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INTEGRATING BACTERIA INTO FOOD WEBS: STUDIES WITH *SARRACENIA PURPUREA* INQUILINES

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Abstract. Predation can have both direct and indirect effects on the population dynamics and community structure of freshwater plankton communities, but its effects on the bacterial component of aquatic systems are less well known. We used a series of laboratory reconstructions of the detritus-based food web in the leaves of the northern pitcher plant *Sarracenia purpurea* to test the hypothesis that interactions at higher trophic levels could control bacterial densities and community structure. The typical pitcher community is composed of a basal-level bacterial assemblage, bacterivorous protozoa and rotifers, and larvae of the pitcher plant mosquito *Wyeomyia smithii.*

Using organisms isolated from natural pitchers, we constructed food webs comprising 1–4 consumer species (all possible combinations of the presence and absence of *Colpoda, Cyclidium, Bodo,* and *Wyeomyia* larvae) along with a constant bacterial species pool in a factorial design experiment. Bacterial community structure was modified by the direct effects of grazing by protozoa and mosquito larvae, by the indirect effects of competitive interactions among the three protozoans, and by the cascading effects of predation by mosquito larvae. Each combination of grazers/predators produced a different, species-specific pattern of bacterial species relative abundances. Changes in nutrient supplies and other abiotic characteristics of the microcosm environment resulting from the feeding activities of *Wyeomyia* and the protozoa also had indirect effects on bacterial species profiles. We found an apparent trophic cascade that was mediated by the species composition and relative abundances of the intermediate-level protozoan grazers.

Our data support the hypothesis that *Wyeomyia smithii* serves as a keystone predator in the pitcher community. Mosquito larvae were responsible for the overall architecture of the pitcher food web and the subsequent interactions among grazers and the basal-level bacterial community. At low densities, mosquito larvae controlled patterns of coexistence among protozoan species by modifying competitive interactions, while at normal field densities, *Wyeomyia* rapidly drove the two ciliates to extinction but permitted continued existence of heterotrophic microflagellates. *Wyeomyia* larvae also played a dominant role in structuring the bacterial assemblage. Bacterial species profiles in food webs containing *Wyeomyia* were more similar to each other than to the patterns observed in webs without mosquito larvae.

Our results suggest that the interactions among members of microbial communities are just as complex as those observed in plant and animal communities and require study at the species level. Because interactions among higher trophic levels can cascade down to the microbial level, it is therefore appropriate to consider the microbes as integral parts of the entire ecosystem, not merely as decomposers or food resources, but as fully interacting members of the community.

Key words: bacteria; food web interactions; keystone predators; microbial community structure; microcosms; pitcher plant; predation, direct and indirect effects of; protozoa; Sarracenia purpurea; *trophic cascade;* Wyeomyia smithii.

Introduction

Predation can have both direct and indirect effects on population dynamics and community structure in freshwater plankton communities (Hrbacek et al. 1961, Carpenter et al. 1987, Vanni 1987, Elser and Carpenter 1988). For example, in some lake food chains the predatory interactions of piscivorous fish cascade down to the phytoplankton producing changes in biomass and community structure (Carpenter et al. 1985, 1987, Carpenter 1988, Elser and Carpenter 1988, Elser et al. 1988). But, much of what we know about the impact of consumers on aquatic ecosystems comes from studying the effects of consumers on only a portion of the planktonic food web. The trophic cascade hypothesis was originally used to explain variability in zooplankton and phytoplankton, but it neglected two major microbial components of freshwater systems, protozoa and bacteria. While the effects of predation on zoo-

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plankton and phytoplankton are well established, the role of consumers in regulating the microbial component is less clear.

The last two decades have seen an increase in research into the role of microbes in aquatic food webs. Bacteria and protozoa (primarily heterotrophic microflagellates and ciliates) account for a major portion of the biomass, respiration, nutrient recycling, and productivity in both marine and freshwater ecosystems (Porter et al. 1979, Azam et al. 1983, Porter et al. 1985, Porter et al. 1988, Sherr and Sherr 1991), and the importance of the bacterial–protozoan–metazoan link in the overall trophic dynamics of pelagic systems is now well established (Fenchel 1982, Riemann 1985, Pomeroy and Wiebe 1988, Sherr et al. 1988, Gifford 1991). Furthermore, Wetzel (1995) has suggested that heterotrophic metabolism of particulate and dissolved detritus by microorganisms may provide long-term ecosystem stability, and in fact, may dampen the effects of short-term fluctuations at higher trophic levels. Thus, our perception of how these ecosystems function and how they respond to perturbations may be flawed if the microbial component is not given the same level of consideration afforded the higher trophic levels.

