

Relative importance of top-down and bottom-up forces in food webs of *Sarracenia* pitcher communities at a northern and a southern site

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Received: 10 May 2010 / Accepted: 23 September 2010 / Published online: 8 October 2010
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Abstract The relative importance of resources (bottom-up forces) and natural enemies (top-down forces) for regulating food web dynamics has been debated, and both forces have been found to be critical for determining food web structure. How the relative importance of top-down and bottom-up forces varies between sites with different abiotic conditions is not well understood. Using the pitcher plant inquiline community as a model system, I examine how the relative importance of top-down and bottom-up effects differs between two disparate sites. Resources (ant carcasses) and top predators (mosquito larvae) were manipulated in two identical 4×4 factorial press experiments, conducted at two geographically distant sites (Michigan and Florida) within the range of the purple pitcher plant, *Sarracenia purpurea*, and the aquatic community that resides in its leaves. Overall, top predators reduced the density of prey populations while additional resources bolstered them, and the relative importance of top-down and bottom-up forces varied between sites and for different trophic levels. Specifically, top-down effects on protozoa were stronger in Florida than in Michigan, while the opposite pattern was found for rotifers. These findings experimentally demonstrate that the strength of predator–prey interactions, even those involving the same species, vary across

space. While only two sites are compared in this study, I hypothesize that site differences in temperature, which influences metabolic rate, may be responsible for variation in consumer–resource interactions. These findings warrant further investigation into the specific factors that modify the relative importance of top-down and bottom-up effects.

Keywords Predator–prey interactions · Top-down effects · Spatial ecology · Food web dynamics · *Sarracenia purpurea*

Introduction

Abiotic factors can change the strength of biotic interactions, with wide-ranging consequences for biological communities (Sanford 1999; Berlow et al. 2004). An important consequence of biotic interaction strength is the relative importance of top-down and bottom-up effects. Populations are affected by lower (bottom-up) and higher (top-down) trophic levels. The relative importance of these forces and how they vary in different systems is debated (Chase 2000). It is broadly recognized that a combination of top-down and bottom-up effects are responsible for determining food web structure and dynamics (Hunter and Price 1992; Moore et al. 2003). However, these effects vary spatially and temporally, and therefore understanding what influences their importance is an important question in community ecology (Gripengberg and Roslin 2007).

The relative importance of top-down and bottom-up effects may be influenced by a number of abiotic variables that vary among systems in time and space (Polis and Winemiller 1996). Sites at different latitudes or elevations can be useful for determining the effects of factors that vary across space (e.g., Belovsky and Joern 1995) and for making predictions about how those effects may change at a

Communicated by Jonathan Shurin.

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given location in the future. Top-down and bottom-up effects may vary spatially in the aquatic inquiline community contained within the purple pitcher plant, *Sarracenia purpurea* (L.) (Kneitel and Miller 2002; Hoekman 2007). Pitcher plant mosquito *Wyeomyia smithii* (Coq.) larvae have been shown to initiate trophic cascades in Florida (Kneitel and Miller 2002) but not in Michigan (Hoekman 2007), suggesting that top-down effects may be stronger in Florida. Buckley et al. (2003) conducted a survey across North America of *Sarracenia purpurea* inquiline food webs in order to quantify spatial patterns of species richness. They found that species richness increased with latitude, and they speculated that this was driven by decreased top-down effects of the pitcher plant mosquito at northern sites, where they are less abundant (Buckley et al. 2003). Higher species diversity can result in compensatory dynamics and weaker top-down control (Frank et al. 2006). However, Buckley et al. (2003) did not quantify top-down or bottom-up effects or demonstrate functional differences in the food web between northern and southern sites.

I conducted a pair of manipulative experiments using the same pitcher plant food web to investigate how consumer–resource interactions differed between two very similar food webs at geographically disparate locations (Michigan and Florida). I expected to find differences between sites in the strength of top-down and bottom-up effects. Because of accelerated metabolism in response to warmer temperatures (Brown et al. 2004), I expected stronger top-down effects at the southern site (O'Connor et al. 2009). In greenhouse experiments, the top-down effect of pitcher plant mosquitoes on protozoa has been shown to increase with temperature (Hoekman 2010). In addition, the reverse latitudinal diversity gradient in pitcher plant communities (Buckley et al. 2003) suggests that top-down effects could be stronger at less diverse southern latitudes (Frank et al. 2006). Therefore, based on metabolic theory, species diversity and previous studies (Kneitel and Miller 2002; Hoekman 2007, 2010), I hypothesized that top-down effects would be stronger at the southern site (Florida).

Methods

A model system: the pitcher plant inquiline community

The purple pitcher plant, *Sarracenia purpurea* (L.), has modified leaves arranged in a rosette that both attract invertebrates with nectar and entrap them in a fluid-filled cavity. It grows in ombrotrophic bogs and other wetlands with infertile soils. A simple aquatic community exists in rainwater that collects in each pitcher (Fig. 1a) (Giberson and Hardwick 1999) and receives all of its energy from exterior sources (mainly arthropods that drown in the pitcher).

