The presence of a processing detritivore (*Metricnemus knabi*) and its effect on top-down and bottom-up forces in the northern pitcher plant (*Sarracenia purpurea*) inquiline food web

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ABSTRACT

Top-down (predator) and bottom-up (resource) forces are believed to influence to all populations. The northern pitcher plant, *Sarracenia purpurea,* houses a dynamic food web of organisms including *Wyeomyia smithii*, the top predator mosquito larvae, which preys on lower trophic levels including protozoa, rotifers, mites and the basal trophic level, bacteria. Also present is *Metriocnemus knabi*, the pitcher plant midge larvae, that has been predicted to act as a processing detritivore that accelerates nutrient cycling within the pitcher plant and therefore increasing densities of those organisms downstream in the food web. Utilizing the pitcher plant inquiline food web for a factorial field experiment, we manipulated three factors including midge (0,16), mosquito (0,15) and dead ant carcass (0, 4, 20 mg) density and evaluated three response variables (rotifer density, average protozoa biovolume, and bacteria density). Top-down effects were observed on all response variables, while the effects of bottom-up forces were completely absent. The effect of midges on the pitcher plant only played a significant effect on bacterial densities but did not affect higher trophic level densities. Therefore our experiment found

inconclusive results on the ability of the processing detritivore midge to increase the effect of bottom-up forces in the pitcher plant.

INTRODUCTION

Food web dynamics, (the flow of energy within a community), can be altered by both top-down effects, controlled by consumers, and by bottom-up effects, that are controlled by resource levels in the community. While both have been observed, what determines the relative importance of each is still debated (Hunter 2001). While studies have demonstrated variation in the relative influence of these two effects in a community, certain biotic factors such as rate of decomposition, may play a role in these differences. I will examine how decomposition rates influence the effect of top-down and bottom-up forces in the northern pitcher plant (*Sarracenia purpurea*) inquiline food web.

Within a community, a population can be controlled by two independent factors; top-down and bottom-up effects. Bottom-up effects concern the alteration of a community structure and dynamic by the level of resources at the base of the food web. When there is higher productivity at the basal trophic level, this alters the distribution of food availability for the rest of the food web, and therefore can result in an increase in biomass at higher trophic levels (Elton 1927, Linderman 1942, Persson et al. 2001).

Top-down theory explains the influence of predators, or consumers, on their prey populations. With relative increases in predator densities in a food web, it is predicted that there is a negative response in prey populations at the lower trophic levels. For example, top-down forces are present in an aquatic environment with the manipulation of piscivores and planktivores and their effect on potential primary production. Carpenter et al., (1985) found the influence of consumers had a significant negative effect on lake primary production by effects propagated through the food web.

An intricate interaction of top-down and bottom-up effects is responsible for determining food web structure and dynamics. Therefore, an important question is: under what conditions does one of these effects have more influence on a given community than the other? Because food web structure and dynamics are affected by biotic and abiotic factors, their relative influences on specific environments may lead to varying levels of top-down and bottom-up effects.

The northern pitcher plant is typically found in low-nutrient wetlands, such as bogs and fens (Schnell 1976). This carnivorous plant acquires some supplemental nutrients from insects that become trapped in its rainwater filled rosette consisting of up to 12 leaves (Miller et al 1994) and provides habitat for a diverse aquatic fauna of inquilines. The symbiotic coexistence of several species include water mites, larval stages of three dipterans, bacteria, protozoa, and a bdelloid rotifer, *Habrorocha rosa,* while nematodes and cladocerans are rarely present (Bledzki and Ellison 1998, D. Hoekman, personal communication). The three obligate symbiotic dipterans include the predaceous pitcher plant mosquito, *Wyeomyia smithii,* the pitcher plant midge, *Metriocnemus knabi,* and the pitcher plant flesh fly, *Blaesoxipha fletcheri* (Miller et al. 1994). While the pitcher plant community has essentially no primary production, the arthropod carcasses that drown in the inquiline fluid provide the plant with essentially all of its nutrient availability. The presence of a processing detritivore, the midge, increases nutrient cycling of allochthonous input, or arthropod carcasses, thereby making it more readily available to higher trophic levels, such as bacteria. The protozoa, mites and rotifers that feed on the bacteria and are all consumed by the omnivorous top predator, the mosquito larvae (Cochran-Stafira et al. 1998).

