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Community Assembly Dynamics and Consequences of Invasion:

Tests with the *Sarracenia purpurea* Model System

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Abstract of the Dissertation

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The central theme of this dissertation research addresses fundamental ecological questions about community assembly (which species are found in a given place). This includes our understanding of the role of abiotic stresses in environments, species invasions, system resistance to invasion and the impacts of invaders. The natural, model aquatic community found in leaves of the pitcher plant, *Sarracenia purpurea,* was used to experimentally test the effects of different biotic interactions, abiotic factors, and characteristics of different species on the success of invasion by a new species in a community. These factors were looked at individually as well as in combination, and their relative impacts on resident community dynamics were also examined. The molecular technique of metagenomics (16s rRNA clone libraries) was incorporated into this work in order to determine the effects of the introduction of consumers with different characteristics on food web structure and species diversity. These experiments revealed that for this system: 1) it is the number of invaders, not their body size, that is important for invasion success, 2) the effects of invasion are dependent upon which species invade, 3) invasion impacts on the resident community depend on whether species are competitors or prey for the invader, 4) invaders in the middle of the food web that are better competitors and arrive in large numbers will have the greatest success, 5) the presence of a top predator controls how the community is affected when species in other levels invade, and 6) the availability of resources changes the susceptibility of a community to invaders. These results are important for understanding and predicting species invasion success and how to mitigate the spread and impact of invaders. By incorporating modern genomics tools, this dissertation research also re-examined results from prior experiments using this system and verified that community level responses observed in experiments using only common culturable bacteria reflected the response of the entire community and that the generalizations found among studies of this community are robust.

I dedicate this dissertation to my parents, Vera and Larry Gray, for their continuous and unconditional love, support and understanding.

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Chapter 1

Introduction

An over-arching theme within community ecology is identifying mechanisms that govern the assembly of communities (e.g., Diamond 1975, Belyea and Landcaster 1999). Properties considered important during community assembly include characteristics of habitats and of the individual species that colonize a given habitat and their interactions. The generalizability of these community assembly properties, or rules, has gained greater attention recently due to concern about species being transported around the world by humans and the impacts they have on environments. However, much of the research on introduced species has been on individual case studies, making it difficult to generalize across taxa and systems. Although many hypotheses have been proposed to explain invasion success, a general theory of community invasiblity or of predictable characteristics of species that successfully invade new communities and their impacts on community structure and dynamics has yet to be developed (Lonsdale 1999, Williamson 1999, Gurevitch et al. 2011).

The complexity of natural systems makes it challenging to design experiments that will allow us to disentangle community interactions and decipher which factors are important for shaping community structure and assembly. Microorganism model systems, which are simplified versions of larger scale communities, have been used to address such questions because they provide the tractability and high statistical power often difficult to obtain in larger scale, more complex systems (Jessup et al. 2004, Srivastava et al. 2004). Because of this, they can be powerful experimental tools, providing predictive power to understand the dynamics occurring in simplified communities, and allowing a better understanding of the relative importance of mechanisms that may drive dynamics in more complex systems. The goal of my dissertation was to use the natural, model aquatic community held within leaves of the carnivorous pitcher plant, *Sarracenia purpurea* to experimentally test the relative importance of different biotic

interactions, abiotic factors, and characteristics of different species that have been hypothesized to be most important for the success of invasion by a new species into a community, and how community structure in the *S. purpurea* system changed due to a particular invasion.

The northern pitcher plant, *S. purpurea,* is a carnivorous plant found in nutrient-limited bogs throughout North America, from Florida to Canada. Its leaves trap rainwater, creating a microscopic aquatic habitat that has dynamics of larger aquatic food webs, but on small spatial and short time scales. Insects, especially ants, fall into the trapped water. Bacteria and yeast colonize the system, decompose the insects, and liberate nutrients for the plant. A variety of protozoans and a rotifer species also colonize this community and consume the bacteria. The highest trophic level is filled by the larvae of the pitcher plant mosquito, *Wyeomyia smithii,* which feeds on the protozoans and rotifers. The mosquito, rotifer and protozoan species, and the phenotypes of the culturable bacteria, are the same across the entire geographic range of the plant (Buckley et al. 2003).

The rapid dynamics of this system (generation time for bacteria 3-4 hrs and protozoans 8-10 hrs), the readily available natural replication (multiple plants in one area), the ability to examine all interactions in the food web, and the fact that the community can be easily replicated and manipulated in both the lab and the field, have greatly facilitated the development of this model system. It has been used to test fundamental questions in community ecology including the importance of top-down and bottom-up forces in regulating community structure, and trophic cascades (Kneitel and Miller 2002), community consequence of invasion (Miller et al. 2002), nutrient limitation (Gray et al. 2006), and commensalisms (Heard 1994).

Traditional work on this model system has used only culturable bacteria, which are only a small subset of species in this community, to serve as a proxy for the bottom trophic level of the

community. This technique is common across ecological systems as it is extremely difficult to capture the dynamics of all species in a system, therefore a smaller group of species is generally followed. However, it has not been clear if this subset of species is an accurate surrogate of species-level diversity for the community of bacteria found in pitcher plants. For the *S. purpurea* system, it is unclear whether the community level responses observed in experiments with the common culturable bacteria reflects the response of the entire community, and thus whether the generalizations found among studies with this community are robust. By incorporating modern genomics tools, my dissertation research has allowed me to test this assumption. Specifically, by using metagenomics (16s rRNA clone libraries) to study the bacterial community within the *S. purpurea* model system, my dissertation directly tested:

How microbial communities varied within two different sites located in the native range of *S. purpurea*, and how food web composition affected the microbial community structure in this system – **Chapter 2**

If succession within this aquatic community is driven by deterministic or stochastic processes, and if resource availability and higher trophic level interactions affected the results – **Chapter 3**

If invasion success within this system is a result of certain species-level characteristics, and whether characteristics predicted to be important for invasion success (initial density, competitive ability, body size) are generalizable across pitcher plant communities with different trophic structure and resource availability. – **Chapter 4**

The effects of the introduction of consumers with differing species-level traits on food web structure of the *S. purpurea* system and how features of the resident community, such as diversity, are affected by invasion – **Chapter 5**

and

If traits that convey invasion success for species in one trophic level are generalizable to species in other trophic levels in the *S. purpurea* system, and if assumed trade-offs between competitive ability and resistance to predators or environmental stress play a major role in shaping invasion success – **Chapter 6**

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Chapter 2

Variability in aquatic microbial community composition and diversity in the pitcher plant *Sarracenia purpurea*

Abstract

Microbes play an essential role in community and ecosystem processes; yet, much is still unknown about their distribution and diversity. In this study, I used the aquatic community held within the cup-shaped leaves of the carnivorous pitcher plant, *Sarracenia purpurea*, to address 1) how microbial communities vary at the local scale at two different sites (southern and mid-regional sites in *S. purpurea*'s native geographic range), and 2) how food web composition affects microbial populations. Differences in the abundance and richness of the non-bacterial members of the community were also determined to assess whether food web dynamics impact microbial community composition. Communities from 6 leaves (one leaf per plant) from New York (mid-regional) and Florida (southern) study sites were analyzed and 508 and 417 16S rRNA gene clones for each population, respectively, were screened. I found very little overlap in bacterial community composition and diversity when the two sites were compared to each other. Each pitcher within each site also had a distinct community; however, there was more overlap in bacterial composition within each site than when communities were compared across sites. There was overlap in the identity of protozoans and metazoans in this community when identity was compared across the two sites, but the abundances and presence/absence of these species within communities varied between sites. Results from this study demonstrate that specific bacteria species or functional groups are not required by the plant in order to decompose trapped insects, and co-evolution between the plant and bacteria may not have occurred as it has for other members of this community.

Introduction

Microbial ecologists strive to understand the complex relationships between microorganisms and their environment. Two long-standing questions for microbial ecologists are: how do microbial communities vary within and across sites, and how are microbial populations affected by food web composition (where the food web includes the protozoans and metazoans, coexisting with prokaryotes). The vast complexity of ecosystems across spatial scales and the complex nature of food webs make addressing these questions a serious challenge.

The carnivorous perennial pitcher plant, *Sarracenia purpurea* L., native to wetlands and bogs throughout the eastern and mid-western portion of the United States and most of Canada, provides an excellent natural habitat to address the variability in microbial communities and food web dynamics. The leaves of *S. purpurea* collect rainwater after opening, providing a micro-environment for an aquatic food web to develop. These spaces are referred to as phytotelmata, or plant-held bodies of water, and are common and widely studied naturally occurring micro-habitats. The community dynamics within these leaves are similar to those of larger aquatic food webs, but on smaller spatial and shorter time scales. The pitcher plant has been developed as a model system to test fundamental questions in community ecology including the importance of top-down and bottom-up forces in structuring communities, and the potential for trophic cascades (Kneitel and Miller 2002), community consequences of invasion (Miller et al. 2002), nutrient limitation (Gray et al. 2006) and commensalisms (Heard 1994).

The development of the aquatic food web within the leaves of *S. purpurea* begins when newly opened leaves collect rainwater and act as a pitfall trap for insects. Captured insects, primarily ants, provide nutrients for the plant and become the basal resource for the food web. Once insects have drowned in the water, aquatic invertebrates that live within the pitcher break

down the dead insects into smaller fragments and bacteria decompose the insects, releasing nutrients in a form that can then readily be taken up by the plant. Unlike other species of pitcher plants, *S. purpurea* does not produce digestive enzymes, except possibly in newly opened leaves (Gallie and Chang 1997), and is therefore largely reliant on bacteria to decompose the captured insects. The degree to which bacteria can decompose insects is dependent on the abundance of protozoans and rotifers in the intermediate trophic level, which feed on the bacteria. The top predator, larvae of the specialist mosquito, *Wyeomyia smithii* (Culicidae), feeds primarily on the protozoans and rotifers, but also occasionally on bacteria (Kneitel and Miller 2002). The midge *Metrocnemus knabi* (Chironomidae), found along the bottom of a pitcher, facilitates the release of nutrients into the food web by breaking the dead insects into smaller pieces (Heard 1994).

Because of the close association between bacteria and the plant, it can be hypothesized that specific bacterial species may be particularly important, and this subset of bacteria should be expected in high frequencies in leaves throughout the geographic range of the plant. This pattern is already found with the rotifer, protozoan, and dipteran species present in this community, which are believed to be the same set of species throughout the entirety of the plant's broad native geographic range (Buckley et al. 2003). Until recently, identifying bacteria in this system has been limited to the phenotypic comparison of culturable bacteria. It is presently not known if these culturable bacteria are the same species across this range, if they dominate the bacterial community, if their responses in ecological experiments are representative of the entire bacterial community, and if a larger focus on unculturable bacteria is needed in this system to understand community dynamics.

By using Terminal Restriction Fragment Length Polymorphisms (T-RFLPs) on the biofilm lining the inside of the leaf, Peterson et al. (2008) took the first step towards characterizing the

variability in composition of the bacterial community in *S. purpurea* leaves in bogs in central Massachusetts. However, little is still known about the bacteria found in the water column of these leaves, which form the base of the food web in this community. The water column bacteria are key members of the community and for decades have played a large part in food web experiments using this system (e.g., Cochran-Stafira and von Ende 1998, Kneitel and Miller 2002, Gray et al. 2006). In this study, I examined the bacterial community composition in the water column of the community at two bogs within *S. purpurea*'s native range: within a bog in Florida (southern site) and within a bog in New York (mid-range site).

Methods

Study system, field site and sampling

Sarracenia purpurea is native to nutrient-limited wetlands and bogs throughout the eastern United States and most of Canada, with the southernmost population located in the panhandle of northern Florida. *S. purpurea* is composed of two subspecies: subspecies *purpurea* (north of New Jersey, including New York) and subspecies *venosa* (south of New Jersey) (Schnell 2002). The population located in north Florida is thought to be a separate variety, var. *burkii* (Godt and Hamrick 1999), but contains the same members of the aquatic community as are seen throughout North America (Buckley et al. 2003). The leaves form in a rosette pattern, with the main morphological difference between the subspecies being the shape of the leaf (diameter of the opening of pitcher, wing size, and size and frill of the hood vary in size according to subspecies) (Schnell 2002). All plants can interbreed, resulting in a variety of hybrids along the border between subspecies distributions (Schnell 2002). The leaves of all subspecies also harbor the same aquatic community (Buckley et al. 2003).

Pitcher plant populations in a single bog in Sumatra, Florida (Apalachicola National Forest (30N, 84W)) and a single bog near Riverhead, New York (Cranberry Bog Preserve (40N, 72W)) were chosen for this study. These sites represent the mid- (New York) and southern range (Florida) of *S. purpurea*. Although *S. purpurea* is found in nitrogen poor soils, characteristics of the local habitat can vary greatly. The New York bog site used in this study is composed of sphagnum moss and contains only one other carnivorous plant species, the roundleaf sundew *Drosera rotundifolia*, which grows on the sphagnum moss alongside cranberry (*Vaccinium macrocarpona)* shrubs and the reed *Phragmites australis*. The most common ant species found inside *S. purpurea*'s leaves at this field site is *Tapinoma sessile* (Gray, personal observation).

The Florida bog site is located in a sandy, open savannah within a long leaf pine (*Pinus palustris*) forest. The habitat is mainly composed of the grass *Aristida stricta* and a large diversity of other carnivorous plant species (*S. flava, S. psitticina, Drosera capillaris* and *Pinguicula* species). The most common ant species found inside the pitcher plant leaves at this field site is the invasive fire ant *Solenopsis* sp. (Gray, personal observation). It is possible that the bacterial community composition is affected by these habitat differences even though the species identity of the remainder of the community appears to be unaffected by habitat (Buckley et al. 2003).

At the beginning of the growing season for each location (May 2008 for Florida and June 2008 for New York), the first fully developed, yet still unopened leaves of that growing season were marked. These plants were randomly selected based on walking through the bog and marking the unopened leaves that were observed on plants. Within two days of being marked, each leaf opened into its characteristic pitcher shape, filled with rainwater, collected insects into its pitfall trap, and the aquatic community of protozoans, rotifers, bacteria, and larvae assembled

in the pitcher. After 14 days (chosen based on the high capture rate of insects by new leaves) one marked leaf per pitcher plant (15 leaves in Florida and 15 leaves in New York) was selected for further analysis of this aquatic community. Six of these collected samples at each site (6 in NY and 6 in FL) were used to develop 16s rRNA clone libraries to assess bacterial composition. The water in each selected leaf was gently mixed with a sterile pipette and placed into a sterile 50 ml centrifuge tube, which was transported on ice back to the laboratory for further processing.

Processing of pitcher plant aquatic community

I assessed the richness and abundance of the common members of the pitcher plant aquatic community according to standard methods (e.g., Kneitel and Miller 2002, Gray et al. 2006). The volume and clarity of the water was recorded for each sample and the number of dead ants and other invertebrates present in the water were counted. I used a compound microscope to determine the richness and densities of protozoan species within a 0.1 ml aliquot of each pitcher plant aquatic community sample. To determine the relative abundance and richness of the culturable bacterial morphotypes, a 10^{-4} dilution of the pitcher plant water was plated onto a half-strength Luria broth plate (Cochran-Stafira and von Ende 1998; Kneitel and Miller 2002; Gray et al. 2006). Plates were incubated at 26°C for 72 hours after which the colony forming units (CFUs) were counted (Kneitel and Miller 2002, Gray et al. 2006).

Environmental clone library construction and phylogenetic analyses

A 1 ml aliquot of the water sample from each pitcher was filtered onto a 0.22 µM Isopore membrane filter (Millipore, Billerica, Massachusetts) to collect bacterial cells. Microbial community DNA was extracted from the filters using the Ultra Clean Soil DNA kit according to

manufacturer's instructions (Mo Bio Laboratories, Solana Beach, California), with the exception of Step 1 of the protocol. Instead of using a soil sample, the filter containing the bacteria was placed in the bead solution tubes with 200 µl of sterile water.

Aliquots of purified DNA were PCR amplified using the *Bacteria* domain-specific SSU rRNA gene primers 27F (5'-AGA GTT TGA TCM TGG CTC AG -3') (Johnson 1994) and 1392R (5'-ACG GGC GGT GTG TAC-3') (Wilson et al. 1990) as previously described (Akob et al. 2007). PCR products were purified using the Qiagen Gel Extraction Kit (QIAGEN, Valencia, CA), then ligated into the TOPO TA cloning vector pCR 2.1 according to manufacturer's instructions (Invitrogen, Carlsbad, CA). Ligation reactions were sent to The Genome Center at Washington University (St. Louis, Missouri), where they were transformed and a total of 96 clones were sequenced for each library. Clones from New York libraries were sequenced using the primers 27F, 907R and 1392R and the Florida clones were sequenced in a single direction with primer 907R.

Sequences were assembled and vector sequences flanking the SSU rRNA gene inserts were removed using Sequencher v4.8 (Gene Codes Corp., Ann Arbor, MI). Clones were grouped into phylotypes based on a sequence similarity cut off of 97% using the program FastGroupII (Yu et al. 2006). All clone sequences were aligned with the alignment tool by Greengenes (DeSantis et al. 2006a), accessible at http://greengenes.lbl.gov, and nearest neighbors were identified using the Classify tool against the Greengenes database (DeSantis et al. 2006b). Rarefaction curves were calculated using Analytic Rarefaction 1.3 (Heck et al. 1975, Holland 2003).

Variation in community composition and Diversity Indices

I calculated diversity indices (Shannon Index and richness) as well as Pielou's evenness with

the statistical program Primer 6.1 (PRIMER 6, Version 6.1.6, Primer E-Ltd. 2006). Nonparametric multivariate statistics (PRIMER 6, Version 6.1.6, Primer E-Ltd. 2006) were used to determine the similarity in bacterial community structure at the local scale within and between two sites. For similarity in abundances, data were first normalized using a square root transformation. Bray-Curtis distances were then calculated, which uses values from 0 (most similar) to 1 (least similar) to determine similarity between samples (Bray and Curtis 1957). To graphically visualize the differences between bacterial communities, non-metric Multi-Dimensional Scaling (MDS) ordination was used. Communities that are more similar are spatially close to each other on a MDS plot and those that are less similar are spatially separated. An Analysis of Similarity (ANOSIM) was performed to calculate a Global R, which determined the overall similarity between communities, with a value of 1 representing extreme dissimilarity and a value of 0 representing complete overlap in community composition. Values between 0 and 1 represent varying degrees of community similarity (high values = more dissimilarity in composition between communities).

Results

Ecological characterization of the aquatic community within Sarracenia purpurea *leaves*

The key members of the pitcher plant food web were highly variable in abundance and diversity within both bogs and when community structure within each bog was compared, suggesting that 14 days subsequent to a pitcher plant leaf opening is not sufficient time for the communities to reach equilibrium. This result is supported by data in Chapter 3. New York pitcher plants contained more water than samples collected in Florida (One-way ANOVA, $F = 3.76$, $p = 0.081$) and had higher average protozoan densities (Mann-Whitney U Test to account

for zeros and high variance, $p = 0.037$) and average number of protozoan species (One-way ANOVA, $F = 7.78$, $p = 0.019$).

Culturable bacterial diversity and evenness were not significantly different in New York than in Florida (Figure 1, One-way ANOVA, Diversity: $F = 0.83$, $p = 0.384$; Evenness: $F = 0.0789$, $p = 0.784$). When culturable bacteria were the only bacteria assessed in the communities (by identifying morphotypes growing on agar), communities were significantly less diverse and even community than if 16S rRNA gene clone data were used (Table 1, Figure 1). This was true for both the Florida and New York site (Table 1, Figure 1; One-way ANOVA comparing culturable bacteria and 16S genes sampling methods in the NY site, Diversity: $F = 72.3$, $p < 0.0001$, Evenness: $F = 4.63$, $p = 0.0568$, and the Florida site, Diversity: $F = 80.15$, $p < 0.0001$, Evenness: $F = 11.59$, $p = 0.00672$).

The similarity in bacterial community composition between pitcher plant leaves within and between sites when only culturable bacteria were considered (agar plate counts) are shown in multi-dimensional scaling plots (Figure 2a,b). This graphical representation of community similarity was done for both the abundance of colonies of individual morphotypes (Figure 2a) and for just presence/absence data (presence/absence of each morphotype, Figure 2b). For culturable bacteria, communities were similar both within a bog and between sites (FL and NY) when either abundances of individual morphotypes data or presence/absence data were used (ANOSIM Global $R = 0.306$ for abundance data and 0.373 for presence/absence data). Culturable bacteria community composition was not completely overlapping in similarity from leaf to leaf within a bog, however, the composition was still more similar within one site than when compared across sites (Figure 2a,b).

Analysis of Florida and New York clone libraries

A total of 12 clone libraries were constructed for the communities of aquatic bacteria present in newly opened leaves of *Sarracenia purpurea* from New York and Florida. For each study site, communities from 6 leaves (one leaf per plant) were analyzed and 508 and 417 clones for New York and Florida populations, respectively, were screened (Table 1). Grouping of phylotypes based on sequence similarity revealed a total of 247 phylotypes, of which 12 phylotypes were found in both Florida and New York clone libraries, and most were closely related to environmental bacteria. Rarefaction curves from each library (Figure 3) did not indicate saturation, i.e., the slope was greater than zero (Heck et al. 1975). However, percent coverage ranged from 55.9% (Florida Leaf 214) to 84.5% (New York Leaf 4) (Table 1). Although additional sampling of clones would be necessary to describe the overall diversity fully, numerically dominant groups from multiple lineages were obtained. Shannon-Wiener diversity and evenness indices were significantly higher in Florida samples than in New York samples (One-way ANOVA, Diversity: $p = 0.047$, $F = 5.11$; Evenness: 0.027, $F = 6.67$; Table 1). The 247 OTUs observed in Florida and New York clone libraries were most closely related to members of the phlya Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes and unclassified Bacteria lineages (Figure 4).

Bacteroidetes was the most abundant phylum detected in both Florida and New York (36 and 34% of total clones, respectively) and contained members of the classes Sphingobacteria and Flavobacteria. Florida Bacteroidetes-related OTUs were related to members of four families, Sphingobacteriaceae, Flexibacteraceae, and Crenotrichaceae, and Flavobacteriaceae, whereas, OTUs from New York were related to members of the families Sphingobacteriaceae and Flavobacteriaceae (data not shown). The abundance of the Bacteroidetes phylum was highly

variable within each site, ranging from 2-52% of total clones for Florida leaves and 8-63% of total clones from New York leaves (Figure 4). The most abundant Bacteroidetes-related phylotype detected in New York libraries was clone NY06_LEAF11_C06 (49 total clones), which was detected in Leaves 1 (1 clone), 8 (19 clones), and 11 (29 clones). Clone NY06_LEAF11_C06 had 99% sequence similarity to the Flavobacteriaceae isolate *Chryseobacterium* sp. M229 (accession number AB461706). Phylotypes FL_LEAF222_CLONE_H11 and FL_LEAF228_CLONE_B04 were the most abundant Bacteroidetes-related phylotypes in Florida libraries representing 20 total clones each. Phylotype FL_LEAF222_CLONE_H11 had 95% sequence similarity to *Pedobacter kwangyangensis* strain CW39 (accession number EF693742). Phylotype FL_LEAF228_CLONE_B04 was most closely related to the uncultured Sphingobacteria bacterium IT-4 (accession number AB491320). Phylotype FL_LEAF228_CLONE_A08 was detected in Florida Leaf 228 and New York Leaf 6 and had 97% sequence similarity to uncultured bacterium clone AB_P5_H06 (accession number GQ328411) within the Sphingobacteria.

Members of the phylum Proteobacteria were detected in aquatic communities from both Florida and New York leaves, however, not all subclasses were detected at both sites. Members of the Alpha-, Beta-, and Gamma-subclasses of the Proteobacteria were detected at both sites and in all leaves, whereas, the Deltaproteobacteria subclass was only found in New York Leaf 8. For Florida libraries, members of the Alpha-, Beta-, and Gammaproteobacteria comprised 27, 19, and 8% of total clones and varied in their abundance for each leaf. The Alphaproteobacteria were most abundant in Florida Leaf 304 (30% of total clones; Figure 4). The most abundant Alphaproteobacteria-related phylotype was FL_LEAF304_CLONE_E11, which was most closely related (99% sequence similarity) to the uncultured Sphingomonadaceae bacterium clone

Plot29-2F10 (accession number EU202874). Betaproteobacteria-related clones were 39% of total clones in Florida Leaf 200 and the highest abundance of Gammaproteobacteria-related clones was observed in Leaf 228 (Figure 4). In New York clone libraries, Gamma-, Beta- and Alphaproteobacteria represented 32, 19, and 14% of total clones. The highest abundance of Gammaproteobacteria-related clones was found in New York Leaf 1 with 69% of clones for this library. The most abundant phylotypes, NY01_LEAF01_H05 and NY03_LEAF06_C06, were related to *Klebsiella* sp. 2009I7 (accession number GU290324; 94% sequence similarity) and *Pseudomonas costantinii* (accession number AB440177; 99% sequence similarity), respectively. Beta- and Alphaproteobacteria- related clones were most abundant in New York Leaf 4 (39% of total clones) and Leaf 11 (29% of total clones), respectively (Figure 4). The most abundant Betaproteobacteria-related clones, NY02_LEAF04_E09 and NY06_LEAF11_H04_B1, were most closely related to the uncultured bacterium clone nbt40f08 (accession number FJ894731; 99% sequence similarity) within the Oxalobacteraceae family.

Four Beta- and 7 Alphaproteobacteria-related phylotypes were observed in both Florida and New York clone libraries. The 4 Betaproteobacteria-related phylotypes grouped into the Oxalobacteraceae, Comamonadaceae and Neisseriaceae families. The Oxalobacteraceae-related phylotype NY02_LEAF04_G01_B2 had 98% sequence similarity to *Herbaspirillum rubrisubalbicans* (accession number AJ238356) and the Comamonadaceae phylotype FL_LEAF222_CLONE_F10 had 98% sequence similarity to *Xylophilus ampelinus* strain DSM 7250 (accession number AJ420330). The Neisseriaceae phylotypes were related to the genus *Aquitalea* with phylotype FL_LEAF200_CLONE_H12 closely related (97% sequence similarity) to the uncultured Aquitalea sp. clone ntu70 (accession number EU159475) and phylotype

NY02_LEAF04_H09 closely related (99% sequence similarity) to *Aquitalea denitrificans* strain 5YN1-3 (accession number EU594330).

The 7 *Alphaproteobacteria*-related phylotypes observed in both Florida and New York clone libraries were related to members of the Sphingomonadaceae and Rhizobiaceae families. Phylotypes NY02_LEAF04_G10 and FL_LEAF222_CLONE_B03, within the family Sphingomonadaceae, were most closely related to uncultured *Sphingomonas* sp. GBb6 (accession number AJ812013; 99% sequence similarity) and uncultured bacterium clone SRODG082 (accession number FM995170; 97% sequence similarity). Rhizobiaceae-related phylotypes included NY04_LEAF07_G09_B1 and NY04_LEAF07_G03_B1, which were related to *Agrobacterium tumefaciens* (accession number GU580896; 97 and 100% sequence similarity). Phylotypes NY05_LEAF08_E09 and NY06_LEAF11_E10 had highest similarity to *Agrobacterium* sp. strain B1490 (accession number GQ169803; 94% sequence similarity) and *Agrobacterium rubi* (accession number GU580894; 100% sequence similarity), respectively. The final Rhizobiaceae-related phylotype, NY02_LEAF04_C08 was related to uncultured Rhizobiales bacterium clone Sto3-1 (accession number AY138237; 97% sequence similarity).

Members of the *Actinobacteria* phylum comprised only 8 and 1% of clones in Florida and New York clone libraries, respectively, whereas, unclassified lineages were only observed in Florida libraries (1% of total clones) (Figure 4). The most abundant *Actinobacteria*-related phylotype was FL_LEAF222_CLONE_A04, which was most closely related to *Microbacterium schleiferi* (accession number EU440992; 99% sequence similarity) within the Microbacteriaceae family.

Similarity in Bacterial OTU community composition

Non-metric multidimensional scaling analyses comparing the similarity between bacterial community composition (two separate analyses based on the abundance and the presence/absence of bacterial OTUs) were constructed using data obtained from aligned sequences inputted into FastGroup II. The resulting MDS plots illustrate that bacterial communities both within a site and between sites are very different from one another (Figure 5a,b; ANOSIM Global R = 0.922 based on abundance data and 0.904 based on presence absence data). Although different in community composition, bacterial communities within a site were more similar to each other than when compared across sites. Pitcher plant bacterial communities within the Florida site clustered closer together in the MDS plots than to the bacterial communities in the New York site (Figure 5a,b). The FastGroup II analysis did find that Florida and New York shared 12 of the same bacterial OTUs, however, this overlap was not large enough to alter the overall differences in community similarity between these sites.

Discussion

Unlike the protozoans and metazoans that are part of the pitcher plant aquatic community, I found surprisingly high diversity and variability in species identity in this microbial community across small spatial scales, within a bog, as well as when the communities were compared across sites. Out of the clones that were screened for the northern (New York, 508) and southern (Florida, 407) populations, 247 different phylotypes were identified based on sequence similarity, and only 12 of these were found in both the Florida and New York sites. Furthermore, some lineages present in the leaves sampled in the Florida site were not found in the leaves sampled in the New York site (Firmicutes and unclassified), and members of the Gamma-subclass of the Proteobacteria were only found in one of the sampled leaves in this study

(New York Leaf 8). Bacterial OTU diversity differed significantly between the two sites, and all sampled pitchers in the Florida site had more diverse bacterial communities than the pitchers in the New York site. Bacterial community composition was more similar among plants within bogs; however, there was still great variation from plant to plant within bogs in the identity of bacterial OTUs as well as their abundance.

The variation in the bacterial community composition at both the local and region scale may partially be explained by the non-bacteria members of the *S. purpurea* community. Unopened pitcher plant leaves are sterile on the inside, with bacteria only present once a leaf opens into its characteristic pitcher shape (Peterson et al. 2008). Bacterial colonization of each new pitcher habitat may result from hitchhiking on insect prey, protozoans and rotifers that enter the aquatic community once the leaf has opened, and dipterans that use this community to deposit their eggs. This mechanism of transport has been found in other aquatic systems (e.g., Grossart et al. 2010), suggesting that it could also be a mechanism in the pitcher plant system.

If insects are an important vector of transport for the bacteria, variation in the abundance and type of insect prey collected in a pitcher would be expected to impact the diversity of bacterial input in each newly developing community. This affect would result in the continual colonization of new bacteria as the pitcher community relies on the continued addition of insects for essential resources. Ants are the most common prey that fall victim to the plant's pitfall traps (Newell and Nastase 1998; Gray, personal observation), with the highest capture rate occurring within the first month after a leaf has opened (Fish and Hall 1978, Wolfe 1981, Heard 1998). However, the passive nature of the trapping mechanism creates high variation in the abundance of ants caught in each leaf and also allows other insects to be captured (Newell and Nastase 1998; Gray, personal observation). Insect abundance varied in each leaf sampled in our study,

and although ants were the most common insect found in leaves, not every leaf had them as prey. Although non-significant, the leaf containing no insect prey (Leaf NY 1) had the lowest diversity and evenness of 16S rRNA phylotypes, while the leaf that trapped a spider instead of ants had the highest diversity and evenness (Leaf FL 214). These results are consistent with the hypothesis that insect prey are an important factor affecting bacterial diversity in the *S. purpurea* community, but more sampling should be done to confirm these results.

Protozoans, rotifers, and dipterans laying their eggs can also influence bacterial composition by either transporting bacteria when entering the leaf or through internal community dynamics once inside the leaf. In this system, protozoans and rotifers consume the bacteria, while the top predator, the mosquito larva *Wyeomyia smithii*, preys on the protozoans and rotifers. The presence of this top predator releases the bottom trophic level (bacteria) from predation pressure and by doing so, greatly influences the abundance (Kneitel and Miller 2002) and diversity (Peterson et al. 2008) of the bacteria. Midge larvae are also thought to affect bacterial dynamics by breaking down insect prey into smaller pieces, facilitating bacterial decomposition (Heard 1994).

Similar to other studies (Buckley et al. 2003), I found the same protozoans, rotifers, and dipteran larvae in the communities at the two sites that were sampled, even though the sites were in the southern and mid-parts of *S. purpurea*'s geographic range; however, the presence of these species varied from pitcher to pitcher both within a site and when the communities were compared across sites. These results suggest that species are entering the community at different abundances and densities, and possibly transporting different bacteria. The large variation in protozoan density and diversity in the community held by each leaf both within and between

sites could also affect bacterial community composition between each leaf as protozoans are the major consumers of bacteria.

Much of the variation in the abundances and densities of the non-bacterial members of the community can be explained by the time period in which the communities were sampled. By sampling two weeks after leaves had opened, I captured community composition early in community assembly (Chapter 3). It would be interesting to re-sample pitcher plant communities at the end of the season to compare the similarity in bacterial composition of mature communities both within (Chapter 3) and across sites.

The molecular methodology used could also help explain the variation found in bacterial community composition. Few studies have characterized the bacterial community of pitcher plants, and studies that have been done have varied in methodology. For example, Koopman et al. (2010) described the bacterial community composition in *Sarracenia alata* with high throughput sequencing. They found extremely high microbial richness in pitcher fluid, with more than 1000 phylogroups identified across at least seven phyla and over 50 families. The majority of OTUs they found were considered novel, undescribed species. Both the differences in the methodology used and fundamental differences between the two species of *Sarracenia* could explain the discrepancy between results. *S. purpurea* is unique among carnivorous plants because it does not rely on digestive enzymes to decompose insects and it has a well defined aquatic community of common protozoans and metazoans (Juniper et al. 1989), both of which could affect the abundance and diversity of bacteria found inside the leaves.

Peterson et al. (2008), used the *S. purpurea* system and described the bacterial diversity found in the biofilm coating the pitcher plant leaves with T-RFLP in different bogs in Massachusetts. They found a total of 133 unique gene fragments across sites. They found that

nearby pitchers shared more similar T-RFLP fingerprints than sites that were further away. Similarly, in this study the bacterial community was more similar for pitchers within a site than among sites.

Although I found more species of bacteria in the community when using bacteria OTU data, patterns of bacterial species evenness were the same for data that included only culturable bacteria or bacteria OTU data. This was surprising, and should be followed up with more replication among more sites. But, just focusing on culturable bacteria, which are relatively easy and inexpensive to study, can provide similar results as the more expensive metagenomics techniques, a result supported by data in Chapter 6.

Conclusions

For the *Sarracenia purpurea* system, the non-bacteria members of the community are the same throughout its geographic range (Buckley et al. 2003), yet the bacteria in these communities do not show this same pattern, at least at the beginning of the season. This suggests one of two possibilities for community development. A strong filter may exist for the non-bacteria trophic levels of this community, but not for the bacteria. Alternatively, the filter occurs later in community development for bacteria and interactions with the higher trophic level as well as resource input shape the bacterial community through time. The large difference in bacterial composition from pitcher to pitcher could also mean that certain bacteria species or functional groups may not be required by the plant for decomposition of the trapped insects, and co-evolution between the plant and bacteria may not have occurred as it has for other members of this community (i.e., mosquito and midge larvae). Future work is needed to explore the importance of this idea in more detail.

Table 1. Statistical analyses of 16S rRNA gene clone libraries using ecological estimates of phylotype diversity. Diversity was significantly higher for the Florida site when compared to the 16s genes for the NY site ($F = 5.11$, $p = 0.047$), however, there was no difference in the Pielou's evenness between the two sites (0.8301, $p = 0.384$).

Location	Leaf	Number of clones	Number of phylotypes	Shannon-Wiener (H')	Pielou's evenness	Percent coverage
New York	1	89	28	1.897	0.6187	76.4
	4	84	22	2.206	0.7989	84.5
	6	95	36	2.570	0.8340	68.4
	7	79	31	2.711	0.8634	75.9
	8	78	32	2.899	0.9158	71.8
	11	83	28	2.547	0.8548	77.1
Florida	200	67	29	2.855	0.9181	70.1
	214	68	41	3.422	0.9658	55.9
	216	74	30	3.134	0.960	79.7
	222	75	25	2.568	0.8843	78.7
	228	68	24	2.647	0.9087	76.5
	304	65	27	2.870	0.9308	73.8

Figure 1. Different measures of diversity for cultural bacteria and bacterial OTUs from pitcher plant communities in New York and Florida. **(A)** Pielou's Evenness (J')**, (B)** Shannon's Diversity (H') between bacterial OTUs and culturable bacteria in Florida and New York. Data for culturable bacteria were obtained from agar plate counts from the same samples used in OTU analysis. Graphed data are the means of samples from 6 plants from Florida (dark bar) and New York (light bar). Diversity (H') is significantly higher in Florida than in New York when OTU data are used (One-way ANOVA, $F = 5.11$, $p = 0.047$) but not when only culturable bacteria were considered (One-way ANOVA, $F = 0.830$, $p = 0.384$). Evenness was not significantly different between sites when only culturable bacteria were sampled ($F = 0.0789$, $p = 0.784$) or when 16s OTU data was used ($F = 0.8301$, $p = 0.384$). For both the New York and Florida site, when culturable bacteria were the only bacteria assessed, the bacterial communities were significantly less diverse and even community than if 16S rRNA gene clone data were used (NY site, Diversity: $F = 72.3$, $p < 0.0001$, Evenness: $F = 4.63$, $p = 0.0568$ (non-significant) and the Florida site, Diversity: $F = 80.15$, $p < 0.0001$, Evenness: $F = 11.59$, $p = 0.00672$).