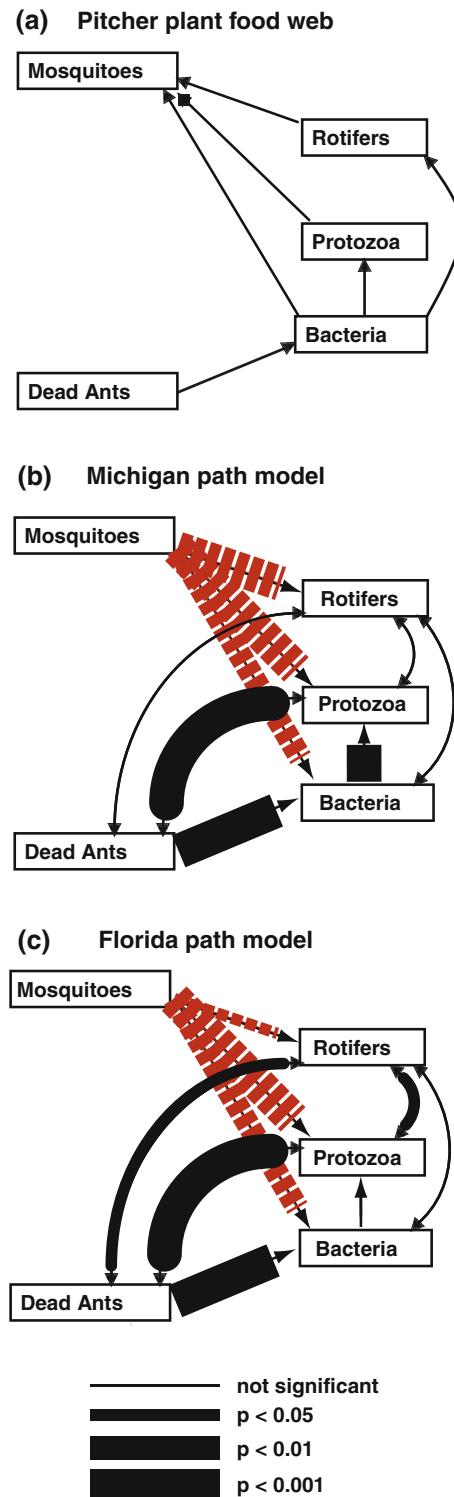


Fig. 1 a Food web of the pitcher plant inquiline community used in experiments at two geographically disparate locations. Path diagrams from b Michigan and c Florida displaying positive (solid) and negative (dashed) effects and their significances (line thickness). Mosquitoes and dead ants are manipulated variables, while rotifer, protozoan and bacterial populations are response variables. Single-headed arrows represent feeding relationships and were modeled as direct effects. Double-headed arrows depict correlations in the model

The food web inside each pitcher includes bacteria, protozoa, bdelloid rotifers, *Habrotrocha rosa* (Donner), water mites, *Sarracenopus gibsoni* (Nesbitt), and three larval dipterans. The dipterans are the pitcher plant mosquito, *Wyeomyia smithii* (Coq.), the pitcher plant midge, *Metriocnemus knabi* (Coq.), and the pitcher plant flesh fly, *Fletcherimyia fletcheri* (Aldrich). All three dipterans are obligate symbionts of the pitcher plant inquiline habitat. The midge, flesh fly and mites feed directly on insect carcasses, comminuting prey and accelerating their digestion by bacteria (Heard 1994; Ellison et al. 2003). Bacteria grow on surfaces and suspended particles in the water and are consumed by rotifers and a diverse group of protozoa. Mosquito larvae are omnivorous top predators, feeding on small rotifers, protozoa, and bacteria (Miller et al. 2002; Hoekman 2007).

There are several advantages to utilizing this inquiline community as a model food web in manipulative experiments. The system is small, relatively simple, easily manipulated, and replicable (each leaf) (Miller and Kneitel 2005). Simple aquatic food webs have been proposed as model systems for studying food webs (Morin and Lawler 1996), and this system is particularly applicable because it is a natural community, not a laboratory construct (Srivastava et al. 2004). In addition, the food web is well suited to experiments manipulating top-down and bottom-up forces. Bacterial productivity, derived from allochthonous input, fuels the system and can be manipulated by adding arthropod carcasses. Top-down processes can be controlled by manipulating top-predator (mosquito larvae) density.

The food web found within the pitchers contains largely the same species across the range of the plant (Buckley et al. 2003), from northern British Columbia to the east coast of North America as far south as Florida. Across this range, many factors vary that may influence the relative importance of top-down and bottom-up effects, including surrounding environment, regional species pool, local genotypes, day length, rainfall, and temperature. For example, Bradshaw and Holzapfel (2001) have shown that the critical photoperiod that triggers seasonal diapause in the pitcher plant mosquito varies with latitude and is shifting toward shorter day lengths (especially in the north, an example of recent evolution driven by climate change). In the inquiline communities used in the current experiments, the insect larvae, rotifers, and most protozoa were the same species at both sites.

Experiments in Michigan and Florida

I conducted a factorial “press” experiment at two sites, one in Michigan (at the University of Notre Dame Environmental Research Center, 46° N) and one in Florida (in the Apalachicola National Forest, 30° N) (hereafter referred to as

MI and FL). Mean daily temperature and total precipitation during the experiments were 15°C, 3.6 cm and 25°C, 3.1 cm, respectively (MI, 21 June–10 July; FL, 18 September–6 October 2004). The temperature ranged from 5 to 28°C in MI and from 15 to 34°C in FL. The 4 × 4 factorial design included four levels of resource addition (0, 5, 10, 20 mg ant carcasses, each ant weighs approx. 1 mg) and four top-predator levels (0, 5, 10, 20 mosquito larvae, third or fourth instar), which reflect common field densities. Predator treatments represent half mean predator density, mean predator density and double mean predator density (mean ± 1SE: mosquitoes 10.5 ± 1.3, $n = 66$). Each of the 16 treatments were replicated three times ($n = 48$ pitchers at each site).

The experiments were initiated by collecting inquiline fluid from approximately 65 randomly chosen pitchers at each site. Insect larvae and carcasses were filtered out using folded 1 mm tulle mesh, and the fluid was pooled to homogenize the rotifer, protozoan, and bacterial communities. In the field, 48 similarly sized new pitchers (<3 weeks old) were chosen from among the emptied pitchers to be rinsed and stocked with 15 mL of liquid (5 mL of filtered fluid and 10 mL of deionized water) and the appropriate number of mosquito larvae and biomass of ant carcasses. Mosquito larvae had been removed from inquiline fluid collected that day and were pooled and randomly assigned to treatments. Dead fire ants, *Solenopsis invicta* (Buren), collected from a single source, were added as resources in both MI and FL. Experimental pitchers were covered with 1 mm tulle mesh to prevent additional prey entry or mosquito colonization. Treatments were assigned in three blocks across a uniform habitat, each containing the 16 treatment combinations.