The pitcher plant provides a model food web system that is easy to observe and manipulate because it has most of the essential features of laboratory aquatic systems. These include functionally isolated leaves, great abundance, as well as a simple fauna and predator-prey trophic system (Addicott 1974). Because there is minimal movement of larval insects between the pitchers, each individual pitcher plant can provide as an experimental unit therefore making an easy subject for replication (Heard 1994).

Pitcher plants are exceptionally useful for manipulation of both bottom-up and top-down food web dynamics. Bottom-up effects can be controlled by the rate of breakdown or presence of allochthonous input, while top-down effects can be controlled by manipulation of the predaceous mosquito larvae density (Kneitel and Miller 2002, Miller et al 1994).

Previous work shows that manipulation of both resource levels (arthropod carcasses) and predators (mosquito larvae) can have significant effects on populations within the inquiline community food web (Heard 1994). The results from Heard's experiment supported bottom-up and top-down theoretical predications previously presented.

One comparable experiment of top-down and bottom-up forces on species abundance and diversity of the inquiline community of pitcher plants was conducted by Kneitel and Miller (2002) in Florida. Miller found a trophic cascade that caused an increase in bacteria densities with an increase in mosquito densities and therefore a relatively more important effect of top-down forces than in Heard's study.

A comparison of these studies shows that the strength of both top-down and bottom-up effects on this pitcher plant community can vary depending on characteristics that differ among locations. From the differences in these experiments, there is room for experimentation on what factors influence the relative strength of these two forces. In order to determine why a trophic cascade was witnessed in the pitcher plants in Florida and not in the northern pitcher plants, my experiment will be conducted to possibly further our understanding of bottom-up and top-down effects on the food web of the inquiline community in pitcher plants.

Hypotheses

Heard (1994) suggested that *W. smithii* and *M. knabi* have a commensal relationship. This indicates that while both species feed on the same carcass material, they prefer it in different stages of decay. Midges accelerate nutrient cycling of whole carcasses into fine particulate detritus that provide nutrients for the prey of mosquitoes. Therefore, mosquitoes directly benefit from the presence of midges through a propagation of nutrients through the pitcher plant food web. Therefore, I hypothesized that the densities of mosquitoes will covary with midge density due to its function as a processing detritivore.

As bottom-up theory predicts, Kneitel and Miller (2002) found that increased resource levels (dead carcasses) significantly increased densities in higher trophic levels. An increase in nutrient availability for basal trophic level densities allows for increased nutrients for the higher trophic levels. Therefore, I hypothesized that an increase in nutrient availability will lead to an increase in densities of bacteria, protozoa, mites and rotifers.

If top-down effects are present within a community, then density manipulation of the top predator will significantly alter the population density of the species that make up the bottom trophic levels. Addicott (1974) also found this pattern; that an increase in top-predator mosquito densities were found to decrease the total number of prey individuals. I hypothesized that mite, rotifer and protozoa densities will decline with increasing densities of mosquitoes.

One possible explanation for the differences in the results of the two studies in the northern pitcher plants and Florida is the presence of the pitcher plant midge, *M. knabi*. The natural environment in Florida where the study was conducted contained such a small percentage of midges in the inquiline community, that Kneitel and Miller excluded them from their study. In the north, the pitcher plant midge is the dominant processing detritivore, and manipulates the processing chain role of releasing nutrients into the nutrient pool (Heard 1994, Hoekman personal communication).

Therefore, tracing the role of the midge and their release of nutrients into the nutrient pool may lead to an explanation in the differences in the two studies relative importance of top-down and bottom-up forces. Midges should play a role of accelerating the effect of bottom-up forces in a pitcher plant by increasing the rate of breakdown of the dead carcasses. I hypothesize that an increase in the abundance of decomposers (midges) will increase nutrient availability and therefore bacterial productivity, increasing the relative importance of bottom-up forces in a food web (detected by an increase in protozoa, rotifer and mite densities).

