

The effects of species properties and community context on establishment success

Sarah M. Gray, Daniel E. Dykhuizen and Dianna K. Padilla

S. M. Gray (sarahmarie.gray@unifr.ch), D. E. Dykhuizen and D. K. Padilla, Dept of Ecology and Evolution, Stony Brook Univ., Stony Brook, NY 11794-5245, USA. Present address for SMG: Dept of Biology, Univ. of Fribourg, Ch. du Musée 10, CH-1700 Fribourg, Switzerland.

Understanding whether factors important for species establishment in a local community are predictable or context-dependent is key for determining the features that affect community stability and species coexistence. A major challenge for scientists addressing this question is that natural systems are complex. This makes it difficult to test multiple properties of species and features of the resident community simultaneously to determine what factors are most important for establishment success of a species into a novel community. We used the model aquatic system inside the leaves of the pitcher plant *Sarracenia purpurea* to test whether properties predicted to be important for establishment success of a species (initial density, competitive ability, body size) are generalizable across communities varying in resource availability and the presence of a top predator. For intermediate trophic-level species, we found that both competitive dominance and initial density were important for establishment success. Although a less competitive species was also able to successfully establish in the communities, high resource availability and high initial density were important for its establishment success. Body size of the introduced species, although correlated with competitive ability, was not an important characteristic for establishment success. The presence of a top predator significantly decreased the densities of the introduced species when resources were low, but did not completely inhibit establishment success. The relative importance of each of these factors, and interactions among them, could not have been discerned through single hypothesis testing. The results from this work show the need for detailed experiments that focus on combinations of factors to understand if mechanisms determining community assembly and species establishment can be generalized across systems.

Identifying mechanisms that govern the assembly of a community has been an over-arching theme within community ecology for decades (Gleason 1927, Clements 1938, Diamond 1975, Samuels and Drake 1997, Belyea and Lancaster 1999). Ecologists have recognized that functional traits and species characteristics are fundamentally important for allowing a species to successfully establish in a community (Götzenberger et al. 2012). These characteristics determine if a species is able to successfully pass through a set of dispersal, biotic and abiotic filters, and thus contribute to the local species assemblage of a particular community (Keddy 1992). These same mechanisms have also recently gained attention as scientists try to determine the causes and effects of non-native species introductions. Like community assembly (Diamond 1975, Belyea and Lancaster 1999, Lonsdale 1999), particular aspects of the species and of the resident community are thought to play key roles in invasion success of a species (Elton 1958, Baker 1974, Davis et al. 2000, Lockwood et al. 2007). Yet, in both community assembly and invasion biology, the ability to predict which of these factors is the most influential to the successful establishment of a species into a community is limited. The reasons behind this limitation are multifaceted. Ecological systems differ, multiple factors may interplay and are difficult to test

simultaneously, and the importance of particular characteristics for the establishment of a species might be context dependent, changing depending on the specific abiotic and biotic factors at play in a system.

Among community assembly and invasion studies, propagule pressure and characteristics such as competitive ability and body size are thought to contribute to the successful establishment of a species into a community and, ultimately, the trajectory of community organization (Diamond 1975, Belyea and Lancaster 1999). It has been hypothesized that a species will successfully establish in a community if it is competitively dominant, displacing current species in a resident community (Diamond 1975, Case 1990, 1991, Cornell and Lawton 1992, Morton and Law 1997, Sakai et al. 2001), or if it enters a community in large numbers (Lockwood et al. 2007). The higher the propagule pressure (i.e. higher abundance or density of individuals introduced), the more likely that some individuals will survive stochastic events, allowing for a successful establishment into a resident community (Colautti et al. 2006, Lockwood et al. 2007, Simberloff 2009, Blackburn et al. 2011).

The relative importance of propagule pressure and species characteristics for colonization and establishment success is also thought to be impacted by properties of the resident

community, such as the stage of community development (Belyea and Lancaster 1999, Olito and Fukami 2009), the availability of resources (Huennike et al. 1990, Burke and Grime 1996, Lonsdale 1999, Davis et al. 2000), and by the presence or absence of predators and pathogens (Hairston et al. 1960, Carpenter et al. 1987, Crawley 1997, Keane and Crawley 2002, Shea and Chesson 2002). For example, when more resources are available in a habitat, competition among species has been predicted to be affected in two ways. First, with an increase in resources, competition is predicted to decrease, and thus increase the types and densities (Huennike et al. 1990, Burke and Grime 1996) of species that can successfully establish in a community (Davis et al. 2000). However, as density increases, competition for previously non-limiting resources such as space or light may increase (Suding et al. 2005). Furthermore, if a species is introduced into a community where it has no enemies, it will not be regulated by consumption or disease and can readily increase in numbers and ultimately successfully establish in the community (Crawley 1997, Keane and Crawley 2002, Shea and Chesson 2002).