The pitchers were sampled six times over the course of three weeks, during the growing season of the plant. Sampling included gently mixing the community, temporarily removing the pitcher contents, counting mosquito larvae, and making appropriate adjustments to maintain treatment levels. Mosquito larvae (third or fourth instar) were added to replace those lost to mortality, and new eggs or young larvae that were previously undetected were removed. Additional prey biomass was added (0, 2, 5, 10 mg) based on treatment type to maintain resource treatments. Small samples (<300 µL) of inquiline fluid were taken for protozoan and bacterial analysis.

Protozoa and rotifers were enumerated live using Palmer counting cells and a compound microscope (×100). From each pitcher sample, 100 µL were pipetted onto a slide, and the abundance and richness of protozoa as well as the abundance of rotifers were recorded. Protozoa were identified to genus. Because protozoa are a disparate group and vary enormously in size, I calculated protozoan biovolume (µm³) based on the density and dimensions of each genus and used this measure as a dependent variable for statistics.

Protozoa size did not vary between sites. To estimate bacterial density, a 25 μL sample from each pitcher was treated with formaldehyde, stained with acridine orange, and strained through a Nuclepore filter with a pore size of 0.2 μm (Pace 1993). The filters were put on slides and later photographed under an epifluorescence microscope. Cell density was counted using the Metamorph[®] imaging system (Molecular Devices Corp., Sunnydale, CA, USA).

Laboratory experiments

Two lab experiments were conducted to address whether differences in treatment effects were due to initial protozoan densities or some unknown feature of the mosquitoes at the two sites. To test the effects of initial stocking density of protozoa on their density over time, protozoa were stocked in 50 mL tubes at three densities (25,000, 2,500, and 250 protozoa per mL), and their population density was measured five times over the following month ($n = 10$, $N = 30$). To compare the top-down effects of mosquitoes (third or fourth instar) from a northern and a southern site, a week-long laboratory experiment was performed in 50 mL tubes at 20°C (intermediate temperature between sites). The tubes were stocked with 2 mL of protozoan solution, 18 mL of deionized water, 20 mg of dead ants, and either zero mosquitoes, two mosquitoes from FL or two mosquitoes from a northern population ($n = 5$, $N = 15$). The northern mosquitoes and protozoa for this experiment were collected from a bog in northern Indiana. Protozoa were enumerated and biovolume calculated as described above.

Statistical analyses

Site and top-down and bottom-up treatment effects

Analysis of covariance (ANCOVA) was used to test for the effects of site (factor), mosquitoes and dead ants (two covariates) on the \log_e -transformed density of rotifers, protozoa and bacteria. Because the experiments were not conducted simultaneously, this analysis confounds space and time. However, it does allow for a direct statistical test for site differences and site-by-treatment interactions. Multiple sample sessions over time were used to ensure adequate estimation of population sizes, but no time series or repeated measures tests were done because temporal dynamics were not of primary interest. Therefore, tests were conducted on either the average values over the six sampling sessions (rotifers) or the final sample session (bacteria and protozoa). Each individual sample session was also tested, and results were consistent in the direction of the effect with the average or final sample session. Spatial block had no effect on any dependent variables and was consequently not considered a factor in the models.

Effect size

Using the following formulae, top-down and bottom-up effects were measured using log response ratios, and the relative strengths of top-down and bottom-up effects were evaluated by calculating relative effect sizes (Denno et al. 2005):

- (1) Effect size = $\ln[(\text{treatment density})/(\text{control density})]$
- (2) Specific effect size = (effect size in each treatment)/(number of predators or milligrams of resources added in that treatment)
- (3) Relative effect size = $\ln[(\text{bottom-up specific effect size})/(\text{top-down specific effect size})]$

“Treatment” refers to ant (5, 10, 20 mg) or mosquito (5, 10, 20 larvae) addition treatments for bottom-up or top-down effects, respectively, while “control” refers to zero ant- or mosquito-addition treatments. Positive bottom-up effects and negative top-down effects are expected. If relative effect size is positive, bottom-up effects had a greater impact on the population density than top-down effects, while if relative effect size is negative, top-down effects were stronger than bottom-up effects, and if relative effect size is zero, population density was influenced equally by top-down and bottom-up treatments. At each site, three effect sizes were calculated for each group (rotifers, protozoa and bacteria) by comparing the mean density of each of the three treatments to the control. Specific effect sizes were calculated by dividing each effect size by 5, 10, or 20 (depending on the treatment) to determine the top-down effect per mosquito predator or the bottom-up effect per mg of ant resources. Mean specific effect sizes were used to calculate relative effect size. A commonly noted problem with effect size measures is the fact that the magnitude of any treatment effect will depend on the magnitude of the experimental manipulation (Wojdak and Luttbeg 2005). For example, a large top-down effect may be indicative of a drastic predator treatment rather than the strength of top-down effects in nature. To address such criticism, I designed the top-down and bottom-up treatments to be representative of the levels of predators and resources found in the field at both sites.