MATERIALS AND METHODS

Survey

The first hypothesis was tested by a broad survey of the pitcher plants in two different bogs, Tender Bog and Tuesday Bog on UNDERC property. The survey was used to help indicate to what extent midge and mosquito larvae covary in pitchers. In a period of one week, pitcher plants were sampled for mosquito and midge density, lip width and clarity of water. Relative densities were measured by pipetting all water contents into a glass bowl and enumerating midge and mosquito larvae. Water clarity was scored on five levels of visibility and contents, one being the most visible.

Lip width was measured by the width of the rosette opening and gave some indication of pitcher plant size.

Experiment

The midge, bottom-up and top-down hypotheses were tested using a fully crossed, 3-way factorial experiment with 12 total treatment levels. The three independent factors were predators, at two levels of 0 and 15 mosquitoes, resource concentration, at 0, 4 and 20 mg of dead ants, and midge density, at 0 and 10 midges present. The pitcher plants selected for the experiment were filled from the base upwards with unscented wax to prohibit the flow of old nutrients into the manipulated inquiline fluid. The pitchers were sampled four times over the course of four weeks by gently mixing and extracting the contents within the leaves. The mosquito larvae and midge larvae were counted and adjusted to maintain the initially applied treatment densities. Larvae were added to replace those lost to mortality and new eggs or young larvae that were previously undetected were removed. Initial amounts of carcasses (mg of ants) were re-added to the pitchers in the second sampling session, while treatments with zero ants were maintained during all sampling sessions. Inquiline fluid was taken in small samples (<300 uL) for enumerating protozoa, bacteria and rotifers.

Using Palmer counting cells containing 100 uL of inquiline fluid and a compound microscope (x100), protozoa, rotifers and mites were enumerated. Protozoa was identified to genus and abundance of mites and rotifers were also recorded. Bacteria densities were estimated by a sample from each pitcher that is treated with formaldehyde and stained with acridine orange. This mixture was then strained through a .2 um pore size Nuclepore and enumerated on slides using a Whipple grid on an epiflourescence microscope (Hobbie et al. 1977, Pace 1992).

Statistical Analysis

The survey was evaluated using a simple correlation between the relative abundances of midge and mosquito larvae present throughout the pitchers.

The abundance of inquilines over the course of the sampling sessions was compared between treatments using repeated measure, multi-way ANOVAs and post-hoc Tukey tests.

RESULTS

Over the course of the experiment, all pitcher plants remained in tact (no holes or damages) up until the fourth sampling. In the fourth sampling session, 32 pitchers were lost to browsing and the remaining pitchers were sampled. Mites were not included in statistics due to their low abundance.

Based on our initial survey, we concluded that the densities of mosquitoes and the midges do not covary in pitcher plants in the field ($r=.263$, $p=.033$). Only 1.5% of 66 pitchers contained no midges or mosquitoes.

Rotifers

I log transformed rotifer density to better conform to statistical assumptions. Midges and mosquitoes had a significant negative effect on rotifer density (df=1, F=5.736, p=0.021; Figure 1), (df=1, F=17.0, $p<0.001$; Figure 1). Ants did not significantly influence rotifer densities (df=2, F=0.285, p=0.753), but mosquitoes, midges and ants had a significant interaction (df=2, F=5.33, p=0.008). All other interactions were insignificant.

Protozoa

We calculated protozoa biovolume using estimated volume in µm^3 of all protozoan genera observed multiplied by their abundance in each of the Palmer cells. On account of a large range of protozoa body sizes, biovolume was used for a more accurate estimate of protozoan influence in the food web based on feeding capabilities and not abundance.

Mosquitoes had a negative effect on protozoa biovolume (df=1, F=9.17, p=0.004; Figure 2). Midges had a marginally negative effect on protozoa biovolume (df=1, F=3.90, p=0.054; Figure 2). Ants had no effect on biovolume of protozoa (df=2, F=0.811, p=0.450). Midges and mosquitoes were found to have an significant interaction (df=1, F=4.26 , p=0.044; Figure 3) while all other interactions were not significant.