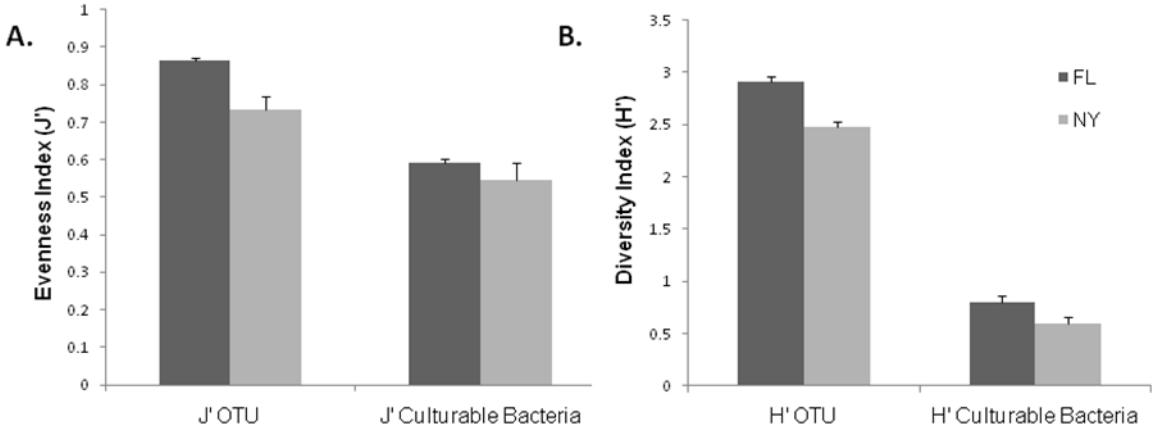


Figure 2. Non-Metric Multi-Dimensional Scaling Plot comparing culturable bacterial community similarity between Florida and New York based on (A) abundance (square-root transformed Bray-Curtis similarity) of individual morphotypes and (B) presence/absence data. Each symbol represents the bacterial community in one pitcher plant leaf. Open triangles - New York, closed triangles - Florida. Analysis of Similarity (ANOSIM) Global R is 0.306 for similarity based on abundances and 0.373 for similarity based on presence/absence data. Thus, the culturable bacteria community composition was not different within and between sites. However, communities were more similar if located within the same site.

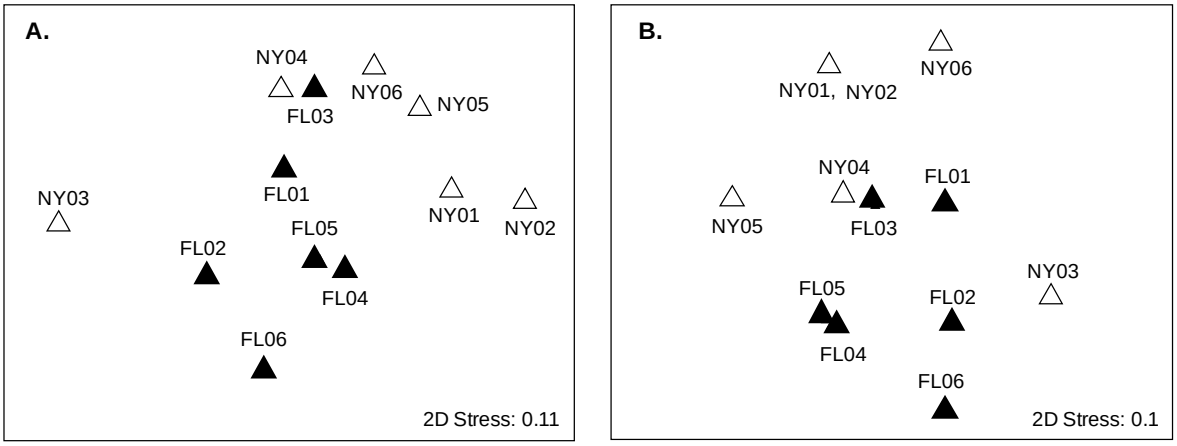


Figure 3. Rarefaction curves determined for the various phylotypes of 16S rRNA gene clones from water samples collected from *Sarracenia purpurea* leaves in Florida and New York. Phylotypes were defined by a 97% sequence similarity cut-off. Rarefaction analysis was performed using equations reported by Heck et al. (1975).

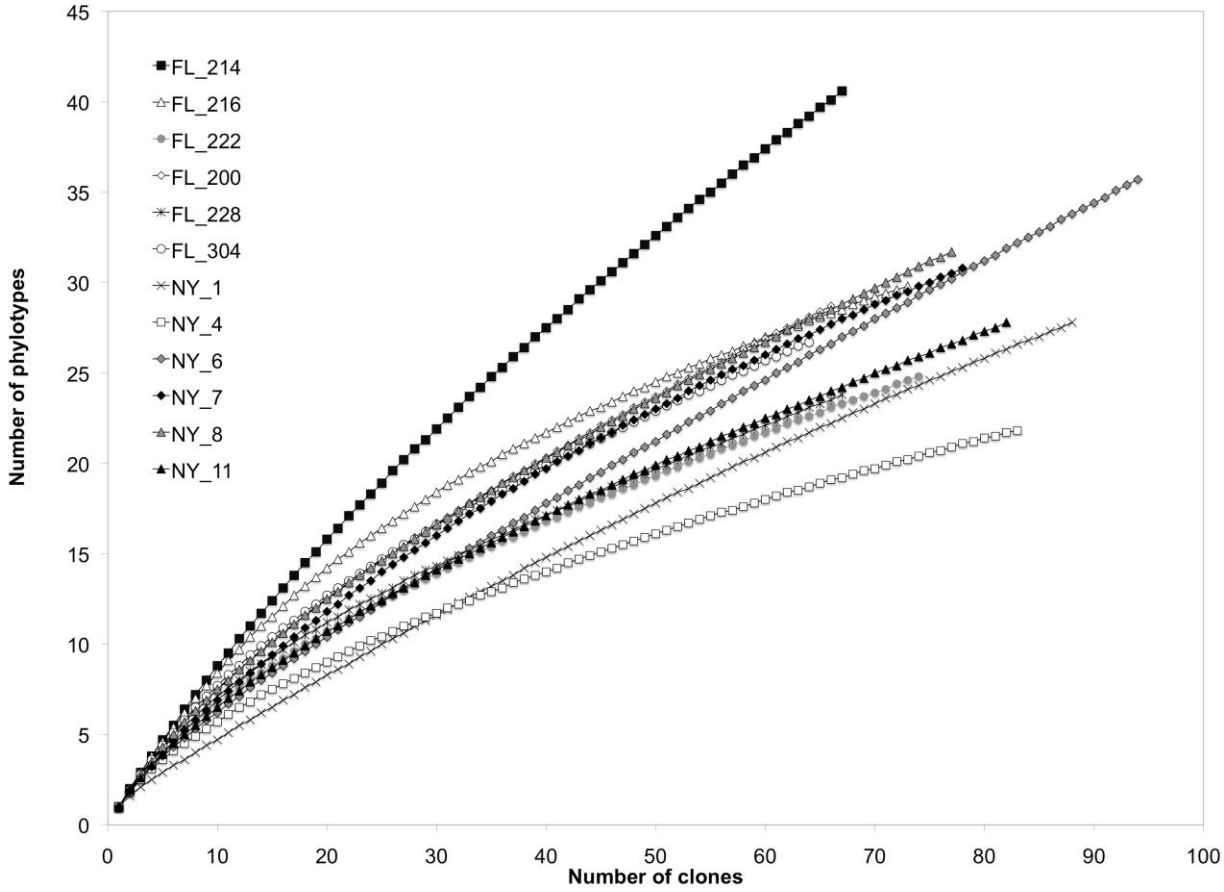


Figure 4. Frequencies of bacterial phylogenetic lineages detected in 16S rRNA gene clone libraries derived from water of *Sarracenia purpurea* leaves collected from Florida and New York. Calculations were made based on the total number of clones associated with a single phylotype.

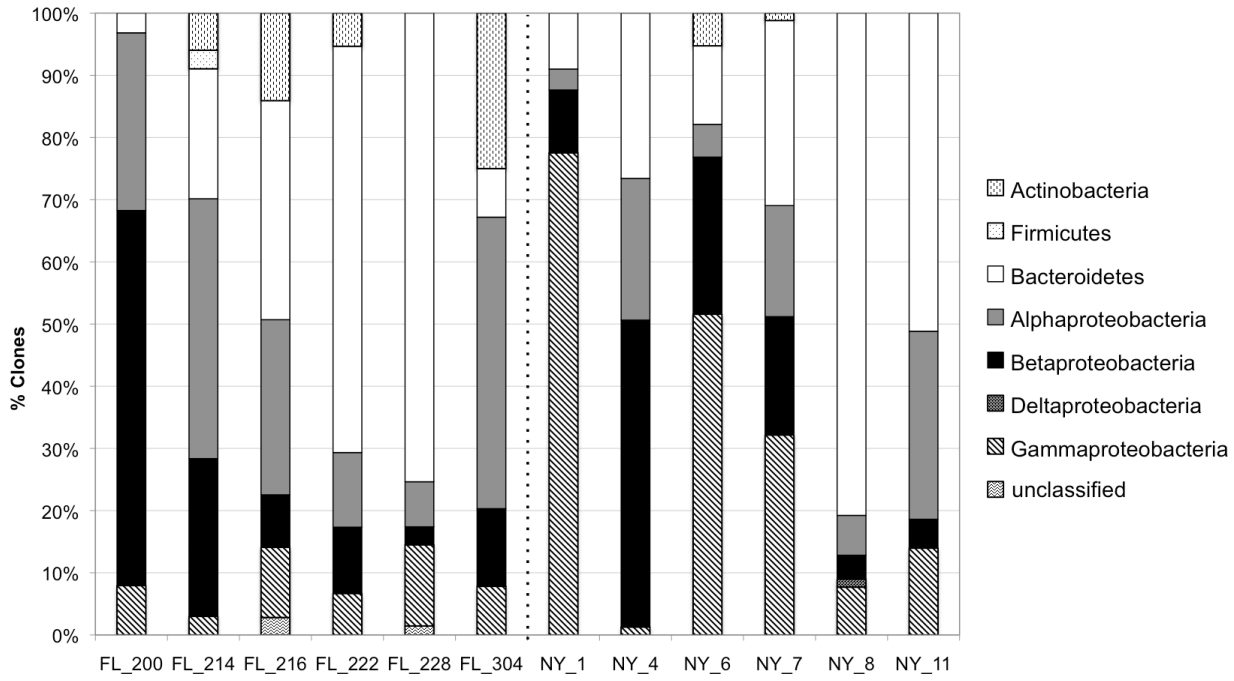
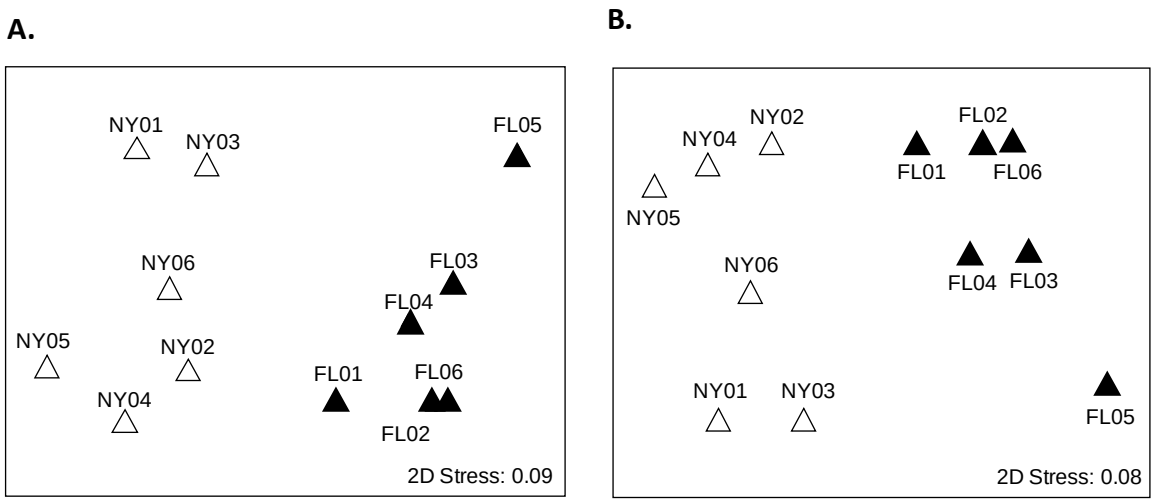


Figure 5. Non-Metric Multi-Dimensional Scaling Plot comparing bacterial OTU community similarity between Florida and New York based on **(A)** abundance (square-root transformed Bray-Curtis similarity) of individual OTUs and **(B)** presence/absence of bacterial OTUs. Each symbol represents the bacterial community held within one pitcher plant leaf. Open triangles - New York, closed triangles - Florida. Analysis of Similarity (ANOSIM) Global R is 0.922 for similarity based on abundances and 0.904 for similarity based on presence/absence data. Based on OTUs, the bacterial community composition was significantly different between sites. Bacterial communities in the Florida pitchers cluster separated from those from New York.



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Chapter 3

Succession in the *Sarracenia purpurea* community: deterministic or driven by contingency?

Abstract

The development of a community through time, or succession, is generally described as the orderly replacement of species until a predictable, stable endpoint is reached. However, stochastic factors, coupled with intrinsic biotic factors, such as herbivory or predation, can cause communities within the same habitat to become highly dissimilar in composition. Much research on the succession of terrestrial systems has been conducted, but factors influencing the succession of a terrestrial system may not apply to aquatic systems. To determine if succession in an aquatic system is driven by deterministic factors, and is predictable or dominated by contingency of stochastic factors, and the role that higher trophic level interactions and resources have in shaping successional patterns, I followed community development and dynamics in the model *Sarracenia purpurea* pitcher plant system throughout an entire growing season. By doing this, I was able to assess changes in community structure and composition through time. By comparing across pitcher plant leaves within the same bog, I was also able to determine if there is a predictable pattern for community assembly in this aquatic community. The results from this study suggest that rather than a sequential replacement of less competitive species (early colonizers) with more competitive species through time, competitively superior species establish in newly formed communities simultaneously with less competitive species, which coexist throughout the growing season. Community assembly in this system can also be altered by stochastic events. Resources and predators did not influence the patterns of community change observed during succession, and patterns of community assembly varied from year to year and depended on the sampling method used.

Introduction

Community change through time, or succession, has been of great ecological interest over the last century. To date, notions of the process of succession within a community have built on those set forth by Clements (1916) who suggested that the first species to arrive in a habitat will modify the environment, facilitating the success of later arriving competitively superior species. By making the environment more suitable for the establishment of competitively-superior species, these early succession species are ultimately replaced in the community (Connell and Slatyer 1977) until the community is dominated by species that can successfully persist (climax community; Clements 1916).

This viewpoint led to the notion that communities have a predictable endpoint, which would drive communities in similar ecosystems to converge in similarity (Clements 1916, Odum 1963). However, in the early 1900's, Gleason (1917, 1927) presented a contrasting viewpoint, suggesting that community assembly is not deterministic and predictable, but instead a stochastic process driven by contingency, allowing communities to diverge throughout succession (Gleason, 1917, 1927; Berlow 1997). Alternative models have also been put forth by Connell and Slatyer (1977), who suggested that early succession species might either have little or no effect on the establishment of new species in a habitat or that they might have a negative effect on both their own persistence and on the establishment success of other species into the community, reducing the predictability of species replacement through time within a community.

Traditionally, research on succession has focused on terrestrial systems, and, in particular, the community assembly dynamics of plants (Connell and Slatyer 1977). However, the factors important for succession in terrestrial systems might not be the same as those in aquatic systems. For example, Huchinson's 'Paradox of the Plankton' (1961) suggested that if species were being

competitively excluded through community development, one would not see such a high diversity of plankton as is observed in aquatic habitats with a limited range of resources. Although the main driver of succession in terrestrial systems is thought to be competition, other important interactions, such as herbivory or predation, are also known to play central roles in shaping community composition and dynamics and could be important during succession. Top predators, for example, are known to greatly affect diversity, abundances and the ability of species to coexist (e.g., Paine 1966), yet how these interactions affect community assembly has rarely been explored in aquatic systems. Chase et al. (2009) found that lakes containing top predator fish were similar in composition, while predator-free lakes greatly varied in composition, suggesting that the presence of predators caused communities to converge in similarity.

To determine if succession in an aquatic system is predictable or dominated by contingency, and the role of higher trophic level interactions and resources in shaping successional patterns, I followed the community structure and dynamics in the model *Sarracenia purpurea* pitcher plant system throughout an entire growing season. By doing this, I was able to determine changes in abundances and species richness through time. By comparing these dynamics across pitcher plant leaves within the same bog, I was also able to determine if there is a predictable pattern for community assembly in the *S. purpurea* aquatic community.

Each leaf of a *S. purpurea* plant represents a habitat island, in which colonization of the aquatic community begins with the opening of a new leaf as a new habitat. No species are present in the community before the leaf opens (Peterson et al. 2009) and each habitat island within a bog has access to the same regional species pool. The species that colonize each habitat island are predominately microorganisms with short generation times and insect larvae, making

this system ideal for following successional patterns through hundreds to thousands of generations and across multiple habitat islands. Larger scale systems contain longer lived organisms, necessitating long-term studies to determine if a community has a successional endpoint, or climax community. Thus, large-scale systems are significantly less tractable for successional studies than microbial communities.

Every month for six months in 2007, I assessed the abundance and richness of the *S. purpurea* community held within pitchers to assess: 1) if succession within this aquatic community is driven by deterministic or stochastic processes, 2) if resource availability and higher trophic level interactions are important factors in the community assembly patterns observed, and 3) the predictability of the observed community development patterns across multiple communities within this system. In 2008, I took advantage of an opportunity to resample the beginning and end time points of the *S. purpurea* aquatic community (June and November). For this sampling, I was able to incorporate metagenomics (16s rRNA cloning) to determine if the successional pattern found when considering a large sample of the bacteria (culturable and unculturable) was the same as when only culturable bacteria were studied, and how different sampling methods for the bacteria may affect the conclusions drawn about the successional trajectory of a system.

Methods

Study System

The pitcher plant *Sarracenia purpurea* is a carnivorous plant found in nutrient poor habitats throughout the eastern and mid-western portion of the United States, and in most of Canada. Each plant forms a rosette of leaves, each with an inquiline aquatic community. Leaf production

begins at the beginning of the season and continues until the onset of fall and winter (Fish and Hall 1978). Since it does not receive an adequate amount of essential nutrients (primarily nitrogen) from the soil, it has evolved pitcher shaped leaves that fill with rainwater and attract and drown insects. These insects die, and bacteria decompose their bodies, releasing the nutrients for the plant to use. The bacteria continuously experience predation from protozoans and rotifers, which form the intermediate trophic level in this community. The larval stage of the endemic mosquito species *Wyeomyia smithii*, which inhabits the top trophic level, consumes the protozoans and rotifers, alleviating predation pressure on the bottom trophic level. An endemic midge, *Metriocnemus knabi,* breaks insects into smaller pieces, facilitating decomposition and release of nutrients for the plant (Heard 1994). Flesh-fly larvae, *Blaesoxipha fletcheri,* are rare members of the pitcher plant community and are thought to consume first instar mosquito larvae and insects as they fall into the pitcher (Gotelli and Ellison 2006). The morphotypes of the culturable bacteria, protozoans, rotifers, and dipteran larvae in this community are thought to be the same throughout the entire geographic range of the plant (Buckley et al. 2003). When metagenomic techniques were used to identify the unculturable and culturable bacteria, the species were different among pitchers within a bog and between bogs found in New York and Florida (Chapter 2).

Field site and aquatic community sampling

The study area was a sphagnum moss bog in the Pine Barrens region near Riverhead, New York, USA (Cranberry Bog Preserve, 40°N, 72°W). The common vegetation found in or surrounding the bog includes pitch pine (*Pinus rigida*), *Sphagnum* sp. moss, sundews (*Drosera* sp.), and the invasive giant reed *Phragmites australis*. This bog includes several hundred

northern pitcher plants, *Sarracenia purpurea*.

I sampled the aquatic community held within the leaves of *Sarracenia purpurea* at this site in two different years. In 2007, I followed the community held within 20 marked leaves at monthly intervals from June (beginning of the growing season) until either the leaf died or to November (end of the growing season, before the first freeze). For each sampling date, the water volume and clarity, accumulated insect input, abundance and richness of culturable bacteria and protozoan species, rotifer (*Habrotrocha rosa*) abundance, the abundance and instar level of mosquito larvae (*Wyeomyia smithii*), and the abundance of the midge (*Metriocnemus knabi*) and flesh fly larvae (*Sarraceniopus gibsoni*) were assessed. In 2008, I sampled the community held within 6 leaves in June and 5 additional leaves in November. For both sampling dates in 2008, I recorded the same data as in 2007, as well as the diversity and abundance of both the culturable and unculturable bacteria. The diversity and relative abundance of the bacterial community including unculturable species was determined with 16s rRNA clone library techniques.

In both 2007 and 2008, I randomly marked unopened leaves (one per pitcher plant) throughout the bog. Two weeks later I designated those leaves that had opened into their characteristic pitcher shape as the leaves used for sampling. In 2008, because 16s rRNA cloning requires using the contents of the entire community without replacement, the communities that were sampled in June were not the same communities sampled in November. However, the communities held within leaves sampled in November were chosen from the same set of leaves marked as unopened at the beginning of the season. Due to herbivory and weather damage, only 5 of the unopened, marked leaves from the beginning of the season were healthy enough for sampling in November. Figure 1 illustrates the geographic distribution of the sampled plants within the field site. Different plants were sampled in 2007 and 2008.

The water collected from pitchers in 2007 was first gently mixed with a sterile pipette, and then transferred into a sterile 50 ml macrocentrifuge tube. The water volume and clarity, the number and instar level of the mosquito larvae, the number of newly captured ants and other insects, and the abundances of midges and flesh flies were recorded. I transferred a 0.2 ml aliquot of the aquatic community to a sterile microcentrifuge tube, which was returned to the laboratory for further analysis. The remainder of the water and community was returned to the pitcher plant leaf and left until the next sampling date. From the water returned to the lab, a 0.1 ml aliquot was used to determine the densities and richness of protozoan species and rotifers with a compound microscope according to standard methods (Kneitel and Miller 2002, Gray et al. 2006). A second aliquot of 0.5 ml was used to plate culturable bacteria for each sample in serial dilutions. These plates were incubated at 27°C for 2 days and then the abundances and richness of the colony forming units (CFUs) were determined from the number of different CFU morphologies that grew on the agar plate (e.g., Kneitel and Miller 2002, Gray et al. 2006).

In 2008, the water in each selected leaf was gently mixed with a sterile pipette and collected into individually labeled, sterile 50 ml centrifuge tubes. The entire content of each sample was then transferred on ice back to the laboratory for further analysis of the aquatic community (using the same methods as in 2007). In addition, I constructed 16s rRNA clone libraries to assess the entire community of bacteria.

Environmental clone library construction

Directly after processing samples at the beginning and end of the season in 2008, a 1 ml aliquot of water from each sample was filtered onto a 0.22 μM Isopore membrane filter (Millipore, Billerica, Massachusetts) to collect all bacteria (both culturable and unculturable

bacterial cells). After filtration, I extracted the DNA of the microbial community found on each filter with the Ultra Clean Soil DNA kit according to manufacturer's instructions (Mo Bio Laboratories, Solana Beach, California), with the exception of Step 1 of the protocol, where instead of using a soil sample, I placed the filter containing the bacteria in the bead solution tubes with 200µl of sterile water. Extracted DNA was stored at -20°C until used for PCR amplification.

During PCR amplification, I used the *Bacteria* domain-specific SSU rRNA gene primers 27F (5'-AGA GTT TGA TCM TGG CTC AG -3') (Johnson 1994) and 1392R (5'-ACG GGC GGT GTG TAC-3') (Wilson et al.1990). The PCR conditions were 95°C for 5 minutes, 94°C for 1 minute, 52°C for 30 seconds, 72°C for 3 minutes, steps 2 – 4 repeated 30 times, and a final step of 72°C for 10 minutes. The PCR products were purified with the Qiagen PCR Purification Kit (QIAGEN, Valencia, CA) and the purified product was ligated into the TOPO TA cloning vector pCR 2.1 according to manufacturer's instructions (Invitrogen, Carlsbad, CA). Ligation reactions were sent to The Genome Center at Washington University (St. Louis, Missouri) where they were transformed and a total of 96 clones were sequenced for each library. Clones were sequenced using the primer 907R (5'-CCG TCA ATT CMT TTG AGT TT 3').

Using Sequencher v4.8 (Gene Codes Corp., Ann Arbor, MI), vector sequences flanking the SSU rRNA gene inserts were removed, sequences were trimmed and sequences below 90% quality threshold and less than 300 bp were deleted. The remaining sequences were then assembled and sent to Greengenes (DeSantis et al. 2006, http://greengenes.lbl.gov) to be aligned. Clone libraries were then developed based on of these aligned sequences. Rarefaction curves were calculated with Analytic Rarefaction 1.3 (Heck et al. 1975, Holland 2003).

Community Composition and Diversity Indices

To calculate Pielou's evenness and Shannon Diversity indices for the communities sampled in 2007 and 2008, I used the multivariate statistical program Primer 6.1 (PRIMER 6, Version 6.1.6, Primer E-Ltd. 2006). Primer 6.1 was also used for statistical analyses to test for similarity in the culturable bacterial community and the assemblage of protozoans among sampling periods in 2007, and for the 16s rRNA bacterial data obtained from the June and November samples of 2008. Community similarity was tested using just presence/absence data, as well as abundances of individual OTUs and culturable bacteria morphotypes within samples. For tests of community similarity based on abundance data, data were normalized with a square root transformation. Bray-Curtis distances were calculated, which use values from 0 (most similar) to 1 (least similar) to determine similarity between samples (Bray and Curtis 1957). To graphically visualize the differences between bacterial communities, a non-metric Multi-Dimensional Scaling (MDS) plot was used. Communities that are more similar are spatially close on a MDS plot; those that are less similar are more spatially separated. Analysis of Similarity (ANOSIM) was used to calculate a Global R, which determines the overall similarity between communities. R will equal 1 when communities within a site are more similar to each other than to replicates across sites, and R will equal 0 when the null hypothesis is true (there is no difference within and among sites) (Clark and Warwick 2001). If the null hypothesis is true, and the Global R value is close to zero, the p value will be non-significant (Clark and Warwick 2001). However, a significant p value can occur even when the null hypothesis is true, and this occurs when there is a large sample size (many replicates) within a site (Clark and Warwick 2001), allowing for a difference among groups to be detected, even when groups have high overlap (low R value).

Results

Monthly comparison of culturable bacteria communities - 2007

When both abundance and presence/absence of species data were used, the similarity among communities of culturable bacterial morphotypes depended on the month sampled. At the beginning of the season (June), both the abundances of individual species and type (morphotype identity) of culturable bacteria were highly variable among the 20 pitcher plant communities sampled (Figure 2). By July, the community of culturable bacteria converged among pitchers and was highly similar in terms of morphotype identity (presence/absence data) and when the abundances of types were included. In August, this pattern shifted, with culturable bacteria diverging again to be different in identity and abundances of morphotypes in the sampled communities (Figure 2). The culturable bacteria communities converged. Community composition was very similar based on presence/absence data as well as abundances of species in September, but then diverged again in October and November (end of the season)(Figure 2).

The observed pattern was not driven by the amount of resources (bottom up effect) in the communities. In each month sampled, those communities that contained newly captured ants were not significantly different in species identity than communities that did not have new ant input (June Abundances: ANOSIM = 0.078, p value = 0.133; June Presence/Absence: ANOSIM = 0.115, p value = 0.09; July Abundances ANOSIM = 0.001, p value = 0.423; July Presence/Absence ANOSIM = 0.04, p value = 0.707; August, no ant input into any community, comparison could not be made; September Abundance ANOSIM = 0.119, p value = 0.283; September Presence/Absence ANOSIM = 0.008, p value = 0.383; October and November, no ant input into the community, comparisons could not be made). Although not significant in the ANOSIM results, communities (abundances of individual culturable bacteria types and their

identity) that did not receive ant input in June appear to be closer to each other than to communities receiving ant input in June in the MDS plots (Figure 2a,b; June).

Similarity in Divergence and Convergence Patterns across Months for Culturable Bacteria

In July and September, the abundances of individual species and the type (morphotype identity) of culturable bacteria were very similar among communities within each month. To test whether the bacterial communities sampled in July converged similarly in September, I used analysis of similarity (ANOSIM). I found that the bacterial community composition present in July was very similar to the bacterial composition present in September (Figure 3a,b; ANOSIM for Abundance = 0.32, p value = 0.001; ANOSIM for Presence/Absence = 0.26, p value = 0.001). This result suggests that communities of culturable bacteria converged to the same subset of species with similar abundances, independent of month sampled.

In the months when the culturable bacteria communities diverged in each pitcher (June, August, October, November), each pitcher contained an unpredictable community composition (Figure 4a,b; ANOSIM for Abundance = 0.503, p value = 0.001; ANOSIM for Presence/Absence = 0.374, p value = 0.001). In the months where communities diverged, the abundances of individual species and species identity of culturable bacteria were most similar in August and November (Figure 4a, ANOSIM for Abundance = 0.038, p value = 0.22; Figure 4b, ANOSIM for Presence/Absence = 0.09, p value = 0.039). The abundances of individual species in the communities in June were significantly different than in the other months (Figure 4b, June/August ANOSIM = 0.583, p value = 0.001; June/October ANOSIM = 0.744, p value = 0.001; June/November ANOSIM = 0.68, p value = 0.001). These patterns suggest that it was difficult to predict which species will be present in a given community or their relative

abundances in the months when the bacterial communities diverged in species composition. However, the species present and their relative abundance at times when communities converge was highly predictable.

Monthly comparison of protozoan communities

The variation in the community composition for protozoans based on abundances of individual species and presence/absence of species among communities also depended on the month, but the convergence and divergence patterns were different than those found for culturable bacteria. Community similarity patterns were independent of whether species abundance or presence/absence data were used (Figure 5a,b). At the beginning of the season (June), community structure was highly variable among individual pitchers. A similar pattern was found for the culturable bacteria (Figure 2). However, as the season progressed, the protozoan assemblage converged among communities (Figure 5a,b). This pattern was seen until November, when the protozoan assemblages in these communities diverged in similarity (Figure 5a,b). The presence of a mosquito larva in the communities (top down predatory control) does not explain the similarities or differences found across communities. Communities containing mosquitoes were never significantly different in composition than those that did not contain mosquitoes, for any month that was sampled (Presence/Absence data; June ANOSIM = 0.041, p value = 0.312; July ANOSIM = 0.085, p value = 0.139; August ANOSIM = 0.01, p value = 0.368; September ANOSIM = 0.026, p value = 0.342; October ANOSIM = 0.142, p value = 0.073; November ANOSIM = 0.137, p value = 0.87).

Similarity in Divergence and Convergence Patterns across Months for Protozoans

Again, similarities and differences among communities were not affected by whether species abundance or presence/absence data were used. There was little variation in community composition for protozoans between the months where the communities converged (Figure 6, Abundance ANOSIM Global R = 0.034, p value = 0.068; Presence/Absence ANOSIM Global R = 0.046, p value = 0.036). In the months where the protozoan communities were divergent from pitcher to pitcher (June and November), communities in June were more divergent than those same communities in November (Figure 7, Abundance ANOSIM Global R = 0.175, p value = 0.002; Presence/Absence ANOSIM Global R = 0.161, p value = 0.002). However, the low ANOSIM values for both presence/absence and abundance data suggest that communities in June and November diverge in a similar manner (Figure 7).

Species Richness and Abundance Dynamics throughout Succession

Figures 8 and 9 show the patterns of community assembly for culturable bacteria and protozoans, respectively. For culturable bacteria, every morphotype except the two least competitive morphotypes (pink and purple morphotype, Chapter 6), were found in these communities throughout the growing season, although their abundances changed (Figure 8). Overall, the density of culturable bacteria decreased throughout the growing season, but species richness remained relatively constant (Table 1).

The protozoans showed similar dynamics as the bacteria. The least competitive species (Chapter 7) was present in communities throughout the entire growing season, and all common species were found in almost every month of the growing season, independent of their competitive ability (Figure 9). However, by the end of the season, the pitcher plant communities had accumulated a high diversity of ciliate protozoans, which are larger in size and are known to

be competitively dominant to flagellates (Kneitel 2002). Yet, the least competitive flagellate, *Bodo* sp., was also found in high abundance at the end of the season (Figure 9). Protozoan densities and species richness had dynamics that were opposite to those found for bacteria. While culturable bacteria densities decreased and species richness remained constant through time, both total protozoan densities and richness increased as succession progressed (Table 1).

Community Similarities - Abundance and Presence/Absence dynamics of 16s rRNA bacterial clones and their comparison to culturable bacteria successional patterns - 2008

Similarities in bacterial community composition at the beginning and end of the season in 2008 are illustrated with the multidimensional scaling (MDS) plots comparing the abundances and presence/ absence of individual bacterial OTUs. The MDS plots revealed that there is a large seasonal difference in the bacterial community when using abundances of individual OTU data (Figure 10a; ANOSIM = 1, p value = 0.002). Bacterial communities were similar at the beginning of the season and at the end of the season, but community structure was very different across time periods (Figure 10a; ANOSIM = 1, p value = 0.002). The types of bacterial species found in the communities (presence/absence data) also depended on the sampling time period. Communities sampled at the beginning of the season were different to one another, but converged by the end of the season such that each community contained a very similar subset of species from the total species pool (Figure 10b). There was also a shift in the identity of the most common species across the season. Species composition at the beginning of the season was significantly different than the species composition in the communities sampled at the end of the season (Figure 10b; ANOSIM = 0.992, p value = 0.002). The community composition of culturable bacteria from the same samples collected for 16s rRNA libraries were also analyzed

for community structure through time. This allowed me to compare the similarity in the successional pattern between the community of unculturable and culturable bacteria to that of just the culturable bacteria, which are only 1% of the bacterial diversity. I found similar community patterns for both methods. For the culturable bacteria, communities were more similar in June than in November (Figure 10a,c; ANOSIM = 0.246, p value = 0.02). However, for the bacteria assessed with clone libraries, communities were less similar than found with the culturable bacteria. When presence/absence data were used communities assessed with clone libraries had greater similarity than those with only culturable bacteria (Figure 10b,d, ANOSIM = 0.133, p value = 0.762). When clone libraries were used, the bacterial communities in June were highly different than those in November when presence/absence data were used (ANOSIM = 0.992, p value = 0.002). The pattern was different for the culturable bacteria. In June and November communities were more similar when presence/absence data were used (ANOSIM = 0.133, p value = 0.762). The difference in pattern found between bacteria OTU data and culturable bacteria could be due to the fact that a greater number of species were found in the clone libraries than on agar plates (how culturable bacteria were assessed).

Diversity and Evenness Patterns of 16s rRNA bacterial clones

Even though communities were different across time periods when considering abundances of individual species or presence/absence data, there was no significant difference in the overall diversity or evenness among communities sampled in the beginning and end of the season (one-way ANOVA, Diversity p = 0.410, F = 0.746; Evenness p = 0.075, F = 4.06).

Overall community succession patterns

Table 2 summarizes the successional patterns for the protozoans and culturable bacteria sampled in 2007 and for the 16s rRNA bacteria sampled in 2008.

Discussion

By following multiple aquatic *S. purpurea* communities within the same habitat throughout a growing season, I found that, contrary the notion of succession as a predictable replacement of species through time proposed by Clements (1916), all pitcher communities maintained the same subset of species throughout succession. These data cannot be used to determine if some species modify the environment and facilitate or inhibit the establishment of other species. However, competitively superior species were found to establish in newly forming communities and less competitive species were able to coexist with competitive dominants throughout the growing season. For both protozoans and culturable bacteria morphotypes, species across the full range of competitive ability were able to coexist in the community during succession, with the exception of the least competitive bacteria morphotype (pink). This finding suggests that there is no requirement for early successional species to first modify the environment for the competitively dominant species to successfully establish. In addition, the competitively superior species did not drive the less competitive species to extinction during succession.

Although a very predictable subset of protozoan species and culturable bacteria morphotypes from the regional pool was found to establish in the pitcher plant community, for the culturable bacteria, communities sampled across the bog varied in an unpredictable manner through time. For months when communities differed in composition (June, August, October, November), six of the eight bacteria morphotypes were found in at least one community each month, however, not all communities contained all six culturable bacteria morphotypes, and densities of individual

types varied considerably among communities. The protozoans, on the other hand, showed very predictable patterns of both community convergence and divergence through time.