Path analysis

Path analysis was used to estimate the strengths and signs of all food web relationships simultaneously and to quantitatively compare the MI and FL food webs. Because the variables are related via known feeding interactions, I produced a path diagram (Fig. 1b, c) that I judged to be most representative of the model food web used in the experiments (Fig. 1a) based on published web topologies (Ellison et al. 2003; Miller and Kneitel 2005) and my own experience with the system (Hoekman 2007). In this model

Table 1 Effect coefficients \pm standard errors for each ANCOVA model (rotifers, protozoa and bacteria)

	Rotifers	Protozoa	Bacteria
Site	0.183 \pm 0.077*	-4.772 \pm 0.312***	-0.630 \pm 0.079***
Mosquitoes	-0.048 \pm 0.010***	-0.384 \pm 0.042***	-0.002 \pm 0.011
Dead ants	0.018 \pm 0.010	0.265 \pm 0.042***	0.055 \pm 0.011***
Site \times mosquitoes	-0.015 \pm 0.010	-0.085 \pm 0.042*	-0.004 \pm 0.011
Site \times dead ants	0.0289 \pm 0.010*	-0.083 \pm 0.042	0.005 \pm 0.011
Mosquitoes \times dead ants	-6.8e-5 \pm 0.001	0.001 \pm 0.006	0.001 \pm 0.001
Site \times mosquitoes \times dead ants	-0.005 \pm 0.001***	-0.012 \pm 0.006*	-0.001 \pm 0.001

This is a summary of the effects of site location, top-down and bottom-up treatments and their interactions on populations sampled from food webs within purple pitcher plants, *Sarracenia purpurea*, in field experiments in Michigan and Florida

Statistical significance is noted with asterisks (* $p < 0.05$, *** $p < 0.001$)

(Fig. 1b, c), variation in mosquito density has direct effects on bacterial, protozoan and rotifer populations. Ants have a direct effect on bacteria, which have direct effects on protozoa and rotifers. Ants are allowed to covary with protozoa and rotifers, and also with mosquitoes (though mosquitoes and ants were factorially manipulated, so the correlation between them is 0). Protozoa and rotifers are also allowed to covary. To better meet the assumptions of linearity and homogeneity of variance, all measured variables were \log_e transformed. Structural equation models were fitted using M-plus (version 3.0, Muthén & Muthén, Los Angeles, CA, USA). The block experimental design was accommodated using the “cluster” option. Partial regression coefficients were first estimated independently for each site (MI and FL). Then, to determine if the food webs were functioning differently between sites, I constrained the path coefficients to be equal and repeated the analysis. The constrained model was compared to the unconstrained model using a hierarchical chi-square test to test the null hypothesis that the models fit the data equally well. The chi-square difference test required a scaling factor (Satorra and Bentler 2001), which did not change the result of the test.

Laboratory experiments

The effects of initial stocking density of protozoa and mosquito origin on the subsequent protozoan biomass (\log_e transformed) were both tested using separate one-way ANOVAs.

Results

Density and diversity differences between sites

Initial densities of bacteria, protozoa and rotifers were similar at both sites. Location (site) was a significant factor in every test (Table 1). By the end of the experiments, rotifer

density was higher in FL, while bacterial density was higher in MI and protozoan density was much higher in MI (Figs. 2 and 3). Protozoa samples were more diverse in MI (2.8 ± 0.4 , 1–8) than in FL (1.8 ± 0.2 , 0–5) (mean richness in fourth sample session \pm SE, range).

Top-down (mosquito treatment)

Increases in mosquito density caused a significant decline in rotifer density in both MI and FL (Fig. 2a, Table 1). Protozoan density was also reduced by mosquitoes in both MI and FL (Fig. 2b, Table 1), though the effect was stronger in FL (two-way interaction between site and mosquitoes, Table 1). In addition, the effect of mosquitoes is stronger at low ant densities in MI, but not in FL (three-way interaction between site, mosquitoes and ants). In contrast, bacteria populations were unaffected by mosquito treatments (Fig. 2c, Table 1).

Bottom-up (ant treatment)

The response of rotifer density to dead ant additions depended on location (site). In FL, rotifer density was positively affected by ant addition (Fig. 3a, Table 1). In MI, in the absence of mosquitoes, there was a negative relationship between rotifers and dead ants. However, in the presence of mosquitoes, rotifer density was reduced so dramatically that they could not respond to ant treatments (Fig. 3a, Table 1, three-way interaction between site, mosquitoes and ants). Protozoan density increased monotonically with ant addition in both MI and FL (Fig. 3b, Table 1). Bacterial density also increased with ant addition at both sites (Fig. 3c, Table 1).

Effect size

Top-down forces (mosquitoes) had negative effects on rotifer and protozoan populations in both MI and FL and no discernable effects on bacteria (Fig. 4a). Bottom-up