Given increased rates of environmental change, it is essential to identify the most important factors influencing establishment success of a species under different abiotic and biotic conditions. By doing this, scientists will gain a better understanding of the mechanisms affecting community assembly and species invasion success, and ultimately, community stability and coexistence under different ecological conditions. Due to the complexity of natural communities and the long generation time of many species, controlled experiments that use species with fast generation times are advantageous to answering questions about the establishment success of species (Catford et al. 2009). We used the model aquatic system inside the leaves of the pitcher plant *Sarracenia purpurea* to test whether initial density (one aspect of propagule pressure), species identity in terms of competitive ability and body size, resource availability, the presence of a top predator, or some combination of these factors, impacted the establishment success of species in the intermediate trophic level (primary consumers) of this community. Using this experimental system allowed us to directly test the generalizability of factors allowing successful establishment across communities within the same system, as well as the effects of resource availability and trophic structure (presence or absence of a predator) on this establishment success.

Methods

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 microcosm community that has dynamics similar to larger aquatic food webs (e.g. such as commensalism, Heard 1994; top-down and bottom-up effects, Kneitel and Miller 2002, nutrient limitation, Gray et al. 2006; succession, Gray 2012), but on small spatial and short time scales. In the native range of North America, insects, primarily ants, fall into the trapped rainwater

(Newell and Nastase 1998). Bacteria and yeast colonize the system, decompose the insects, and liberate nutrients for the plant (Kneitel and Miller 2002). A variety of protozoans (flagellates and ciliates) and a rotifer species also colonize this community and consume the bacteria (Kneitel and Miller 2002). The highest trophic level is filled by the larvae of the endemic pitcher plant mosquito *Wyeomyia smithii* which feed on the protozoans and rotifers (Kneitel and Miller 2002). An endemic midge, *Metriocnemus knabi*, found at the bottom of the leaf, also decomposes 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 similar across the entire native geographic range of the plant (Buckley et al. 2010).

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), competition and evolution (terHorst et al. 2010), food web diversity (Baiser et al. 2011), and invasion success (Miller et al. 2002) on community dynamics and structure. This background knowledge of community composition, species interactions and community dynamics allow us to investigate mechanisms driving establishment success of species with different characteristics in different environments.

We used this system to test the relative importance of species identity in terms of body size (small and large) and competitive ability (least or most competitive), initial density (an aspect of propagule pressure), and the combinations of these factors, on the establishment success of intermediate trophic level species (primary consumers). We also tested whether features of the resident community (resource availability or presence of a top predator) affected establishment success (Fig. 1). Due to the large number of treatments and

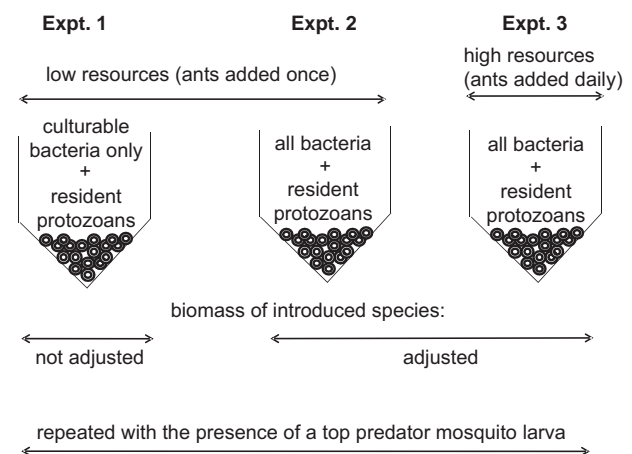


Figure 1. Experimental design. Test tubes represent the resident communities and are aligned according to resource availability (low resources and high resources) and experiment number. The circles in the test tubes represent the beads that were used for habitat complexity and are used as refugia for 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. If the biomass of the introduced species was equal or not was also specified according to experiment, as well as if a predator was present or absent.

replication needed, we conducted three separate experiments. Experiment 1, which was conducted in July 2009, tested the effect of low resources, initial density, top predator presence, and competitive ability on establishment success. Experiment 2, which was conducted in July 2010, had the same treatments as experiment 1, except the size difference of the two introduced species was adjusted. Experiment 3, which was conducted in August 2010, had the same treatments as experiment 2, except that the resident community had high resource availability.

Intermediate trophic level species

Five of the most common protozoan species found in pitcher plant aquatic communities in North America (Buckley et al. 2003, 2010, Gray 2012, Miller and terHorst 2012) were collected from Cranberry Bog Preserve in Riverhead, NY (40°90'N, 72°67'W) and isolated into monocultures that were maintained on a 12 h light / dark cycle in a growth chamber (27°C). These five species included three ciliates (*Colpidium* sp., *Colpoda* sp., *Cyclidium* sp.) and two flagellates (*Bodo* sp. and *Chrysonomad* sp.).

Through preliminary pairwise experiments, we determined the relative competitive ability of these species, and found a competitive hierarchy. *Colpidium* sp. was the competitive dominant followed by *Cyclidium* sp. > *Colpoda* sp. > *Chrysonomad* sp. > *Bodo* sp. This competitive ranking is supported by results of other studies using these same species collected from *S. purpurea* 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 with larger protozoans more competitively dominant than smaller protozoans. Based on these results, we selected the competitively-dominant, large-sized protozoan (the ciliate *Colpidium* sp.) and the least competitive, smaller protozoan (the flagellate *Bodo* sp.) to test for species and community-specific properties of establishment 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).