Interestingly, top down predator-control or bottom up resource-control do not seem to drive the patterns of succession observed in the *S. purpurea* communities, contrary to predictions by Chase and Leibold (2003) and Chase et al. (2009). In the *S. purpurea* system, ant input dramatically decreased to zero in August and remained either at zero or very low throughout the rest of the growing season. If bacteria community dynamics were to follow those predicted by Chase and Leibold (2003), the lack of ant input should have created a low resource habitat, making it more likely for communities to diverge in similarity. Instead, the divergent and convergent patterns of the culturable bacteria communities occurred independent of the amount of resources available (communities converged in September even though only 2 communities out of 16 received ant input and the ant input that was received was extremely low). The presence of the top predator, which was predicted to drive similarity in communities (Chase et al. 2009), did not drive convergence in the protozoan communities in the *S. purpurea* system. More research needs to be done to understand what mechanisms drive the patterns of community succession in both the *S. purpurea* system and or the aquatic systems before generalizations about predictable patterns in aquatic community assembly can be made.

When following succession in the *S. purpurea* community, for both the bottom trophic level and the intermediate trophic level, communities among pitchers were highly variable at the beginning of the season, in June, and at the end of the season, in November. This result suggests that intrinsic and extrinsic factors may be very important for the initial community development (e.g., propagule pressure, disturbance, size of regional pool, Chase 2003) and for communities to destabilize at the end of the season (e.g., temperature, disturbance). However, the major drivers

of community succession in middle of the growing season are still unknown, but do not appear to be due to predation and resource availability alone. Disturbance, which is also known to be a very influential factor affecting community structure (Sousa 1984, Connell 1978 'Intermediate Disturbance Hypothesis'), may be playing a major role in shaping whether community assembly in this system leads to single or multiple stable equilibria. More experiments testing the relative importance of disturbance (e.g., desiccation or flooding of the pitchers, temperature), coupled with predation and resource availability, need to be conducted to fully understand their roles in shaping the community patterns observed. Interestingly, protozoan abundances and species richness increased as the season progressed. This increase in protozoan species richness through time has been found in other studies (e.g., McCormick et al. 1988), and may suggest that not all niches in this trophic level are filled during succession in the *S. purpurea* system, or that bacteria are abundant enough that competition is not a driver of succession for protozoans.

When OTU data were used in 2008, communities at the beginning of the season were highly dissimilar as compared to communities at the end of the season, both when presence/absence and abundance data were used. A similar, but weaker pattern was found for community similarities based on abundance data for the culturable bacteria collected from the same samples, but this could be an effect of sample size (Chapter 5). Unlike 2007 when bacterial communities diverged at the end of the season, in 2008, bacterial communities converged at the end of the season. Year-to-year variation in temperature and in the regional species pool as well as other intrinsic and extrinsic factors may influence the successional trajectory, making seasonal dynamics unpredictable. This high year-to-year variation in community dynamics during succession of new communities has been found for other microorganism systems (e.g., phytoplankton,

Soininen et al. 2005). Longer term data sets for the *S. purpurea* system are needed to understand the variability community dynamics among years.

Table 1. The averaged log densities and the average species richness of culturable bacteria and protozoans in the 20 communities sampled through one growing season. Average log culturable bacteria densities (per 0.1 ml) were high in June and decrease throughout the growing season. Variation between samples (standard error) also decreased throughout the growing season. Protozoans showed the opposite pattern as the culturable bacteria. Protozoan average log densities (per 0.1 ml) increased through time. Culturable bacteria species richness remained relatively constant throughout the growing season; however, protozoan species richness increased through time.

Month	Avg. Log Density of culturable bacteria		Avg. culturable bacteria richness		Avg. Log Density of protozoans		Avg. protozoan richness	
		SE +/-		SE +/-		SE +/-		SE +/-
June	10.86	0.282	3	0.072	1.19	0.132	0.667	0.065
July	6.40	0.263	2.26	0.089	1.91	0.091	1.74	0.093
August	4.49	0.121	3.55	0.061	1.56	0.126	1.61	0.089
Sept.	4.21	0.111	2.69	0.067	1.56	0.121	1.67	0.112
October	4.54	0.126	3.26	0.073	2.66	0.132	2.27	0.102
Nov.	4.23	0.126	3.64	0.091	2.49	0.124	2.21	0.0894

Table 2. Overall summary of successional patterns observed for the culturable bacteria and protozoans in 2007 and for the 16s rRNA bacteria OTUs in 2008.

	Protozoans - 2007	Culturable Bacteria - 2007	16s rRNA Bacteria - 2008
Species Replacement Through Time	No	No	Yes
Community Convergence Through Time	Yes, until November	Oscillation pattern throughout season	Yes
Community Convergence/Divergence due to Top Predator	No	Not tested	Not tested
Community Convergence/Divergence due to Resource Input	Not tested	No, but non-significant community separation in June between ant and no ant communities	Not tested
Similar convergence patterns in months when communities converged	Yes	Yes	N/A
Similar divergence patterns in months when communities diverged	Yes, but communities in June are more variable	No	N/A

Figure 1: Pitcher plants were sampled within Cranberry Bog near Riverhead NY. The relative spatial distribution of sampled pitcher plants is shown. Open circles symbolize the 20 plants sampled in 2007 for community composition (protozoan, rotifer, culturable bacteria, and dipteran larvae richness and abundance), black circles (NY 1 through 6) represent pitcher plants sampled at the beginning of season in 2008 and gray circles (NY A through E) indicate the plants sampled at the end of the 2008 season. In 2008, in addition to quantifying the culturable bacteria, 16s rRNA bacterial clone libraries were used to examine the entire bacterial community.

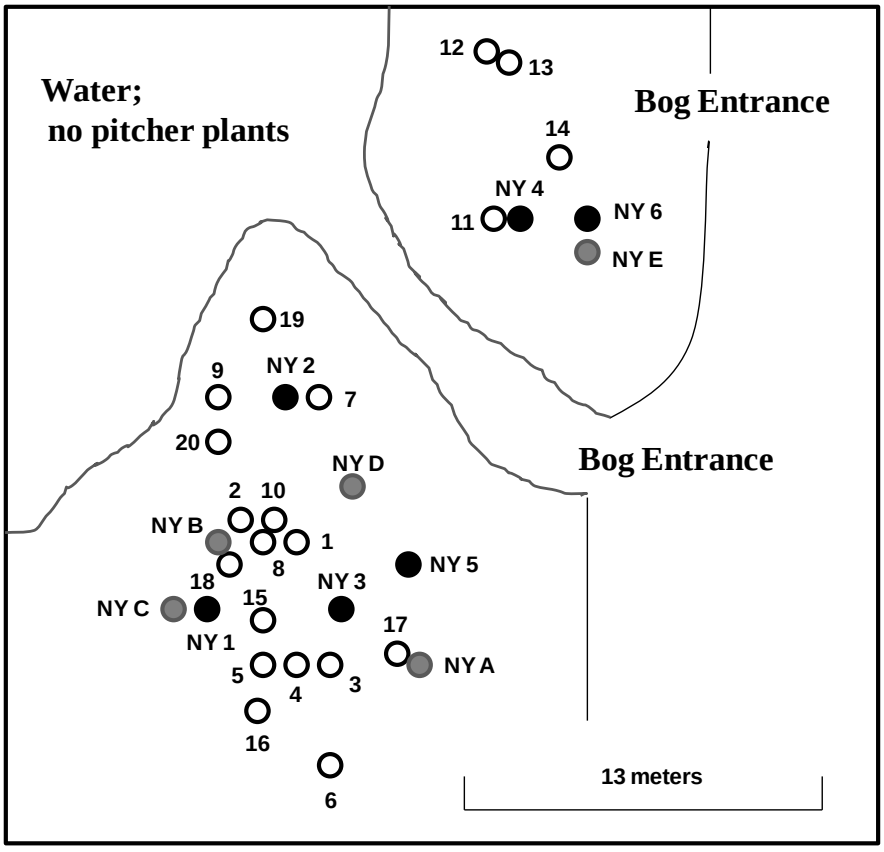
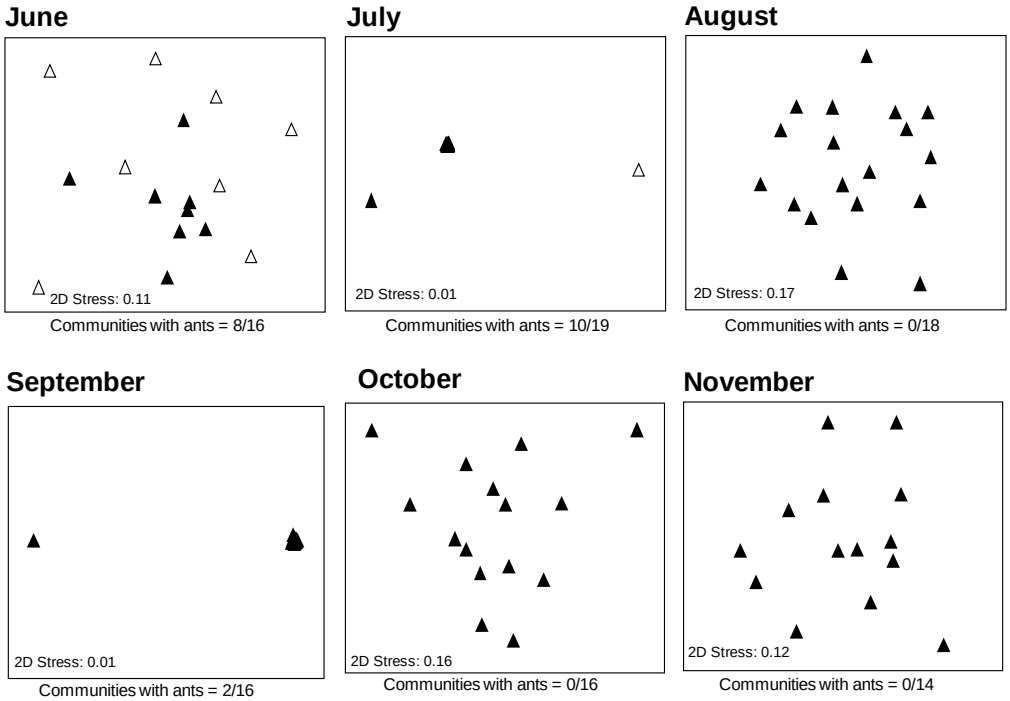


Figure 2. Multi-Dimensional Scaling (MDS) Plots illustrate community similarity for culturable bacteria morphotypes found within 20 pitcher plant leaves (1 leaf per plant) sampled monthly throughout one growing season. For **A)** abundances of individual morphotypes data were used and for **B)** presence/absence data were used. 2D stress indicates how well multi-dimensional groupings are represented in a two dimensional graph. In general, a stress less than 0.2 is considered an adequate representation. Open triangles indicate communities with ants found in the water (indicating high resource availability for bacteria), and closed triangles indicate those communities where ants were not found at the time of sampling. Ant capture rate dramatically decreased to zero by August. Communities were not significantly different within each month sampled; however, there was large variation in community structure in June, August, October and November. Culturable bacteria communities were highly similar within the July and September sampling dates.

A) Abundance Data



B) Presence/Absence Data

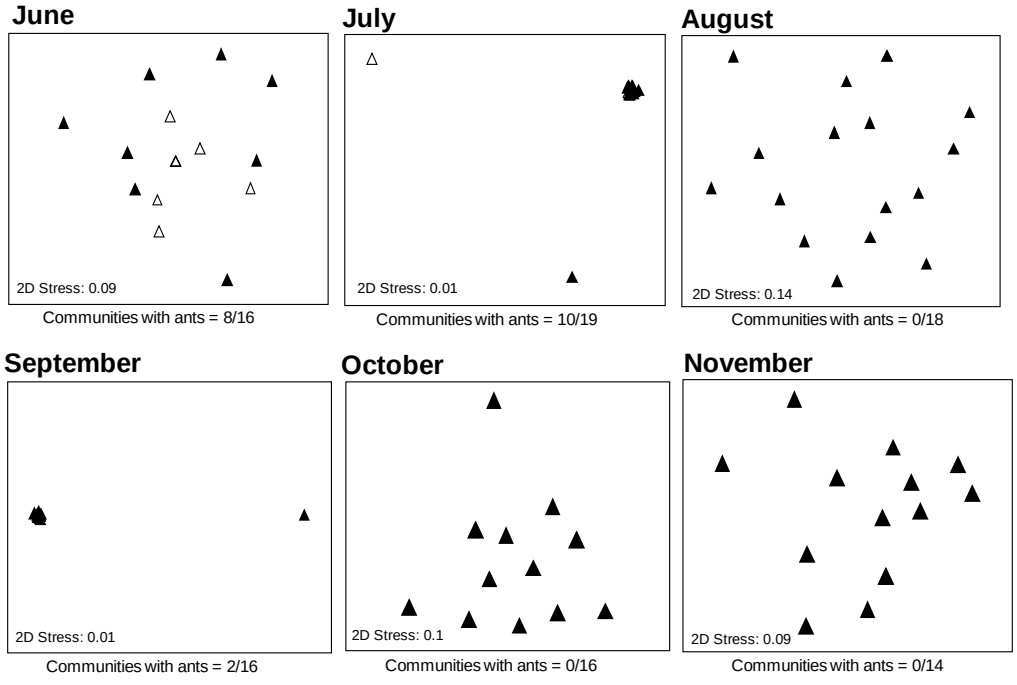


Figure 3. Multi-Dimensional Scaling (MDS) plots for the two months (July and September) when culturable bacterial communities converged, based both on the abundances of individuals of each species as well as presence/absence data. The black triangles represent communities in July and gray triangles represent communities in September. The communities of culturable bacteria within each pitcher converged at both sampling periods (July and September). **A)** Community similarity based on abundances of individual culturable bacteria morphotypes among the 19 leaves in July and 16 leaves in September (ANOSIM = 0.32, p value = 0.001). **B)** Community similarity based on presence/absence data for culturable bacteria among the 19 leaves in July and 16 leaves in September (ANOSIM = 0.26, p value = 0.001). 2D stress indicates how well multi-dimensional groupings are represented in a two dimensional graph. In general, a stress less than 0.2 is considered an adequate representation.

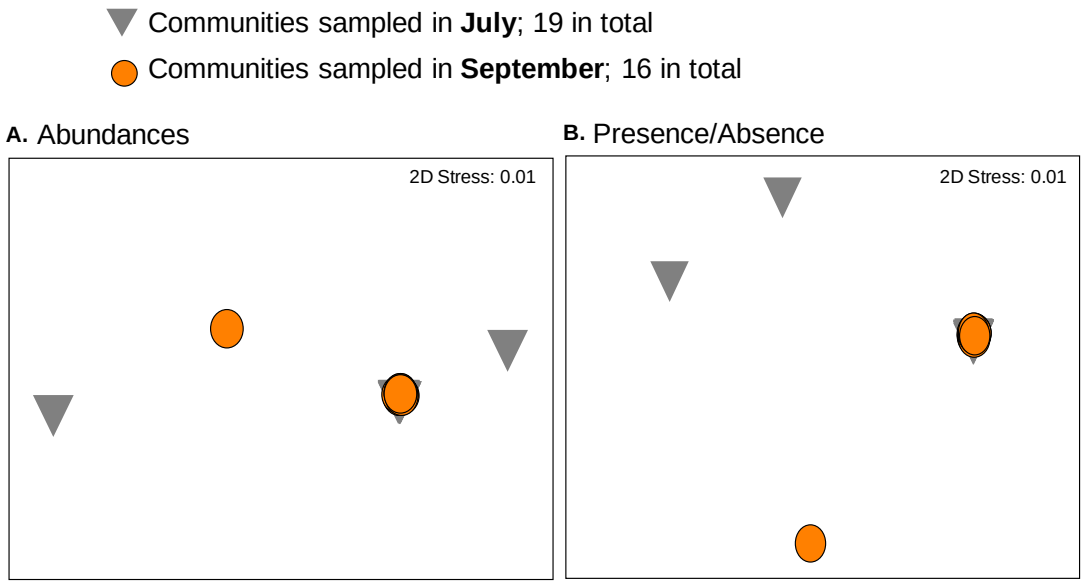


Figure 4. Multi-Dimensional Scaling (MDS) Plots illustrating community similarity among months (June, August, October, November). For these months, the culturable bacteria communities were highly divergent between leaves. For **A)** abundance of individuals of bacteria morphotypes data were used and for **B)** presence/absence data were used. Analysis of similarity among communities based on individual species abundance data- ANOSIM = 0.503, p value = 0.001. Analysis of similarity among communities using presence/absence of species data - ANOSIM = 0.374, p value = 0.001**.** 2D stress indicates how well multi-dimensional groupings are represented in a two dimensional graph. In general, a stress less than 0.2 is considered an adequate representation. June (black triangle), August (green square), October (purple cross), November (blue diamond).

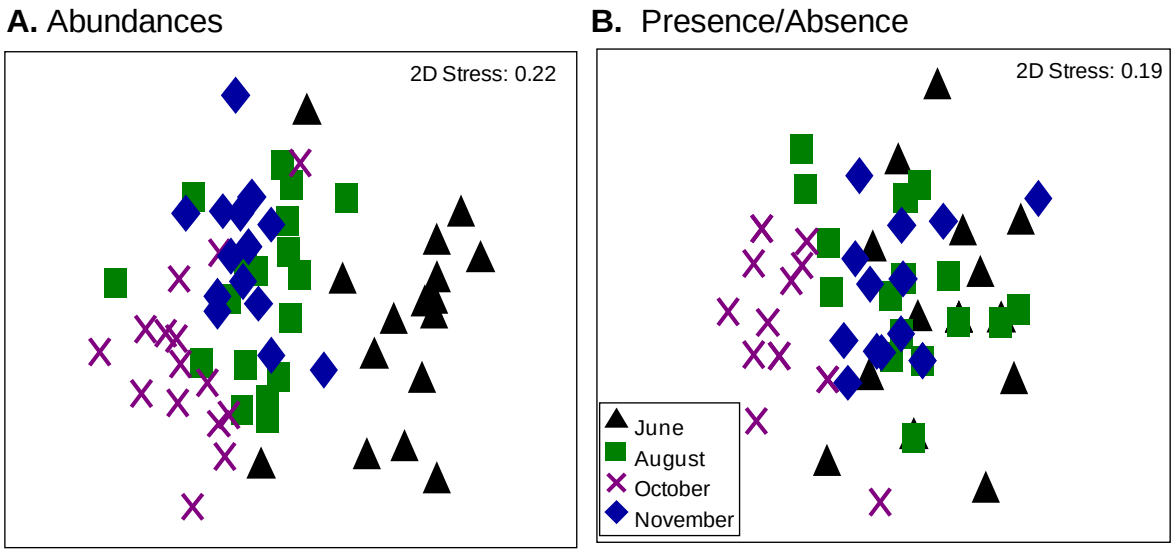
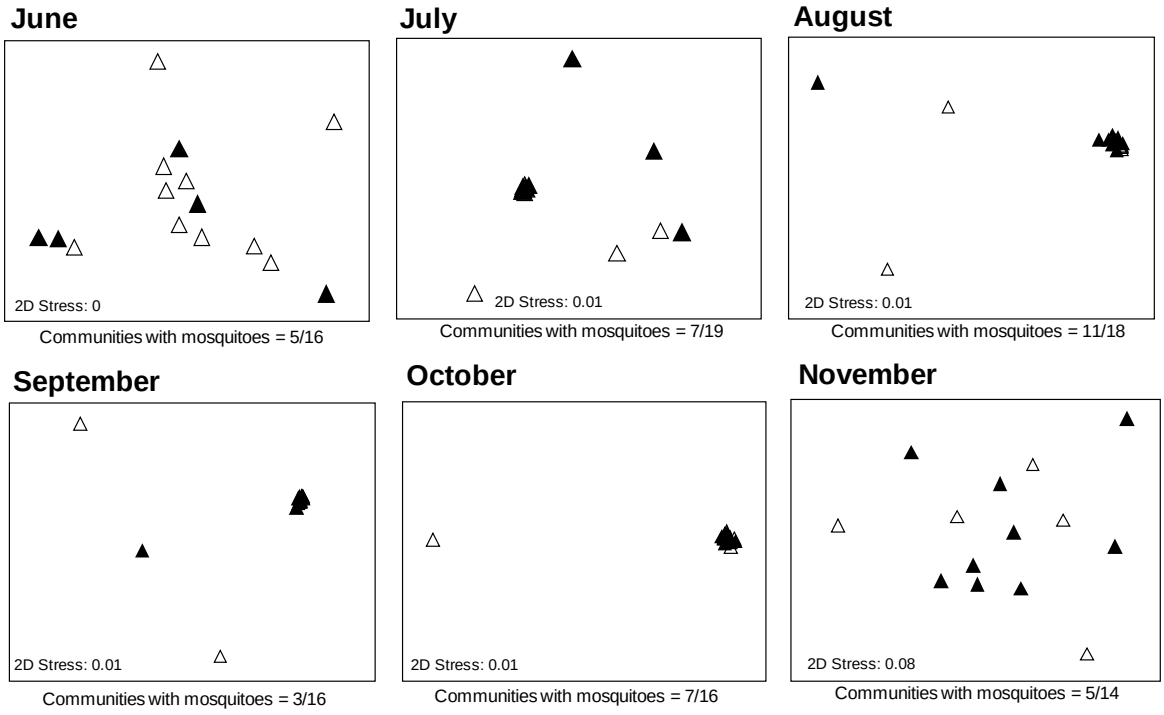


Figure 5. Multi-Dimensional Scaling (MDS) Plots illustrate community similarity for protozoan species found within 20 pitcher plant leaves (1 leaf per plant) sampled monthly throughout one growing season. For **A)** abundances of individuals of protozoan species data were used and for **B)** presence/absence data were used. 2D stress indicates how well multi-dimensional groupings are represented in a two dimensional graph. In general, a stress less than 0.2 is considered an adequate representation. Each MDS plot represents a different month. Open triangles indicate communities with mosquito larvae present (indicating top-down predatory forces), and closed triangles indicate those communities where mosquito larvae were not found at the time of sampling. For both abundances of individual species and presence/absences, protozoan communities were highly variable when compared to each other in June (beginning of the season) and November (end of the season). Communities were highly similar to one another in July, August, September, and October. The presence of mosquito larvae did not affect the similarity in composition between protozoan communities.

A) Abundance Data △ Mosquito ▲ No Mosquito



B) Presence/Absence Data

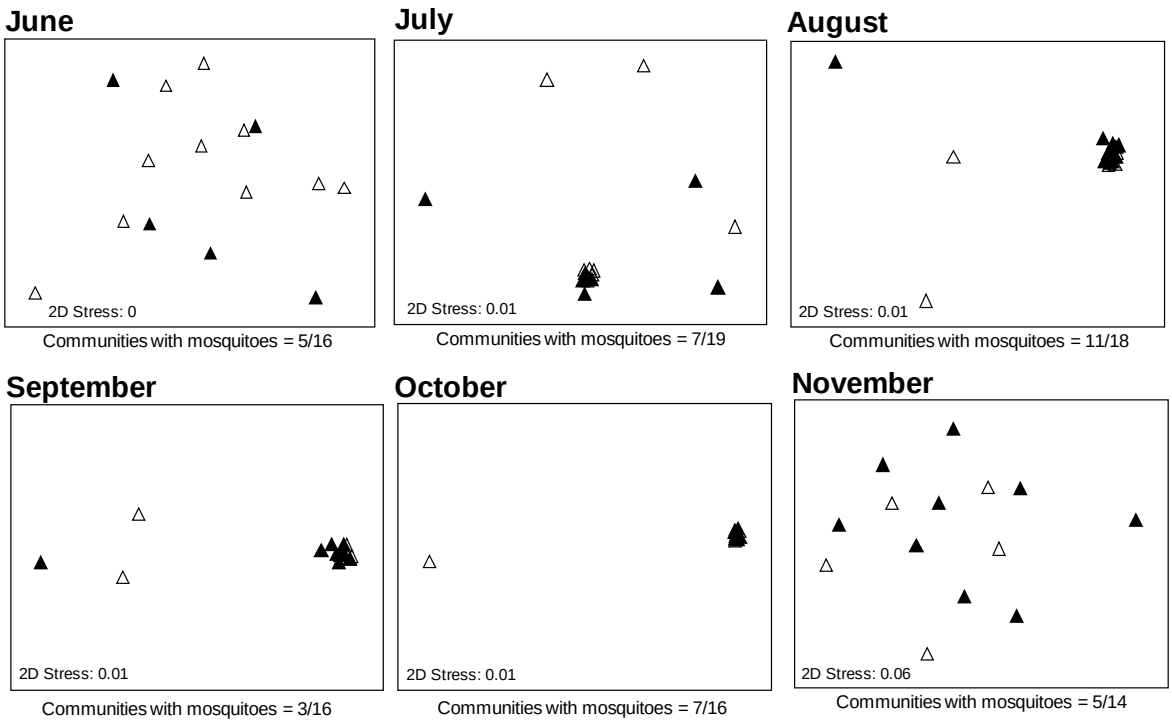
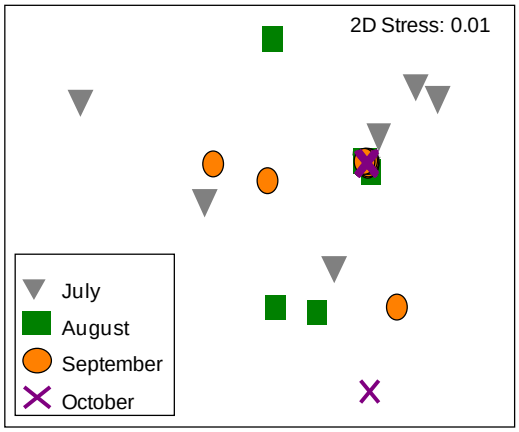


Figure 6. Multi-Dimensional Scaling (MDS) Plots for the four months (July, August, September, and October) when protozoan communities converged, based both on the abundances of individual species as well as presence/absence data. July = gray triangle, August = green square, September = orange circle, October = purple cross. 2D stress indicates how well multidimensional groupings are represented in a two dimensional graph. In general, a stress less than 0.2 is considered an adequate representation. The communities in July were the most divergent. Overall, the pattern of convergence was similar when **A)** abundances of individual protozoans data were used (ANOSIM = 0.034, p value = 0.068) and when the **B)** presence/absence of species data were used (ANOSIM = 0.046, p value = 0.036).

A. Abundances



B. Presence/Absence

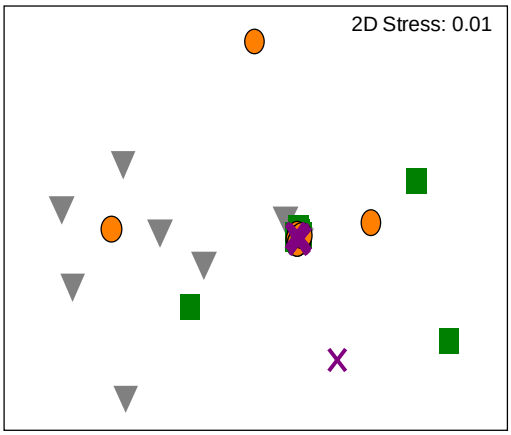


Figure 7. Multi-Dimensional Scaling (MDS) Plots illustrating community similarity among the months (June, November). For these months, the protozoan communities were highly divergent between leaves. For **A)** abundance of individual protozoan species data were used and for **B)** presence/absence data were used. Analysis of similarity among communities based on individual species abundance data- ANOSIM = 0.175, p value = 0.002). Analysis of similarity among communities using presence/absence of species data - ANOSIM = 0.161, p value = 0.002. Communities are more variable in June than when sampled in November, but overall communities in June and November are similar to each other. 2D stress indicates how well multidimensional groupings are represented in a two dimensional graph. In general, a stress less than 0.2 is considered an adequate representation. June (black triangle) and November (blue diamond).

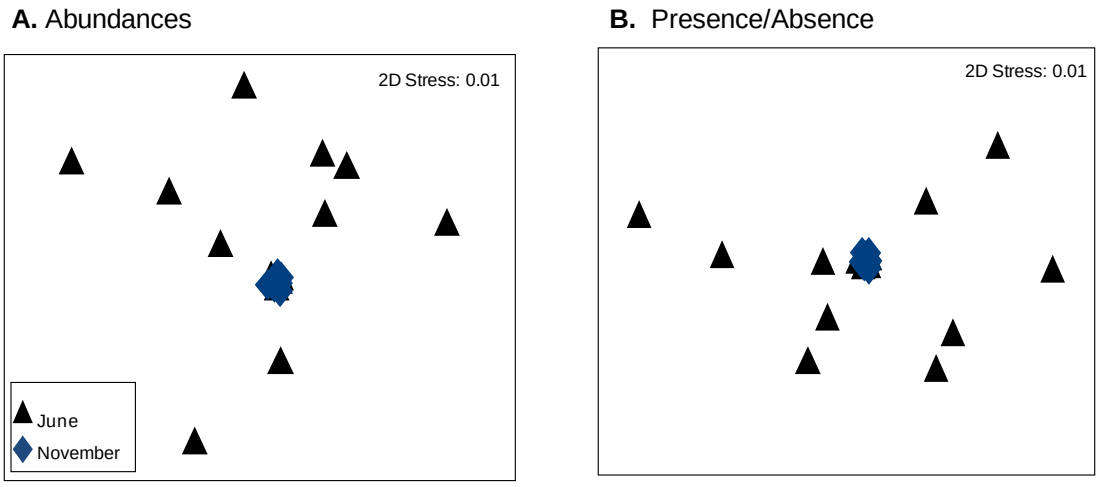


Figure 8. Abundances and species richness of culturable bacteria averaged across 20 pitcher plant communities sampled monthly from June to November in 2007. Each bar on the graph represents the average log bacteria abundance (per 0.1 ml) per month. The abundance and richness were obtained by counting bacteria on agar plates. The culturable bacteria morphotypes highlighted in colors are ordered according to competitive ability with 'Pink' as the least competitive and 'Cloudy' as the most competitive (Chapters 4, 5 and 6). The bacteria represented by shades of gray at the top of each bar are unknown morphotypes with undetermined competitive ability.

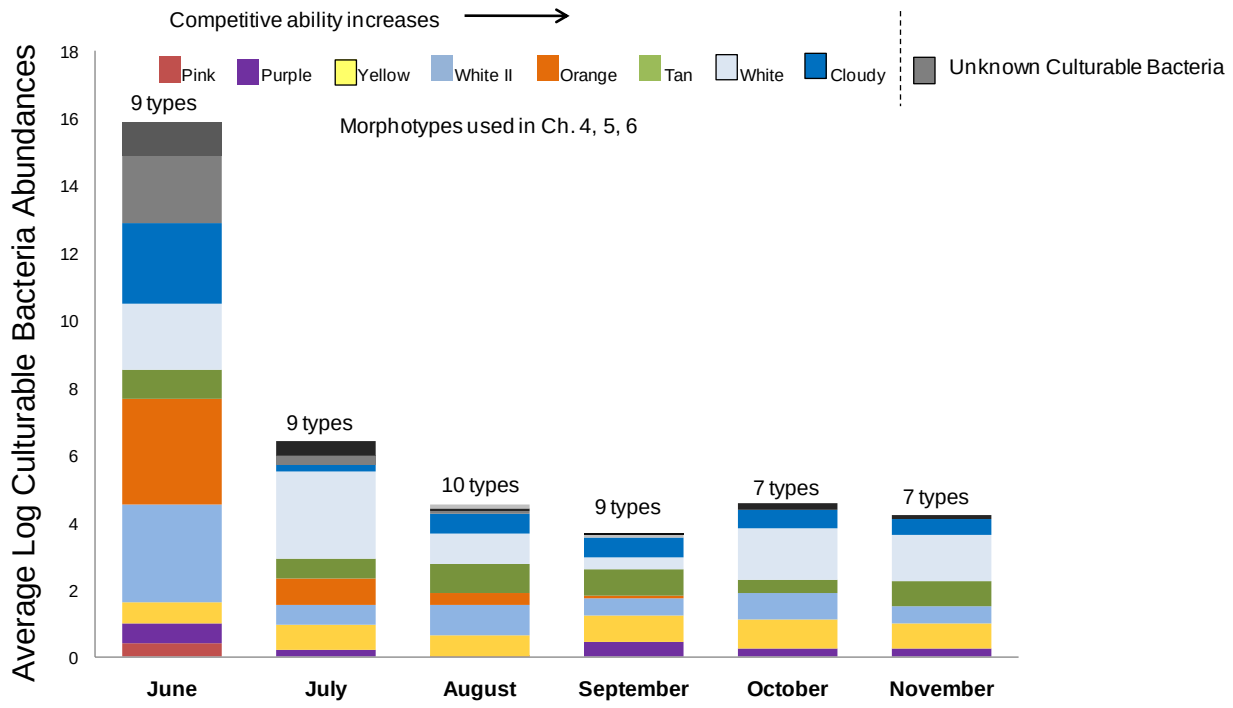


Figure 9. Composition of 20 protozoan communities sampled monthly from June to November in 2007. Each bar on the graph represents the average log protozoan densities per month. Each stacked bar represents the density of a specific protozoan species. Densities and richness were obtained by counting protozoans in 0.1 ml aliquots. The bottom and upper gray bars in each month represent the densities and species richness of flagellate and ciliate protozoans not used in experiments in Chapter 4, 5, and 6. These protozoans have unknown competitive ability. The colored bars represent the protozoan species used in experiments in Chapter 4, 5 and 6 and are organized according to the competitive ability (determined in Chapter 6 and supported by results found in Kneitel 2002), with *Bodo* sp. (purple bar) as the least competitive and *Colpidium* sp. (green bar) as the most competitive (*Bodo* sp. < *Chrysomonad* sp. < *Colpoda* sp. < *Cyclidium* sp. < *Colpidium* sp.).

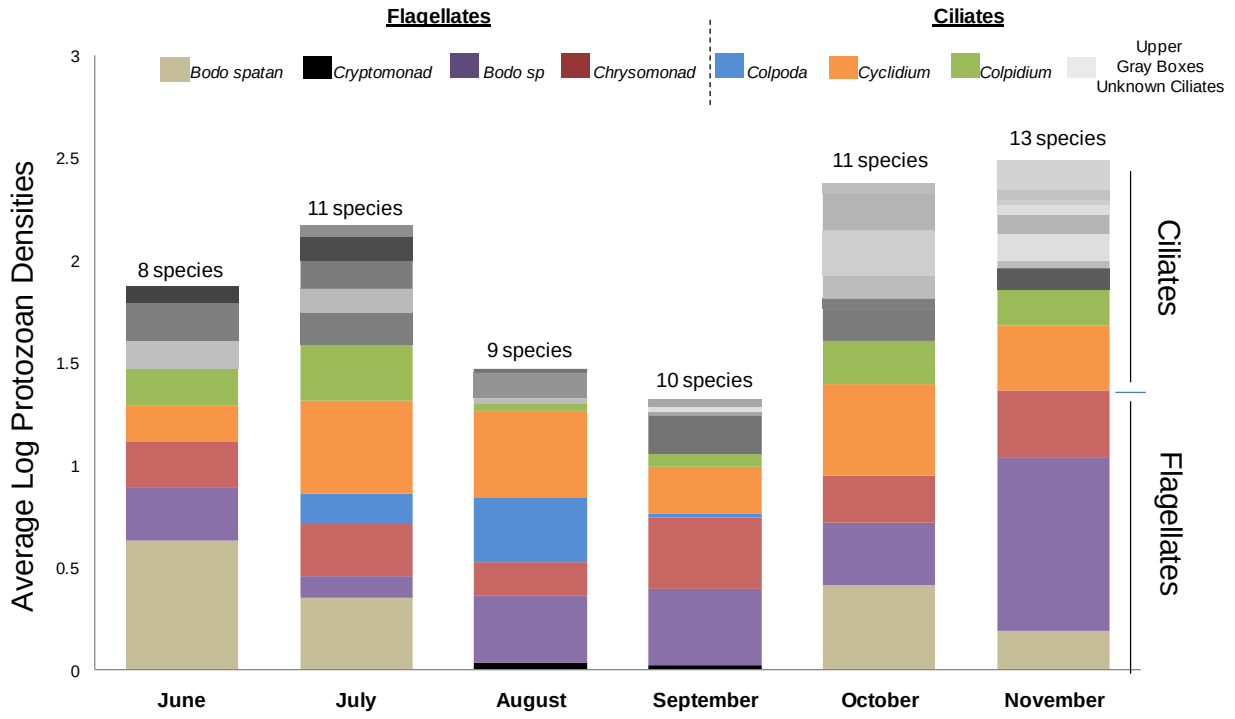
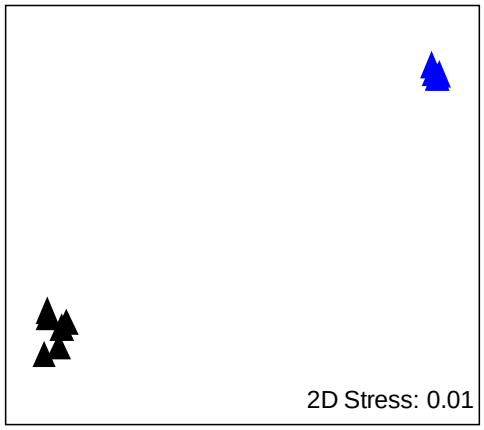


Figure 10. Non-Metric Multi-Dimensional Scaling (MDS) Plots comparing similarity between communities sampled at the beginning (black triangles) and end (blue triangles) of the season in 2008. MDS plots are based on **A)** the abundances of individual bacterial OTUs identified from the 16s rRNA libraries (square-root transformed Bray-Curtis similarity) and **B)** on the presence/absence of bacterial OTUs found in each pitcher. Each symbol represents the bacterial community in one leaf. Analysis of Similarity (ANOSIM) Global R is 1.0 and 0.992 (p values = 0.002) for similarity based on abundances of individual OTUs and presence/absence data, respectively. The bacterial communities based on OTUs were significantly different between the beginning of the season and the end of the season. The particular OTUs present (presence/absence data) was more variable among communities at the beginning of the season, but decreased by the end of the season, with communities converging **(B)**. **C)** and **D)** represent the community similarity based on abundance of individual species data and presence/absence data for the culturable bacteria from these same communities. The black triangle represents communities sampled at the beginning of the season and gray triangles represent communities sampled at the end of the season. ANOSIM for culturable bacteria, abundance data 0.246, p value = 0.02, presence/absence data 0.133, p value = 0.762. 2D stress indicates how well multidimensional groupings are represented in a two dimensional graph. In general, a stress less than 0.2 is considered an adequate representation.