Bacteria

Bacteria densities were only enumerated during sample session two, due to lack of adequate replication in the fourth sampling session. Mosquitoes had a significant negative effect on bacteria density (df=1, F=5.158, p=0.028; Figure 4) while midges had a significant positive effect on bacteria density (df=1, F=4.92, p=0.031). Ants had no significant influence on bacteria densities (df=2, F=1.01, p= 0.372). None of the interaction effects were significant.

DISCUSSION

The strongest impact on the food web in pitcher plants on UNDERC property is the top-down effects produced by the presence of *Wyeomyia smithii*, the pitcher plant mosquito, while unexpectedly the effect of bottom-up forces were absent throughout this experiment. The presence of *Metriocnemus knabi* (the midge) had a positive effect on the densities of bacteria by supplying an increase in nutrient availability. However, bottom-up effects were not propagated further up the food web to protozoa or rotifers.

Heard (1994) suggested that midges and mosquitoes interact in northern pitcher plants through a processing chain commensalism. He predicts that mosquito densities positively respond to the accelerated nutrient cycling provided by midges. In contrast to Heard's predictions, our general survey provides evidence that mosquito densities do not covary with midge densities and casts doubt on a strong processing chain commensalism. The significant p-value given by the correlation falsely describes our data because a large percent of pitchers that were sampled contained low midge and mosquito densities. The low r-value gives a better depiction of the lack of covariance we found between the two consumer densities.

Top-down theory suggests that the presence of a top predator can control the populations of lower trophic levels. In our top-down manipulation experiment, we found that the presence of *Wyeomyia smithii* (the mosquito) had a significantly negative effect on rotifer densities, average protozoa biovolume and bacteria densities. In the northern pitcher plants, mosquitoes act as an omnivorous top predator that filter feeds on bacterivores (protozoa and rotifers) and bacteria themselves. Addicott (1974) found similar results that support top-down theoretical predictions that the presence of *Wyeomyia smithii* plays a prominent role in the northern pitcher plants food web structure and dynamics.

In previous studies including Knietel and Miller (2002), and Hoekman (*unpublished manuscript*), the effects of bottom-up forces had a strong influence on the inquiline food web in the northern pitcher plant (*Sarracenia purpurea)*. In each of these experiments, an increase in nutrient availability (drowned carcasses) resulted in an increase in densities of higher trophic levels and therefore suggested that the northern pitcher plant food web is resource limited.

In our experiment, an increase in resource availability (ants) was not found to significantly increase densities of organisms in higher trophic levels (bacteria, protozoa and rotifers). The discrepancy of our results compared to previous studies may be explained by our experimental methods. The pitcher plants we sampled contained accumulated nutrients in their trumpet shaped base and to avoid the exchange of old nutrients into the experimental fluid, each pitcher was plugged with wax. We believe that this method, being implemented for the first time, did not completely separate the inquiline fluid from the old nutrients. Therefore the ability of accumulated nutrients to move into the water column may have lead to an indistinct difference between pitcher plants with and without experimentally added ants. With the presence of some nutrients in all treatment levels, there was an inability to find significant treatment effects of ants.

Heard (1994) suggests that the presence of *Metriocnemus knabi,* the processing detritivore midge, accelerates nutrient cycling by comminuting whole carcasses into particles and therefore providing increased resource availability to higher trophic levels. Increased resource availability in the food web will result in increased densities of higher trophic levels. In contrast to our hypothesis, our experiment showed that midges have a variable effect on organisms in higher trophic levels, therefore leaving its ability to increase the effect of bottom-up forces in the northern pitcher plant inconclusive.

Supported by Heard's prediction, we found that midges have a strong positive response on bacteria density due to its ability to accelerate nutrient cycling (Heard 1994). In this treatment effect, midges comminuting contribution to downstream consumers outweighs the overall loss of resources by upstream consumption (Heard 1994). Therefore, midges increase the net resource availability for downstream consumers and allows for higher densities of those organisms, as shown by the higher densities of bacteria.