Bottom trophic level species

To determine the impact of the bottom trophic level (bacteria) on the establishment success of intermediate trophic level consumers, the bottom trophic level of the resident communities was inoculated in two different ways. For experiment 1, we used seven isolated culturable bacteria (able to grow on agar in the laboratory) obtained from *S. purpurea* water collected in the field and maintained in monocultures in the laboratory. In this experiment, all bacteria were collected at the same time and from the same communities as the protozoans that were used in the experiments. After plating *S. purpurea* water on luria-bacto agar media in serial dilutions, we allowed the bacteria to grow in the incubator at 27°C for 72 h. We then selected the morphotypes that were the most phenotypically distinct from one another and created cultures of these isolates which we maintained in the incubator for the same amount of time as the isolated protozoan species. We identified the seven culturable phenotypes of bacteria with sequence analysis of the 16S rRNA gene. The

two isolates 'Cloudy' and 'White' were Gammaproteobacteria with 'Cloudy' having a 100% sequence similarity to *Enterobacter* sp. DB32 (accession number JN975206) and 'White' having a 99% sequence similarity to *Serratia* sp. 9A_5 (accession number AY689057). The isolates 'Purple' and 'Tan' were both Betaproteobacteria with 'Purple' having a 99% sequence similarity to *Chromobacterium violaceum* strain ESBV (accession number EU93450) and 'Tan' having a 99% sequence similarity to *Aquitalea magnusonii* (accession number NR_043475). The two isolates 'Orange' and 'Pink' were from the Phylum Bacteroidetes with 'Orange' in the Class Flavobacteria with a 100% sequence similarity to *Chryseobacterium* sp. COLI2 (accession number EF442766) and 'Pink' in the Class Sphingobacteria with 99% sequence similarity to *Pedobacter* sp. PBI 19 (accession number HM204921). The isolate 'Yellow' was an Actinobacteria with 99% sequence similarity to *Leifsonia* sp. TP2ME (accession number GU272377).

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. We collected *S. purpurea* water from leaves until we obtained enough water to create all resident communities for the experiments. This water was collected the same day as the resident communities were built for each experiment. In the laboratory, the water from the leaves was filtered multiple times to remove all detritus, invertebrates, and protozoans. A sterilized 233 µm sieve was used for the first filtration. A vacuum pump was then used to pass the water through successively smaller filters (sterilized 8 µm and 0.8 µm Millipore filters, and a final 0.7 µm Glass Fiber GF / F Millipore filter). After the final filtration, water containing only bacteria, and no other members of the food web from *S. purpurea* communities, remained.

Formation of the resident community

For all three experiments, we built resident communities that contained the three isolated protozoans, *Cyclidium* sp., *Colpoda* sp. and *Chrysonomad* sp., in the same average densities as these species are naturally found in the communities within *S. purpurea* leaves in the field (Gray 2012). *Colpidium* sp. and *Bodo* sp. were excluded, as they were used as the introduced species treatments. For each experiment, the protozoan and bacterial species were pooled into one large, sterilized (autoclaved) container. For experiment 1 (only culturable bacteria), this container was filled with autoclaved deionized water to a volume that allowed for the appropriate number of replicate communities to be created. For experiments 2 and 3 (with both culturable and unculturable bacteria), filtered pitcher plant water was used instead. The three species of resident protozoans from laboratory cultures were then added to the bacterial communities. In all cases, this pooled community was mixed continuously to homogenize the community, and 10 ml aliquots were distributed into 50 ml experimental sterilized macrocentrifuge tubes. Two milliliters of autoclaved glass beads (3 mm diameter with 1 mm 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 unpubl., terHorst 2010). Four dead, autoclaved *Tapinoma sessile*, the most common ant found in pitcher plants at Cranberry Bog Preserve (Gray et al. unpubl.), were added to each resident community as the nutrient source.

The completed resident communities (ants, bacteria and three protozoan species) were allowed to stabilize in the growth chamber for 72 h at 27°C (12 h light / dark cycle) before the addition of the experimental treatments. This allowed a turnover of approximately 18 bacterial generations and seven protozoan generations.

Initiation of the experiments

After resident communities developed for 72 h, we then introduced the test protozoan species (as individual treatments, never in combination). For all experiments, *Colpidium* sp. (competitive-dominant and large body size) and *Bodo* sp. (least competitive and small body size) remained in isolated cultures until the start of the experiment and had not interacted with other *S. purpurea* protozoan species for thousands of generations.

Experiment 1

Experiment 1 was designed to test the effect of initial density on the establishment success of each of the two intermediate trophic level species, and if a top predator and low resource availability affected establishment success. In this experiment, the body size difference of the two species was not adjusted. Four autoclaved ants were added to each replicate of each treatment when the resident communities were built, before the initiation of the experiment. No ants were added during the experiment, producing low resource availability conditions. We manipulated three factors in a fully factorial design: 1) introduced species identity (*Bodo* sp. or *Colpidium* sp.), 2) initial introduction density (high = 1000 individuals, 100 / ml, medium = 500 individuals, 50 / ml, low = 50 individual, 5 / ml), and 3) predator absence or presence (one *W. smithii*). This produced a total of 12 treatment combinations, each replicated four times (Fig. 1). Each second instar mosquito larva was double rinsed in sterilized deionized water (30 min per rinse) to remove any protozoans before being added to experimental communities.