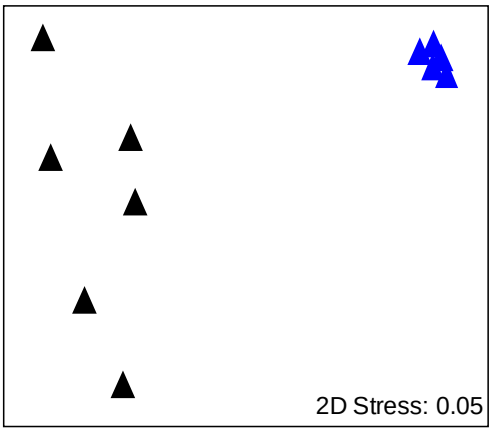
Bacteria OTUs

June
November

A. Abundance

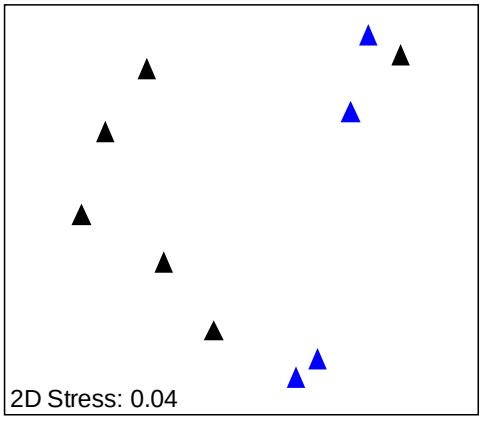


B. Presence/Absence

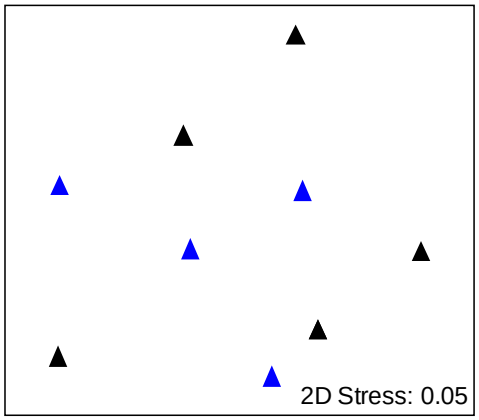


Culturable Bacteria

C. Abundance



D. Presence/Absence



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Chapter 4

Are there predictable patterns for invader success and the vulnerability of a community to invasion? Using *Sarracenia purpurea* as a model system

Abstract

With an escalation in the number of communities in which non-native species are introduced, ecologists are increasingly interested in factors that affect the susceptibility of natural communities to invasion, and the characteristics of species most likely to become invasive in order to determine if generalizable properties for invasion exist across trophic levels and systems. To-date, results from studies of invasive species have yet to provide a general theory predicting the characteristics of communities that impact their invasiblity. By using the model aquatic system inside the leaves of the pitcher plant, *Sarracenia purpurea,* I tested whether characteristics predicted important for invasion success (initial density, competitive ability, body size) are generalizable across resident communities varying in resource availability, and the presence of a top predator. For intermediate trophic level species in the *S. purpurea* system, I found that both competitive dominance and a high initial density (high propagule pressure) were important for a successful invasion. Less competitive species also invaded, but were more successful invading communities with high resource availability (less resource competition) and if they were introduced at high densities. The presence of a top predator significantly decreased the densities of intermediate trophic level species, but did not inhibit them from successfully invading and was most likely to affect invasion success if species were introduced at a high initial density.

Introduction

Identifying mechanisms that govern the assembly of a community has been an over-arching theme within community ecology for decades (e.g., Gleason 1927, Clements 1938, Samuels and Drake 1997, Diamond 1975, Belyea and Landcaster 1999). The concept of community assembly includes characteristics of localities and of individual species that have the opportunity to colonize a given area. A habitat must first successfully be invaded and colonized by species (Belyea and Landcaster 1999, Tilman 2004) and these species invasions are predicted to continue to occur until all niches or functional groups of the community are filled (Hutchinson 1957, Fox 1987). Particular characteristics of the species and of the resident community can play key roles, determining the invasion success of a species and explaining the trajectory of community development (Lonsdale 1999).

Characteristics such as competitive ability, growth rate and body size, and the numbers in which individuals of a given species arrive in a community are thought to be factors especially important for invasion success during community assembly (Diamond 1975, Belyea and Landcaster 1999). The relative importance of each of these characteristics for the colonization and establishment of a species is also thought to be impacted by properties of the resident community such as the stage of community development (e.g., Belyea and Landcaster 1999, Olito and Fukami 2009), the availability of resources (e.g., Lonsdale 1999, Davis et al. 2000), and by the presence or absence of predators and pathogens (e.g., Hairston et al. 1960, Carpenter et al. 1987). The interactions between properties of the resident community and characteristics of potential invaders can determine the community's invasibility, or susceptibility to the colonization and establishment of new species (Lonsdale 1999, Davis et al. 2000).

Determining the factors that can explain the success of colonizers into a community is not new to ecology, but has gained recent attention because of the focus on species that are transported by humans and are impacting environments around the world. To date, much of the research on invasive species have been on individual case studies, and although many hypotheses explaining invasion success have been proposed, results from invasive species research remains difficult to generalize, and has not allowed for a general theory of community invasiblity or of predictable characteristics of invasive species across systems and taxa (Lonsdale 1999, Williamson 1999), although recent progress has been made in developing a general conceptual framework (Gurevitch et al. 2011) .

Among studies, several factors have emerged as important for the invasion success of species, and these share similarities with factors that have been predicted to be important in community assembly. One large focus in invasion research is identifying species-level traits that are important for invasion success (e.g., Baker 1974, Newsome and Noble 1986, Noble 1989). It is thought that a successful invader must displace current species in a resident community, making the competitive ability of the invading species important for invasion success (e.g., Case 1990, 1991; Cornell and Lawton 1992; Morton and Law 1997). Propagule pressure, which has several definitions, is defined here as the number of individuals that are introduced to a community, and is also considered important for invasion success. The larger the number of individuals released (i.e., the higher the propagule pressure or initial density), the more likely at least several individuals of the invading species will survive stochastic events, allowing for successful establishment into a resident community. Although intrinsically considered important for invasion success of both terrestrial and aquatic organisms, empirical evidence regarding its

relative importance is still lacking due to the difficulty of knowing how many individuals enter a particular community during an invasion event (Lockwood et al. 2007).

Properties of the invaded community are also considered important for a successful invasion event to occur. For example, it has been predicted that when more resources are available in a habitat, competition among species decreases, increasing the range of species that can successfully invade and establish in the community (Davis et al. 2000). High resource availability has also been found to allow invading species to establish into a resident community at a higher biomass (e.g., Burke and Grime 1996, Huenneke et al. 1990). However, to date, most work examining the effect of nutrient availability on invasion success has been conducted in terrestrial systems; similar research in aquatic systems is lacking.

The presence or absence of predators in the resident community is also thought to influence the invasion success of real-world invaders (e.g., Crawley 1997, Keane and Crawley 2002, Shea and Chesson 2002). The intuitive reasoning behind this concept is that when there are no species present in the resident community that are able to consume the invading species, that invading species will not be regulated by predation and therefore will be able to increase in numbers and ultimately successfully establish in the community. Although many studies have found support for this concept and it fundamentally important in ecological research, there has been little experimental evidence determining how predators affect the likelihood of a successful invasion (e.g., Levins and Heatwole 1973, Miller et al. 2002) or if an invader's success is solely or primarily due to its escape from predators (Colautti et al. 2004).

To-date, the relative importance of these factors and if a single species-level or community-level property or some combination of properties is the most important for predicting invasion success of a species is not known. By using the model aquatic system inside the leaves of the

pitcher plant, *Sarracenia purpurea,* I tested whether resource availability, number of prey items, propagule pressure, competitive ability, and presence of a top predator impacted the invasion success of species in the intermediate trophic level (primary consumers). These experiments allowed me to test if traits important for invasion success of intermediate trophic level species in this system are generalizable across resident *S. purpurea* system communities of varying resource availability and trophic structure (presence or absence of a predator). The results from these experiments allowed me to determine if factors considered important in the invasion literature for a successful invasion are also of key importance in the model *Sarracenia* system.

Study System

Sarracenia purpurea is a plant found in nutrient poor environments that relies on the capture of insects for its essential nutrients. Its leaves form a pitcher shape and trap rainwater, creating an aquatic habitat that is colonized by bacteria, yeast, protozoans, rotifers and insect larvae. This microscopic community has the dynamics of larger aquatic food webs (e.g., Heard 1994, Kneitel and Miller 2002, Gray et al. 2006), but on small spatial and short time scales. In the native range of North America, insects, primarily ants, fall into this trapped rainwater. Bacteria and yeast colonize the system, decompose the insects, and liberate nutrients for the plant. A variety of protozoans and a rotifer species also colonize this community and consume the bacteria. The highest trophic level is filled by the larvae of the endemic pitcher plant mosquito, *Wyeomyia smithii*, which feed on the protozoans and rotifers. An endemic midge species, found at the bottom of the leaf, breaks apart insects and facilitates the release of nutrients to the plant (Heard 1994). The mosquito, midge, rotifer and protozoan species, as well as the phenotypes of the culturable bacteria, are the same across the entire native geographic range of the plant (Buckley

et al. 2003).

This system has previously been used to examine the role of trophic cascades (Kneitel and Miller 2002), omnivory (Kneitel 2007), commensalism (Heard 1994), top down and bottom up forces (Cochran-Stafira and von Ende 1998, Kneitel and Miller 2002, Gray et al. 2006, Hoekman 2007, Mouquet et al. 2008), competition and evolution (terHorst 2010), and invasion success (Miller et al. 2002) on community dynamics and structure. These previous studies have greatly increased knowledge of the food web dynamics, community composition, and predator/prey/competition relationships in the *S. purpurea* system, and allow me to advance research using this model system by investigating the mechanisms driving invasion success of species with different traits.

In this study, I used this system to test the relative importance of species level traits (competitive ability and the number of individuals introduced, here defined as propagule pressure) as well as system level properties (the presence or absence of a predator and resource availability) on introduction success for two species of protozoans. To test properties of the resident community that may affect its invasibility, I developed three types of resident communities in a laboratory setting. Each of the three resident community types contained one of two levels of resource availability (low and high), and a top predator was either present or absent. These resident communities included three common species of protozoans, isolated from the field, and either culturable bacteria isolated from the field, or unculturable and culturable bacteria collected from pitcher plants in the field. I then introduced into the resident communities either the competitively-dominant or least competitive protozoan species (as individual treatments, never in combination), which allowed me to test the effect of competitive ability on invasion success. The two species were introduced at multiple densities to test the

effect of initial density (propagule pressure) on invasion success. I also tested whether body size, independent of density, is a characteristic important for invasion success. In all cases, the densities of the two invading species were followed throughout both the initial invasion and establishment stages.

The protozoan species that were used as the invaders in the resident communities are naturally found in the *S. purpurea* system and are therefore native "invaders". Using native species as invaders into experimentally established communities has been used with success to address questions about invasion in other studies (e.g., Crawley et al. 1999, Hector et al. 2001, Troumbis et al. 2002, Meiners et al. 2004, France and Duffy 2006), including studies with this system (Miller et al. 2002). Invasion can, therefore, be thought of as a part of the community assembly process. The knowledge gained from experiments that experimentally use native species as introduced species address properties important in community assembly and help increase our understanding of the community level processes that occur and result from species introductions.

Methods

Experimental Design

I tested the relative importance of body size (small and large), initial density (propagule pressure), competitive ability (least or most competitive), and combinations of these factors, on the invasion success of intermediate trophic level species, and whether features of the resident community (resource availability or presence of a top predator) affected invasion success. I conducted three experiments with different resident communities (see below). A full factorial design was used for all treatments in all three experiments (Tables 1, 2, 3). Due to the large

number of treatments and replication, each experiment was conducted at a separate time. Experiment 1 was conducted in July 2009, Experiment 2 was conducted in July 2010 and Experiment 3 was conducted in August 2010.

The species used in the three experiments

Five of the most common protozoan species found in pitcher plant aquatic communities (Buckley et al. 2003) were collected from Cranberry Bog Preserve in Riverhead, NY (40.90°, 72.67°) and isolated into monocultures. These monocultures were maintained on a 12hr light/dark cycle in a growth chamber (27°C) and were the same isolates used in experiments in Chapters 5 and 6 and were present in the communities studied in Chapters 2 and 3. These five protozoan species included three ciliates (*Colpidium* sp., *Colpoda* sp., *Cyclidium* sp.) and two flagellates (*Bodo* sp. and *Chrysomonad* sp.).

Through preliminary pairwise experiments, I determined the competitive ability of these protozoan species. I found a competitive hierarchy where *Colpidium* sp. was the competitive dominant followed by *Cyclidium* sp. > *Colpoda* sp. > *Chrysomonad* sp. > *Bodo* sp.. This competitive ranking is supported by results from similar competition experiments using these same species collected from pitcher plant water in Florida (Kneitel 2002). In all cases, ciliates have been found to be better competitors than flagellate protozoans, and competitive ability is size dependent (larger protozoans are more competitively dominant than smaller protozoans). Based on these results, I selected the competitively-dominant protozoan (the ciliate *Colpidium* sp.) and the least competitive protozoan (the flagellate *Bodo* sp.) as the target introduced species used to test species and community-specific properties of invasion success. In addition, *Bodo* sp. had been used before for studies of the role of propagule pressure on invasion success in this

community (Miller et al. 2002). Before the initiation of experimental treatments, I created resident communities that contained all of the remaining three isolated protozoans (*Colpidium* sp. (competitive dominant) and *Bodo* sp. (least competitive) were excluded). These resident communities were created with the same average densities of *Cyclidium*, *Colpoda*, and *Chrysomonad* as are naturally found in the communities held within *S. purpurea* leaves in the field (Chapters 2 and 3).

To determine the impact that bottom trophic level (bacterial) diversity had on the invasion success of intermediate trophic level consumers (protozoans), the bottom trophic level of the resident communities were created in two different ways. For Experiment 1 I used 7 isolated culturable bacteria (able to grow in the laboratory) obtained from *S. purpurea* water collected in the field and maintained in the laboratory in monocultures. The culturable bacteria used were two Gammaproteobacteria (*Enterobacter* sp. AR19, *Serratia* sp. 9A_5), two Betaproteobacteria (*Chromobacterium violaceum*, *Aquitalea magnusonii*), and one Bacteroidetes (*Chryseobacterium* sp. COLI2) and one Actinobacteria (*Leifsonia xyli*). For Experiment 2 and 3, all bacteria (both culturable and unculturable) present in water collected from randomly selected leaves of *S. purpurea* in the field were used.

When only culturable bacteria were used (Experiment 1), all bacteria were collected from *S. purpurea* water in the field at the same time and from the same communities as the protozoans that were used in the experiments. After growing the bacteria and plating them on agar, I selected the morphotypes that were the most distinct from one another. By using only these culturable, yet morphologically different bacteria, I was able to correctly assess changes in both species richness and abundance of the seven culturable bacteria with plate count techniques (Chapter 5).

When the entire bacterial community (both culturable and unculturable bacteria) was used to form the bottom trophic level of the resident community (Experiments 2 and 3), I collected *S. purpurea* water from leaves randomly selected in the field until I obtained enough water to create all resident communities for the experiment. The water from the leaves was pooled and filtered multiple times (sterilized 233 µm, 8 µm and 0.8 µm Millipore filters) to remove all detritus, invertebrates, and protozoans. After the final filtration (0.7 µm Glass Fiber GF/F Millipore filter), water containing only bacteria from *S. purpurea* communities remained.

For all experiments, the protozoan and bacterial species that formed the resident communities for a specific experiment were pooled into one large, sterile (autoclaved) container. For the resident communities containing only culturable bacteria (Experiment 1), this container was filled with autoclaved deionized water to a volume that allowed for the appropriate number of replicate communities to be created. When the container had protozoans and both culturable and unculturable bacteria (Experiments 2 and 3), the filtered pitcher plant water was used instead. In both cases, this pooled community of protozoans and bacteria was mixed continuously to homogenize the community, and 10 ml aliquots were distributed into 50 ml experimental macrocentrifuge tubes. 2 ml of glass beads (3mm diameter with 1mm hole in the center) were added to the bottom of each tube to mimic the environmental complexity found in the bottom of pitcher plant leaves as a result of the exoskeletons of decomposed insects. This environmental complexity reduced the rate at which the top predator, the larval stage of the mosquito *Wyeomyia smithii*, consumed the protozoans in the community (Gray, unpublished data; terHorst 2010). Four dead, sterilized *Tapinoma sessile*, the most common ant found in pitcher plants at Cranberry Bog Preserve (personal observation), were added to each experimental resident community as the nutrient source.

The completed experimental resident communities consisting of ants, either seven culturable bacteria species (Experiment 1) or a bacterial soup of culturable and unculturable pitcher plant bacteria (Experiment 2 and 3), and three protozoan species, were allowed to stabilize in the growth chamber for 72 hours at 27°C (12h light/dark cycle) before the addition of the experimental treatments. This allowed a turnover of approximately 18 bacterial generations and 7 protozoan generations. Because of this, it can be argued that it is appropriate to use *Colpidium* sp. and *Bodo* sp. to test questions about invasion because even though these species are naturally found in the pitcher plant community, the protozoans and bacteria in the resident communities I created for my experiments had not encountered *Colpidium* sp. or *Bodo* sp. for many generations.

Experiment 1: Culturable Bacteria, Low Resources, Size Difference of Invaders Not Adjusted, With and Without Predators (Table 1)

In this experiment, the resident community consisted of 3 protozoan species and culturable bacteria only and had low resource availability. I tested if the density of the invader, independent of size, affected the invasion and establishment success of the larger, competitively-dominant (*Colpidium* sp.) and smaller, least competitive (*Bodo* sp.) protozoan species. There were a total of 14 treatments (Table 1), replicated 4 times each. The following experimental treatments were applied after the resident communities stabilized: 1) high *Colpidium* sp. density (1000 individuals, 100 / ml), 2) medium *Colpidium* sp. density (500 individuals, 50 / ml), 3) low *Colpidium* sp. density (50 individuals, 5 / ml), 4) high *Bodo* sp. density (1000 individuals, 100 / ml), 5) medium *Bodo* sp. density (500 individuals, 50 / ml), 6) low *Bodo* sp. abundance (50 individuals, 5 / ml), 7) control with no addition of *Colpidium* sp. or *Bodo* sp. All seven

treatments were repeated with one *Wyeomyia smithii* mosquito larva added to each replicate of each treatment (treatments 8-14, Table 1) to test if the presence of the top predator affected invasion success. Each second instar mosquito larva was double rinsed in sterilized deionized water (30 minutes per rinse) to remove any protozoans or bacteria before being added to experimental communities. To test the effect of low resource availability on invasion success and establishment, resources (4 autoclaved ants) were only added when the resident communities were created, before the initiation of the treatments. These treatments allowed me to test the effect of initial density (propagule pressure) independent of body size on the invasion success of each of the two intermediate trophic level species (*Colpidium* sp. and *Bodo* sp.) and if predation by a top predator affected invasion success.

*Experiment 2: All Bacteria, Low Resource*s, *Size Difference of Invaders Adjusted, With and Without Predator* (Table 2)

and

*Experiment 3: All Bacteria, High Resource*s, *Size Difference of Invaders Adjusted, With and Without Predator* (Table 3)

Experiments 2 and 3 used the resident communities that contained pitcher plant water with both culturable and unculturable bacteria. In these experiments, the propagule pressure treatments included approximately equal biomass of *Colpidium* sp. and *Bodo* sp. rather than equal numbers, as in the first experiment, to take into account the differences in size of these two protozoans. *Colpidium* sp. is approximately 10 times larger than *Bodo* sp.. For Experiments 2 (Table 2) and 3 (Table 3) there were a total of 12 treatments: 1) high initial density of *Colpidium* sp. with 100 individuals, 2) high initial density of *Bodo* sp. with 1000 individuals, 3) low initial

density of *Colpidium* sp. with 10 individuals, 4) low initial density of *Bodo* sp. with 100 individuals, 5) control without protozoans added. Treatments 6 - 10 were identical to Treatments 1 - 5, but one mosquito larva was added as in Experiment 1. The Treatments were identical in Experiments 2 and 3, except that due to contamination, the Controls in Experiment 3 could not be used. To determine if resource availability was important, Experiment 2 used the same resource input as in Experiment 1 (4 ants at the start of the experiment). For Experiment 3 (high resource availability), 4 ants were added to initiate the resident community, and then one autoclaved ant was added daily throughout the experiment to all replicates of all treatments. Each treatment was replicated 4 times in Experiment 2 and 3 times Experiment 3.

Sampling Methods

The same sampling protocol was used for all three experiments. All replicates of each treatment were sampled every two days for six days in order to follow the densities of the introduced *Colpidium* sp. and *Bodo* sp. through time. On each sampling day, communities and beads were gently mixed and a 0.1 ml aliquot from each tube was used to count the densities of both *Colpidium* sp. and *Bodo* sp. with a compound microscope. The treatments containing a mosquito larva were checked daily to make sure that mosquitoes had not died or pupated. No mosquitoes needed replacement during the time course of the experiments.

Statistical Analysis

Because I was interested in invasion success through time, repeated measures ANOVAs were performed on *Colpidium* sp. and *Bodo* sp. densities for days 0 (initial introduction densities), 2, 4, and 6 of the experiment. Time was treated as the within-in subject factor . The assumptions

of ANOVA (independence of treatments, the residuals were normally distributed, and variances were equal) were tested before the ANOVAs were performed using Statistica 6.1. Data were normalized with either log or square root transformations.

Results

The full repeated measures ANOVA tables for the three experiments are in Table 4 (Experiment 1), Table 5 (Experiment 2), and Table 6 (Experiment 3). In all experiments, invasion success was affected by: 1) competitive ability of the invader, 2) initial density at which the invader was introduced, and 3) the presence of the top predator, whose affect increased through time (Table 7). The densities of the invaders were also affected differently as the experiment progressed through time (Time*Species interaction)(Table 7). In resident communities with low resources, the effect of the predator differed for the success of the two invaders (Species*Predator), the predator had a different effect depending on the initial densities of the invaders (Initial Density*Predator), and there was a Time*Species*Predator interaction (Table 7). In communities containing all bacteria and the biomass difference of the invaders was adjusted (Experiment 2 and 3), there was a Time*Initial Density interaction (Table 7).

Invader Type (The Species Factor)

In all experiments, *Colpidium* sp., the competitive dominant, was significantly more successful at invading the resident communities than *Bodo* sp., across all densities, even when densities were adjusted to take into account the difference in biomass between these two species (Table 7; Figure 2, 3 and 4).

Initial Density

The initial density of a species was important for invasion success for both *Colpidium* sp. and *Bodo* sp.. Invasion success was better for both *Colpidium* sp. and *Bodo* sp. when introduced at high initial densities in all experiments (Table 7; Figure 2, 3, and 4). This result suggests that the number of individuals, and not just the total biomass of the invader, is important for invasion success.

Presence of a Top Predator

The presence of a top predator significantly affected invasion success of both intermediate trophic level species (Table 7; Figure 2, 3, and 4). In all experiments, for all treatements with the predator present, densities at which the invaders established were significantly lower than when no top predator was present (Figure 3, 4 and 5).

When resource availability was low (Experiment 1: culturable bacteria/size difference of invaders not adjusted and Experiment 2: all bacteria/size difference of invaders adjusted), the density of *Colpidium* sp. was more affected by the presence of the predator than was *Bodo* sp. (Predator * species interaction, Figure 2 and 3). However, *Bodo* sp. never reached high densities in low resource experiments, with or without predators (Figure 2 and 3). Therefore, the effect of the top predator on *Bodo* sp. may not be as large as that on *Colpidium* sp. because only a few *Bodo* sp. were available in the community. When resources in the resident community were high, there was no significant difference in the effect of the predator on *Colpidium* sp. and *Bodo* sp. (no significant species*predator interaction, Table 6 and 7). When resources were low, predators had a greater impact when propragule pressure was high for both species (Initial Density*Predator, Table 7; Figure 2 and 3).

The presence of a mosquito as a predator also significantly decreased the densities of both *Colpidium* sp. and *Bodo* sp. through time compared to predator-free communities. This result was found across all experiments (Table 7, Figure 3, 4, and 5).

Discussion

Species characteristics such as competitive ability, growth rate and body size, and the numbers in which individuals of a given species arrive in a community (propagule pressure) have been considered factors that facilitate invasion success during community assembly (Diamond 1975, Belyea and Landcaster 1999). A successful invasion is also thought to be dependent on properties of the community, such as high or low resource availability (e.g., Lonsdale 1999, Davis et al. 2000) and the presence or absence of predators (e.g., Hairston et al. 1960, Carpenter et al. 1987). These same factors have recently gained attention because of the recent focus on species that are transported by humans around the world and are impacting communities globally. Although many hypotheses have been proposed to explain the success of these invasive species, the presence of a predator, the availability of a predator and species-level traits such as competitive ability and the initial density of a species in a community, have been thought to be of major importance for the success of species invasions.

I found that both competitive dominance and high propagule pressure (high initial density) were important for the successful invasion of a intermediate trophic level species. The less competitive species was able to invade, but invasion success was greater with high propagule pressure and in communities with high resource availability and limited resouce competition. The less competitive species was always achieved significantly lower densities in the experimental communities than the competitively dominant invader, independent of the initial

density introduced into the community. These results are not an artifact of the size difference between the two invader species used in these experiments and were robust when species differences in biomass were taken into account. The competitively dominant species remained the better invader.

Resources availability and prey species richness in the resident communities also played a key role in determining the invasion success of *Colpidium* sp. and *Bodo* sp.. Both *Colpidium* sp. and *Bodo* sp. were able to successfully establish in all communities, but the densities that they attained were dependent on the amount of resources available. Both species were present in higher densities in resident communities containing high resources. Although *Bodo* sp. was successful at invading all communities, its persistance in a community was strongly dependent on the amount of resources available. Similar results have been found in other studies testing the invasion success of other intermediate trophic level species. Romanuk and Kolasa (2005) found that the invasion success of the competitive midge *Dasyhelea* sp. in rock pool communities increased with increasing resources. When modeling food webs, Baiser et al. (2010) found that the invasion success of an intermediate trophic level species was dependent on the amount of prey items (resources) and the presence or absence of potential predators.

The results from the three experiments presented here suggest that even though high resources do in fact allow both highly competitive and less competitive species to invade, the competitively dominant species is still more successful at invading than a less competitive species, even in high resource communities. These results imply that intermediate trophic level species with high competitive ability are better at invading a community than less competitive species, independent of the resource availability.

The presence of a top predator also had a clear impact on the invasion success of the intermediate trophic level species. In all experiments, the presence of the mosquito larva *Wyeomyia smithii* decreased the densities that the invaders were found in the community, but did not prevent the species from successfully invading. Therefore, invasion success was not the results of enemy release. Counter-intuitively, the predator had a greater impact on both the invaders when propagule pressure was high than when propagule pressure was low. The top predator significantly decreased the densities of both *Colpidium* sp. and *Bodo* sp. in the resident community when these invaders were introduced at high initial densities. This result was also the case when *Colpidium* sp. and *Bodo* sp. were introduced into communities with high resource availability. By not being resource limited, *Colpidium* sp. and *Bodo* sp. densities quickly increased to high numbers, allowing them to be equally impacted by the presence of a top predator. In communities with low resources, *Colpidium* sp. was more affected by the presence of a top predator than the less competitive *Bodo* sp. However, in these conditions *Bodo* sp. failed to establish at high densities in the resident communities, which may explain why the top predator appears to have no impact on *Bodo* sp. densities in low resources. From these results, it appears that although the presence of a top predator will always have a negative effect on the establishment success of intermediate trophic level species, it will have the largest impact on species that are introduced at a high propagule pressure or are in the resident community in high densities, independent of their competitive ability or body size.

All factors tested in this study, which have been considered important for a successful invasion - competitive ability, propagule pressure, resource availability and predation risk - affected invasion and establishment success. Although body size was correlated with competitive ability, body size per se was not an important factor for invasion success. The

relative contributions of each of these factors could not have been discerned from experiments testing single hypotheses. There is a need for more studies to consider all of these hypotheses, rather than single hypothesis testing. Had a single factor been tested, a full understanding of the relative importance of each factor would be missed. Further work on more systems is needed to assess the relative roles of propagule pressure, competition, resource availability and predators in a multi-trophic context (Baiser et al. 2010).

Table 1. Experiment 1 Treatments. The resident community in Experiment 1 included three species of protozoans and culturable bacteria only, and had low resource availability. Each treatment was replicated 4 times. Changes in the densities of the introduced protozoans (either *Colpidium* sp. or *Bodo* sp. added as separate treatments) were quantified every 2 days, and analyzed using repeated measures ANOVA. *Colpidium* sp. and *Bodo* sp. were introduced at the same initial densities across treatments, which did not account for differences in their biomass. The controls (Treatment 7 and 14) were not included in the analyses in this chapter, but were used in Chapter 5 to test whether the densities of the resident protozoans and resident culturable bacteria were different in uninvaded communities.

Treatment	Invader	Initial Density	Presence of a Predator
1	*Colpidium* sp.	High	No Predator
2	*Colpidium* sp.	Medium	No Predator
3	*Colpidium* sp.	Low	No Predator
4	*Bodo* sp.	High	No Predator
5	*Bodo* sp.	Medium	No Predator
6	*Bodo* sp.	Low	No Predator
7	Control	-	No Predator
8	*Colpidium* sp.	High	Predator
9	*Colpidium* sp.	Medium	Predator
10	*Colpidium* sp.	Low	Predator
11	*Bodo* sp.	High	Predator
12	*Bodo* sp.	Medium	Predator
13	*Bodo* sp.	Low	Predator
14	Control	-	Predator

Table 2. Experiment 2 Treatments. The resident community in Experiment 2 contained three species of protozoans and both culturable and unculturable bacteria, and had low resource availability. Each treatment was replicated 4 times. The densities of the introduced protozoans (either *Colpidium* sp. or *Bodo* sp. added as separate treatments) were quantified every 2 days, and analyzed using repeated measures ANOVA. The densities of *Colpidium* sp. and *Bodo* sp. introduced were adjusted to take into account the ~10 fold difference in their size, so that the low and high density treatments had a similar biomass of *Colpidium* sp. or *Bodo* sp added. The controls (Treatment 5 and 10) were not included in the analyses in this chapter, but were used in Chapter 5 to test whether the densities of the resident protozoans and resident culturable bacteria were different in uninvaded communities.

Treatment	Invader	Initial Density	Presence of a Predator
1	*Colpidium* sp.	High	No Predator
2	*Colpidium* sp.	Low	No Predator
3	*Bodo* sp.	High	No Predator
4	*Bodo* sp.	Low	No Predator
5	Control	-	No Predator
6	*Colpidium* sp.	High	Predator
7	*Colpidium* sp.	Low	Predator
8	*Bodo* sp.	High	Predator
9	*Bodo* sp.	Low	Predator
10	Control	-	Predator

Table 3. Experiment 3 Treatments. The resident community in Experiment 3 contained three species of protozoans and both culturable and unculturable bacteria, and were provided with high resource availability. Each treatment was replicated 3 times. The densities of *Colpidium* sp. and *Bodo* sp. introduced were adjusted to take into account the ~10 fold difference in their size, so that the low and high density treatments had a similar biomass of *Colpidium* sp. or *Bodo* sp added. All controls treatments (no *Colpidium* sp. or *Bodo* sp added) in this experiment were contaminated and could not be used in analyses.

Treatment	Invader	Initial Density	Presence of a Predator
1	*Colpidium* sp.	High	No Predator
2	*Colpidium* sp.	Low	No Predator
3	*Bodo* sp.	High	No Predator
4	*Bodo* sp.	Low	No Predator
5	*Colpidium* sp.	High	Predator
6	*Colpidium* sp.	Low	Predator
7	*Bodo* sp.	High	Predator
8	*Bodo* sp.	Low	Predator

Table 4. Experiment 1 results of repeated measures ANOVA. Factors included Species (*Colpidium* sp. or *Bodo* sp.), initial density (low, medium, high), and predator presence or absence. The communities were sampled every 2 days for 6 days. Time was treated as a within-subject factor. Red represents significant p values.

	SS	df	MS	F	p
Intercept	25.78	1	25.78	616.76	P < 0.00001
Species	1.40	1	1.40	33.60	**P < 0.00001**
Initial Density	2.87	1	2.87	68.70	**P < 0.00001**
Predator	2.06	1	2.06	49.37	**P < 0.00001**
Species*Initial Density	0.007	1	0.007	0.18	0.68
Species*Predator	1.13	1	1.13	27.11	**0.000025**
Initial Density*Predator	0.44	1	0.44	10.47	**0.0035**
Species*Initial Density*Predator	0.06	1	0.060	1.35	0.26
Error	1.00	24	0.041		
TIME	2.71	3	0.902	27.35	**P < 0.00001**
TIME*Species	0.99	3	0.33	9.98	**0.000014**
TIME*Initial Density	2.29	3	0.76	23.10	**P < 0.00001**
TIME*Predator	0.97	3	0.32	9.75	**0.000018**
TIME*Species*Initial Density	0.14	3	0.04521	1.3702	0.26
TIME*Species*Predator	1.66	3	0.55179	16.7243	**P < 0.00001**
TIME*Initial Density*Predator	0.41	3	0.13662	4.1408	**0.0091**
TIME*Species*Initial Density*Predator	0.062	3	0.02061	0.6248	0.601
Error	2.37	72	0.03299		

Table 5. Experiment 2 results of repeated measures ANOVA. Factors included Species (*Colpidium* sp. or *Bodo* sp.), initial density (low, high), and predator presence or absence. The communities were sampled every 2 days for 6 days. Time was treated as a within-subject factor. Red represents significant p values.

	SS	df	MS	F	p
Intercept	25.78	1	25.78	616.76	**P < 0.00001**
Species	1.40	1	1.40	33.60	**P < 0.00001**
Initial Density	2.87	1	2.87	68.70	**P < 0.00001**
Predator	2.06	1	2.06	49.37	**P < 0.00001**
Species*Initial Density	0.007	1	0.007	0.18	0.68
Species*Predator	1.13	1	1.13	27.11	**0.000025**
Initial Density*Predator	0.44	1	0.44	10.47	**0.0035**
Species*Initial Density*Predator	0.06	1	0.060	1.35	0.26
Error	1.00	24	0.041		
TIME	2.71	3	0.902	27.35	**P < 0.00001**
TIME*Species	0.99	3	0.33	9.98	**0.000014**
TIME*Initial Density	2.29	3	0.76	23.10	**P < 0.00001**
TIME*Predator	0.97	3	0.32	9.75	**0.000018**
TIME*Species*Initial Density	0.14	3	0.04521	1.3702	0.26
TIME*Species*Predator	1.66	3	0.55179	16.7243	**P < 0.00001**
TIME*Initial Density*Predator	0.41	3	0.13662	4.1408	**0.0091**
TIME*Species*Initial Density*Predator	0.062	3	0.02061	0.6248	0.601
Error	2.37	72	0.03299		

Table 6. Experiment 3 results of repeated measures ANOVA. Factors included Species (*Colpidium* sp. or *Bodo* sp.), Initial Density (low, high), and predator presence or absence. The communities were sampled every 2 days for 6 days. Time was treated as a within-subject factor. Red represents significant p values.