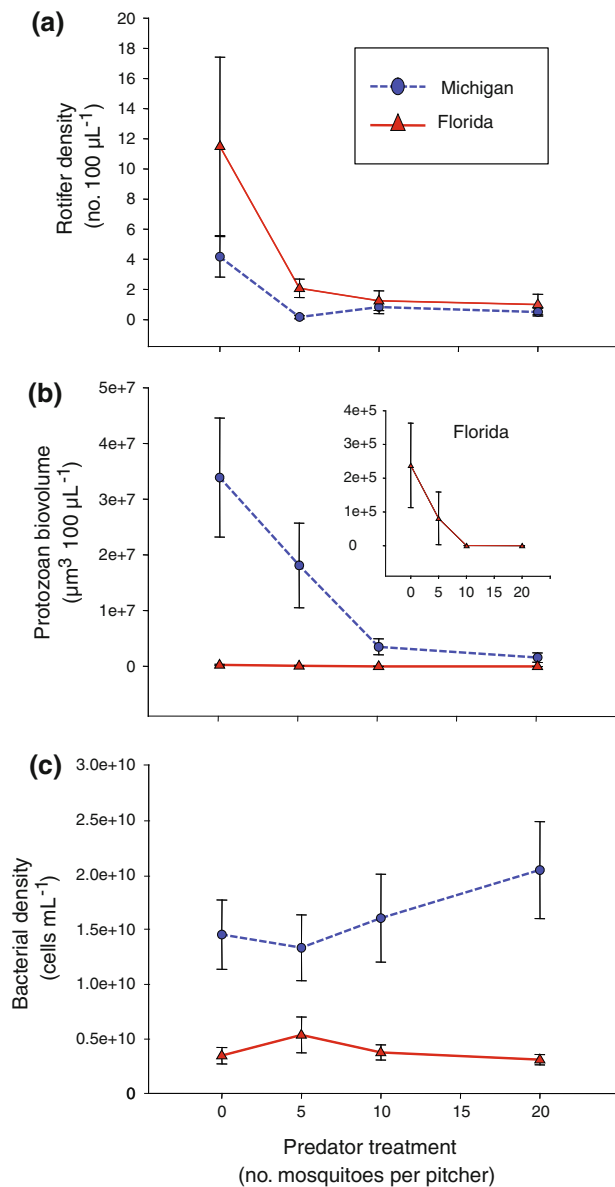


Fig. 2 The influence of the top-down predator treatment (mosquito larvae) on the population densities of **a** rotifers, **b** protozoa, and **c** bacteria in pitcher plants in Michigan and Florida. **a** Rotifer density is totaled over all sampling sessions; the *inset* in **b** shows Florida protozoa alone with an adjusted y-axis scale for greater visibility; **c** bacterial density is shown for the last sampling session. Datapoints are the mean \pm SE, pooled across all four resource-addition treatments

forces (dead ants) had positive effects on bacterial and protozoan populations in both MI and FL, and positive effects on rotifers in FL, but negative effects on rotifers in MI (Fig. 4b). Relative effect size showed that top-down effects predominated in rotifer populations in MI and protozoa in FL, while bottom-up effects were more important than top-down effects for bacterial populations at both sites (Fig. 4c).

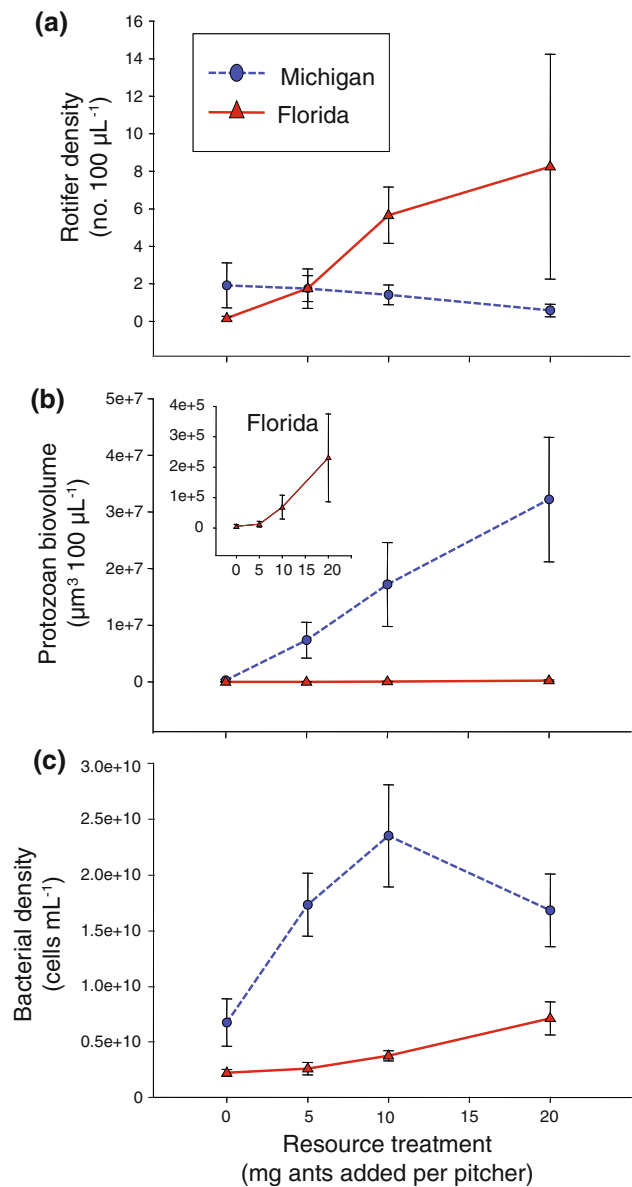


Fig. 3 The influence of the bottom-up resource treatment (dead ants) on the population densities of **a** rotifers, **b** protozoa, and **c** bacteria in pitcher plants in Michigan and Florida. **a** Rotifer density is totaled over all sampling sessions; the *inset* in **b** shows Florida protozoa alone with an adjusted y-axis scale for greater visibility; **c** bacterial density is shown for the last sampling session. Datapoints are the mean \pm SE, pooled across all four predator-addition treatments

Path analysis

The path coefficients for each variable and the standard error of the estimate summarize the effects and confirm ANCOVA results in terms of both direction and significance. Standardized z-scores were computed for each path coefficient and used to determine significance (if the absolute value of the z-score is >1.96 , the effect is signifi-