Experiment 2

Experiment 2 was designed to test the effects of initial density in term of biomass of the introduced species (rather than its numerical abundance) on establishment success. *Colpidium* sp. is approximately 10 times larger than *Bodo* sp., therefore the densities of individuals that were added were adjusted so that their biomasses were approximately equal. We manipulated three factors in a fully factorial design: 1) introduced species identity (*Bodo* sp. or *Colpidium* sp.), 2) initial introduction density with biomass differences taken into account (high = 100 individuals of *Colpidium* sp. and 1000 individuals of *Bodo* sp., low = 10 individuals of *Colpidium* sp. and 100 individuals of *Bodo* sp. and 3) predator absence or presence (one *W. smithii*). This produced a total of 8 treatment combinations, each replicated four times

(Fig. 1). This experiment had the same low resource availability as experiment 1.

Experiment 3

The treatment combinations in experiment 3 were identical to those in experiment 2, except that resource availability was greater throughout the experiment. In this case, four ants were added to initiate the resident community, and then one autoclaved ant was added daily to all replicates of all treatments throughout the experiment (Fig. 1). All treatments in experiment 3 were replicated three times.

Sampling methods

The same sampling protocol was used for all three experiments. All replicates of each treatment were sampled on days 2, 4 and 6 to determine the densities of the introduced *Colpidium* sp. and *Bodo* sp. through time. On each sampling day, communities were gently mixed and the densities of both *Colpidium* sp. and *Bodo* sp. in a 0.1 ml aliquot from each tube was counted at 40× magnification with a compound microscope. No mosquitoes died or pupated in any replicate during the experiments.

Statistical analysis

Because we were interested in the establishment success of the test species through time, we used a linear mixed effects model (lme) to analyze the establishment success of *Colpidium* sp. and *Bodo* sp. All data were boxcox transformed to create a normal distribution and the residuals were checked and determined to be normal. The analysis was performed in R (ver. 2.13.1) with the function `lme` (package `nlme`; Pinheiro et al. 2011) with the REML method to fit the model by maximizing the restricted log-likelihood. Each experiment was analyzed separately. We set the fixed effects in the model as invader identity, initial introduction density, and mosquito presence / absence, plus the interaction terms of all factors. As random effects, we used time since the start of the experiment (days 2, 4 and 6), which takes into account the lack of independence among the repeated observations. For each experiment, we present the results of the model containing the lowest AIC value.

Results

For the full linear mixed effect model tables of the three experiments see Supplementary material Appendix 1–3. In all three experiments, establishment success was affected by: 1) the identity of the invader, 2) propagule pressure (initial density), and 3) the presence of the top predator (Table 1). In communities with low resources (experiments 1 and 2), the effect of the predator differed for the two introduced species (species–predator interaction). Experiment 1 also contained a significant Species × Initial density interaction, experiment 2 contained a significant Initial density × Predator interaction, and experiment 3 contained a significant three-way interaction. However, these significant reactions were only found within one experiment and were not generalizable across experiments.

Table 1. Summary of the similarities in results across experiments. Results were obtained from the linear mixed effect (lme) models testing the properties important for establishment success of the competitively dominant protozoan, *Colpidium* sp., and the least competitive protozoan, *Bodo* sp. The significant results are displayed by experiment. Significant p-values are in bold. Non-significant results are represented in the table as 'NS'. The highlighted results are those that are similar across experiments. The slope, standard error, and t-test value are reported in parentheses under all significant p-values. For 'Species', the positive slopes found across experiments indicates that *Colpidium* sp. is present in the resident communities in a higher density than *Bodo* sp. For 'Initial density', the negative slopes indicates that the treatment 'low density' had less of an effect on establishment success than 'high density' (the introduced species were found at a higher density in communities when they were introduced at high density, than when they were introduced at low density). The negative slopes for the 'Predator' treatment indicate that *Colpidium* sp. and *Bodo* sp. densities were lower when a predator was present. The negative slopes of 'Species × Predator' indicate that *Colpidium* sp. density was more negatively affected by the presence of a predator than *Bodo* sp. in the low resource experiments.

	Experiment 1	Experiment 2	Experiment 3
Species	p < 0.00001 (1.33, 0.205, 6.47)	p < 0.00001 (1.21, 0.256, 4.70)	p < 0.043 (0.611, 0.296, 2.07)
Initial density	Low density: p < 0.00001 (-0.79, 0.177, -4.46) Med. density: p = 0.0028 (-0.541, 0.177, -3.05)	Low density: p = 0.0022 (-0.87, 0.256, -3.15)	Low density: p = 0.0035 (-0.897, 0.296, -3.03)
Predator	p = 0.0179 (-0.347, 0.145, -2.40)	p = 0.064 (-0.481, 0.256, -1.88)	p = 0.0001 (-1.22, 0.296, -4.11)
Species×Initial density	Low density: NS Med. density: p = 0.0416 (0.516, 0.251, 0.0426)	NS	NS
Species×Predator	p < 0.00001 (-0.946, 0.205, -4.614)	p < 0.00001 (-1.31, 0.296, -4.43)	NS
Initial density×Predator	NS	Low density: P = 0.019 (0.705, 0.296, 2.38)	NS
Species×Initial density×Predator	NS	NS	p = 0.0257 (-1.35, 0.592, -2.28)

Introduced species type (the Species factor)

In all three experiments, *Colpidium* sp., the competitive dominant with a large body size, was significantly more successful at establishing in 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 1, Fig. 2–4).