	SS	df	MS	F	p
Intercept	12015.07	1	12015.07	309.15	P < 0.00001
Species	817.33	1	817.33	21.03	**0.000305**
Initial Density	562.28	1	562.28	14.47	**0.001561**
Predator	1278.58	1	1278.58	32.90	**0.000031**
Species*Initial Density	4.56	1	4.56	0.12	0.736511
Species*Predator	48.08	1	48.08	1.24	0.282473
Initial Density*Predator	1.18	1	1.18	0.03	0.863780
Species*Initial Density*Predator	125.14	1	125.14	3.22	0.091663
Error	621.84	16	38.87		
TIME	3160.62	3	1053.54	72.55	**P < 0.00001**
TIME*Species	299.56	3	99.85	6.88	**0.000604**
TIME*Initial Density	238.94	3	79.65	5.48	**0.002535**
TIME*Predator	704.50	3	234.83	16.17	**P < 0.00001**
TIME*Species*Initial Density	98.02	3	32.67	2.25	0.094504
TIME*Species*Predator	87.41	3	29.14	2.01	0.125580
TIME*Initial Density*Predator	9.32	3	3.11	0.21	0.886178
TIME*Species*Initial Density*Predator	57.55	3	19.18	1.32	0.278437
Error	697.01	48	14.52		

Table 7. Summary of the results from repeated measures ANOVAs testing the properties important for introduction success of the competitively dominant protozoan, *Colpidium* sp., and the least competitive protozoan, *Bodo* sp. The results are displayed by experiment, with the significant results from *Experiment 1: Culturable Bacteria, Low Resources, Size Difference of Introduced Species Not Adjusted* in the first column, significant results from *Experiment 2: All Bacteria, Low Resources, Size Difference of Introduced Species Adjusted* in the second column, and significant results from *Experiment 3: All Bacteria, High Resources, Size Difference of Introduced Species Adjusted* in the third column. Significant p values are in red and p values marginally significant are in blue. When a significant result was found in all three experiments, the row is highlighted in light blue. When a significant result was found in 2 experiments, the row is highlighted in light purple. Significant results that were only found in one experiment are not highlighted. When results were non-significant, they are represented in the table as 'NS'.

	Low Resource, Culturable Bacteria, Size Difference of Introduced Species Not Adjusted	Low Resource, All Bacteria, Size Difference of Introduced Species Adjusted	High Resource, All Bacteria, Size Difference of Introduced Species Adjusted
Species	P < 0.00001 F(1, 36) = 108.02	P < 0.00001 F(1, 24) = 33.60	P = 0.000305 F(1,16) = 21.03
Initial Density	P < 0.00001 F(2, 36) = 25.47	P < 0.00001 F(1, 24) = 68.70	P = 0.001561 F(1,16) = 114.47
Predator	P < 0.00001 F(1, 36) = 84.9	P < 0.00001 F(1, 24) = 49.37	P = 0.000031 F(1,16) = 32.9
Species*Initial Density	NS	NS	NS
Species*Predator	P < 0.00001 F(1, 36) = 45.08	P = 0.000025 F(1, 24) = 27.11	NS
Initial Density*Predator	P = 0.0611 F(2, 36) = 3.02	P = 0.0035 F(1, 24) = 10.47	NS
Species*Initial Density*Predator	NS	NS	NS
Time	P < 0.00001 F(3, 108) = 10.90	P < 0.00001 F(3, 72) = 27.35	P < 0.00001 F(3, 48) = 72.55
Time*Species	P < 0.00001 F(3, 108) = 19.78	P = 0.000014 F(3, 72) = 9.98	P = 0.000604 F(3, 48) = 6.88
Time*Initial Density	NS	P < 0.00001 F(3, 72) = 23.10	P = 0.002535 F (3, 48) = 5.48
Time*Predator	P < 0.00001 F(3, 108) = 17.65	P = 0.000018 F(3, 72) = 9.75	P = 0.000747 F(3, 48) = 9.09
Time*Species*Initial Density	NS	NS	NS
Time*Initial Density*Predator	NS	P = 0.0091 F(3, 72) = 4.14	NS
Time*Species*Predator	P < 0.00001 F(3, 108) = 9.14	P < 0.00001 F(3, 72) = 16.6	NS
Time*Species*Initial Density*Predator	NS	NS	NS

Figure 1. Experimental design. Test tubes represent the resident communities and are aligned according to resource availability (low resources and high resources) and experiment number. The circles in the test tubes represent the beads that were used for habitat complexity and are used as refugia for protozoans to escape predation from mosquito larvae. Similar refugia are found in natural pitcher plants due to ant exoskeletons and detritus that accumulate in the water held by the pitcher plant leaves.

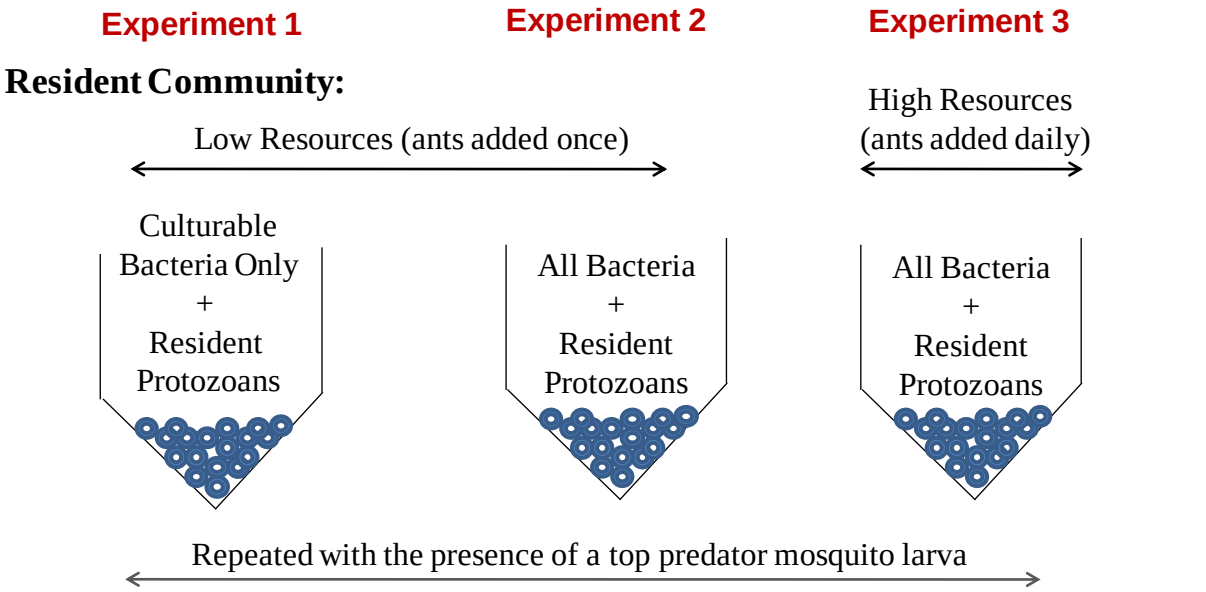


Figure 2. Average Densities of *Colpidium* sp. (orange line) and *Bodo* sp. (green line) in Experiment 1 through time. *Colpidium* sp. and *Bodo* sp were added in the same initial densities in separate treatments in the low, medium and high initial density treatments. The graph on the left represents the densities of *Colpidium* sp. or *Bodo* sp. when introduced into separate predator-free communities. The graph on the right represents *Colpidium* sp. or *Bodo* sp. when they are introduced into separate communities that contain a predator. Triangles symbolize when the species was introduced at *high initial densities*, circles symbolize introduction at *medium initial densities*, and squares symbolize introduction at *low initial densities*. Vertical lines are standard error bars.

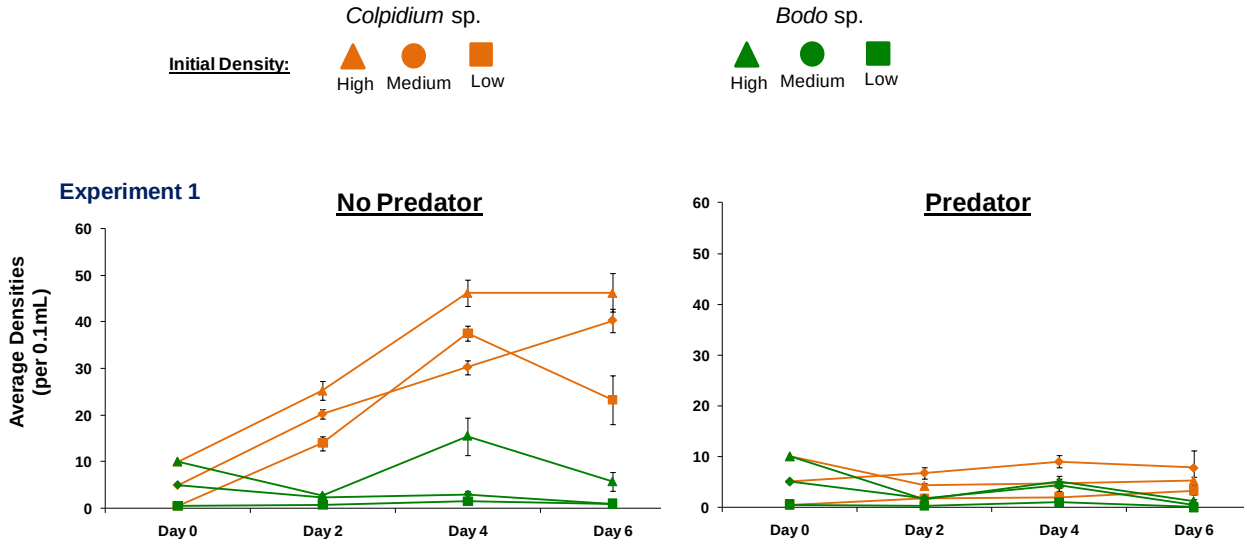


Figure 3. Average Densities of *Colpidium* sp. (orange line) and *Bodo* sp. (green line) in Experiment 2 through time. The graph on the left represents the densities of *Colpidium* sp. or *Bodo* sp. when introduced into separate predator-free communities. The graph on the right represents *Colpidium* sp. or *Bodo* sp. when they are introduced into separate communities that contain a predator. Triangles symbolize when the species was introduced at *high initial densities* and squares symbolize introduction at *low initial densities*. Vertical lines are standard error bars.

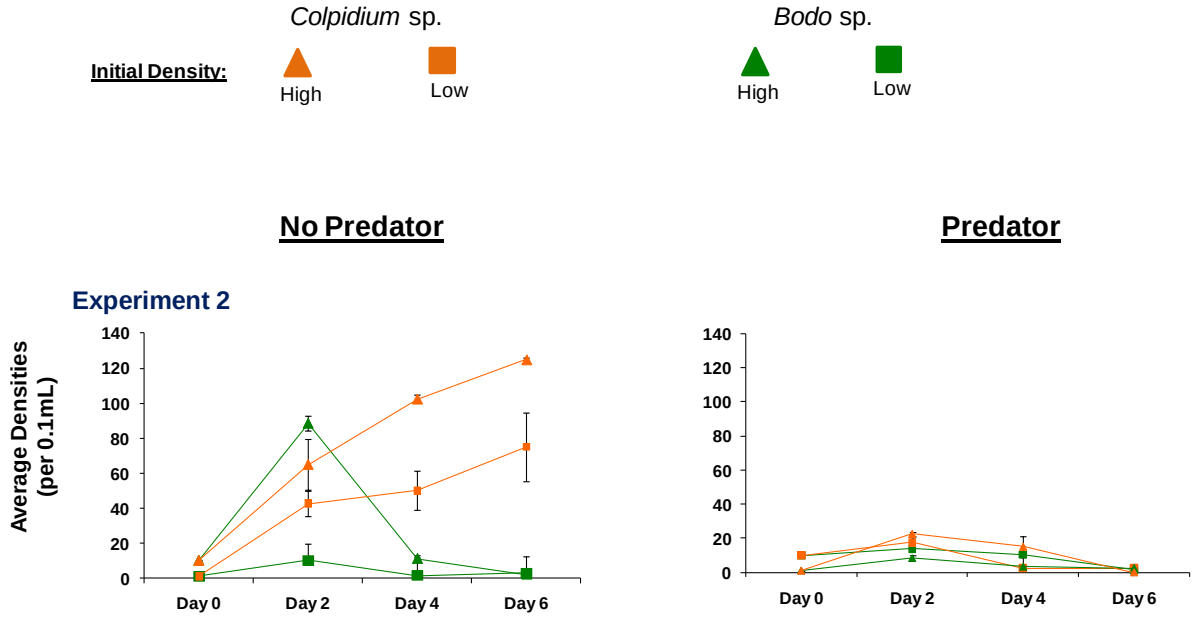
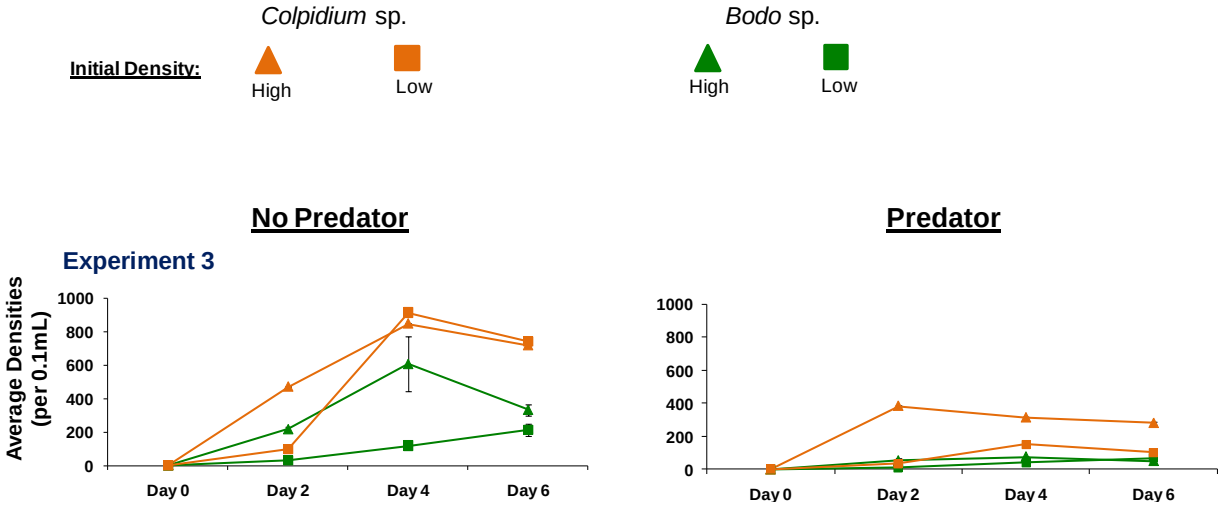


Figure 4. Average Densities of *Colpidium* sp. (orange line) and *Bodo* sp. (green line) in Experiment 3 through time. The graph on the left represents the densities of *Colpidium* sp. or *Bodo* sp. when introduced into separate predator-free communities. The graph on the right represents *Colpidium* sp. or *Bodo* sp. when they are introduced into separate communities that contain a predator. Triangles symbolize when the species was introduced at *high initial densities* and squares symbolize introduction at *low initial densities*. Vertical lines are standard error bars.



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Chapter 5

Community Consequence of Invasion by Primary Consumers

Abstract

Understanding how introduced species will impact community structure and dynamics once they have successfully invaded is complicated by the large combination of possible biotic and abiotic interactions occurring within a specific community and by the properties of the invading species. The large combination of possible outcomes have limited our ability to develop a generalizable framework for predicting the impact that newly introduced species will have on communities. By using the model system of the aquatic community held within the leaves of the pitcher plant *Sarracenia purpurea*, I tested the impacts that intermediate trophic level species (primary consumers) with different levels of competitive ability had on resident community dynamics and structure and whether the propagule pressure of the invading species and characteristics of the resident community affected the magnitude of impact. Results from this study showed that the impacts of species on a community it invades are case study specific and depend on resource availability and the trophic structure of the community and the competitive ability and initial density of the invader. The competitive dominant invader in the intermediate trophic level out-competed species in the resident community and decreased the density of its prey, however, it did not impact prey diversity, evenness or richness. The least competitive intermediate trophic level invader had no effect on the densities of the resident species, but did affect the evenness and diversity of prey (the bacteria). The effect it had on bacterial diversity and evenness depended on the resource availability in the community, the presence of a top predator and the initial density that the invader entered the community. In order to develop a general framework that can predict the effects of introduced species, more research needs to be done to understand the impacts of species with different traits and how characteristics of the resident community affect these impacts.

Introduction

The foundations of community ecology lie in understanding species interactions within a community, and the factors that drive general patterns across taxa and systems. Our understanding of community ecology has built on several classic ecological studies, which paved the way for ecologists to test the predictability of patterns across a large array of communities. Elton (1927) developed the concept of the niche, which led to research defining and describing the properties of species that drive competitive exclusion of species in a community (Volterra 1931, Lotka 1932, Gause 1932). Hutchinson (1957), MacArthur (1958), and Connell (1961) refocused research on the importance of the niche and niche overlap for determining whether species are able to coexist in a community. Hairston et al. (1960) showed the importance of top predators in regulating community dynamics, followed by Paine (1966, 1969) who demonstrated the importance of top predators in maintaining diversity in natural systems.

The exponential increase in the movement of species around the world due to human transport is resulting in new species being added to existing communities, potentially changing community structure and dynamics. To date, research has shown that introduced species can displace native species or reduce their abundance through both competition and predation, and disrupt ecological processes found in natural communities (e.g., Mack et al. 2000). However, most research examining the impact that introduced species have on community structure and dynamics have been case studies, field observations or anecdotal, limiting our ability to develop a generalizable framework for predicting the impact that newly introduced species will have on communities.

Determining the generalizability of the impact that species introductions will have on community structure and dynamics is complicated by the large combination of possible biotic interactions within a community. Similarly, introduced species with different traits can have

very different impacts on communities they enter. Competitive ability and susceptibility to predation have been highlighted as possible key traits of introduced species that are important for causing large impacts on resident community dynamics (e.g., Mack et al. 2000, Bohn et al. 2008). However, our ability to predict the community effects of specific traits of an invading species is limited.

By using the model system of the aquatic community held within the leaves of the pitcher plant *Sarracenia purpurea*, I tested the impacts that intermediate trophic level species (primary consumers) with different levels of competitive ability had on resident community dynamics and structure and whether the propagule pressure of the invading species affected the magnitude of impact. To test whether a top predator regulated the changes in community structure and dynamics produced by the introduced species, the impact of invasion on community structure and dynamics was tested in experimental communities with or without the presence of a predator. Experiments were also designed to test whether the amount of resources available to the lowest trophic level (bacteria) influenced the community-wide effects of introduced species.

Study System

The model aquatic system held within the leaves of *Sarracenia purpurea* (described in detail in Chapters 1, 2, 3, 4, and 6) was used for all experiments. This carnivorous plant is located in nitrogen poor habitats, and captures insects in pitcher-shaped leaves that fill with rainwater. Bacteria, protozoans, a rotifer species and dipteran larvae colonize this miniature aquatic habitat, and form a microscopic community and food web that resembles that of other aquatic systems, but on shorter temporal and spatial time scales (e.g., Heard 1994, Kneitel and Miller 2002, Gray et al. 2006).

Methods

Using natural bacteria and protozoans from pitcher plants in the field, replicate experimental communities were built in 50 ml macrocentrifuge tubes in the laboratory (Chapter 4). These communities served as resident communities in which either a competitively dominant protozoan or less competitive protozoan were introduced in high, medium or low densities (propagule pressure), either with or without a top predator, one larva of the mosquito, *Wyeomyia smithii,* in a factorial design (Chapter 4). Because I was also interested in testing whether the impacts of invasion by the competitive dominant and leastcompetitive species were due to the density or the biomass of the introduced species, and if resources of the resident community affected the impacts due to invasion, I conducted three different experiments. The invasion success of the competitive dominant and least competitive protozoan were assessed in Chapter 4. The impact that these protozoan invaders had on the resident community (densities of resident protozoans, resident culturable bacteria, and diversity, richness and evenness of resident culturable bacteria and bacteria OTUs) was assessed in this chapter.

Experiment 1 (conducted in 2008) examined invasion success of a competitively dominant protozoan and a less competitive protozoan under low resource conditions with a community composed of only culturable bacteria. Invaders were introduced into the resident communities at the same densities, with size difference of these two species not taken into account (Chapter 4). Experiment 2 was also under low resource conditions, but a larger subset of bacteria (culturable and unculturable) found in the pitcher plant community in the field were used instead of just culturable bacteria, and the densities of the invaders was adjusted to take into account a 10 fold difference in biomass between these two species (Chapter 4). Experiment 3 had the same

experimental treatments as Experiment 2 except that resource availability was high (Chapter 4). The experimental setup for all three experiments is illustrated in Figure 1 of Chapter 4.

Experimental Treatments

In Chapter 4 I used these same experimental communities to test factors that have been hypothesized to affect the invasion success of intermediate trophic level species (primary consumers). Here I used these same treatments to examine the impact of the two introduced protozoans, *Colpidium* sp. and *Bodo* sp., on the communities they invaded. Experiment 1 had a total of 12 treatments plus controls, replicated 4 times each, and were monitored every other day for six days. The following experimental treatments were applied after the resident communities stabilized: 1) high *Colpidium* sp. density (1000 individuals, 100 / ml), 2) medium *Colpidium* sp. density (500 individuals, 50 / ml), 3) low *Colpidium* sp. density (50 individuals, 5 / ml), 4) high *Bodo* sp. density (1000 individuals), 5) medium *Bodo* sp. density (500 individuals), 6) low *Bodo* sp. abundance (50 individuals), plus a control with no addition of *Colpidium* sp. or *Bodo* sp. All six treatments were repeated and one *Wyeomyia smithii* mosquito larva was added to each replicate of each treatment (treatments 7-12) to test the effects of a top predator in this system (Chapter 4, Table 1).

In Experiment 2 and 3, because *Colpidium* sp. is approximately 10 times larger than *Bodo* sp., the propagule pressure treatments included equal biomass of *Colpidium* sp. and *Bodo* sp. rather than equal numbers to take into account the 10 fold differences in size of these two protozoans. There were a total of 8 treatments for Experiments 2: 1) high initial density of *Colpidium* sp. with 100 individuals (10 / ml), 2) high initial density of *Bodo* sp. with 1000 individuals (100 / ml), 3) low initial density of *Colpidium* sp. with 10 individuals (1 / ml), 4) low

initial density of *Bodo* sp. with 100 (10 / ml), plus a control without protozoans added. Treatments 5 - 8 were identical to Treatments 1 - 4, but one mosquito larva was added to each as in Experiment 1. A control community with a mosquito present was also used (Chapter 4, Table 2). Experiment 3 had the same treatments as Experiment 2, but, unfortunately, all replicates of the controls in Experiment 3 were contaminated with *Bodo* sp., and could not be used for analyses (Chapter 4, Table 3). Experiment 2 used the same resource input as in Experiment 1 (4 ants at the start of the experiment). For Experiment 3 (high resource availability), 4 ants were added to initiate the resident community, and then one autoclaved ant was added daily throughout the experiment to all replicates of all treatments. The treatments were replicated 4 times in Experiment 2 and 3 times in Experiment 3 due to contamination and financial limitations with genomic sequencing.

Development of Resident Communities

The development of the resident communities and the initiation of the treatments are described in Chapter 4, and summarized here. Protozoan species and culturable bacteria were collected from water inside pitcher plant leaves in Cranberry Bog Preserve in Riverhead, NY (40.90°, 72.67°). Five protozoan species and seven culturable morphotypes of bacteria that are common in pitcher plant aquatic communities throughout the plant's native geographic range (Buckley et al. 2003) were isolated from the collected water and kept in monocultures maintained on a 12hr light/dark cycle at 27°C. These are the same isolates used in experiments for Chapters 4 and 6, and were present in data in Chapters 2 and 3.

The relative competitive ability of the 5 isolated protozoan species were determined (Chapter 6) and the competitive dominant *Colpidium* sp. and least competitive *Bodo* sp. were used as the

invader treatment in the three experiments. The three remaining protozoans, *Chrysomonad* sp., *Colpoda* sp., and *Cyclidium* sp., were used to form the resident community. The bottom trophic level of the community contained either the 7 culturable bacteria obtained from the field and held in monocultures (Experiment 1) or a large diversity of both culturable and unculturable bacteria that were collected from water held within pitcher plant leaves at the field site just prior the start of Experiments 2 and 3.

When only culturable bacteria were used (Experiment 1), the monocultures that were selected contained morphotypes that were the most distinct when grown on agar plates, allowing me to correctly assess changes in diversity, evenness, species richness and abundances of the bacteria with plate count techniques. This technique was also used to assess culturable bacteria dynamics in Chapters 2, 3 and 6. The culturable bacteria used were two Gammaproteobacteria (*Enterobacter* sp. AR19, *Serratia* sp. 9A_5), two Betaproteobacteria (*Chromobacterium violaceum*, *Aquitalea magnusonii*), and one Bacteroidetes (*Chryseobacterium* sp. COLI2) and one Actinobacteria (*Leifsonia xyli*).

When a larger subset of the bacterial community (both culturable and unculturable bacteria) was used to form the bottom trophic level of the resident community, water held within randomly selected leaves of *S. purpurea* was collected until enough water was obtained to create all resident communities for the experiment. In order to remove detritus, insect larvae, or other members of the aquatic pitcher plant community and just retain the bacteria, the water collected in the field was pooled and filtered through 4 different sized filters (sterilized 233 µm, 8 µm and 0.8 µm Millipore filters, and a final filtration of 0.7 µm Glass Fiber GF/F Millipore filter).

For each experiment, the species forming the resident communities were pooled into one large, sterile (autoclaved) container. In Experiment 1, autoclaved deionized water was used.

Filtered pitcher plant water was used in Experiments 2 and 3. In all experiments, the pooled community of protozoans and bacteria was mixed continuously to homogenize the community, and 10 ml aliquots were distributed into sterile 50 ml experimental macrocentrifuge tubes. The macrocentrifuge tubes contained 2 ml of glass beads (3mm diameter with 1mm hole in the center), mimicking the habitat complexity found in the bottom of pitcher plant leaves as a result of the exoskeletons of decomposed insects, which reduces the rate at which the top predator consumes the protozoans in the community (Gray, unpublished data; terHorst 2010). Four dead, autoclaved ants, *Tapinoma sessile*, were added initially to each experimental resident community as a nutrient source.

The completed experimental resident communities consisted of ants, either seven culturable bacteria species with deionized water or a bacterial soup of pitcher plant water and culturable and unculturable pitcher plant bacteria, and three protozoan species. These communities were then incubated in a growth chamber for 72 hours at 27°C (12h light/dark cycle) before the addition of the experimental treatments, allowing for a turnover of approximately 18 bacterial generations and 7 protozoan generations.

Sampling Methods of the Resident Community

The treatments containing a mosquito larva were checked daily to ensure mosquitoes had not died or pupated. No mosquitoes required replacement during any of the experiments. All experiments had the same sampling protocol for the resident protozoan and culturable bacteria, and Experiment 2 and 3 used the same sampling protocol for producing the 16s rRNA clone libraries. To assess the dynamics of resident protozoans and culturable bacteria (including Experiments 2 and 3, where field-collected bacterial communities were used), experimental

communities were sampled every 2 days for 6 days. For sampling, communities and beads were gently mixed and an aliquot (0.1 ml) from each tube was used to count the densities of each species of protozoan with a compound microscope. The culturable bacteria were sampled in the same manner as in Chapter 2, 3, and 6, in which serial dilutions of a 0.1 ml aliquot of each resident community were plated on agar plates. The culturable bacteria were allow to grow on the agar plates for 72 hours (27°C) and then densities of each morphotype and morphotype richness was assessed by counting the bacteria that grew on the plates.

16s rRNA Bacteria Cloning

To determine the relative abundance and diversity of bacterial species (OTUs) in Experiment 2 and 3, I used 16s rRNA clone libraries. Due to financial limitations restricting the number of clone libraries that could be sequenced, clone libraries were generated for only two replicates of each treatment in Experiments 2 and 3. The resident communities in these experiments contained a high diversity of environmental bacteria, who could only be identified through genomic methods (Chapter 2). Clone libraries were developed for 2 replicates of each treatment in both experiments on the last day of the experiment (Day 6). A Day 0 Control resident bacterial community (2 replicates each) was also used for cloning in both experiments to assess variability in experimental bacterial communities before the treatments were initiated.

On Day 6 of Experiments 2 and 3, for each replicate of each treatment, after the aliquot for quantifying protozoans was collected, the entire experimental community was filtered onto a 0.22 µM Isopore membrane filter (Millipore, Billerica, Massachusetts) to collect bacterial cells. I then extracted microbial community DNA from the filters using the Ultra Clean Soil DNA kit

according to manufacturer's instructions (Mo Bio Laboratories, Solana Beach, California), with the exception that instead of using soil, I used the filter containing the bacteria.

After DNA extraction, aliquots of purified DNA were PCR amplified using the *Bacteria* domain-specific SSU rRNA gene primers 27F (5'-AGA GTT TGA TCM TGG CTC AG -3') (Johnson 1994) and 1392R (5'-ACG GGC GGT GTG TAC-3') (Wilson et al. 1990) as previously described by Akob et al. (2007). PCR products were purified with the Qiagen Gel Extraction Kit (QIAGEN, Valencia, CA), and ligated into the TOPO TA cloning vector pCR 4.1. Ligations were then cloned according to manufacturer's instructions (Invitrogen, Carlsbad, CA). Clones containing the insert of pitcher plant bacteria DNA had successfully grown on agar plates after 24 hrs. A subset of these clones was selected and PCR amplified to check for false-positive inserts. Forty-eight clones per replicate where an insert of pitcher plant bacteria DNA had been verified were picked from the agar plates and placed into 96 well plates. These clones were sent to Sequetech Sequencing Center (Mountain View, CA), where they were sequenced with a one-way read using the primer 907R.

Using Sequencher v4.8, vector sequences flanking the SSU rRNA gene inserts were removed (Gene Codes Corp., Ann Arbor, MI). All clone sequences were aligned with the alignment tool by Greengenes (DeSantis et al. 2006), accessed at http://greengenes.lbl.gov. Clones were grouped into phylotypes based on a sequence similarity cut off of 97% using the program FastGroupII (Yu et al. 2006), which allowed for an Analysis of Similarity (ANOSIM) to be conducted in Primer 6.1 (PRIMER 6, Version 6.1.6, Primer E-Ltd.) to determine the community similarity of bacterial OTUs among treatments within and across Experiment 2 and 3.

Community Composition and Diversity Indices

I used Primer 6.1 to calculate both culturable bacteria and bacterial OTU diversity (Shannon Index), species (OTU and culturable) richness, and Pielou's evenness for treatments and across experiments. Nonparametric multivariate statistics were used to determine the similarity in bacterial community structure among treatments within experiments and to test for generalizable patterns for bacteria community composition across experiments. For similarity in abundances of individual bacteria species, data were first normalized using a square root transformation and Bray-Curtis distances were then calculated (Bray and Curtis 1957). A non-metric Multi-Dimensional Scaling (MDS) ordination was used to graphically visualize differences among bacterial communities. Communities that were more similar are spatially close to each other on a MDS plot and those that were less similar are spatially separated. An Analysis of Similarity (ANOSIM) was performed to calculate a Global R, which determined the overall similarity between communities, with a value of 1 representing extreme dissimilarity and a value of 0 representing complete overlap in community structure.

Statistical Analysis

Factorial ANOVAs with data from the last day of each experiment (Day 6) were used to test for treatment effects on the densities of resident protozoans and culturable bacteria. Data were normalized with log or square root transformations and assumptions for ANOVA (independence of samples, residuals are normally distributed, and homoscedasticity) were checked before the ANOVAs were performed using the program Statistica 6.1. Because the clone library technique provides relative abundances of OTUs, not absolute abundances, similar analyses could not be used for the bacterial OTU part of the community in Experiments 2 and 3. Because only 2 replicates were used for OTU data, bonferroni corrected pairwise T-Tests were used.

Results

Resident Protozoan Density

In all experiments, resident protozoan density was lower in communities invaded by the competitive dominant *Colpidium* sp. relative to communities invaded by *Bodo* sp. (Table 1, 2, 3, 4, Figure 1; Experiment 1 $p = 0.000022$, Experiment 2 $p = 0.071$ (marginally significant), Experiment 3 $p = 0.000029$). In Experiment 1, resident protozoan densities in predator-free communities invaded by *Colpidium* sp. were also significantly (or marginally significant) lower than resident protozoan communities in predator-free Control communities (Figure 1, one way ANOVA Resident Protozoan Densities in predator-free Control communities vs. predator-free communities with *Colpidium* sp. introduced at a high initial density: $F(1, 6) = 6.10$, $p = 0.048$; Control community vs. *Colpidium* sp. introduced at medium initial density: $F(1, 6) = 5.69$, $p = 0.054$ (marginally significant); Control community vs. *Colpidium* sp. introduced at low initial density: $F(1, 6) = 3.79$, $p = 0.09$). In Experiment 2, resident protozoan densities in predator-free communities invaded by *Colpidium* sp. were not significantly different than resident protozoan densities in predator-free control communities (Figure 1, one way ANOVA Resident Protozoan Densities in predator-free control communities vs. predator-free communities with *Colpidium* sp. introduced at a high initial density: $F(1, 6) = 0.949$, $p = 0.36$; Control community vs. *Colpidium* sp. introduced at low initial density: $F(1, 6) = 0.42$, $p = 0.54$). No controls were available for comparison in Experiment 3.

In Experiment 1 (Low Resource) and Experiment 3 (High Resource) the density of resident protozoans was different in the presence versus absence of a predator, and depended on which species (*Colpidium* sp. or *Bodo* sp.) invaded (Species*Predator) (Table 1, 3, 4, Figure 1a,c; Experiment 1 p value = 0.007, Experiment 3 p value = 0.012). Resident protozoan density was

higher when a top predator was present in communities invaded by *Colpidium* sp. compared to resident protozoan densities in communities invaded by *Bodo* sp. (Figure 1). When a predator was present in Experiment 1, the resident protozoan densities in communities invaded by either *Bodo* sp. or *Colpidium* sp. were not significantly different than resident protozoan densities in predator-present Control communities (Figure 1, Resident protozoan densities in predator-present control communities vs. predator-present *Bodo* sp. low initial density communities: $F(1, 6) = 4.08$, $p = 0.089$; Control vs. *Bodo* sp. medium initial density: $F(1, 6) = 0.86$, $p = 0.39$; Control vs. *Bodo* sp. high initial density: $F(1, 6) = 0.14$, $p = 0.72$; Resident protozoan densities in predator-present control communities vs. predator-present communities invaded by *Colpidium* sp. at low initial density: $F(1, 6) = 0.61$, $p = 0.46$; Control vs. *Colpidium* sp. medium initial density: $F(1, 6) = 1.40$, $p = 0.28$; Control vs. *Colpidium* sp. high initial density: $F(1, 6) = 0.89$, $p = 0.38$). The same comparison could not be made in Experiment 3 because there were no controls in this experiment due to contamination. These same effects were not found in Experiment 2, which could be due to the overall low densities of resident protozoans across treatments and the variation among replicates.

In Experiment 1, the resident protozoan densities in communities invaded by *Colpidium* sp. were significantly higher when a predator was present than when a predator was absent from the community (Figure 1a, Factorial ANOVA, Predator Main Effect: $F(1, 18) = 14.27$, $p = 0.0014$). The presence of a predator in communities invaded by *Bodo* sp. had no effect on resident protozoan densities (Figure 1a, Factorial ANOVA, Initial Density: $F(1, 18) = 0.34$, $p = 0.72$; Predator: $F(1, 18) = 2.65$, $p = 0.12$; Initial Density*Predator: $F(1, 18) = 1.29$, $p = 0.29$).

Tukey results can be found in Appendix 1 for Experiment 1 and Appendix 2 for Experiment 3. There were no significant Tukey results for Experiment 2.

Culturable Bacteria Density

In low resource environments (Experiment 1 and 2), the density of resident culturable bacteria was lower when the competitive dominant *Colpidium* sp. was introduced to the community than when *Bodo* sp. invaded the community (Table 5, 6, 8, Figure 1a,b, Experiment 1 $p < 0.00001$, Experiment 2 $p = 0.019$). This effect was not found in high resource environments (Table 7, 8, Figure 1c, Experiment 3 $p = 0.21$).

In low resource communities with only culturable bacteria (Experiment 1), there was a significant effect of the predator on the density of bacteria, but the direction of this effect depended on which protozoan consumer invaded the community (Species*Predator interaction, Table 5, Figure 1). In Experiment 1, culturable bacteria increased in density when the community had a top predator and was invaded by *Colpidium* sp. but this same effect was not found when *Bodo* sp. was introduced (Figure 1a). In predator-present communities invaded by either *Colpidium* sp. or *Bodo* sp., bacterial densities were not significantly different than bacterial densities in predator-present Control communities (Experiment 1, one-way ANOVA, bacteria densities in predator-present control communities vs. communities with *Bodo* sp. introduced at low initial density: $F(1, 6) = 0.62$, $p = 0.46$; Control vs. *Bodo* sp. medium initial density = $F(1, 6) = 0.0003$, $p = 0.99$; Control vs. *Bodo* sp. high initial density = $F(1, 6) = 1.18$, $p = 0.32$), Control vs. *Colpidium* sp. low initial density = $F(1, 6) = 0.97$, $p = 0.36$; Control vs. *Colpidium* sp. medium initial density = $F(1, 6) = 0.605$, $p = 0.47$; Control vs. *Colpidium* sp. high initial density = $F(1,6) = 0.15$, $p = 0.71$). Experiment 2 also had a main predator effect, but no significant interaction as was found in Experiment 1 (Table 6, $p = 0.037$).