Despite the positive effect that midges had a bacteria density, we found that midges still had a negative effect on rotifer densities. Our results contradicted our hypothesis that the presence of midges would increase nutrient availability for bacteria and therefore increase densities of bacterivorous rotifers. There are two possible explanations as to why midges had a significantly negative effect on rotifer density: predation and competition. We observed that rotifers are most frequently found on large pieces of detritus in the pitcher plants that midges also feed on; therefore midges may unintentionally feed on rotifers and deplete their population. Midges may also compete with rotifers for bacteria in the pitcher plants and therefore may hinder their growth and survivorship.

Midges also had a marginally negative effect on average protozoa biovolume. This result may be explained by the strong influence of mosquitoes on the top-down effects in pitcher plant. This strong top-down force coupled with a strong mosquito-midge interaction may be the reason why midges have a weak negative effect on average protozoa biovolume.

While midges had a positive effect on bacteria densities in the northern pitcher plant, this effect did not propagate throughout the food web. Therefore, while there was an increased nutrient availability for bacteria detected by their increased densities, midges did not necessarily increase the relative importance of bottom-up effects in the entire food web of the northern pitcher plant.

Conclusion

The influence that *Wyeomyia smithii* (the mosquito) has on top-down forces and its strong influence on food web structure and dynamics in the northern pitcher plant is well supported by previous studies, but the absence of bottom-up effects in our experiment is unexpected and may be attributed to our experimental methods. *Metriocnemus knabi* (midges) played an inconclusive role in increasing the effect of bottom-up forces in the northern pitcher plant. The strong positive effect that midges have on bacteria density suggests that midges play a significant role in accelerating nutrient cycling, but the varying results that midges had on other trophic levels (protozoa and rotifers) leaves room for further experimentation for clarity on their function in the northern pitcher plant community.

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FIGURES

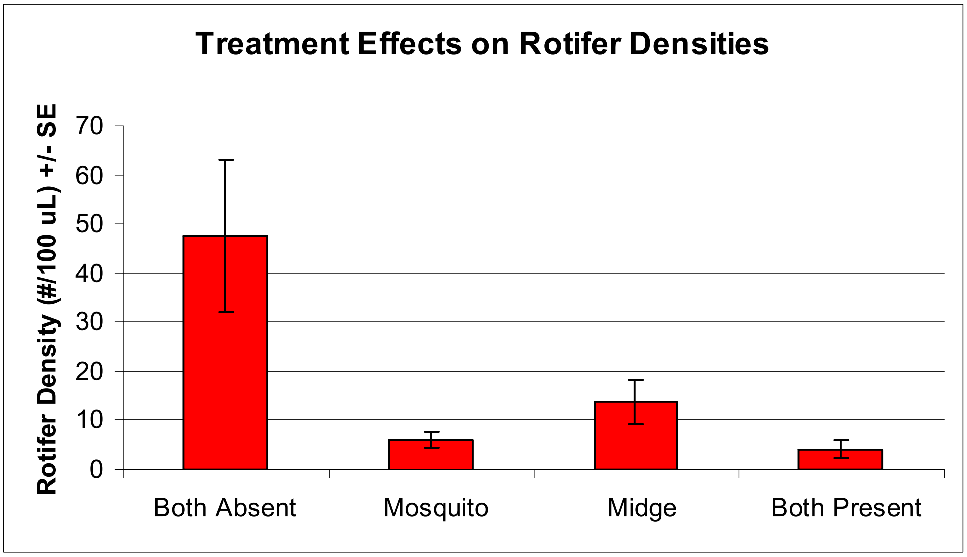

Figure 1: This graph represents the four treatment levels used in our experimental design and their effect on rotifer density. Both mosquitoes and midges had a significantly negative effect on rotifer density ($p<0.001$, $p=0.021$). Error bars were calculated using standard error of the data.