Initial density

The initial density (propagule pressure) was important for establishment success for both *Colpidium* sp. and *Bodo* sp.; establishment success was greater for both species at high initial densities in all experiments, and also at medium initial density when this density was tested in experiment 1 (Table 1, Fig. 2–4).

Enemy release

The presence of a top predator significantly affected the establishment success of both intermediate trophic level species (Table 1, Fig. 2–4). In all three experiments, for all treatments with the predator present, densities at which the introduced species established were significantly lower than when a top predator was absent (Fig. 2–4). This was especially the case for experiment 1 and 3, while showing a trend towards significance in experiment 2.

The density of *Colpidium* sp. was more affected by the presence of the predator than was *Bodo* sp. when resource availability was low (results from the individual analyses of experiment 1 and experiment 2; Predator × Species interaction, Fig. 2–3). However, the density of *Bodo* sp. in the

low resource experiments was always low, and did not differ with predators present or absent (Fig. 2–3). When resources in the resident community were high (experiment 3), there was no significant difference in the effect of the predator on *Colpidium* sp. and *Bodo* sp. (no significant Species × Predator interaction).

Discussion

The initial density at which a species arrives in a community (an aspect of propagule pressure) and properties of species such as competitive ability and body size have been considered factors that facilitate establishment success during community assembly (Diamond 1975, Belyea and Lancaster 1999). Successful establishment of a species into a community is also thought to be dependent on properties of the community, such as high or low resource availability (Lonsdale 1999, Davis et al. 2000) and the presence or absence of predators (Hairston et al. 1960, Carpenter et al. 1987). These same factors have gained recent attention because of the increase in the number of species that are transported by humans around the world and are negatively impacting communities globally. The absence of predators (enemy release, Jones and Lawton 1991, Shea and Chesson 2002), the availability of resources (Davis et al. 2000), competitive ability (Sakai et al. 2001) and the propagule pressure of a species (Lockwood et al. 2007), are all thought to be of major importance for the success of species invasions. Because natural ecosystems are highly complex, however, research in invasion biology rarely considers factors at the species' level and at the community level simultaneously (Catford et al. 2009, Lowry et al. 2013), even though multiple hypotheses

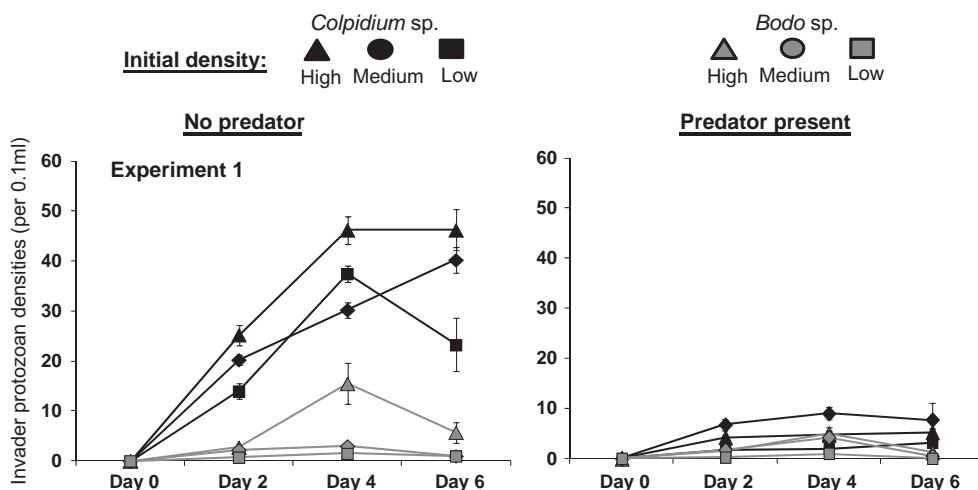


Figure 2. Average densities of *Colpidium* sp. (black line) and *Bodo* sp. (gray line) in experiment 1 through time. *Colpidium* sp. and *Bodo* sp. were added in the same initial densities in separate treatments in the low, medium and high initial density treatments. The graph on the left represents 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.

are likely to explain invasion success and the assembly of a community (Lowry et al. 2013).

By conducting experiments with the model *Sarracenia* system, we were able to test the the importance of specific species-level and community-level features on establishment success, simultaneously. We found that the identity of a species, in terms of specific species characteristics, and high initial density were important for the successful establishment of intermediate trophic level species. The less competitive species was able to invade, but establishment success depended on high propagule pressure and high resource availability. The less competitive species always showed 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 was always better able to establish in a community.