In Experiment 1, in communities invaded by *Colpidium* sp., independent of the initial density that *Colpidium* sp. was introduced, bacterial densities increased when a predator was present in

the community (Figure 1a, Day 6 Factorial ANOVA, $F(1, 18) = 8.96$, $p = 0.007$). This same effect was not seen in communities invaded by *Bodo* sp. (Figure 1a, Day 6 Factorial ANOVA, Initial Density: $F(1,18) = 1.37$, $p = 0.28$; Predator: $F(1, 18) = 0.93$, $p = 0.34$; Initial Density*Predator: $F(1, 18) = 0.75$, $p = 0.48$).

Tukey results can be found in Appendix 3 for Experiment 1 and Appendix 4 for Experiment 2. There were no significant Tukey results for Experiment 3.

Culturable bacteria community similarity

In Experiment 1, the community structure for culturable bacterial (abundance data) was different for communities invaded by *Colpidium* sp. than for communities invaded by *Bodo* sp. (ANOSIM Global R = 0.375, p value = 0.001; Figure 2a). Although not statistically significant (ANOSIM Global R = 0.005, p value = 0.489), a similar pattern was seen for community structure in the high resource experiment (Experiment 3; Figure 2c). Communities were not significantly different in Experiment 2 (Low Resource).

Bacteria OTU community similarity

In the low resource experiment (Experiment 2), the presence of a top predator had a significant effect on bacterial community structure (Figure 3, Presence/Absence ANOSIM = 0.462, p value = 0.004; Abundances ANOSIM = 0.46, p value = 0.005), with each community containing a top predator having similar bacteria abundances and species identity than when compared to communities that were predator free. This was the case for all predator-free communities, except when *Bodo* sp. was invaded at a high propagule pressure (Figure 3), which were more similar in bacterial structure to communities that contained a top predator. When

resources were high (Experiment 3), there were no significant differences in bacterial OTU community composition and structure across all treatments (Experiment 3, Figure 3, Presence/Absence ANOSIM = 0.117, p value = 0.21; Abundance ANOSIM = 0.102, p value = 0.242).

Bacteria Diversity, Evenness and Richness

In general, the patterns of diversity, evenness and species richness among treatments were similar when only culturable bacteria were examined (from agar plate counts) or when both culturable and unculturable bacteria were included (metagenomic clone libraries). As expected, the number of OTUs found by metagenomics was higher than the number of culturable species (7 total) found on agar plates. In most cases, the variance among replicates was large for the culturable bacteria, especially for the few situations when the trend in diversity, evenness and richness patterns differed between culturable bacteria and bacteria OTUs and when culturable bacteria evenness was assessed. This result suggests that, in general, many more replicates are needed if culturable bacteria are to be used to detect patterns of diversity for field-collected bacterial samples.

Bacteria OTU Diversity

The presence of *Colpidium* sp. did not have a significant effect on the diversity of bacteria within experimental communities at any density for low and high resource conditions (Figure 4b, T-Test Results in Appendix 5). Bacterial diversity was significantly lower in low resource communities when a predator was present compared to communities with high resource

availability, independent of the initial density that *Colpidium* sp. was introduced. (Figure 4b, Appendix 6).

The experimental results were different for *Bodo* sp.. Overall, the diversity of bacteria was significantly lower in low resource communities invaded by *Bodo* sp. than high resource communities (Appendix 6, Figure 4a). This result was consistent for both densities of initial introduction and if a predator was present or absent (Appendix 6, Figure 4a).

Evenness

As was found for diversity, *Colpidium* sp. had no significant effect on bacterial evenness in predator-free environments with either high or low resource availability, independent of the initial density it is introduced (Figure 5b, Appendix 7). Bacterial evenness was significantly lower in low resource communities containing a predator, independent of the initial density that *Colpidium* sp. was introduced in (Figure 5b, Appendix 8).

When resources were high, the presence of *Bodo* sp. did not affect evenness for either density of introduction, in the presence or absence of the predator (Figure 5a, Appendix 7). When resources were low, bacterial evenness was significantly lower in communities invaded by *Bodo* sp. at a high initial density than in communities invaded by *Colpidium* sp. at a high initial density. This result was found both in the presence and absence of a top predator (Figure 5a, Appendix 7, Appendix 8).

Species Richness

Bacterial richness was unaffected by any treatment except for resource availability (Figure 6a, b, Appendix 9, Appendix 10). Bacterial richness was significantly lower in low resource

communities than high resource communities across all treatments, except when *Bodo* sp. invaded at a low initial density into a predator free community (Figure 6a,b, Appendix 10).

Discussion

Introduced species are thought to be one of the greatest threats to native species biodiversity, community structure and dynamics (Elton 1958, Vitousek et al. 1996, Mack et al. 2000). Yet, to date, the ability to predict which species will have the largest impact on a system is lacking (Moyle and Light 1996, Parker et al. 1999). Although progress has been made, it is still not known if or under what conditions the impacts of an invader will be due to the traits of that species or specific conditions within the system that is invaded.

By using an experimental *S. purpurea* model system, I found that when the competitive dominant protozoan, *Colpidium* sp., was introduced into a community, with or without a top predator, the abundance of resident protozoans declined compared to communities invaded by the least competitive species. This decline in resident protozoan density was most likely due to competition from *Colpidium* sp.. When a top predator was present in the community and resource availability was low, the densities of the resident protozoans depended on the competitive ability of the invader. In low resource communities invaded by the competitive dominant *Colpidium* sp., resident protozoan densities increased when a predator was present. The resulting densities matched those of control communities, not invaded by *Colpidium* sp. or *Bodo* sp.. In these low resource communities, the presence of a predator reduced the densities of *Colpidium* sp. (Chapter 4), and created a trophic cascade in which the bacteria increased in abundance. The magnitude of this trophic cascade was greatest in communities with the greatest *Colpidium* sp. propagule pressure. Even with a trophic cascade occurring, in low resource

communities the bacterial densities were higher in communities invaded by the least competitive consumer, *Bodo* sp., independent of the presence of a predator. When resource availability was high, bacterial density was unaffected by the competitive ability of the invading consumer or the presence of a predator.

Unlike *Colpidium* sp., introduction of the competitive subordinate, *Bodo* sp., did not have an effect on resident protozoan densities. This result was consistent, whether introduction was at a low density and established densities of *Bodo* sp. were low, (Chapter 4), or when *Bodo* sp. was introduced at high densities or into a high resource community, and its establishment success increased (Chapter 4). Predation pressure by the top predator on primary consumers increased when *Bodo* sp. was present in the community in high densities (Chapter 4), which resulted in lower density of the resident protozoan. Unfortunately, it is not possible to determine if these results are different than the control. However, results from Chapter 4 suggest that the top predator in this system, the larva of the mosquito *Wyeomyia smithii*, feeds at a greater rate when food is readily available and easily accessible.

The effect on bacterial diversity, evenness, or richness also depended on the type of consumer that invaded and the resource availability of the community. When the competitive dominant *Colpidium* sp. invaded, it decreased culturable bacteria densities, but had no affect on bacteria diversity, evenness, or richness. This result occurred in all cases except when resources were low and a top predator was present in the resident community. In these communities, bacterial diversity and evenness was significantly lower compared to communities with high resource availability, independent of the initial density that *Colpidium* sp. was introduced.

The experimental results were different for *Bodo* sp.. Overall, the diversity of bacteria was significantly lower in low resource communities invaded by *Bodo* sp. than high resource

communities. This result was consistent for both densities of initial introduction and if a predator was present or absent. Bacterial evenness was only affected in communities invaded by *Bodo* sp. if resources were low and *Bodo* sp. was introduced at a high propagule pressure, irrespective of the presence or absence of a predator.

Neither *Colpidium* sp. nor *Bodo* sp. affected bacterial richness. Richness was affected by the resource availability of the community. Bacterial richness was significantly lower in low resource communities than when compared to high resource communities.

These results show that although certain properties of a species and a community are needed for a successful invasion to occur (Chapter 4), the affect that a species has on the community once it has invaded depends on the resource availability and trophic structure of the community and the competitive ability and initial density of the invader. The impact of invasion on community structure, at least as shown with the *S. purpurea* system, is specific to the particular properties of the community and of the invader. To develop a general framework that can predict the effects of introduced species we need to understand the impacts of species with different traits, which will allow us to understand whether there are commonalities in how invaders impact the structure of communities they successfully invade.

The role of competition in driving community structure and dynamics has guided ecological research since Volterra (1931), Lotka (1932), and Gause (1932), and has been assumed to be an important driver of community assembly patterns (Diamond 1975). Competitive exclusion has also been shown to occur in communities when a competitive dominant introduced species successfully establishes in a community (e.g., Sanders et al. 2003, Bohn et al. 2008). Similar results were found here for the *S. purpurea* system. Invasive species are also thought to impact community structure by consuming prey and reducing the abundance and diversity of species in

lower trophic levels. In the study presented here, the competitive dominant invader did reduce the abundance of species in the lower trophic level, but had little effect on diversity, evenness or richness of its prey. Invasion by the least competitive species had a different impact on community structure. For example, by being present in high densities, the presence of the less competitive species triggered the top predator to increase its feeding rate, decreasing the density of resident protozoans and affecting the evenness of diversity of the bottom trophic level species.

To begin to build a general framework to predict the impacts that introduced species will have on communities they invade, we need more research that includes testing of the relative roles of multiple factors including propagule pressure, enemy release, the role of competitive ability and resource availability, rather than single hypothesis testing. Especially needed are studies that examine both the impacts of propagule pressure and competitive ability of an invader, alone and together on communities they invade. It is also important to determine if similar or different impacts are seen across trophic levels within communities invaded by species with these important traits.

Table 1. Experiment 1 ANOVA results. Factors included Species (*Colpidium* sp. or *Bodo* sp.), Initial Density (low, medium, high), and predator (presence or absence). The response variable was the total density of resident protozoans on day 6 of the experiment. Significant p values are in red.

	SS	df	MS	F	p
Intercept	378.79	1	378.78	327.14	0.000000
Species	27.59	1	27.59	23.83	0.000022
Initial Density	2.33	2	1.16	1.01	0.38
Predator	0.048	1	0.048	0.04	0.84
Species*Initial Density	0.90	2	0.45	0.38	0.68
Species*Predator	9.39	1	9.39	8.11	0.007
Initial Density*Predator	1.54	2	0.77	0.66	0.52
Species*Initial Density*Predator	4.72	2	2.36	2.04	0.14
Error	41.68	36	1.18		

Table 2. Experiment 2 ANOVA results. Factors included Species (*Colpidium* sp. or *Bodo* sp.), Initial Density (low, high), and predator (presence or absence). The response variable was the total density of resident protozoans on day 6 of the experiment. None of the main effects or interactions were statistically significant. The only factor with a marginally significant p value was the invading species, which is in blue.

	SS	df	MS	F	p
Intercept	82.10	1	82.10	67.04	0.00
Species	4.38	1	4.38	3.58	0.07
Initial Density	0.49	1	0.47	0.40	0.53
Predator	0.003	1	0.003	0.002	0.96
Species*Initial Density	0.009	1	0.009	0.007	0.93
Species*Predator	0.20	1	0.20	0.16	0.69
Initial Density*Predator	1.24	1	1.24	1.01	0.32
Species*Initial Density*Predator	1.20	1	1.20	0.98	0.33
Error	29.39	24	1.22		

Table 3. Experiment 3 ANOVA results. Factors included Species (*Colpidium* sp. or *Bodo* sp.), Initial Density (low, high), and predator (presence or absence). The response variable was the total density of resident protozoans on day 6 of the experiment. Significant p values are in red.

	SS	df	MS	F	p
Intercept	371.32	1	371.32	112.23	0.00
Species	110.25	1	110.25	33.32	0.000029
Initial Density	0.052	1	0.052	0.016	0.90
Predator	24.00	1	24.00	7.25	0.016
Species*Initial Density	0.23	1	0.23	0.070	0.79
Species*Predator	26.53	1	26.53	8.019	0.012
Initial Density*Predator	2.42	1	2.42	0.73	0.41
Species*Initial Density*Predator	0.26	1	0.26	0.08	0.78
Error	52.93	16	3.31		

Table 4. Summary of the ANOVA results for experiments 1, 2 and 3. The results are displayed by experiment, with the significant results from *Experiment 1: Culturable Bacteria, Low Resources, Size Difference of Introduced Species Not Adjusted* in the first column, significant results from *Experiment 2: All Bacteria, Low Resources, Size Difference of Introduced Species Adjusted* in the second column, and significant results from *Experiment 3: All Bacteria, High Resources, Size Difference of Introduced Species Adjusted* in the third column. Significant p values are in red and p values considered marginally significant are in blue. When a significant result was found in all three experiments, the row is highlighted in light blue. When a significant result was found in 2 experiments, the row is highlighted in light purple. Significant results that were only found in one experiment are not highlighted. When results were non-significant, they are represented in the table as 'NS'.

	Low Resource, Culturable Bacteria, Size Difference of Introduced Species Not Adjusted	**Low Resource, All Bacteria, Size Difference of Introduced Species Adjusted**	**High Resource, All Bacteria, Size Difference of Introduced Species Adjusted**
Species	P = 0.000022 F(1, 36) = 23.8	P = 0.071 F(1, 24) = 3.58	P = 0.000029 F(1, 16) = 33.3
Initial Density	NS	NS	NS
Predator	NS	NS	P = 0.0160 F(1, 16) = 7.25
Species*Initial Density	NS	NS	NS
Species*Predator	P = 0.00723 F(1, 36) = 8.02	NS	P = 0.0120 F(1, 16) = 8.11
Initial Density*Predator	NS	NS	NS
Species*Initial Density*Predator	NS	NS	NS

Table 5. Experiment 1 ANOVA results. Factors included Species (*Colpidium* sp. or *Bodo* sp.), Initial Density (low, medium, high), and predator (presence or absence). The response variable was the total density of culturable bacteria on day 6 of the experiment. Significant p values are in red.

	SS	df	MS	F	p
Intercept	12829.86	1	12829.86	732.0828	0.000000
Species	563.79	1	563.79	32.17	0.000002
Initial Density	70.26	2	35.13	2.00	0.15
Predator	5.25	1	5.25	0.30	0.59
Species*Initial Density	29.27	2	14.64	0.84	0.44
Species*Predator	88.86	1	88.86	5.07	0.03
Initial Density*Predator	53.68	2	26.84	1.53	0.23
Species*Initial Density*Predator	3.12	2	1.56	0.089	0.91
Error	630.91	36	17.53		

Table 6. Experiment 2 ANOVA results. Factors included Species (*Colpidium* sp. or *Bodo* sp.), Initial Density (low, high), and predator (presence or absence). The response variable was the total density of culturable bacteria on day 6 of the experiment. Significant p values are in red.

	SS	df	MS	F	p
Intercept	1227.652	1	1227.65	233.79	0.000000
Species	33.210	1	33.21	6.32	0.019
Initial Density	8.687	1	8.69	1.65	0.21
Predator	25.458	1	25.46	4.85	0.037
Species*Initial Density	3.878	1	3.88	0.74	0.40
Species*Predator	0.516	1	0.52	0.098	0.76
Initial Density*Predator	2.626	1	2.63	0.50	0.49
Species*Initial Density*Predator	15.946	1	15.95	3.037	0.094
Error	126.027	24	5.25		

Table 7. Experiment 3 ANOVA results. Factors included Species (*Colpidium* sp. or *Bodo* sp.), Initial Density (low, high), and predator (presence or absence). The response variable was the total density of culturable bacteria on day 6 of the experiment. None of the main effects or interactions were statistically significant. P values that were considered marginally significant are in blue.

	SS	df	MS	F	p
Intercept	4598.45	1	4598.45	241.62	0.000000
Species	31.70	1	31.70	1.67	0.21
Initial Density	**71.73**	**1**	**71.73**	**3.77**	**0.07**
Predator	3.10	1	3.10	0.16	0.69
Species*Initial Density	43.20	1	43.20	2.27	0.15
Species*Predator	37.86	1	37.86	1.99	0.18
Initial Density*Predator	43.53	1	43.52	2.29	0.15
Species*Initial Density*Predator	0.92	1	0.92	0.048	0.83
Error	304.51	16	19.032		

Table 8. Summary of the ANOVA results from experiments 1, 2 and 3. The results are displayed by experiment, with the significant results from *Experiment 1: Culturable Bacteria, Low Resources, Size Difference of Introduced Species Not Adjusted* in the first column, significant results from *Experiment 2: All Bacteria, Low Resources, Size Difference of Introduced Species Adjusted* in the second column, and significant results from *Experiment 3: All Bacteria, High Resources, Size Difference of Introduced Species Adjusted* in the third column. Significant p values are in red and p values considered marginally significant are in blue. When a significant result was found in all three experiments, the row is highlighted in light blue. When a significant result was found in 2 experiments, the row is highlighted in light purple. Significant results that were only found in one experiment are not highlighted. When results were non-significant, they are represented in the table as 'NS'.

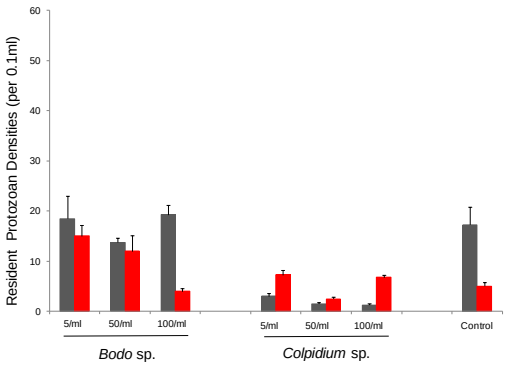
	Low Resource, Culturable Bacteria, Size Difference of Introduced Species Not Adjusted	**Low Resource, All Bacteria, Size Difference of Introduced Species Adjusted**	**High Resource, All Bacteria, Size Difference of Introduced Species Adjusted**
Species	P < 0.00001 F(1, 36) = 32.2	P = 0.0190 F(1, 24) = 6.32	NS
Initial Density	NS	NS	P = 0.070 F(1, 16) = 3.77
Predator	NS	P = 0.0375 F(1, 24) = 4.85	NS
Species*Initial Density	NS	NS	NS
Species*Predator	P = 0.030 F(1, 36) = 5.07	NS	NS
Initial Density*Predator	NS	NS	NS
Species*Initial Density*Predator	NS	NS	NS

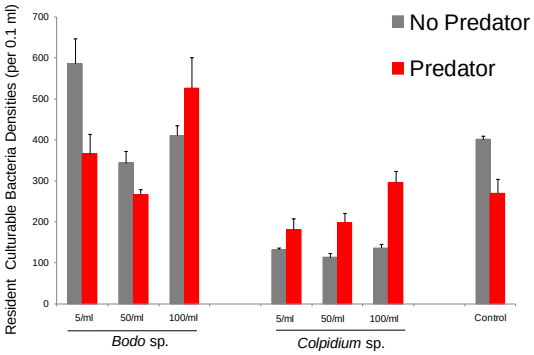
Figure 1. Resident protozoan densities (left column) and culturable bacteria densities (right column) when either *Bodo* sp. or *Colpidium* sp. are added to the community, with and without a top predator. **A)** Experiment 1: Low Resources, **B)** Experiment 2: Low Resources, and **C)** Experiment 3: High Resources. Gray bars represent resident protozoan and culturable bacteria densities (average number per 0.1 ml) when a predator was absent, and red bars represent average densities when a predator was present. Four replicates were used in Experiment 1 and Experiment 2 and three replicates were used in Experiment 3. Vertical lines are standard error bars. In general, the competitive dominant *Colpidium* sp. suppressed the densities of the resident protozoans. Bacteria were released from predation by the protozoans in the low resource communities invaded by *Colpidium* sp. when a predator was present in the community. Culturable bacteria in high resource communities were unaffected by treatments.

Resident Protozoan Densities

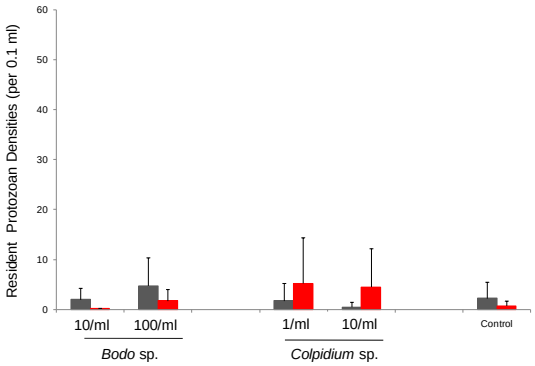
Resident Culturable Bacteria Densities

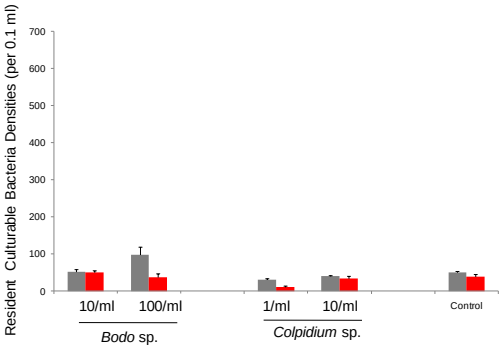
A) Experiment 1: Low Resources



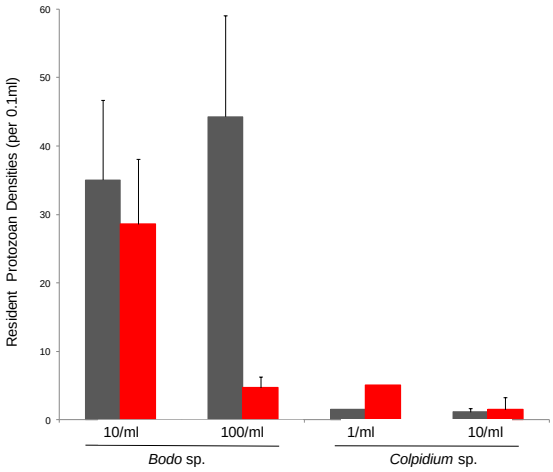


B) Experiment 2: Low Resources





C) Experiment 3: High Resources



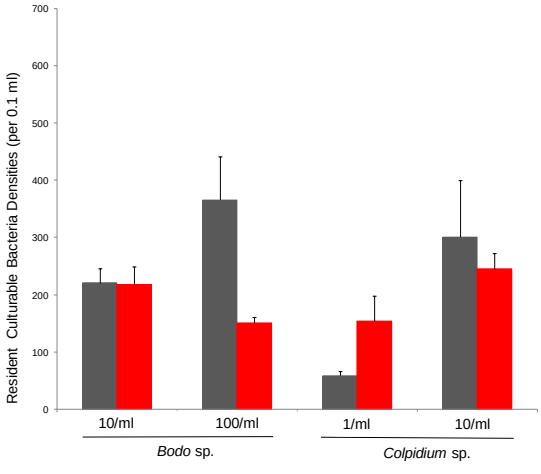
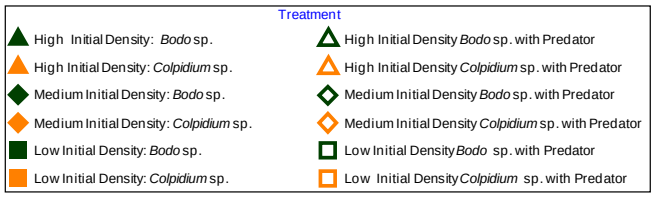
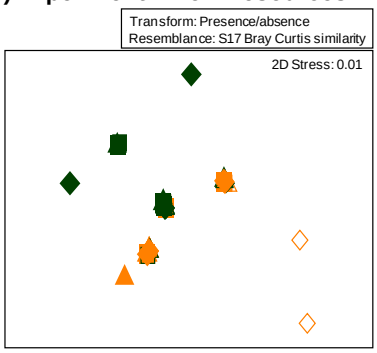


Figure 2. Multi-Dimensional Scaling (MDS) Plots illustrating community similarity for culturable bacteria morphotypes among replicates of all treatments on Day 6. **A)** Experiment 1: Low Resource. **B)** Experiment 2: Low Resource. **C)** Experiment 3: Low Resource. MDS plots in the left column are for presence/absence data of culturable morphotypes and MDS plots in the right column are for abundances of individual morphotypes data. 2D stress indicates how well multi-dimensional groupings are represented in a two dimensional graph. In general, a stress less than 0.2 is considered an adequate representation. Orange data points represent communities invaded by *Colpidium* sp. and green data points represent communities invaded by *Bodo* sp. Open symbols represent communities with a top predator. *Bodo* sp. or *Colpidium* sp. were introduced at high propagule pressure (triangle symbols), medium propagule pressure (diamond symbols), or low propagule pressure (square symbols). Invasion by *Bodo* sp. resulted in different community structure for the culturable bacteria (based on abundance data) than did invasion by *Colpidium* sp. in Experiment 1: Low Resource. A similar pattern, although non-significant, was found in Experiment 3: High Resource.

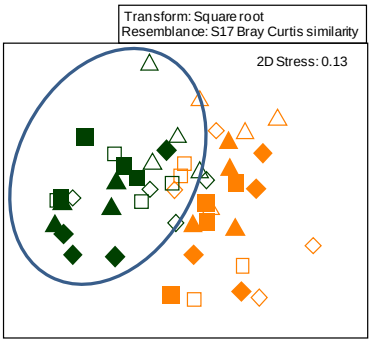


Presence/Absence **Abundances**

A) Experiment 1: Low Resources

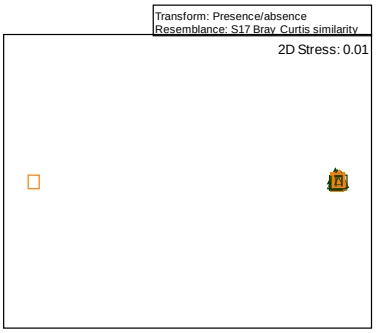


ANOSIM Global R: 0.05, P value: 0.124

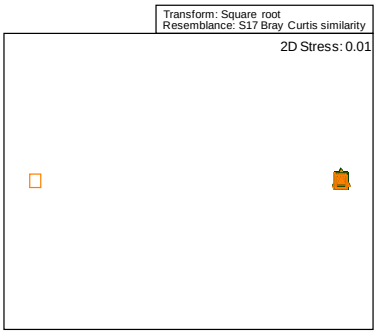


ANOSIM Global R: 0.375, P value: 0.001

B) Experiment 2: Low Resources

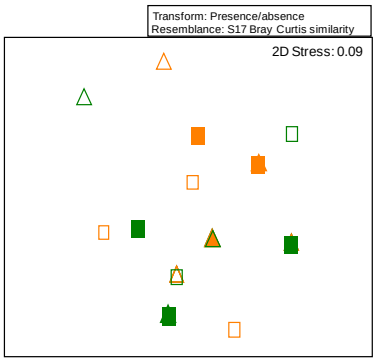


ANOSIM Global R: 0.003, P value: 0.437

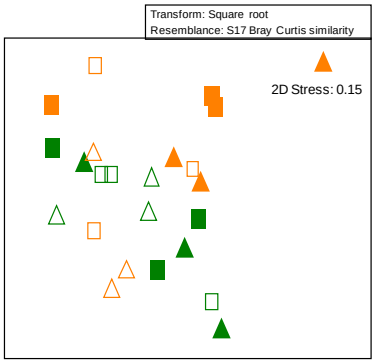


ANOSIM Global R: 0.022, P value: 0.36

C) Experiment 3: High Resources



ANOSIM Global R: 0.048, P value: 0.667

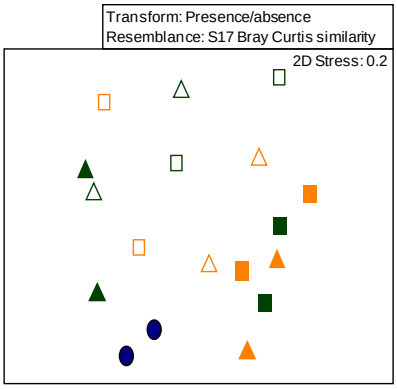


ANOSIM Global R: 0.005, P value: 0.489

Figure 3. Multi-Dimensional Scaling (MDS) Plots illustrating community similarity for bacteria from clone libraries for all treatments on Day 6 of the experiments. **A)** Experiment 2: Low Resource. **B)** Experiment 3: High Resource. MDS plots in the left column were generated with presence/absence data of OTUs and those in the right column were generated with abundances of individual OTUs data. 2D stress indicates how well multi-dimensional groupings are represented in a two dimensional graph. In general, a stress less than 0.2 is considered an adequate representation. Orange points represent communities invaded by *Colpidium* sp. and green points represent communities invaded by *Bodo* sp. Open symbols represent communities with a top predator present. *Bodo* sp. or *Colpidium* sp. were introduced at high (triangle symbols) or low (square symbols) propagule pressure. The blue circle symbol represents Day 0 Control bacteria communities, sampled at the beginning of the experiment, before the addition of treatments. When resources were high (B), there was no effect of any treatment on community structure. When resources were low, bacterial community structure was significantly different when a top predator was present versus when it was absent, both when presence/absence data or relative abundances of OTUs were used. There were no differences in community structure due to the species of the invader, or propagule pressure, except when *Bodo* sp. was introduced at a high propagule pressure. Communities invaded by *Bodo* sp. at a high propagule pressure were more similar to communities containing a predator than communities without a predator. Analysis of similarity reults and p values are given for each plot and also in the results section of this chapter.

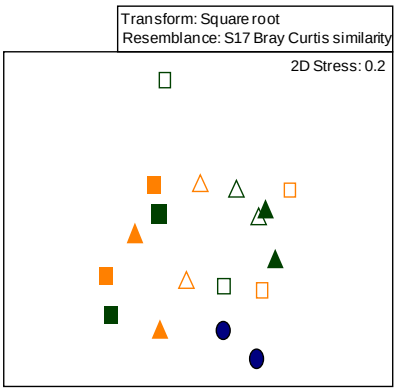
A) Experiment 2: Low Resource – Bacteria OTUs

Presence/Absence

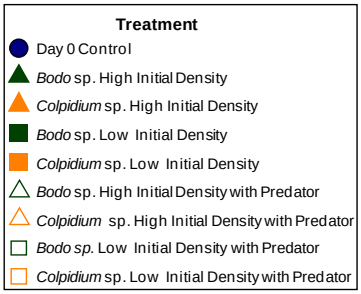


ANOSIM Global R: 0.462
P value = 0.004

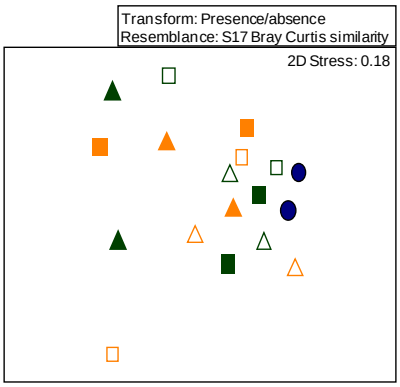
Abundances



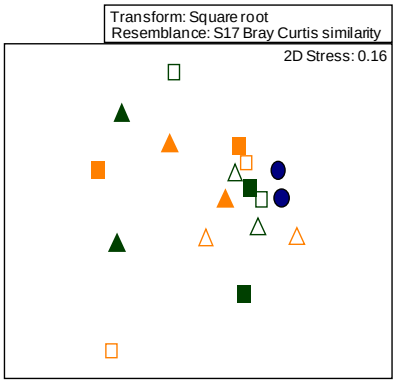
ANOSIM Global R: 0.46
P value = 0.005



B) Experiment 3: High Resource - Bacteria OTUs



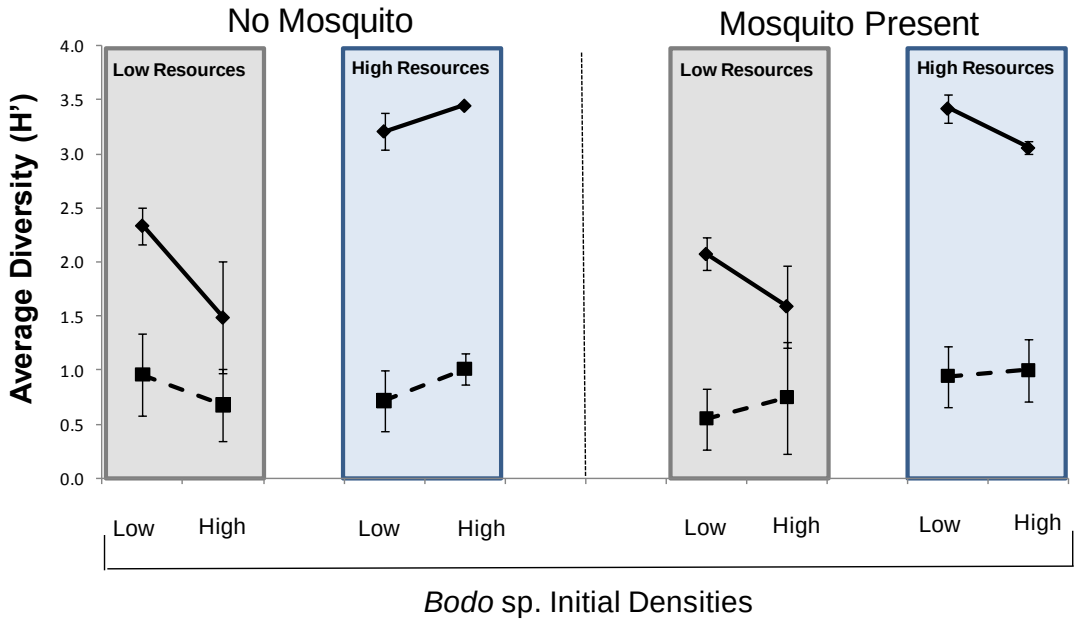
ANOSIM Global R: 0.117
P Value = 0.21



ANOSIM Global R: 0.102
P Value = 0.242

Figure 4. Shannon Diversity Index (H'). Bacteria OTU (solid line) and culturable bacteria (dashed line) diversity when **A)** *Bodo* sp. and **B)** *Colpidium* sp. were introduced at different initial densities into the community without (left) and with (right) a top predator. Data highlighted in purple are from Experiment 2: Low Resources and data highlighted and blue are from Experiment 3: High Resources. As expected, overall, diversity was higher for bacteria OTUs, but the general patterns and trends are similar for both bacteria OTUs and culturable bacteria. N = 2 for OTU data, for culturable bacteria N = 4 for low resources, and N = 3 for high resources. Error bars are standard errors.

A) *Bodo* sp.



B) *Colpidium* sp.

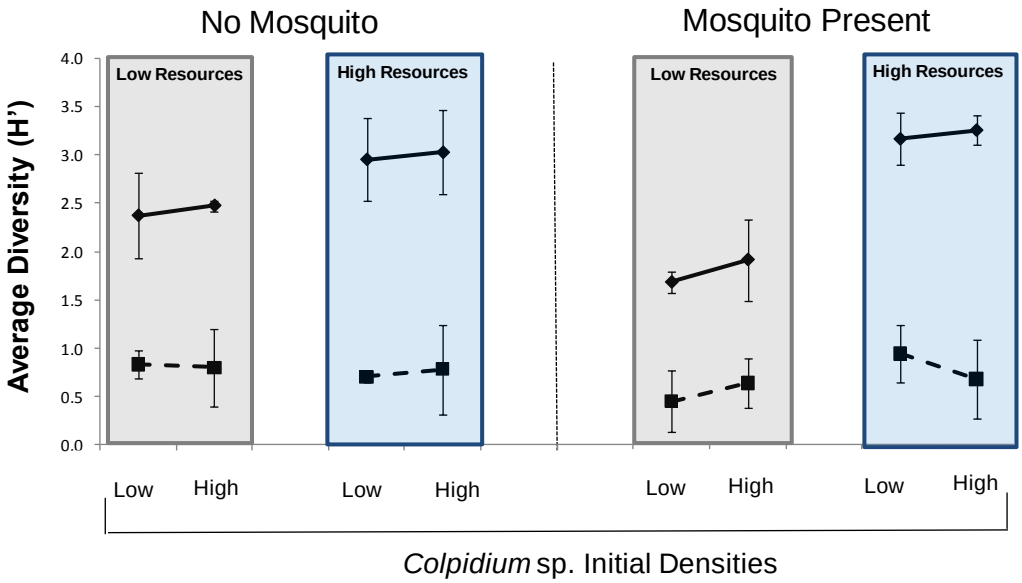
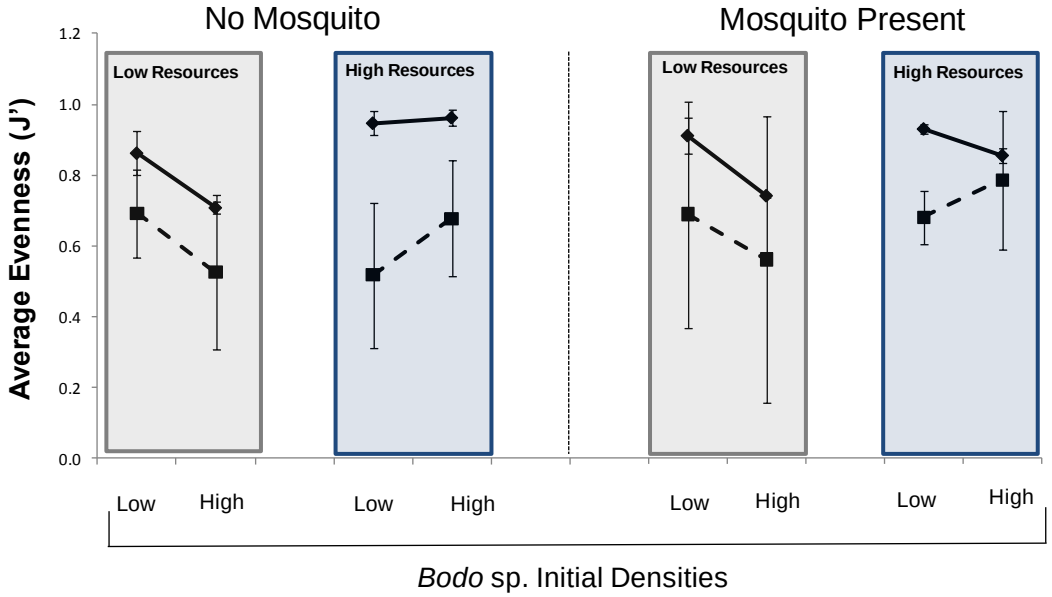


Figure 5. Pielou's Evenness. Bacteria OTU (solid line) and culturable bacteria (dashed line) evenness when **A)** *Bodo* sp. and **B)** *Colpidium* sp. were introduced at different initial densities into the community without (left) and with (right) a top predator. Data highlighted in purple are from Experiment 2: Low Resources and data highlighted and blue are from Experiment 3: High Resources. Evenness was higher for bacteria OTUs, but the general patterns and trends are the same for both bacteria OTUs and culturable bacteria, except that variation among replicates was larger when only data for culturable bacteria was used. N = 2 for OTU data, for culturable bacteria N = 4 for low resources, and N = 3 for high resources. Error bars are standard errors.