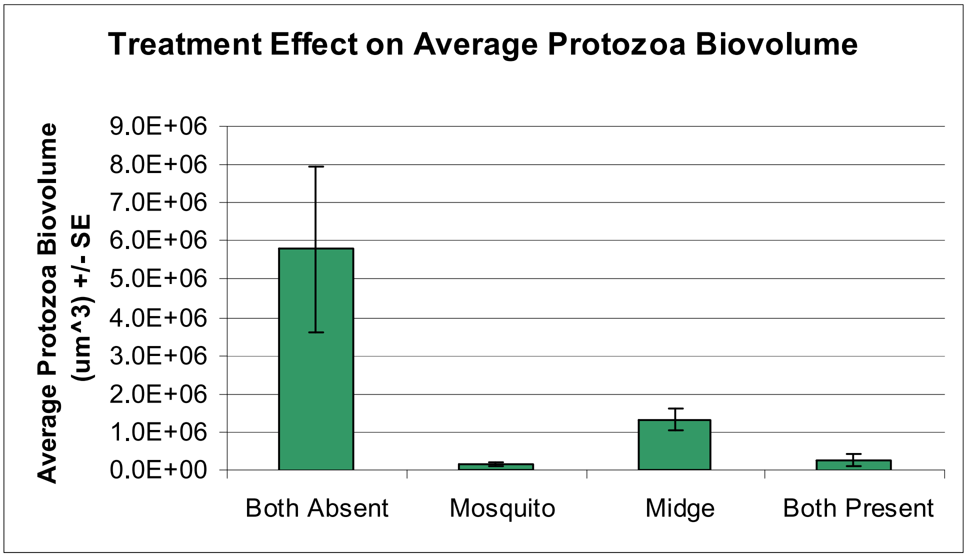

Figure 2: This graph shows the four treatment levels and their effect on average protozoa biovolume. The presence of mosquitoes strongly suppressed the average biovolume of protozoa while midges had a marginally significant negative effect ($p=0.004$, $p=0.054$). Error bars were calculated using standard error of the data.