Resource availability in the resident communities also played a key role in determining the establishment success of *Colpidium* sp. and *Bodo* sp. Both species were present in higher densities when resource availability was high. Although *Bodo* sp. was successful at invading all communities, its persistence in a community was strongly dependent on the amount of resource available. The results from the three experiments presented here suggest that even though

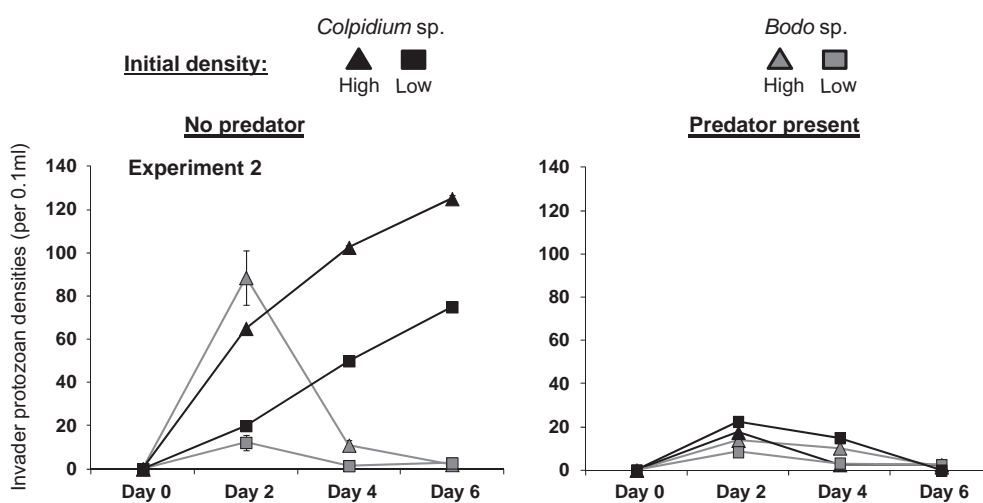


Figure 3. Average densities of *Colpidium* sp. (black line) and *Bodo* sp. (gray line) in experiment 2 through time. The graph on the left represents the densities of *Colpidium* sp. or *Bodo* sp. when introduced into separate predator-free communities. The graph on the right represents *Colpidium* sp. 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.

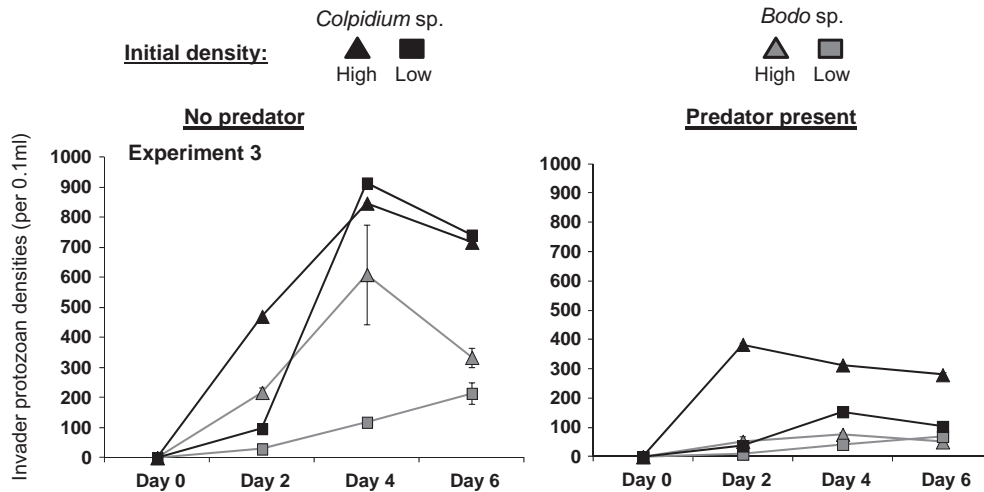


Figure 4. Average densities of *Colpidium* sp. (black line) and *Bodo* sp. (gray line) in experiment 3 through time. The graph on the left represents the densities of *Colpidium* sp. or *Bodo* sp. when introduced into separate predator-free communities. The graph on the right represents *Colpidium* sp. 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.

high resource availability will allow both highly competitive and less competitive species to invade, the competitively dominant species is still more successful at establishing in a community than a less competitive species. These results imply that for intermediate trophic level species in this system, competitive dominants may be better at establishing in a community than less competitive species, independent of 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 of the introduced species in the resident community. When resources were not limited, *Colpidium* sp. and *Bodo* sp. densities quickly increased to high numbers, and they were both 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 the low resource environment. These results suggest that the presence of a top predator will always have a negative effect on the establishment success of intermediate trophic level species in this system. In addition, the composition of the bacterial community did not affect the overall results. The patterns observed were similar in the experiment with only culturable bacteria and the two experiments with additional species of non-culturable bacteria present.