A) *Bodo* sp.



B) *Colpidium* sp.

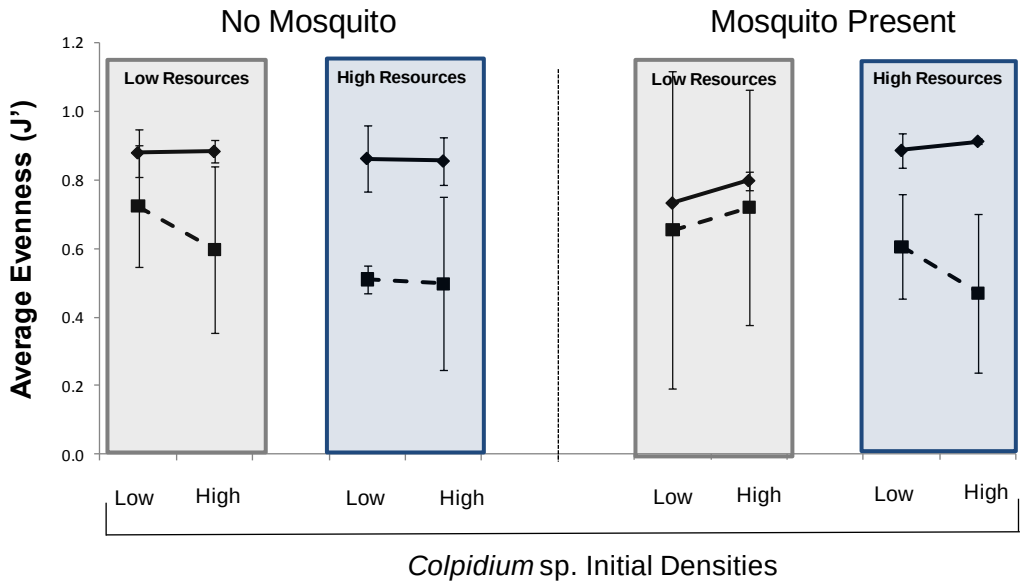
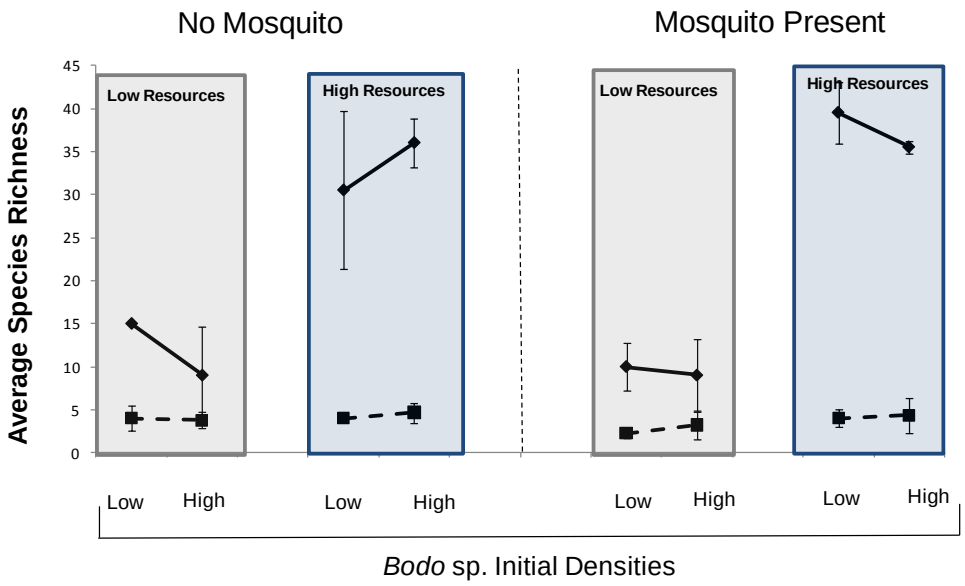
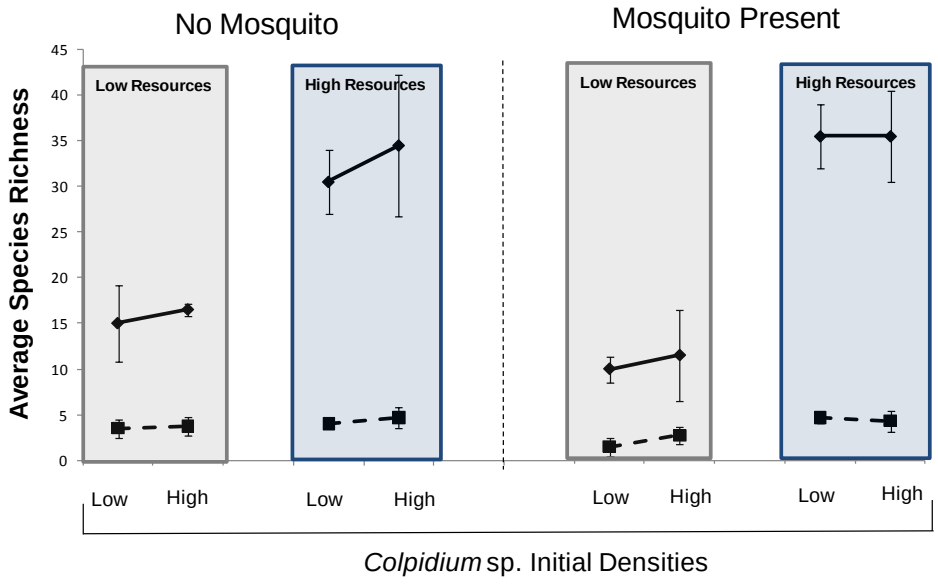


Figure 6. Species Richness. Bacteria OTU (solid line) and culturable bacteria (dashed line) richness when **A)** *Bodo* sp. and **B)** *Colpidium* sp. were introduced at different initial densities into the community without (left) and with (right) a top predator. Data highlighted in purple were obtained from Experiment 2: Low Resources and data highlighted and blue were obtained from Experiment 3: High Resources. As expected, richness was higher for bacteria OTUs than for culturable bacteria. Culturable bacteria richness did not differ among treatments because only 7 culturable bacteria were present versus 48 OTUs per replicate, which did vary among replicates. N = 2 for OTU data, for culturable bacteria N = 4 for low resources, and N = 3 for high resources. Error bars are standard errors.

A) *Bodo* sp.



B) *Colpidium* sp.



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Chapter 6

Trophic position determines if the jack-of-all-trades is the master-of-none

Abstract

Assumed trade-offs between competitive ability and resistance to predators or environmental stressors are considered to play a major role in shaping community dynamics. Due to limited available energy, the best competitors should be more vulnerable to predation and environmental stressors, following the adage 'jack-of-all-trades is the master-of-none'. However, the complexity of large scale, natural systems makes it extremely difficult to test for the universality of this notion of trade-offs with competitive ability across multiple trophic levels simultaneously. I used the microscopic food web found in the pitcher plant *Sarracenia purpurea* to simultaneously test for trade-offs among resistance to consumers, stress and competitors for two competitively dominant species in two different trophic levels: the most competitive protozoan (intermediate trophic level) and the most competitive bacterium (basal trophic level). The results show that the introduced intermediate trophic level species, *Colpidium* sp., defied all trade-offs associated with competitive dominance. *Colpidium* sp. established in the community, was not vulnerable to predation, and survived better in a stressful environment than the less competitive resident protozoans. The competitive dominant bottom trophic level species ('Cloudy' morphotype bacterium), however, conformed to such trade-offs. The success of the competitively dominant intermediate trophic level species, even in the face of predators and stress, indicates that it may show characteristics similar to a 'super species'. This work indicates the need for further testing of species in intermediate trophic levels in other systems to determine the generality of these results and the potential threat of the introduction of a competitively dominant intermediate trophic level species to a novel community.

Introduction

The concept of trade-offs is a fundamental assumption underlying a number of major ecological and evolutionary theories. Levins (1968) was one of the first to articulate the notion of trade-offs through the energy-based 'Principle of Allocation'. According to this principle, organisms have a limited amount of energy to allocate to traits that maximize fitness. It is this finite amount of energy that makes it impossible to optimize all traits simultaneously, resulting in trade-offs between traits (Levins 1968).

This notion of trade-offs, especially for competitive ability, is paralleled in ecological theory. Grime (1974, 1977, 1979) articulated trade-offs as the basis for three life strategies of plants (C-S-R Theory). These strategies originally described plant habitats, but were later adapted to test for trade-offs between species' traits (Grime 1988a,b). Under these trade-offs, an organism must either be a superior competitor, be able to avoid predators, or be able to survive in a stressful environment (ruderal). The organism could not be superior in more than one of these traits, following the adage 'jack-of-all-trades is the master-of-none'.

Trade-offs with competitive ability have been the basis of explanation for fundamental patterns of species diversity along both disturbance and predator gradients. The classic Intermediate Disturbance Hypothesis (Connell 1978) depends on such a trade-off, where the competitively dominant species is the most susceptible to a disturbance and therefore the most likely to be eliminated from the habitat when a disturbance occurs, ultimately resulting in an increase in diversity of the system. Lubchenco (1978) hypothesized a similar pattern for responses to gradients in predation risk in which the top competitive species will be more vulnerable to predators than less competitive species.

Since the development of these pioneering ideas, researchers have tested for trade-offs in a

wide variety of systems and species (e.g., amphibians, Werner and McPeek 1994; algae, Airoldi 1998; plants, Turnbull et al. 1999; acacia-ants, Stanton et al. 2002; bacteria, Jessup and Bohannan 2008). However, in most cases it has only been feasible to test for trade-offs between only two of the three (competition, predation, stress) axes. Grime's C-S-R theory, which was specifically developed to predict trade-offs between all three of these axes, has mainly only been used by Grime and colleagues and has rarely been extrapolated to organisms other than plants (Wilson and Lee 2000; but see Bestelmeyer 2000, Fynn et al. 2005, Hartley and Mitchell 2005), making the universality of this trade-off questionable.

The complexity of large-scale natural systems inhibits our ability to fully examine simultaneous trade-offs in competition, predation, and stress. It is known that competition plays a major role in shaping community dynamics, but the complexity of communities makes it nearly impossible to tease apart all species interactions and conduct direct tests for the presence of trade-offs with competition. It is even more difficult to test for such trade-offs in multiple trophic levels simultaneously, making it unknown if the trophic position of a competitive species will impact the magnitude of trade-offs and how community dynamics will be altered if trade-offs are either present or absent.

The microscopic community that forms within the cup-shaped leaves of the pitcher plant, *Sarracenia purpurea*, provides an ideal experimental system for overcoming the limitations in testing Grime's strategies at all three axes (competition, predation, stress). By using this system, trade-offs with competition can be tested not only in one trophic level, but across multiple trophic levels of the same community and the potential effect of these trade-offs on overall community dynamics can be determined. This system has proven to be a powerful model for ecological studies for decades (e.g., Addicott 1974, Heard 1994, Kneitel and Miller 2002, Gray

et al. 2006). The community has all of the dynamics of other natural food webs, but at a smaller spatial and shorter temporal scale and because of these features, it is ideal for testing questions that are difficult if not impossible in larger scale systems.

Insects, especially ants, fall into the trapped water in the leaves, as do bacteria and yeast, which then decompose the insects, liberating nutrients for the plant. Unlike other carnivorous plants, this species does not produce digestive enzymes in its leaves, except possibly in newly opened leaves (Gallie and Chang 1997). This makes bacterial decomposition extremely important in providing the much needed nutrients to this plant. A variety of protozoan species and a rotifer species (*Habrotrocha* cf. *rosa* Donner) also colonize this community and consume the bacteria and yeast. The highest trophic level is filled by the larvae of the pitcher plant mosquito, *Wyeomyia smithii*, which feeds primarily on protozoans and rotifers (e.g., Addicott 1974), but to some extent also on the bacteria (Kneitel and Miller 2002). However, predation pressure on the protozoans and rotifers has a much stronger effect, causing a trophic cascade which enables the bacteria to increase in abundance (Kneitel and Miller 2002). The rapid dynamics of this system (generation times of approximately 3-4 hours for bacteria and 8-10 hours for protozoans) and the fact that the community can be easily recreated and manipulated in the lab have greatly facilitated the development of this model system.

I used this experimental system to simultaneously test for trade-offs between competitive ability, predation resistance and stress. Specifically, I looked at resistance to consumers, stress and competitors for two competitively dominant species in two different trophic levels: the most competitive protozoan (intermediate trophic level) and the most competitive bacterium (basal trophic level). This allowed me to test whether trade-offs associated with competitive ability were affected by the trophic level position of the competitively dominant species. I also assessed

the full community response to the introduction of these competitively dominant species.

Methods

The protozoan species and bacterial morphotypes used for this experiment were isolated from pitcher plant water collected from Cranberry Bog Preserve in Riverhead (Long Island), NY and maintained in a growth chamber on a 12 hr light/dark cycle at 27°C in a laboratory at Stony Brook University, Long Island, NY. The isolated protozoans were the most common species found in pitcher plant water throughout the geographic range of *S. purpurea* (Buckley et al. 2003): the ciliates *Colpidium* sp., *Colpoda* sp., *Cyclidium* sp., and the flagellates *Bodo* sp. and *Chrysomonad* sp. Bacterial morphotypes were selected from pitcher plant water plated on agar plates and were chosen because they were visually distinct from one another. By choosing only morphologically distinct, culturable bacteria (able to grow in the lab), errors were minimized in identification and during estimates of diversity in the experiment (e.g., Cochran-Stafira and von Ende 1998). The isolated protozoan cultures maintained in the growth chamber were fed only the isolated resident bacterial morphotypes used in the experiment so that only these bacterial morphotypes were present when the experimental communities were formed.

I conducted preliminary competition experiments to identify the competitively dominant protozoan species and bacterial morphotype without the presence of predators. All species/morphotypes were tested for competitive rank by being grown alone, in all pair-wise combinations, as well as in mixture with all species combined. The species/morphotype with the resulting higher density at the end of the experiments was chosen as the introduced competitive dominant in the experiment. I found a clear competitive hierarchy among the protozoans with *Colpidium* sp. being the competitive superior and was 5 times more abundant than the next best

competitor in the pairwise experiments and 4 times more abundant than all species when combined together (*Colpidium* > *Cyclidium* > *Colpoda* > *Bodo* > *Crysomonad).* There was a similar clear competitive hierarchy among the bacteria, with the 'Cloudy' morphotype dominant.

I created an experimental community, which included all of the isolated protozoans and bacteria, except the competitive dominants, at the densities they are normally found at the cranberry bog site (Chapters 2 and 3; and similar design to Cochran-Stafira and von Ende 1998): approximately 200 *Bodo* sp., 20 *Chrysomonad* sp., 2 *Cyclidium* sp., and 1 *Colpoda* sp. per 0.1mL. The seven bacterial morphotypes that had been kept in isolated cultures (and checked for contamination) were then added to the pooled community at densities that correlated to their natural densities in the field.

Replicate experimental communities were created in 50mL macrocentrifuge tubes that were filled with 2 ml of 3mm glass beads in order to provide refugia for the protozoans (terHorst et al. 2010). The glass beads are analogous to the exoskeletons of insects found at the bottom of pitcher plant leaves. There were a total of 5 replicates for each of the 8 treatments, resulting in 40 experimental tubes. I used a sterile pipet to add 10 ml of the pooled community (mixed continually) into these macrocentrifuge tubes. Five dead, sterilized ants (*Tapinoma sessile*) were added into each tube to provide the nutrient source for each community. This ant species is the most common ant found in the cranberry bog pitcher plants in Riverhead, New York (personal observation). Although these communities do not contain all members of *S. purpurea*'s food web, they still provide a model for a three trophic level food web containing species that naturally occur together in the field.

The experimental communities were allowed to stabilize in the growth chamber for 12 hrs before the addition of the top predator, the mosquito larva *Wyeomyia smithii*. I double rinsed

each mosquito larva in sterilized deionized water to remove any protozoan or bacterial contamination, and then placed one larva into each tube. No contamination of additional species was found during the time course of the experiment. The entire resident community was allowed to stabilize for 72 hrs, which is equivalent to approximately 7 generations for protozoans and 10 generations for bacteria. After this stabilization time period, a small aliquot of each replicate was collected to determine the initial density and richness of the resident protozoans and bacteria.

Experimental Treatments

The following treatments were used in a 2 x 2 x 2 full factorial design: pH stress (yes, no), competitive dominant protozoan (yes, no), and competitive dominant bacterium (yes, no). For the low pH stress, a 0.1mL sterilized drop of a NH_4Cl aqueous solution at a pH of 4 was added. I added a 0.1mL drop of each culture of the competitively dominant bacterium and protozoan to each appropriate replicate such that ~75 *Colpidium* individuals and ~ 1000 bacteria were added. Sterilized deionized water was added to tubes as necessary so that each treatment received a total of 0.3mL liquid.

Sampling Methods

After the initial sampling prior to the addition of treatments, I sampled each replicate of each experimental community every two days for eight days. On the sampling days, a small aliquot was collected from each tube, of which 0.15mL was used. I used 0.1mL of this aliquot to count the protozoan density and richness with a compound microscope, and 0.05mL of the aliquot was used to plate bacteria on agar plates.

To determine the relative density and richness of bacterial morphotypes, 0.05mL of the aliquot was used to make a 10^{-4} dilution. I then spread 0.1mL of that dilution on a half-strength Luria broth plate (Cochran-Stafira and von Ende 1998, Kneitel and Miller 2002, Gray et al. 2006). Plates were incubated at 26 °C for 72 hours and then a direct count of the colony forming units (CFUs) growing on each plate was conducted to determine the density and richness of the bacterial morphotypes (Kneitel and Miller 2002, Gray et al. 2006).

On every sampling day, each tube was checked to determine if any mosquito larvae had morphed into the non-feeding pupae stage. Pupae that had metamorphosed were replaced with a third instar mosquito larva conditioned in the same growth chamber with the same food source as the experimental tubes. Each replacement mosquito larva was double rinsed with sterile, deionized water. All communities had one replacement mosquito larva event take place during the course of the experiment.

After inverse square root transformation to normalize the data, I analyzed the treatment effects on the density of resident protozoans and bacteria in two separate full-factorial repeated measures ANOVAs with time as the repeated measure (Statistica 6.1, Statsoft Inc., Sokal and Rohlf 1995). The main fixed effects in the model were low pH, addition of the competitively dominant protozoan, and addition of the competitively dominant bacteria treatments. Pielou's species evenness (Pielou 1966) was calculated with the multivariate statistical program Primer 6.1 (Primer-E Ltd.). I used the program EcoSim ver. 7 (Gotelli and Entsminger 2004) to calculate rarefaction to determine differences in species richness across treatments. Rarefaction corrected for the variation in abundances of species which could artificially alter species richness of a sample.

Results

Changes in resident species densities

Resident bacterial density was affected by the low pH stress ($p = 0.0092$), the addition of the competitively dominant protozoan species ($p = 0.0487$) and time ($p < 0.0001$, Table 1). No other treatments or combinations of treatments had any effect on bacterial density (Table 1). When compared to the control, the resident bacteria had a higher average density through time when in a low pH treatment (Figure 1). The addition of a competitively dominant protozoan species decreased the average density of the resident bacterial species relative to the control and the low pH stress (Figure 1).

Only the low pH treatment ($p = 0.0382$) and time ($p < 0.001$) had a significant effect on resident protozoan density (Table 2). The abiotic stress of a low pH greatly reduced the density of the resident species to zero by the last day of the experiment (Day 8, Figure 2). No other treatments or combinations of treatments had a significant effect on resident protozoan density.

Changes in resident species diversity indices

Time had a significant effect on bacterial morphotype richness (repeated measures ANOVA, $p = 0.007$, $F = 8.395$, Figure 3). The type (protozoan or bacterium) of introduced competitively dominant species marginally affected (though not statistically significant) how many resident bacterial morphotypes were present on the last day of the experiment (repeated measures ANOVA, $p = 0.082$, $F = 2.44$, $df = 3$). By the last day of the experiment in a non-stressed environment, resident bacterial richness was higher in the presence of the competitively dominant protozoan, but, interestingly, the pattern was reversed when the competitive dominant bacterium was the introduced species (Figure 3). A low pH stress maintained bacterial richness

at a similar level as was present at the beginning of the experiment, both when added alone and in combination with the bacterial invader (Figure 3).

The evenness of the resident bacterial morphotypes also significantly changed through time (repeated measures ANOVA, $p < 0.0001$, $F = 68.17$, $df = 1$). The evenness decreased in every treatment by the end of the experiment; however, no treatments were significantly different from the control on Day 8.

Resident protozoan species richness and evenness significantly decreased by the end of the experiment (repeated measures ANOVA, Time, $p < 0.0001$ for both richness ($F = 46.5$, $df = 1$) and evenness ($F = 19.96$, $df = 1$)). Richness reduced to zero on the last day of the experiment as all resident species were undetectable in all treatments except the control, low pH and competitive bacteria introduction combined, competitive bacteria alone, and the introduction of both competitive species together (Figure 4a). Evenness also decreased to zero in all treatments except the control and when the competitively dominant bacterium was introduced alone (Figure 4b).

Introduction of the competitively dominant protozoan and bacterial species

The competitively dominant protozoan species (*Colpidium* sp.) established in every community in which it was introduced even though its predator, *Wyeomyia smithii*, was also present (Figure 5a). While establishing, *Colpidium* sp. still managed to decrease bacterial density through consumption ($p = 0.0487$, Figure 1), suggesting that predation by *W. smithii* is not limiting *Colpidium* sp.'s ability to establish and consume bacteria at such a rate that it affects bacterial abundance. Although the density of this ciliate was reduced by the abiotic stressor, it still became established, persisted at a higher abundance than all resident protozoans combined

and resisted predation by the top predator (Figure 5b). The competitively dominant bacterial morphotype, however, was not as successful as its intermediate trophic level counterpart (the protozoan *Colpidium* sp.). The introduced bacterial morphotype was only able to persist in the absence of its consumers and abiotic stressors, but only in low numbers and results were not significantly different among treatments (Figure 6).

Discussion

Surprisingly, counter to predictions, the competitive dominant protozoan *Colpidium* sp. established, resisted predators while simultaneously decreasing bacterial abundance, and successfully survived in a stressful environment. Due to assumed trade-offs associated with competitive ability, this would not be expected, following the adage 'jack-of-all-trades is the master-of-none'. By defying these trade-offs, the results suggest that *Colpidium* sp. may show characteristics similar to that of a 'super species' (Tilman 1982). However, further tests should be conducted to fully understand the limitations of this competitively dominant species.

The competitively dominant bacterial morphotype in the bottom trophic level only established in the community in the absence of its consumers and abiotic stressors, but only in low numbers, which is predicted in Grime's C-S-R theory (Grime 1988a,b). The resident bacteria, which are less competitive, conformed to these trade-offs, and survived better in the community than the competitively dominant species when faced with predation and an abiotic stress. However, in a non-stressful environment, the resident bacterial density was greatly reduced by the competitively dominant predator, the introduced protozoan *Colpidium* sp.

The results of this experiment suggest that for bottom trophic level species, predation may have a greater impact on the persistence of these species than does a stressful environment. For

intermediate trophic level species, it is the stressful environment that may have a greater impact on survival than predation by a top trophic level species. The resident protozoan species may have also been affected by the presence of food. All protozoan densities decreased throughout the time course of the experiment, presumably because the resources (ants) for their bacterial prey were added only at the beginning of the experiment. The evenness and richness of these species increased due to the introduction of the competitively dominant bacteria as an additional food source. By the end of the experiment, the density of *Colpidium* sp. matched densities naturally found in the field. However, the decrease in its density after persisting in the community for 40 generations (time course of the experiment) may have been due to lack of resources. Further experiments should be conducted to test if an increase in basal resources would alter the results presented here.

Even with a lack of resources, *Colpidium* sp. still showed potential 'super-species' characteristics by being competitively dominant in preliminary experiments, simultaneously surviving predation and an abiotic stress, and significantly reducing the density of the resident bacteria. This statement is made with caution though, because the resident protozoans were not significantly reduced in this experiment by the presence of *Colpidium* sp. It should be noted that this potential 'super-species' characteristic of *Colpidium* sp. is not just an artifact of the type of stress used in this experiment. *Colpidium* sp. of the pitcher plant food web also shows no trade-off in temperature stressed environments (ranging from 10 to 35 °C) while under similar predation pressure and resource availability as used in this experiment (D. Hoekman, personal communication; but see Jiang and Morin 2004 for the freshwater *Colpidium striatum*). This experiment demonstrates that it may be possible for a 'super-species' to exist in an intermediate trophic level, and may explain some highly successful invasive species (e.g., the bullfrog *Rana*

catesbeiana, Kruse ad Francis 1977; and the crayfish *Orconectes rusticus,* Berrill et al. 1985, Hill et al. 1993, Hill and Lodge 1999), which are not only competitively dominant, but have also managed to colonize a novel habitat, survive predation and stress, and out-compete local species. However, very few other studies have investigated such characteristics for potential invasive species, where research has mainly focused on species in top and bottom trophic levels. The results from this experiment show that these intermediate trophic level species have the potential to become 'super weeds' that can persist even in the face of disturbance and predation. These results call for more studies to further investigate the importance of processes leading to the dominance of invaders at intermediate trophic levels.

Table 1. Results of Full Factorial Repeated Measures ANOVA testing the effects of a low pH stress, the introduction of the competitive dominant protozoan (*Colpodium* sp.) and the introduction of the competitive dominant bacterium ('Cloudy' morphotype), in all combinations, on resident bacterial density. There were five replicates of each treatment. Statistically significant p-values are in bold.

	df	**F value**	**p-value**
Low pH stress	1	7.691	**0.0092**
Competitive Bacterium	1	2.889	0.0989
Competitive Protozoa	1	4.200	**0.0487**
Low pH*Competitive Bacteria	1	0.752	0.3924
Low pH*Competitive Protozoa	1	3.298	0.0787
Bacteria*Protozoa	1	2.027	0.1641
Low pH*Bacteria*Protozoa	1	1.241	0.2735
Time	4	23.932	<**0.0001**
Time*Low pH	4	1.281	0.281
Time*Bacteria	4	2.110	0.0833
Time*Protozoa	4	0.633	0.640
Time*Low pH*Bacteria	4	0.588	0.672
Time*Low pH*Protozoa	4	0.405	0.805
Time*Bacteria*Protozoa	4	1.563	0.188
Time*Low pH*Bacteria*Protozoa	4	1.742	0.145

Table 2. Results of Full Factorial Repeated Measures ANOVA testing the effects of a low pH stress, the introduction of the competitive dominant protozoan (*Colpodium* sp.) and the introduction of the competitive dominant bacteria ('Cloudy' morphotype), in all combinations, on resident protozoan density. There were five replicates of each treatment. Statistically significant p-values are in bold.

	df	**F value**	**p-value**
Low pH stress	1	4.675	**0.0382**
Competitive Bacteria	1	0.013	0.909
Competitive Protozoa	1	0.508	0.481
Low pH*Competitive Bacteria	1	0.762	0.389
Low pH*Competitive Protozoa	1	0.005	0.945
Bacteria*Protozoa	1	2.37	0.133
Low pH*Bacteria*Protozoa	1	0.010	0.919
Time	4	29.265	< **0.001**
Time*Low pH	4	0.472	0.756
Time*Bacteria	4	0.299	0.878
Time*Protozoa	4	0.163	0.956
Time*Low pH*Bacteria	4	2.101	0.0844
Time*Low pH*Protozoa	4	0.269	0.897
Time*Bacteria*Protozoa	4	1.038	0.3901
Time*Low pH*Bacteria*Protozoa	4	1.345	0.257

Figure 1. The average density of resident bacteria (log number / 0.1 ml) through time for the low pH stress (diamond) and the addition of the competitive dominant protozoan (*Colpidium* sp.) (square) treatments, compared to the control (hashed line, triangle). Symbols are the means of 5 replicates, with standard error bars. Treatments were significantly different from the control.

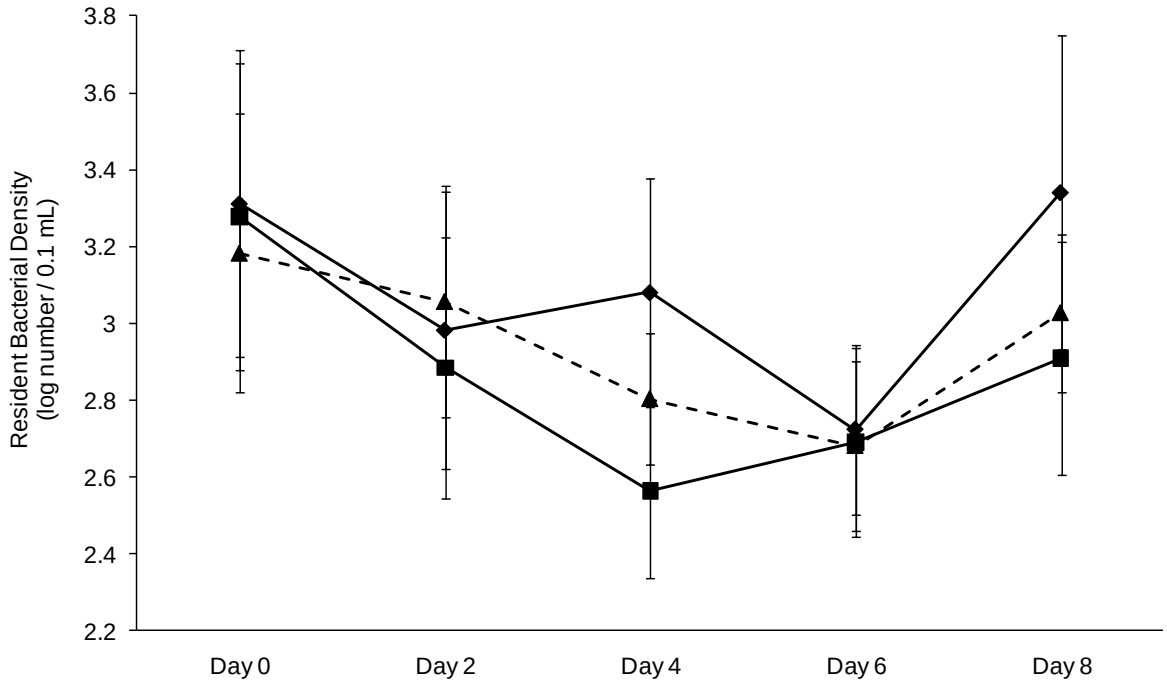


Figure 2. Total resident protozoan density (number / 0.1 ml) through time for the low pH stress treatment (diamond) and the control (hashed line, triangle). The low pH stress treatment was the only statistically significant treatment in this experiment. Symbols are the means of 5 replicates, with standard error bars.

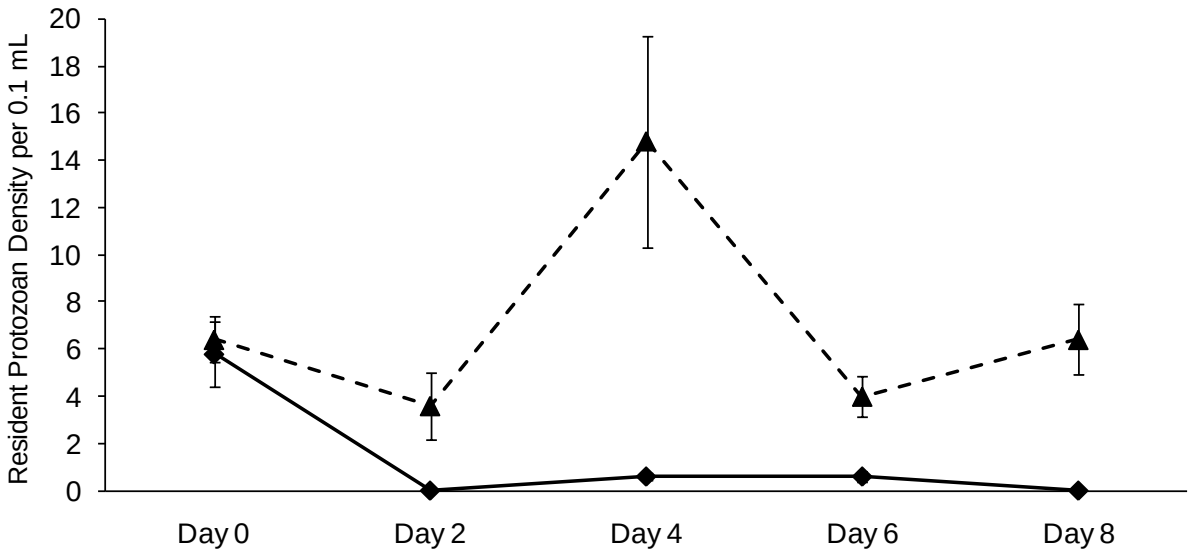


Figure 3. Change in resident bacterial morphotype richness among treatments: no pH stress control, pH stress; introduction of the most competitive protozoan (*Colpidium* sp.), low pH stress and the introduction of *Colpidium* sp.; introduction of the most competitive bacterial morphotype ('Cloudy'), low pH stress and the introduction of 'Cloudy'; *Colpidium* sp. and 'Cloudy' introduced together, low pH stress and *Colpidium* sp. and 'Cloudy' introduced together (average of 5 replicates with error bars.). Day 0 (the initiation of the treatments): filled bars; Day 8 (end of the experiment): white bars. Morphotype richness was significantly affected by the length of the experiment (Time, p = 0.007, F = 8.395) and the type (protozoan or bacterium) of introduced competitively dominant species also affected bacterial richness, although the pattern was non-significant statistically (p = 0.082, F = 2.44).