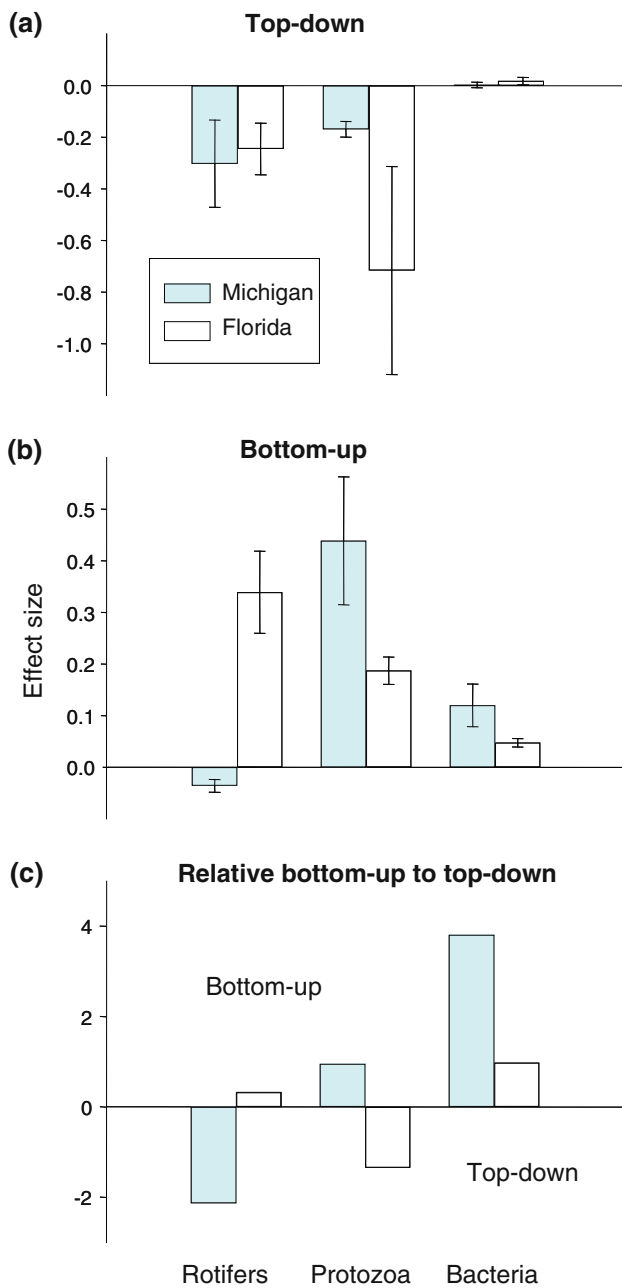


Fig. 4 Specific effect sizes (per mosquito or mg of ants) of **a** top-down or **b** bottom-up factors for rotifers, protozoa, and bacteria in pitcher plants in Michigan (shaded bars) and Florida (open bars), mean \pm SE. The influence of each factor (top-down or bottom-up) on the population density determines the sign of the effect (positive or negative). **c** Relative effect size indicates the relative importance of top-down and bottom-up treatments on population density; e.g., if relative effect size is positive, bottom-up effects had a greater impact on the population density than top-down effects. See main text for calculations

cant at the $p = 0.05$ level). The null hypothesis that the models (i.e., constrained and unconstrained) fit the data equally well was rejected (hierarchical $X^2 = 90.4$, $df = 10$, $p < 0.001$) (constrained model RMSEA 0.29).

This suggests that the MI and FL food webs have different parameter values. This is confirmed by a comparison of some path coefficients. For example, bacteria had a strong effect on protozoa in MI, but this effect is absent in FL (Fig. 1b, c).

Laboratory experiments

When protozoa were stocked at three different densities, the initial stocking differences persisted for a few days, but protozoan biovolume was no longer significantly different between stocking-density treatments by day five of the experiment ($ms = 3.78 \times 10^{12}$, $F_{2,27} = 0.16$, $p = 0.85$) and remained so ($p > 0.05$) for the remainder of the experiment. In the second lab experiment, top-down effects of mosquitoes from northern and southern pitcher plants were compared. Mosquito predation significantly reduced protozoan biovolume ($ms = 4.02 \times 10^{15}$, $F_{1,13} = 9.36$, $p = 0.004$), but mosquito origin did not change the magnitude of the effect (Tukey HSD, $p = 0.61$).

Discussion

Although it has long been recognized that top-down and bottom-up effects in food webs are likely to vary across space, there have been few studies that have experimentally examined these factors in different environmental contexts. In this study, I found that in food webs composed of the same species, the strengths of top-down and bottom-up effects varied between trophic levels and sites (Michigan versus Florida). I hypothesized that top-down effects would be stronger in Florida, but only protozoan populations supported this prediction.

Different densities and dynamics in Michigan and Florida

The components of the food webs and the top-down and bottom-up treatments applied were the same at each site, but the strength of trophic interactions varied. In most cases, adding resources bolstered consumer population density and predators depressed prey populations. The protozoan community exemplifies these trends, showing a strong positive response to resources and a negative response to predators in both MI and FL (Figs. 2b and 3b). In contrast, the rotifer populations responded differently to treatments in MI and FL (Fig. 3a). The bottom-up treatment had a positive effect on rotifer density in FL, but an unexpected negative effect on rotifer density in MI (Figs. 3a and 4b). At both sites, mosquitoes reduced rotifer density to near zero (Fig. 2a), so the divergent responses to resource addition (Fig. 3a) are most apparent

when only the zero-mosquito treatment is considered (data not shown, Fig. 3 includes all mosquito treatment levels). In the zero-mosquito treatment, rotifers increased 30-fold with resource addition in FL, while rotifer density declined across resource addition treatments in MI (three-way interaction, $p < 0.001$). The negative effect of resources on rotifer density in MI may be due to competition with protozoa. Though rotifer density was low in both MI and FL, the density of protozoa was more than 100 times higher in MI. Because protozoan populations were so large, they may have been able to competitively suppress rotifer populations when resources were added to the system. This is a plausible scenario considering that protozoa can respond numerically to resource addition faster than rotifers on account of their smaller body size and shorter generation time (Ellison et al. 2003). Though exploitative competition is expected between rotifers and protozoa, rotifers are superior competitors when stocked at equal densities (Kneitel 2002). However, in this case, protozoan density was so high in MI that they appear to have overwhelmed the rotifer population by their abundance and faster numeric population response to resource availability.

Comparing relative effect size between Michigan and Florida

Relative effect size was positive for bacteria at both sites (Fig. 4c), indicating that bottom-up forces had a greater impact on bacterial populations than top-down forces. This is consistent with expectations for bacteria because they feed directly on detrital resources. The top-down effect of mosquito predation on bacteria was non-existent. The top-down effect may have been mitigated by the extremely high growth rate of bacteria, and also indirectly by mosquito feeding on bacterivorous rotifers and protozoa. In this system, bacteria are prey for both intermediate consumers and top predators, though their importance in the mosquito diet is unknown. Indirect effects (e.g., intraguild predation) may counteract direct effects (Morin and Lawler 1996), and these competing influences cannot be distinguished by effect size calculations.

