]

These findings show that the larvae of *W. smithii* are very effective at filtering out *Euglena* from the liquid of the pitcher plant. A less intuitive finding was that the larvae of the pitcher plant midge *M. knabi* also contribute to controlling the growth of *Euglena* in all likelihood by grasping, shredding, and ingesting clumps of *Euglena.*

Microscopic Evidence

Microscopic observations of the larvae of *W. smithii* and those of *M. knabi* raised in the presence of *Euglena* revealed that the guts of both taxa turned green, indicating both were ingesting *Euglena.* These visual observations confirm the findings of the preceding *in vitro* controlled experiment.

IV. CONCLUSION

The i*n vitro* controlled experiment of this research demonstrated that *Euglena*-growth is suppressed to near zero by the larvae of the pitcher-plant mosquito *Wyeomyia smithii*. This finding is complemented by field observations of the abundant presence of *Euglena* in pitcher-liquid when *W. smithii* larvae are absent and essentially no algae are found in pitchers in which they are present.

Together these field and laboratory findings support the conclusion that *W. smithii* mosquito larvae prevent *Euglena*, and by inference, planktonic algae in general (personal observations) from overwhelming the phytotelmic community of *S. purpurea*.

The larvae of the pitcher-plant midge *Metriocnemus knabi* also suppressed the growth of *Euglena*. Presumably, they do this, by feeding on detritus including frass upon which the *Euglena* that has settled and even proliferated. In support of this assumption Berg (1995), in a review of the chironomid literature,

states that while several chironomid genera are predators, including the genus *Metriocnemus*, most chironomid larvae are opportunistic feeders whose diet includes detritus and associated microorganisms (Johnson, Bostrom and W. van de Bund, 1989). Algae, also, are an important food for many chironomid species (Johnson et al, 1989).

This finding of algal-growth suppression was supported by directly observation – the guts of pitcher-plant mosquito and midge larvae turned green when placed in a suspension of *Euglena*. Based on these experimental findings and field observations, it is concluded that the larvae of *W. smithii* and *M. knabi* prevent *Euglena* proliferation and algal-blooms in general in the phytotelmic community of *S. purpurea*.

What are the implications of this conclusion for the pitcher plant and its phytotelmic community? By feeding on algae the larvae of the pitcher-plant mosquito and midge are preventing algae from over-running the phytotelmic community of the pitcher plant. Domination by algae would possibly result in contents of the pitchers going from aerobic to anaerobic. This would lead to at best the disruption of the pitcher plant phytotelmic community or at worst its destruction. The disruption/destruction of the phytotelmic community would impede the breakdown of insect prey and uptake of nutrients by the plants. Bacteria do the main job of decomposing the prey – the midges help with shredding the prey, the mosquito has the critical role of reducing algae by filtering feeding in the leaf. As such, these dipteran larvae have a vital role in preserving and maintaining the phytotelmic community of the *S. purpurea*. These issues of species dominance, eutrophication and preservation of community structure and their implications for the well-being of the pitcher plant have been addressed by others.

Miller *et al.* (2002) used the pitcher plant phytotelms as a model for investigating the population ecology of invasive species. They did this by manipulating a natural community of pitcher plants. They found that when the larvae of the pitcher-plant mosquito *W. smithii* were removed from the pitcher community three out of six protozoan species and one rotifer succeeded in establishing themselves. These researchers concluded that the presence of larvae of *W. smithii* was necessary to prevent or, at least, suppress the occurrence of protozoa and rotifers in the pitcher-plant phytotelmic community, thus, by implication, preserving the characteristic community composition and structure of the pitcher-plant phytotelms. Other researchers point to the role the pitcher plant mosquito and midge larvae have in preserving its phytotelm community (Petersen et al., 1995). The authors reported that the larvae of the pitcher plant midge *M. knabi* were agile swimmers and voracious predators, killing and consuming the larvae of non-*Wyeomyia* mosquitoes (i.e., a species each of *Aedes* and *Anopheles*). This finding, in conjunction with few reports of other kinds of mosquito larvae in the pitcher plant phytotelms, led these researchers to conclude that the larvae of *M. knabi* are preventing the establishment of non-pitcher plant mosquitoes in the *S. purpurea* phytotelms, thus, preserving its community of organisms only for one species of mosquito larva, that of *W. smithii*. This particular dipteran symbiosis is further exemplified by *W. smithii* females preferentially ovipositing in leaves containing *M. knabi* (Heard, 1994 b). As early as 1905 Knab (1905**)** reported that, with an 'over-abundance' of insect prey, the content of pitcher plant leaves would turn rancid and foul. He attributed this to a lack of oxygen in the fluid of the pitchers. A number of researchers have reported on the characteristically high oxygen content of the pitcher fluid and the role this oxygen plays in the breakdown and assimilation of nutrients by the plant (Cameron, Donald and Paterson, 1977). Further, Bradshaw and Creelman (1984) found that the presence of these mosquito and midge larvae in the pitchers of *S. purpurea* enhanced ammonia and carbon dioxide production in the pitcher fluid and their uptake by the plant.

Hardwick and Giberson (1996) in a field-assessment of pitcher-plant dipteran densities in natural and transplanted populations found that *W. smithii* larvae were rarely present in transplanted populations. These researchers suggested that the absence / lower-density of the pitcher plant mosquito larva might have a detrimental effect on the pitcher plants, presumably expressed as a reduction in pitcher-plant growth and colony success. They recommended that when transplanting pitcher plants that the intact inquiline community accompany the plants.

From our findings reported herein and with those of others a clearer picture emerges of the roles the species-specific mosquito and midge larvae have in the phytotelm community of the purple pitcher plant. One of these roles seems to be excluding non-adaptive organisms, addressed in this report on planktonic algae. Organisms that would disrupt or even destroy the pitcher plant phytotelm community by disrupting the breakdown and assimilation of insect prey.

ACKNOWLEDGMENT

We thank Mr. William McLaughlin, Horticulturalist for access to the pitcher plants of the Unites Sates Botanic Production Facility.

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