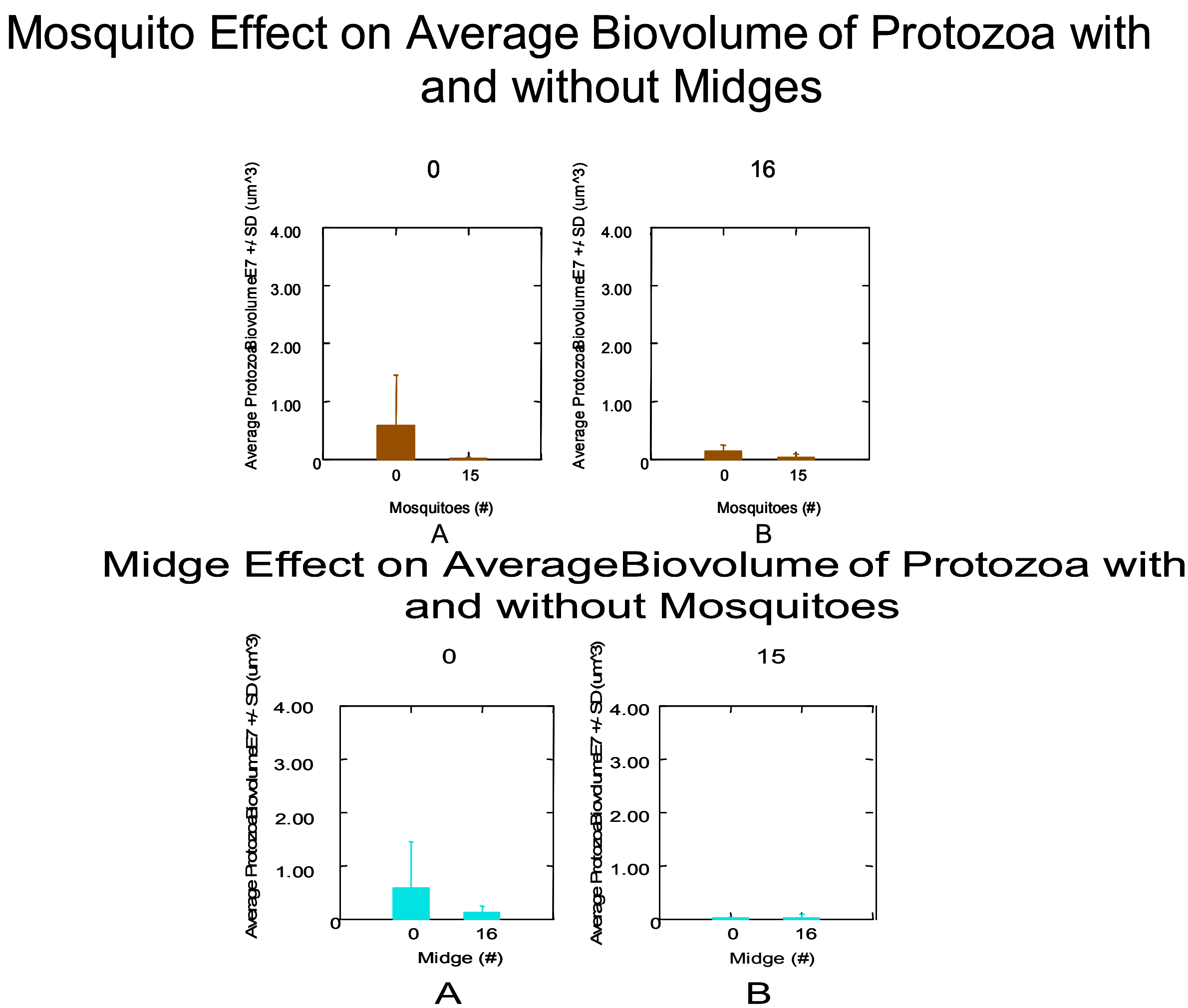

Figure 3: These graphs indicate the significant midge-mosquito interaction effect on average protozoa biovolume (p=0.044). The A graphs indicate the absence of the other major consumer (midge or mosquito) in the food web, while B graphs indicate their presence. Both mosquito and midge effects are significantly suppressed when both of the consumers are present within the pitchers (B graphs). Error bars are a measure of the standard deviation of the data.

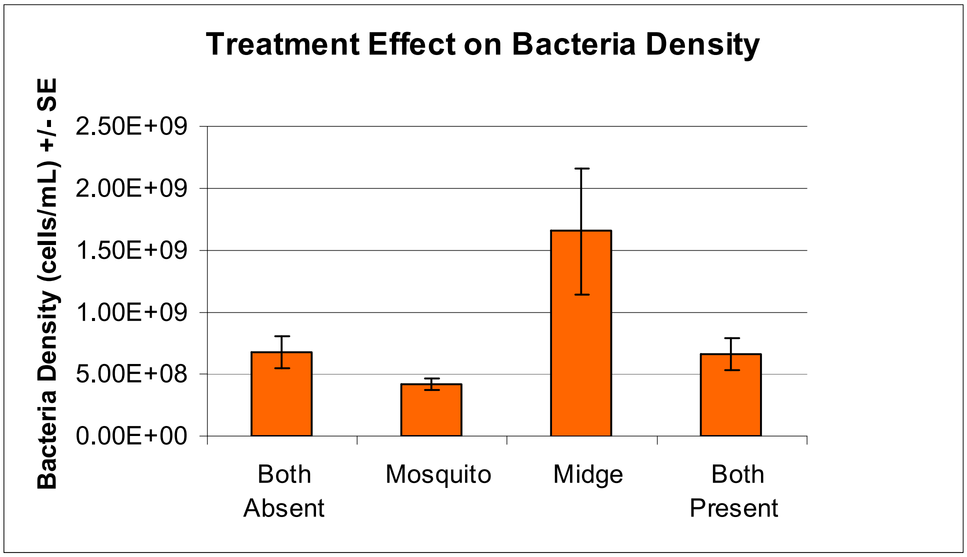

Figure 4: This graph represents the opposite effects that midges and mosquitoes had on bacteria density. Midges had a significant positive effect on bacteria density while mosquitoes had a significant negative effect on bacteria density ($p=0.031$, $p=0.028$). Error bars were calculated using standard error of the data.