Bottom-up effects were more important for the numerically dominant middle trophic level group at each site, protozoa in MI and rotifers in FL, whereas top-down effects were more important for the subdominant group, rotifers in MI and protozoa in FL (Fig. 4c). Whichever group was at lower density at a site seemed to be more susceptible to top-down effects of mosquitoes; in fact, predation may be the reason for their lower density. However, it is unknown why either protozoa or rotifers were numerically dominant.

Between-site differences

Many differences between the sites may have contributed to the community response to treatments. Heterogeneity in temperature can influence the relative importance of top-down and bottom-up effects in a food web at a single site (Ritchie 2000; Chase 2000; Barton et al. 2009), and higher temperatures have been shown to increase the top-down effects of pitcher plant mosquito larvae on protozoa (Hoekman 2010). Ambient temperature differed between sites by about 10°C, and may have influenced the rates of predator grazing and bacterial production, even though the density of predators and the biomass of resources added were the same for both sites.

The metabolic theory of ecology (MTE) describes how metabolic rate increases with temperature (Allen et al. 2002; Brown et al. 2004), which can influence food web structure (O'Connor et al. 2009) and metabolic balance (Yvon-Durocher et al. 2010). The increased metabolic rate of the predatory mosquito at warmer temperatures in FL could have resulted in an increased foraging rate that outpaced any increase in protozoan population growth rate, resulting in a stronger relative importance of top-down effects in FL. At a given resource supply rate, increased temperature will result in higher individual metabolic rates and a lower population density. As MTE predicts, bacteria were less abundant in FL than in MI (on average by an order of magnitude). This same effect of temperature is expected for protozoan and rotifer populations, and because their resources (bacteria) are at a lower density in FL, protozoa and rotifers would be expected to be even more depressed. This is consistent with lower protozoa densities in FL than in MI (on average by two orders of magnitude), but not rotifer densities. Though it is unknown why rotifers were more abundant in FL, their densities were relatively low at both sites (MI: 0.29 ± 0.07 , FL: 0.79 ± 0.23 , mean per 100 μL sample \pm SE).

Although temperature is an important difference between these disparate sites, temperature was not controlled, and other site and seasonal differences may have contributed to the results. The experiments were not conducted at the same time, though pitchers (newly opened) and mosquitoes (third and fourth instars) of the same developmental stage were used in both experiments to reduce seasonal differences. In addition, because there was no replication at the site level, it is uncertain whether the observed variation in response was due to environmental differences related to latitude, or if the same amount of variation would have been observed at replicate sites in MI or FL. The hypothesized role of temperature has some experimental support (Hoekman 2010), but is only one of many potential contributing factors.

Inherent interpopulation differences in life-history traits may contribute to the observed site differences in top-down and bottom-up effects. Microbial communities are diverse and can adapt quickly to local conditions (Strickland et al. 2009). The microbial communities within pitcher plants at disparate sites could have evolved different characteristics, and because microbes form the base of the food web, their traits can influence the flow of energy to higher trophic levels. Mosquitoes could also differ between geographically separated populations. Top-down effects on mosquito prey were similar between the sites, though on average stronger on protozoa in FL (Fig. 4a). Previous studies have suggested that pitcher plant mosquitoes vary across their geographical range (e.g., in life history characteristics; Bradshaw and Holzapfel 2001). This may be due to genotypic differences between local mosquito populations. However, a lab experiment suggested that northern and southern mosquitoes at the same temperature have equal effects. Therefore, I assume that MI and FL mosquitoes are equivalent in predation ability, and that any differences in the relative importance of top-down and bottom-up effects are due to other differences between sites.

Another explanation for differences in results between MI and FL is initial stocking conditions. I used local in situ pitcher fluid to start both experiments. Because the natural initial density was lower, FL inquiline populations started at lower densities, and this may explain why they remained low throughout the experiment. However, the experiments both lasted for 3 weeks and identical allochthonous resources were added. Considering the generation time of the organisms in the food web (a day to a few hours for rotifers and protozoa; less than an hour for bacteria) and the fact that their populations reached stable levels within each treatment group by the end of the experiment, initial conditions are not likely to be responsible for the different results. A laboratory experiment confirmed that initial stocking densities only persisted for 5 days. Therefore, initial stocking densities do not explain the lower densities of bacteria and protozoa in FL pitchers.

Conclusions

An important conclusion that can be drawn from these experiments is that the relative importance of top-down and bottom-up effects varies between different sites of even a well-conserved food web, and can also vary between trophic levels of a food web in one location. How any given factor may influence the relative importance of top-down and bottom-up effects may be contingent upon the specific location or trophic level of the population under consideration. Finally, rising temperature, which accelerates basic metabolic processes in all systems, may have implications

for population densities, species interactions and the relative importance of top-down and bottom-up effects.

Acknowledgments Special thanks to T. Miller for hosting the field work in Florida. Bacteria slides were analyzed at Florida State University, Department of Biological Science, Biological Science Imaging Resource facility with the help of R. Hoekman. This paper was improved by helpful comments from G. Belovsky, J. Hellmann, D. Lodge, G. Lamberti, T. Miller, C. Gratton, A. Laws, K. Anderson, J. Kneitel, H. Buckley and N. Gotelli. P. Deboeck, J. Rausch, J. Grace and T. Meehan provided statistical assistance. B. Mahon helped with the lab experiments. These experiments were funded by a fellowship from the University of Notre Dame's Environmental Research Center and by an NSF Doctoral Dissertation Improvement Grant (DEB-0608143). Additional funding was provided by an Arthur J. Schmitt Presidential Fellowship to D. Hoekman and NSF grant DEB-0717148 to C. Gratton.