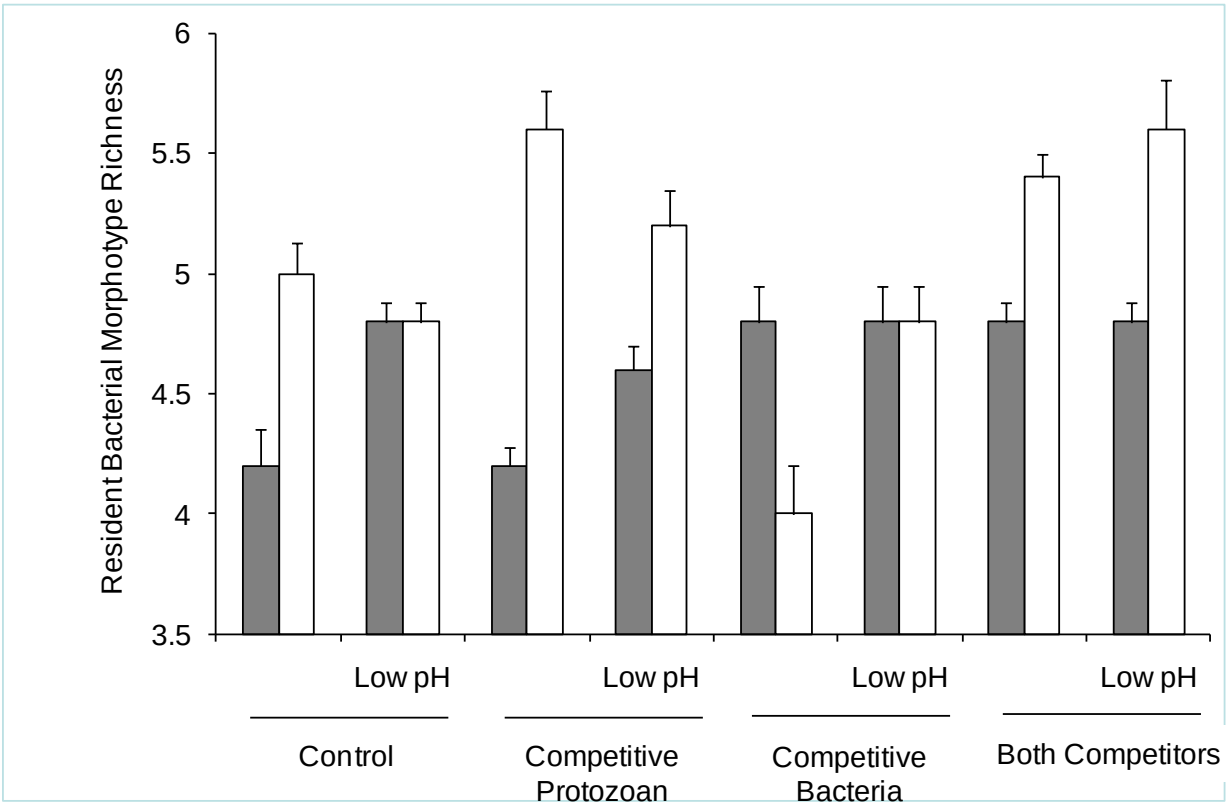


Figure 4. Change in resident protozoan community in terms of species richness **(A)** and Pielou's Evenness (J') **(B)** among treatments: no pH stress control, pH stress; introduction of the most competitive protozoan (*Colpidium* sp.), low pH stress and the introduction of *Colpidium* sp.; introduction of the most competitive bacterial morphotype ('Cloudy'), low pH stress and the introduction of 'Cloudy'; *Colpidium* sp. and 'Cloudy' introduced together, low pH stress and *Colpidium* sp. and 'Cloudy' introduced together. Bars are averages of 5 replicates, with standard error bars. Day 0 (the initiation of the treatments): filled bars; Day 8 (end of the experiment): white bars. Time had a statistically significant effect on all treatments, as protozoan richness and evenness was greatly reduced by the end of the experiment (repeated measures ANOVA).

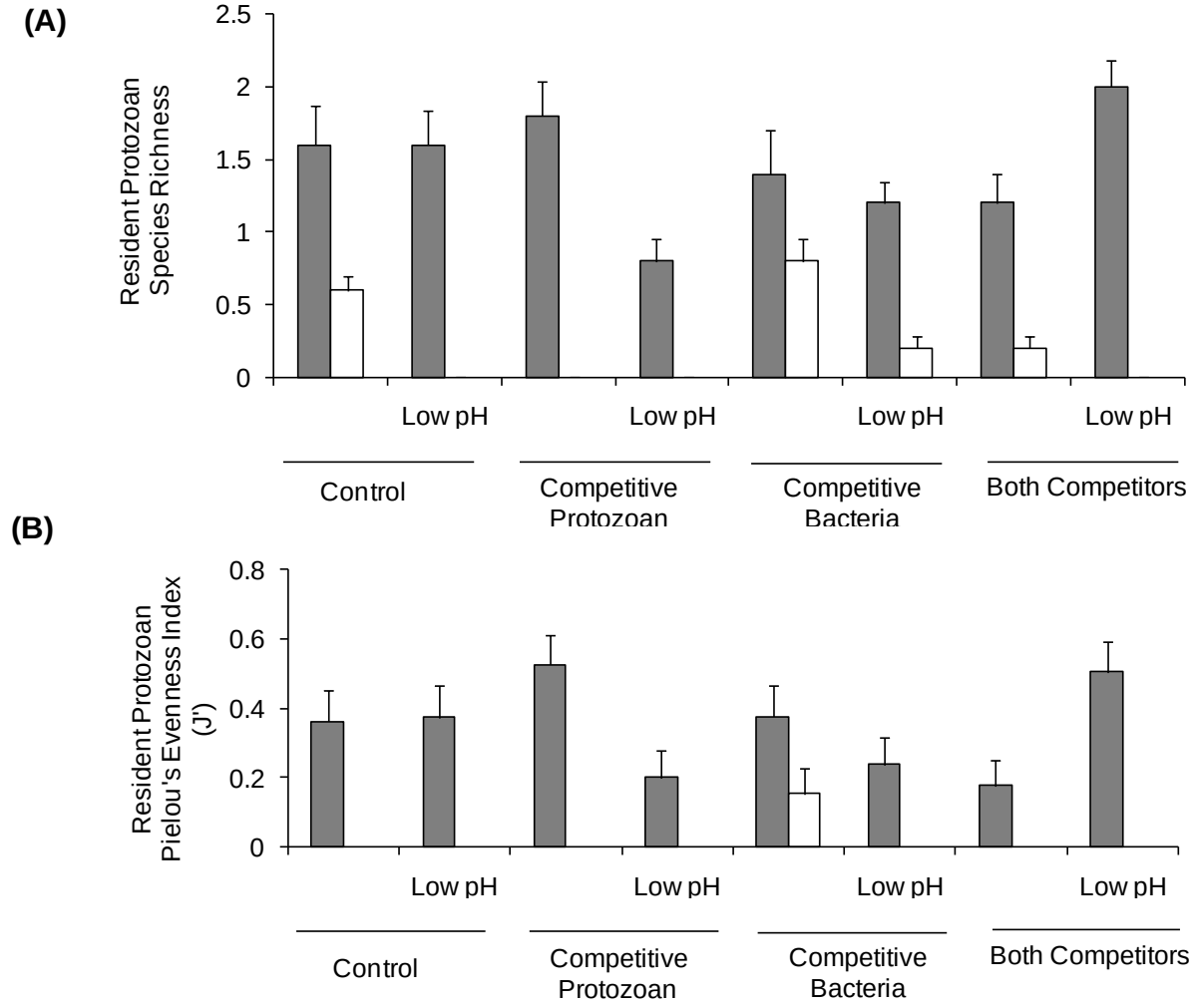


Figure 5. The density of the most competitive protozoan, *Colpidium* sp., through the time course of the experiment. **(A)** The density of *Colpidium* sp. in each of the treatments: Triangle = Introduction of *Colpidium* sp. alone, Cross = Both bacterial and protozoan top competitors introduced, Diamond = Low pH stress and *Colpidium* sp. introduction, Square = Introduction of *Colpidium* sp. in presence of pH stress and introduced bacterium, **(B)** The effect of low pH stress on the density of the *Colpidium* sp. (solid line, diamond) and the resident protozoans (hashed line, diamond). Symbols are the means of 5 replicates, with standard error bars.

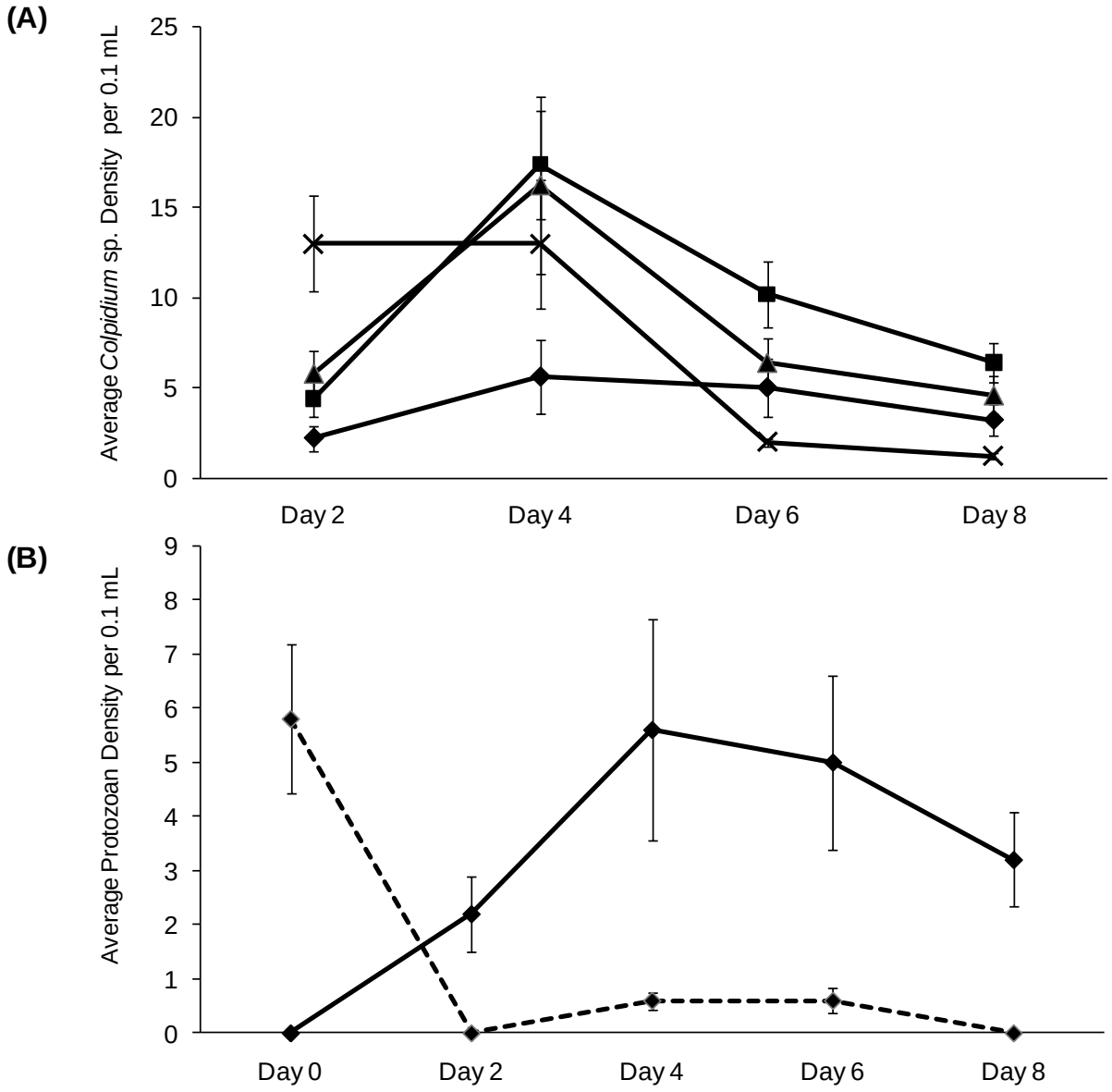
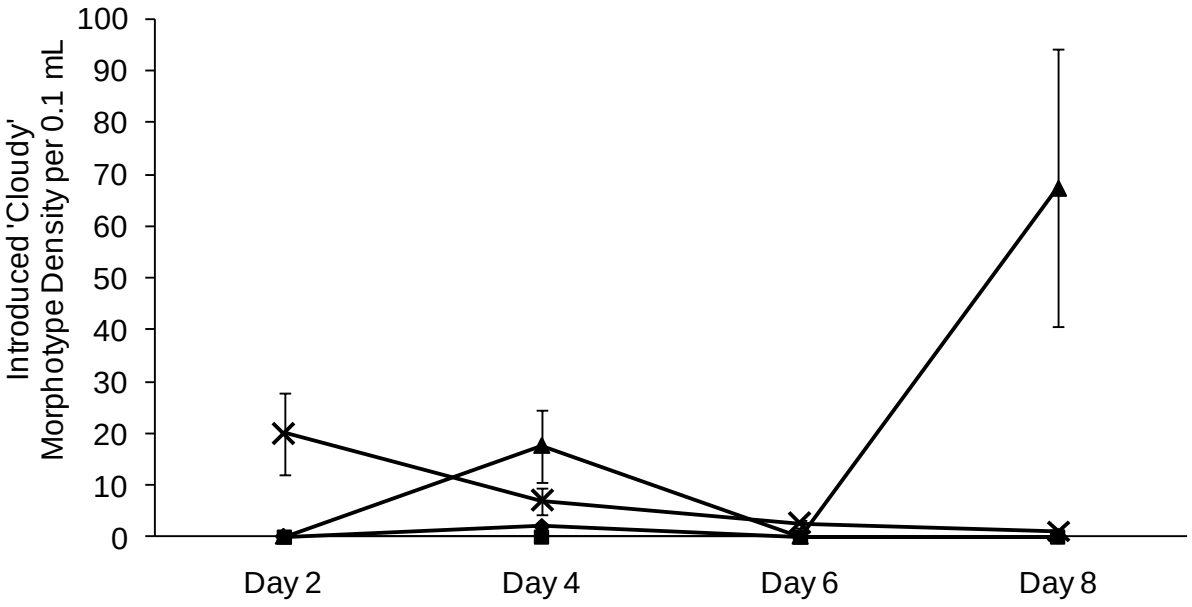


Figure 6. Establishment success of the most competitive bacterial morphotype (‘Cloudy’) through the time course of the experiment. Triangle = Introduction of ‘Cloudy’ morphotype alone, Cross = Both bacterial and protozoan top competitors introduced, Diamond = Low pH stress and ‘Cloudy’ morphotype introduction, Square = Introduction of ‘Cloudy’ morphotype in presence of pH stress and introduced protozoan. Symbols are the means of 5 replicates, with standard error bars.



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Chapter 7

Conclusions

Understanding what factors govern changes in community structure and composition has been a central theme of research in community ecology. The development of a community relies on the introduction and successful establishment of new species (Belyea and Lancaster 1999), and the identity and traits of colonizing species important for shaping community structure throughout succession (e.g., Collinge and Ray 2009). Similarly, as communities develop, they change, and those changes can influence the likelihood that other species can colonize, as well as the impacts that new introductions will have on the existing community. In this dissertation, I used the model *Sarracenia purpurea* system to test the relative importance of species properties thought to be important during community development, and the impact that species with these properties have on community structure and composition.

I found that, contrary to the idea of predictable replacement of species through succession proposed by Clements (1916), the aquatic community held within the leaves of *S. purpurea* maintained the same subset of species from initial development to the end of the growing season. Although there was high variability in species composition and structure among communities initially (Chapter 2 and 3), both the competitive dominant and least competitive species arrived early in community development and co-existed throughout community assembly (Chapter 3).

Properties considered important during community assembly and succession, such as competitive ability, are also considered important for the invasion success of species introduced into a community. To date, it has been difficult to test if a single property is the most important for allowing a species to invade a community or if several factors acting in combination are more likely to allow a successful invasion. In this dissertation, I used the *S. purpurea* system to test the relative importance of these species properties on invasion success and if properties of the community alter the invasion success of a species. The four properties I tested, which are

considered important for invasion to successfully occur, are: high competitive ability, high initial density (propagule pressure), and arriving into a predator-free community or into a community with high resource availability. I found that for the *S. purpurea* system, all four properties (competitive ability, high initial density, predator-free community, high resource availability) are important for invasion success.

The presence of a top predator (the mosquito larva, *Wyeomyia smithii*), determined the relative importance of the initial density that a species in the intermediate trophic level must enter a community in order to successfully invade such that when a top predator was present, the density needed for an introduced species to establish was greater (Chapter 4). Both competitive ability and propagule pressure (high initial density) were found to be important for invasion and establishment success of protozoans, independent of the presence or absence of a top predator (Chapter 4). This result was generalizable across pitcher plant aquatic communities with different resource availability and prey diversity (Chapter 4). These results suggest that intermediate trophic level species that are highly competitive and introduced at high initial density will be the most successful at establishing in a community, independent of resource availability or predation pressure (Chapter 4). Therefore, rather than a single property being supported by this research, all properties tested in this dissertation that are thought to be important for invasion success of intermediate trophic level species in this system.

These same patterns did not hold for introduction of species at the bottom trophic level, the bacteria (Chapter 6). Although the competitive dominant primary consumer was a successful invader in all types of communities (Chapter 4), the same was not true for invasion success of the competitive dominant bottom trophic level species (Chapter 6). It was found that although the

bottom trophic level competitive dominant established in the community, it only ever established in low numbers and was greatly suppressed by a predator and an abiotic stress (Chapter 6).

The impact of introduced species on communities was less predictable. I found that the competitive dominant intermediate trophic level species out-competed resident species irrespective of propagule pressure, the presence of a top predator or the resource availability of the resident community (Chapter 5). This was surprising because of the general expectation of tradeoffs between competitive ability and resistance to predators or stress (Paine 1966, Connell 1961, Grime 1974). Introduction of the less competitive species had no impact on community structure, unless it was introduced at a high initial density (propagule pressure) or in a community with high resource availability, which allowed it to reach high densities (Chapter 5). When the less competitive species was at high densities, the top predator not only consumed more of it but also consumed more of the resident species (Chapter 5). This resulted in consumer release for bacteria and an increase in the abundance of bacteria overall. A similar pattern was found when the competitive dominant species invaded the community, resulting in a trophic cascade (Chapter 5).

By using metagenomic techniques, I was able to determine how aspects of community structure and diversity of the bottom trophic level in this community (bacteria) were impacted by the introduction of consumers (protozoans). I found that invasion by the competitive dominant consumer had no impact on bacterial diversity at any level of propagule pressure or resource availability, with or without a top predator present. This was not the case for invasion by the less competitive consumer. When this species was introduced at high propagule pressure, the diversity of bottom trophic level species decreased (Chapter 5). This was the case independent of the presence or absence of a top predator or level or resources in the community. These

results suggest that although the factors important for invasion success can be highly predictable, the impact that a successful invader has on community dynamics is dependent on the characteristics of the invader and of the resident community.

A major critique of the use of this model system to address questions about community structure and dynamics has been that traditional studies use culturable bacteria. Following culturable bacteria is inexpensive (relative to metagenomics) and allows great experimental control for manipulating and following both bacterial species composition and density. Culturable bacteria are a natural component of the community, but may not be informative, as culturable bacteria are expected to represent about 1% of bacteria in a community. I found that in experiments where I followed both the culturable bacteria (with traditional plating techniques) and a larger subset of the bacterial community (both culturable and unculturable bacteria using metagenomics), the culturable bacteria had the same patterns of diversity and evenness as bacteria OTUs from the metagenomics technique. Although the culturable bacteria were present in the community, none of the culturable bacteria were the dominant players in the natural bacteria community. This result suggests that culturable bacteria can be used as effective surrogates for assessing bacterial community dynamics and that there may be great deal of functional duplication among bacteria (Dykhuizen 1998). More experiments in other systems should be done to compare diversity patterns for culturable bacteria using plating techniques and diversity patterns using metagenomics in order to test the generalizability of this result.

The research in this dissertation has highlighted factors thought to be most important for invasion success, and has shown that the impact of invasion on community structure is dependent on the characteristics of the invader. However, many important questions remain. For example, the dispersal ability of a species is thought to be important for altering community

dynamics (Belyea and Landcaster 1999). The dispersal ability of a species is of fundamental importance to invasion success because, by definition, an introduced species must be able to successfully move to the new location before it can have an impact on the community. Both the distance between habitats and underlying characteristics of the species (e.g., competitive ability, biomass) have the ability to affect whether a species can successfully disperse to a novel community and impact the resident community. Experiments to examine the dispersal and colonization of intermediate trophic level species are especially needed. Future research using this system should also investigate the notion that any bacteria, not specific species, are needed to perform essential ecosystem processes. A big question remains: If bacterial species are just functional duplicates of one another, why are there so many bacterial species?

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Appendices

Appendix 1. Experiment 1 Tukey Test Results for Factorial ANOVA using Day 6 Resident Protozoan Densities as the response variable. Significant results are in red. N = Predator absent, Y = Predator present.

Tukey HSD test; Approximate Probabilities for Post Hoc Tests Error: Between MS = 1.1579, df = 36.000															
	Species	**Initial Density**	**Predator**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1	Colpidium	High	N		0.849	1.000	1.000	1.000	0.832	0.019	0.998	0.123	0.451	0.096	0.111
2	Colpidium	High	Y	0.849		0.893	0.983	0.993	1.000	0.584	1.000	0.960	1.000	0.933	0.950
3	Colpidium	Medium	N	1.000	0.893		1.000	1.000	0.880	0.025	0.999	0.153	0.518	0.120	0.139
4	Colpidium	Medium	Y	1.000	0.983	1.000		1.000	0.979	0.064	1.000	0.315	0.766	0.258	0.291
5	Colpidium	Low	N	1.000	0.993	1.000	1.000		0.991	0.085	1.000	0.384	0.832	0.320	0.357
6	Colpidium	Low	Y	0.832	1.000	0.880	0.979	0.991		0.607	1.000	0.967	1.000	0.942	0.958
7	Bodo	High	N	0.019	0.584	0.025	0.064	0.085	0.607		0.170	1.000	0.927	1.000	1.000
8	Bodo	High	Y	0.998	1.000	0.999	1.000	1.000	1.000	0.170		0.589	0.950	0.514	0.559
9	Bodo	Medium	N	0.123	0.960	0.153	0.315	0.384	0.967	1.000	0.589		1.000	1.000	1.000
10	Bodo	Medium	Y	0.451	1.000	0.518	0.766	0.832	1.000	0.927	0.950	1.000		0.999	1.000
11	Bodo	Low	N	0.096	0.933	0.120	0.258	0.320	0.942	1.000	0.514	1.000	0.999		1.000
12	Bodo	Low	Y	0.111	0.950	0.139	0.291	0.357	0.958	1.000	0.559	1.000	1.000	1.000	

Appendix 2. Experiment 3 Tukey Results for Factorial ANOVA using Day 6 Resident Protozoan Densities as the response variable. Significant results are in red. N = Predator absent, Y = Predator present.

Tukey HSD test; Approximate Probabilities for Post Hoc Tests Error: Between MS = 3.3084, df = 16.000											
	Species	**Initial Density**	**Predator**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	Bodo	High	N		0.064	0.993	0.125	0.006	0.004	0.004	0.008
2	Bodo	High	Y	0.064		0.237	1.000	0.906	0.816	0.816	0.946
3	Bodo	Low	N	0.993	0.237		0.403	0.026	0.017	0.017	0.034
4	Bodo	Low	Y	0.125	1.000	0.403		0.734	0.608	0.608	0.807
5	Colpidium	High	N	0.006	0.906	0.026	0.734		1.000	1.000	1.000
6	Colpidium	High	Y	0.004	0.816	0.017	0.608	1.000		1.000	1.000
7	Colpidium	Low	N	0.004	0.816	0.017	0.608	1.000	1.000		1.000
8	Colpidium	Low	Y	0.008	0.946	0.034	0.807	1.000	1.000	1.000	

Appendix 3. Experiment 1 Tukey Test Results for Factorial ANOVA using Day 6 Resident Culturable Bacteria Densities as the response variable. Significant results are in red. N = Predator absent, Y = Predator present.

Tukey HSD test; Approximate Probabilities for Post Hoc Tests Error: Between MS = 17.525, df = 36.000															
	Species	**Initial Density**	**Predator**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1	Colpidium	High	N		0.793	1.000	0.100	1.000	0.100	0.186	0.0709	0.482	0.934	0.011	0.454
2	Colpidium	High	Y	0.793		0.555	0.993	0.771	0.962	0.994	0.926	1.000	1.000	0.530	1.000
3	Colpidium	Medium	N	1.000	0.555		0.992	1.000	0.999	0.0831	0.028	0.267	0.769	0.004	0.246
4	Colpidium	Medium	Y	1.000	0.993	0.992		0.100	1.000	0.581	0.307	0.900	1.000	0.070	0.883
5	Colpidium	Low	N	1.000	0.771	1.000	0.100		1.000	0.172	0.065	0.457	0.923	0.010	0.429
6	Colpidium	Low	Y	1.000	0.962	0.999	1.000	0.100		0.409	0.188	0.773	0.996	0.036	0.746
7	Bodo	High	N	0.186	0.994	0.0831	0.581	0.172	0.409		1.000	1.000	0.955	0.987	1.000
8	Bodo	High	Y	0.0709	0.926	0.0280	0.307	0.0646	0.188	1.000		0.996	0.777	1.000	0.997
9	Bodo	Medium	N	0.482	0.100	0.2666	0.900	0.457	0.773	1.000	0.996		0.999	0.832	1.000
10	Bodo	Medium	Y	0.934	1.000	0.769	0.100	0.923	0.996	0.955	0.777	0.999		0.326	0.999
11	Bodo	Low	N	0.011	0.530	0.004	0.070	0.0102	0.037	0.987	1.000	0.832	0.326		0.854
12	Bodo	Low	Y	0.454	0.100	0.246	0.883	0.429	0.746	1.000	0.997	1.000	0.999	0.853	

Appendix 4. Experiment 2 Tukey Test Results for Factorial ANOVA using Day 6 Resident Culturable Bacteria Densities as the response variable. Significant results are in red. N = Predator absent, Y = Predator present.

Tukey HSD test; Approximate Probabilities for Post Hoc Tests Error: Between MS = 4.7960, df = 24.000											
	Species	**Initial Density**	**Predator**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	Bodo	High	N		0.340	0.786	0.818	0.585	0.327	0.265	0.011
2	Bodo	High	Y	0.340		0.994	0.990	1.000	1.000	1.000	0.693
3	Bodo	Low	N	0.786	0.994		1.000	1.000	0.992	0.981	0.264
4.	Bodo	Low	Y	0.818	0.990	1.000		1.000	0.988	0.973	0.237
5	Colpidium	High	N	0.585	1.000	1.000	1.000		1.000	0.999	0.437
6	Colpidium	High	Y	0.327	1.000	0.992	0.988	1.000		1.000	0.709
7	Colpidium	Low	N	0.265	1.000	0.981	0.973	0.999	1.000		0.785
8	Colpidium	Low	Y	0.011	0.693	0.264	0.237	0.437	0.709	0.785	

Appendix 5. Experiment 2 (Low Resource) T Test Bacteria OTU Diversity Results. Significant results are in red. Results considered marginally significant are in blue.

Diversity										
Bodo sp.		**t-value**	**df**	**p**	**Valid N**	**Valid N**	**Std.Dev.**	**Std.Dev.**	**F-ratio variance**	**p-value variance**
No Predator	Low Initial Density vs High Initial Density	-2.11	2.00	0.17	2.00	2.00	0.16	0.05	12.98	0.35
Predator	Low Initial Density vs High Initial Density	-1.65	2.00	0.24	2.00	2.00	0.12	0.04	7.59	0.44
Predator vs. No Predator	High Initial Density	0.24	2.00	0.83	2.00	2.00	0.12	0.16	1.95	0.79
Predator vs. No Predator	Low Initial Density	-1.66	2.00	0.24	2.00	2.00	0.04	0.05	1.14	0.96
Colpidium sp.		**t-value**	**df**	**p**	**Valid N**	**Valid N**	**Std.Dev.**	**Std.Dev.**	**F-ratio variance**	**p-value variance**
No Predator	Low Initial Density vs. High Initial Density	0.35	2.00	0.76	2.00	2.00	0.02	0.12	61.82	0.16
Predator	Low Initial Density vs. High Initial Density	-0.75	2.00	0.53	2.00	2.00	0.03	0.12	13.55	0.34
Predator vs. No Predator	High Initial Density	1.82	2.00	0.21	2.00	2.00	0.02	0.12	65.89	0.16
Predator vs. No Predator	Low Initial Density	-2.25	2.00	0.15	2.00	2.00	0.03	0.12	12.71	0.35
Bodo sp. vs. *Colpidium* sp.		**t-value**	**df**	**p**	**Valid N**	**Valid N**	**Std.Dev.**	**Std.Dev.**	**F-ratio variance**	**p-value variance**
No Predator	High Initial Density	-2.51	2.00	0.13	2.00	2.00	0.16	0.02	116.75	0.12
No Predator	Low Initial Density	-0.10	2.00	0.93	2.00	2.00	0.05	0.12	6.87	0.46
Predator	High Initial Density	-0.82	2.00	0.50	2.00	2.00	0.12	0.12	1.10	0.97
Predator	Low Initial Density	3.00	2.00	0.10	2.00	2.00	0.04	0.03	1.62	0.85

Appendix 6. T Test results comparing bacteria OTU diversity in Experiment 2 (Low resource communities) to bacteria OTU diversity in Experiment 3 (high resource communities). Significant results are in red. Results considered marginally significant are in blue.

Diversity										
Bodo sp.		**t-value**	**df**	**p**	**Valid N**	**Valid N**	**Std.Dev.**	**Std.Dev.**	**F-ratio variance**	**p-value variance**
No Predator	Low Initial Density	-5.13	2	0.04	2.00	2.00	1.83	2.05	1.16	0.95
No Predator	High Initial Density	-4.63	2	0.04	2.00	2.00	1.57	2.11	48.79	0.00
Predator	Low Initial Density	-9.32	2	0.01	2.00	2.00	1.75	2.10	1.81	0.81
Predator	High Initial Density	-4.87	2	0.04	2.00	2.00	1.61	2.01	69.71	0.15
Colpidium sp.		**t-value**	**df**	**p**	**Valid N**	**Valid N**	**Std.Dev.**	**Std.Dev.**	**F-ratio variance**	**p-value variance**
No Predator	Low Initial Density	-1.33	2	0.31	2.00	2.00	1.83	1.99	1.21	0.94
No Predator	High Initial Density	-1.83	2	0.21	2.00	2.00	1.86	2.01	50.60	0.18
Predator	Low Initial Density	-7.74	2	0.02	2.00	2.00	1.64	2.04	3.83	0.60
Predator	High Initial Density	-3.93	2	0.06	2.00	2.00	1.71	2.06	10.76	0.38

Appendix 7. Experiment 2 (Low Resource) T Test Bacteria OTU Evenness. Significant results are in red. Results considered marginally significant are in blue.

Evenness										
Bodo sp.		**t-value**	**df**	**p**	**Valid N**	**Valid N**	**Std.Dev.**	**Std.Dev.**	**F-ratio variance**	**p-value variance**
No Predator	Low Initial Density vs High Initial Density	-3.44	2	0.07	2.00	2.00	0.01	0.02	10.96	0.37
Predator	Low Initial Density vs High Initial Density	-4.91	2	0.04	2.00	2.00	0.00	0.02	59.89	0.16
Predator vs. No Predator	High Initial Density	2.55	2	0.13	2.00	2.00	0.00	0.01	8.76	0.41
Predator vs. No Predator	Low Initial Density	-0.88	2	0.47	2.00	2.00	0.02	0.02	1.60	0.85
Colpidium sp.		**t-value**	**df**	**p**	**Valid N**	**Valid N**	**Std.Dev.**	**Std.Dev.**	**F-ratio variance**	**p-value variance**
No Predator	Low Initial Density vs High Initial Density	0.08	2	0.94	2.00	2.00	0.01	0.03	4.18	0.58
Predator	Low Initial Density vs High Initial Density	3.41	2	0.08	2.00	2.00	0.01	0.00	124.20	0.11
Predator vs. No Predator	High Initial Density	2.79	2	0.11	2.00	2.00	0.01	0.01	1.47	0.88
Predator vs. No Predator	Low Initial Density	3.06	2	0.09	2.00	2.00	0.03	0.00	762.86	0.05
Bodo sp. vs. *Colpidium* sp.		**t-value**	**df**	**p**	**Valid N**	**Valid N**	**Std.Dev.**	**Std.Dev.**	**F-ratio variance**	**p-value variance**
No Predator	High Initial Density	5.26	2	0.03	2.00	2.00	0.02	0.00	380.88	0.07
No Predator	Low Initial Density	0.26	2	0.82	2.00	2.00	0.03	0.02	1.25	0.93
Predator	High Initial Density	5.26	2	0.03	2.00	2.00	0.02	0.00	380.88	0.07
Predator	Low Initial Density	-2.81	2	0.11	2.00	2.00	0.00	0.01	19.53	0.28

Appendix 8. T Test results comparing bacteria OTU evenness in Experiment 2 (Low resource communities) to bacteria OTU evenness in Experiment 3 (high resource communities). Significant results are in red. Results considered marginally significant are in blue.

Evenness										
Bodo sp.		**t-value**	**df**	**p**	**Valid N**	**Valid N**	**Std.Dev.**	**Std.Dev.**	**F-ratio variance**	**p-value variance**
No Predator	Low Initial Density	-1.68	2	0.23	2.00	2.00	0.02	0.01	3.33	0.64
No Predator	High Initial Density	-12.89	2	0.01	2.00	2.00	0.01	0.01	1.27	0.92
Predator	Low Initial Density	-0.52	2	0.65	2.00	2.00	0.02	0.00	13.59	0.34
Predator	High Initial Density	-7.59	2	0.02	2.00	2.00	0.00	0.01	10.68	0.38
Colpidium sp.		**t-value**	**df**	**p**	**Valid N**	**Valid N**	**Std.Dev.**	**Std.Dev.**	**F-ratio variance**	**p-value variance**
No Predator	Low Initial Density	0.20	2	0.86	2.00	2.00	0.03	0.04	1.98	0.79
No Predator	High Initial Density	0.53	2	0.65	2.00	2.00	0.01	0.02	4.04	0.59
Predator	Low Initial Density	-4.43	2	0.05	2.00	2.00	0.00	0.02	399.37	0.06
Predator	High Initial Density	-5.67	2	0.03	2.00	2.00	0.01	0.00	13.59	0.34

Appendix 9. Experiment 2 (Low Resource) T Test Bacteria OTU Richness Significant results are in red. Results considered marginally significant are in blue.

Richness										
Bodo sp.		**t-value**	**df**	**p**	**Valid N**	**Valid N**	**Std.Dev.**	**Std.Dev.**	**F-ratio**	**p**
No Predator	Low Initial Density vs High Initial Density	-1.40	2.00	0.30	2	2.00	0.91	0.00	0.00	1.00
Predator	Low Initial Density vs High Initial Density	-0.31	2.00	0.78	2	2.00	0.68	0.43	2.51	0.72
Predator vs. No Predator	High Initial Density	-0.04	2.00	0.97	2	2.00	0.91	0.68	1.81	0.81
Predator vs. No Predator	Low Initial Density	2.30	2.00	0.15	2	2.00	0.00	0.43	0.00	1.00
Colpidium sp.		**t-value**	**df**	**p**	**Valid N**	**Valid N**	**Std.Dev.**	**Std.Dev.**	**F-ratio**	**p**
No Predator	Low Initial Density vs High Initial Density	0.53	2.00	0.65	2	2.00	0.08	0.53	39.72	0.20
Predator	Low Initial Density vs High Initial Density	0.36	2.00	0.75	2	2.00	0.71	0.21	10.98	0.37
Predator vs. No Predator	High Initial Density	1.36	2.00	0.31	2	2.00	0.08	0.71	69.99	0.15
Predator vs. No Predator	Low Initial Density	1.65	2.00	0.24	2	2.00	0.53	0.21	6.23	0.49
Bodo sp. vs. *Colpidium* sp.		**t-value**	**df**	**p**	**Valid N**	**Valid N**	**Std.Dev.**	**Std.Dev.**	**F-ratio**	**p**
No Predator	High Initial Density	-0.53	2.00	0.65	2	2.00	0.53	0.08	39.72	0.20
No Predator	Low Initial Density	0.05	2.00	0.97	2	2.00	0.00	0.53	0.00	1.00
Predator	High Initial Density	-0.54	2.00	0.64	2	2.00	0.68	0.71	1.09	0.97
Predator	Low Initial Density	-0.03	2.00	0.98	2	2.00	0.43	0.21	4.03	0.59

Appendix 10. T Test results comparing bacteria OTU richness in Experiment 2 (Low resource communities) to bacteria OTU richness in Experiment 3 (high resource communities). Significant results are in red. Results considred marginally significant are in blue.

Richness										
Bodo sp.		**t-value**	**df**	**p**	**Valid N**	**Valid N**	**Std.Dev.**	**Std.Dev.**	**F-ratio variance**	**p-value variance**
No Predator	Low Initial Density	-2.72	2	0.11	2.00	2.00	0.00	0.82	0.00	1.00
No Predator	High Initial Density	-4.48	2	0.05	2.00	2.00	0.91	0.23	15.43	0.32
Predator	Low Initial Density	-8.47	2	0.01	2.00	2.00	0.43	0.28	2.37	0.73
Predator	High Initial Density	-6.05	2	0.03	2.00	2.00	0.68	0.06	134.49	0.11
Colpidium sp.		**t-value**	**df**	**p**	**Valid N**	**Valid N**	**Std.Dev.**	**Std.Dev.**	**F-ratio**	**p**
No Predator	Low Initial Density	-3.71	2	0.07	2.00	2.00	0.53	0.32	2.86	0.68
No Predator	High Initial Density	-3.76	2	0.06	2.00	2.00	0.08	0.65	60.00	0.16
Predator	Low Initial Density	-4.43	2.00	0.05	2.00	2.00	0.00	0.02	399.37	0.06
Predator	High Initial Density	-4.38	2	0.05	2.00	2.00	0.71	0.41	2.97	0.67