All factors tested in this study have been considered important during community assembly – species identity in terms of competitive ability and body size, propagule pressure, resource availability and predation risk – and all affected invasion and establishment success. Similar results to this study have been found in other studies of invasion success for intermediate trophic level species. Romanuk and Kolasa (2005) found that the invasion success of the top

competitor, the 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. In experiments with aquatic microorganisms, Mächler and Altermatt (2012) found that species identity was important for determining invasion success, but that it was necessary to test the effects of both species characteristics and environmental factors simultaneously to understand invasion success. Within the *Sarracenia* system, Miller et al. (2002) found that invasion success of an intermediate trophic level species was influenced by migration, predation and resource availability.

Although we found that *Colpidium* sp. was better at establishing in a community than *Bodo* sp., we only tested one competitively dominant species and one least competitive species in this system. However, across experiments, the competitively-dominant protozoan species showed continuously consistent results, whether numbers or biomass were considered as the initial density. The least competitive species was much more likely to establish in a community if the introduced biomass and resource availability was high. In this system, among protozoans that have been tested, body size dictates competitive ability, and ciliates are more competitive than flagellates (Kneitel 2002), therefore, we predict that these results will apply to other species with these same characteristics. However, from the scope of the experiment presented here we cannot say if it is only the competitive ability of *Colpidium* sp. that allowed this species to be better at establishing than *Bodo* sp., or if some untested species' characteristic also played a role. Further experimentation will be needed to determine if it is indeed competitive ability that is the driving characteristic for establishment success and if this result is general across systems.

The results of the experiments we present here demonstrate the importance of multiple factors, and interactions

among factors, that could not have been discerned from experiments testing single hypotheses. Further work on more species and systems is needed to assess the relative roles of propagule pressure, species identity, resource availability and predators in a multi-trophic context (Baiser et al. 2010). We also argue that simultaneous testing of multiple hypotheses will be critical for understanding the contributions of species-level characteristics and the features of communities on the establishment success of species. This information will be essential for understanding the most important factors contributing to community assembly, which can ultimately help determine the factors influencing invasion success of real-world invaders.

Acknowledgements – Statement of authorship: SMG designed and conducted the experiments, analyzed the results of the project, and wrote the manuscript. DED and DKP provided input on the design of the experiment and scientific question, interpretation of the results, and contributed substantially to revisions. We would like to thank Jessica Gurevitch and Shahid Naem for helpful comments on experimental design and earlier versions of this manuscript. This project was funded by the National Science Foundation Doctoral Dissertation Improvement Grant no. DEB-0909830 and the Stony Brook University Slobodkin Award. This manuscript is contribution no. 1233 from the Dept of Ecology and Evolution at Stony Brook University.