References

- Allen AP, Brown J, Gillooly J (2002) Global biodiversity, biochemical kinetics, and the energetic-equivalence rule. *Science* 297:1545–1548
- Barton B, Beckerman A, Schmitz O (2009) Climate warming strengthens indirect interactions in an old-field food web. *Ecology* 90:2346–2351
- Belovsky GE, Joern A (1995) The dominance of different regulating factors for rangeland grasshoppers. In: Cappuccino N, Price P (eds) *Population dynamics*. Academic, San Deigo, pp 359–385
- Berlow EL, Neutel A, Cohen J, de Ruiter P, Ebenman B, Emmerson M, Fox J, Jansen V, Jones J, Kokkoris G, Logofet D, McKane A, Montoya J, Petchey O (2004) Interaction strengths in food webs: issues and opportunities. *J Anim Ecol* 73:585–598
- Bradshaw WE, Holzapfel CM (2001) Genetic shift in photoperiodic response correlated with global warming. *Proc Natl Acad Sci USA* 98:14509–14511
- Brown J, Gillooly J, Allen A, Savage V, West G (2004) Toward a metabolic theory of ecology. *Ecology* 85:1771–1789
- Buckley H et al (2003) Reverse latitudinal trends in species richness of pitcher-plant food webs. *Ecol Lett* 6:825–829
- Chase J (2000) Are there real differences among aquatic and terrestrial food webs? *Trends Ecol Evol* 15:408–412
- Denno R, Lewis D, Gratton C (2005) Spatial variation in the relative strength of top-down and bottom-up forces: causes and consequences for phytophagous insect populations. *Ann Zool Fenn* 42:295–311
- Ellison AM, Gotelli N, Brewer J, Cochran-Stafira D, Kneitel J, Miller T, Worley A, Zamora R (2003) The evolutionary ecology of carnivorous plants. In: Caswell H (ed) *Advances in ecological research*. vol 33. Advances in ecological research, Academic Press, San Diego, pp 1–74
- Frank KT, Petrie B, Shackell NL, Choi JS (2006) Reconciling differences in trophic control in mid-latitude marine ecosystems. *Ecol Lett* 9:1096–1105
- Giberson DJ, Hardwick ML (1999) Pitcher plants (*Sarracenia purpurea*) in Eastern Canadian peatlands. In: Batzer D, Rader R, Wissinger S (eds) *Invertebrates in freshwater wetlands of North America*. Wiley, New York, pp 401–422
- Gripenberg S, Roslin T (2007) Up or down in space? Uniting the bottom-up versus top-down paradigm and spatial ecology. *Oikos* 116:181–188
- Heard SB (1994) Pitcher-plant midges and mosquitoes: a processing chain commensalism. *Ecology* 75:1647–1660

- Hoekman D (2007) Top-down and bottom-up regulation in a detritus-based aquatic food web: a repeated field experiment using the pitcher plant (*Sarracenia purpurea*) inquiline community. *Am Midl Nat* 157:52–62
- Hoekman D (2010) Turning up the heat: temperature influences the relative importance of top-down and bottom-up effects in pitcher plant inquiline communities. *Ecology* (in press)
- Hunter M, Price P (1992) Playing chutes and ladders—heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. *Ecology* 73:724–732
- Kneitel JM (2002) Species diversity and trade-offs in *Sarracenia purpurea* inquiline communities (Ph.D. dissertation). Florida State University, Tallahassee
- Kneitel J, Miller T (2002) Resource and top-predator regulation in the pitcher plant (*Sarracenia purpurea*) inquiline community. *Ecology* 83:680–688
- Miller KM, Kneitel JM (2005) Inquiline communities in pitcher plants as a prototypical metacommunity. In: Holyoak M, Leibold MA, Holt RD (eds) *Metacommunities: spatial dynamics and ecological communities*. University of Chicago Press, Chicago, pp 122–145
- Miller TE, Kneitel J, Burns J (2002) Effect of community structure on invasion success and rate. *Ecology* 83:898–905
- Moore J, McCann K, Setälä H, De Ruiter P (2003) Top-down is bottom-up: does predation in the rhizosphere regulate aboveground dynamics? *Ecology* 84:846–857
- Morin PJ, Lawler SP (1996) Effects of food chain length and omnivory on population dynamics in experimental food webs. In: Polis G, Winemiller K (eds) *Food webs, integration of patterns and dynamics*. Chapman and Hall, New York, pp 218–230
- O'Connor MI, Piehler MF, Leech DM, Anton A, Bruno JF (2009) Warming and resource availability shift food web structure and metabolism. *PLoS Biol* 7:e1000178
- Pace ML (1993) Heterotrophic microbial processes. In: Carpenter S, Kitchell J (eds) *The trophic cascade in lake ecosystems*. Cambridge University Press, Cambridge, pp 252–277
- Polis GA, Winemiller KO (1996) *Food webs, integration of patterns and dynamics*. Chapman and Hall, New York
- Ritchie ME (2000) Nitrogen limitation and trophic vs. abiotic influences on insect herbivores in a temperate grassland. *Ecology* 81:1601–1612
- Sanford E (1999) Regulation of keystone predation by small changes in ocean temperature. *Science* 283:2095–2097
- Satorra A, Bentler PM (2001) A scaled difference chi-square test statistic for moment structure analysis. *Psychometrika* 66:507–514
- Srivastava DS, Kolasa J, Bengtsson J, Gonzalez A, Lawler S, Miller T, Munguia P, Romanuk T, Schneider D, Trzcinski M (2004) Are natural microcosms useful model systems for ecology? *Trends Ecol Evol* 19:379–384
- Strickland MS, Lauber C, Fierer N, Bradford M (2009) Testing the functional significance of microbial community composition. *Ecology* 90:441–451
- Wojdak JM, Luttbegg B (2005) Relative strengths of trait-mediated and density-mediated indirect effects of a predator vary with resource levels in a freshwater food chain. *Oikos* 111:592–598
- Yvon-Durocher G, Jones JI, Trimmer M, Woodward G, Montoya JM (2010) Warming alters the metabolic balance of ecosystems. *Philos Trans R Soc B Biol Sci* 365:2117–2126