References

- Baiser, B. et al. 2010. Connectance determines invasion success via trophic interactions in model food webs. – *Oikos* 119: 1970–1976.
- Baiser, B. et al. 2011. Species richness and trophic diversity increase composition in a co-evolved food web. – *PlosOne* 6: e20672.
- Baker, H. 1974. The evolution of weeds. – *Annu. Rev. Ecol. Syst.* 5:1–24.
- Belyea, L. R. and Lancaster, J. 1999. Assembly rules within a contingent ecology. – *Oikos* 86: 402–416.
- Blackburn, T. M. et al. 2011. Passerine introductions to New Zealand support a positive effect of propagule pressure on establishment success. – *Biodivers. Conserv.* 10: 2189–2199.
- Buckley, H. L. et al. 2003. Reverse latitudinal trends in species richness of pitcher-plant food webs. – *Ecol. Lett.* 6: 825–829.
- Buckley, H. L. et al. 2010. Local- to continental-scale variation in the richness and composition of an aquatic food web. – *Global Ecol. Biogeogr.* 19: 711–723.
- Burke, J. W. and Grime, J. P. 1996. An experimental study of plant community invasibility. – *Ecology* 77: 776–790.
- Carpenter, S. R. et al. 1987. Regulation of lake primary productivity by food web structure. – *Ecology* 68: 1863–1876.
- Case, T. J. 1990. Invasion resistance arises in strongly interacting species-rich model competition communities. – *Proc. Natl Acad. Sci. USA* 87: 9610–9614.
- Case, T. 1991. Invasion resistance, species build-up and community collapse in metapopulation models with interspecies competition. – *Biol. J. Linn. Soc.* 42: 239–266.
- Catford, J. A. et al. 2009. Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. – *Divers. Distrib.* 15: 22–40.
- Clements, F. E. 1938. Nature and structure of the climax. – *J. Ecol.* 24: 252–282.
- Cochran-Stafira, D. L. and von Ende, C. E. 1998. Integrating bacteria into food webs: studies with *Sarracenia purpurea* inquilines. – *Ecology* 79: 880–898.
- Colautti, R. I. et al. 2006. Propagule pressure: a null model for biological invasions. – *Biol. Invas.* 8: 1023–1037.
- Cornell, H. V. and Lawton, J. H. 1992. Species interactions, local and regional processes, and limits to the richness of ecological communities: a theoretical perspective. – *J. Anim. Ecol.* 61: 1–12.
- Crawley, M. J. 1997. Plant ecology. – Blackwell.
- Davis, M. A. et al. 2000. Fluctuating resources in plant communities: a general theory of invasibility. – *J. Ecol.* 88: 1602–1610.
- Diamond, J. M. 1975. Assembly of species communities. – In: Cody, M. L. and Diamond, J. M. (eds), *Ecology and evolution of communities*. Univ. Press, pp. 342–344.
- Elton, C. S. 1958. The ecology of invasions of plants and animals. – Univ. of Chicago Press.
- Gleason, H. A. 1927. Further views on the succession-concept. – *Ecology* 8: 299–326.
- Gray, S. M. 2012. Succession in the aquatic *Sarracenia purpurea* community: deterministic or driven by contingency? – *Aquat. Ecol.* 46: 487–499.
- Gray, S. M. et al. 2006. Nutrient limitation in *Sarracenia purpurea* microcosms. – *Hydrobiologia* 573: 173–181.
- Götzenberger, L. et al. 2012. Ecological assembly rules in plant communities – approaches, patterns and prospects. – *Biol. Rev.* 87: 111–127.
- Hairston, N. G. et al. 1960. Community structure, population control and competition. – *Am. Nat.* 94: 421–425.
- Heard, S. B. 1994. Pitcher-plant midges and mosquitoes: a processing chain commensalism. – *Ecology* 75: 1647–1660.
- Huenneke, L. F. et al. 1990. Effects of soil resources on plant invasion and community structure in Californian serpentine grassland. – *Ecology* 71: 478–491.
- Jones, C. G. and Lawton, J. H. 1991. Plant chemistry and insect species richness of British umbellifers. – *J. Anim. Ecol.* 60: 767–777.
- Keane, R. M. and Crawley, M. J. 2002. Exotic plant invasions and the enemy release hypothesis. – *Trends Ecol. Evol.* 17: 164–170.
- Keddy, P. A. 1992. Assembly and response rules: two goals for predictive community ecology. – *J. Veg. Sci.* 3: 157–165.
- Kneitel, J. M. 2002. Species diversity and trade-offs in pitcher plant (*Sarracenia purpurea*) inquiline communities. – PhD thesis, Florida State Univ.
- Kneitel, J. 2007. Intermediate-consumer identity and resources alter a food web with omnivory. – *J. Anim. Ecol.* 76: 651–659.
- Kneitel, J. M. and Miller, T. E. 2002. The effects of resource and top-predator addition to the inquiline community of the pitcher plant *Sarracenia purpurea*. – *Ecology* 83: 680–688.
- Lockwood, J. L. et al. 2007. Invasion ecology. – Blackwell.
- Lonsdale, W. M. 1999. Global patterns of plant invasions and the concept of invasibility. – *Ecology* 80: 1522–1536.
- Lowry, E. et al. 2013. Biological invasions: a field synopsis, systematic review, and database of the literature. – *Ecol. Evol.* 3: 182–196.
- Mächler, E. and Altermatt, F. 2012. Interaction of species traits and environmental disturbance predicts invasion success of aquatic microorganisms. – *PLoS ONE* 7: e45400.
- Miller, T. E. and terHorst, C. P. 2012. Testing successional hypotheses of stability, heterogeneity, and diversity in pitcher-plant inquilines communities. – *Oecologia* 170: 243–251.
- Miller, T. E. et al. 2002. Effect of community structure on invasion success and rate. – *Ecology* 83: 898–905.
- Morton, R. D. and Law, R. 1997. Regional species pools and the assembly of local ecological communities. – *J. Theor. Biol.* 187: 321–331.

- Newell, S. J. and Nastase, A. J. 1998. Efficiency of insect capture by *Sarracenia purpurea* (Sarraceniaceae), the northern pitcher plant. – *Am. J. Bot.* 85: 88–91.
- Olito, C. and Fukami, T. 2009. Long-term effects of predator arrival timing on prey community succession. – *Am. Nat.* 173: 354–362.
- Pinheiro, P. et al. 2011. nlme: linear and nonlinear mixed effects models. – R package ver. 3.1–101.
- Romanuk, T. N. and Kolasa, J. 2005. Resource limitation, biodiversity and competitive effects interact to determine the invasibility of rock pool microcosms. – *Biol. Invas.* 7: 711–722.
- Sakai, A. K. et al. 2001. The population biology of invasive species. – *Annu. Rev. Ecol. Syst.* 32: 305–332.
- Samuels, C. L. and Drake, J. A. 1997. Divergent perspectives on community convergence. – *Trends Ecol. Evol.* 12: 427–432.
- Shea, K. and Chesson, P. 2002. Community ecology as a framework for biological invasions. – *Trends Ecol. Evol.* 17: 170–176.
- Simberloff, D. 2009. The role of propagule pressure in biological invasions. – *Annu. Rev. Ecol. Evol. Syst.* 40: 81–102.
- Suding, K. N. et al. 2005. Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. – *Proc. Natl Acad. Sci. USA* 102: 4387–4392.
- terHorst, C. P. 2010. Evolution in response to direct and indirect ecological effects in pitcher plant inquiline communities. – *Am. Nat.* 176: 675–685.

Supplementary material (available online as Appendix oik.01550 at <www.oikosjournal.org/readers/appendix>). Appendix 1–3.