Studies of consumer effects on bacterial populations in lakes have produced conflicting results. Some studies show clear evidence of cascading trophic effects. For example, Hessen and Nilssen (1986) showed that bacterial populations in eutrophic lakes increased in the presence of planktivorous fish, and Jurgens and Stolpe (1995) observed zooplankton-mediated changes in bacterial biomass and community structure. In contrast, Pace and Funke (1991) found that bacterial populations in more oligotrophic lakes were controlled primarily by bottom-up forces. Protozoa responded to predation by *Daphnia,* but bacterial populations were regulated by nutrients. In a second study, Pace (1993) found that although fish caused significant changes in zooplankton which then produced changes in pelagic protozoa populations, predation effects did not cascade directly down to the bacterial level. Bacteria experienced effects of fish additions only indirectly via changes in phytoplankton. Hence, the evidence for top-down control in microbial food webs is ambiguous.

One explanation for these inconsistencies may be the nature of the food webs/chains that have been studied. Evidence from both theoretical and empirical studies suggests that the relative importance of top-down control in a particular ecosystem can be difficult to predict without an understanding of the characteristics of the species involved (Vanni 1987, Leibold 1989, Vadas 1989, Leibold and Wilbur 1992, Richardson and Threlkeld 1993, Balčiūnas and Lawler 1995). In order to construct realistic models that can be used to make predictions about the dynamics of natural communities, the nature and consequences of the interactions among individual species must be understood. Much aquatic microbial ecology suffers from a lack of detailed knowledge about the species that make up microbial food webs. Most studies focus on aggregated webs in which these organisms are treated as functional groups or ''trophic species,'' e.g., heterotrophic bacteria, phagotrophic protozoa, and so on. In worst case scenarios, the entire microbiota is aggregated such that eukaryotic (including small metazoans) and prokaryotic taxa are lumped together into a ''microbial community.'' While arguments can be made for the validity of this approach (Christian 1994), we can learn little about the dynamics of microbial communities and the forces regulating species abundance patterns by ignoring heterogeneity within trophic levels. Nevertheless, a few studies have examined the effects of consumers on microbial food webs at the species level. Grazing can influence the size distributions or species diversity in aquatic bacterial communities (e.g., Federle et al. 1983, Turley et al. 1986, Rashit and Bazin 1987, White and Findlay 1988, Sinclair and Alexander 1989, Epstein and Shiaris 1992, Balčiūnas and Lawler 1995). In addition, Balčiūnas and Lawler (1995) showed that differences among species within trophic levels in microcosms containing predatory and bacterivorous protozoa plus a basal bacterial assemblage produced complex interactions among taxa that could change the relative importance of top-down and bottom-up effects.

In this paper we examine the effects of consumers and complex species interactions among intermediate-level bacterivores on the basal-level microbial community in a natural detritus-based aquatic food web. For our model system we chose the phytotelm community that develops in the water-filled leaves of the northern pitcher plant *Sarracenia purpurea* L. (Sarraceniaceae). Small bodies of water found in or upon plants are referred to as phytotelmata (Kitching 1971, Maguire 1971), and the decomposer communities that develop in these miniature aquatic habitats have been the focus of numerous studies of community dynamics (e.g., Maguire et al. 1968, Seifert and Seifert 1976, Naeem 1988, 1990). Like other phytotelmata, *Sarracenia* pitchers provide natural microcosms that are amenable to both laboratory studies and field manipulations (Addicott 1974, Cochran-Stafira 1993, Heard 1994), and the system is simple enough that the community can be simulated in the laboratory with a high degree of realism.

The pitcher food web consists of essentially three trophic levels: a basal-level bacterial assemblage, intermediate-level bacterivores consisting of protozoa and rotifers, and a top-level predator, the larvae of the pitcher plant mosquito *Wyeomyia smithii* Coq. (Culicidae). It resembles a system under the control of a keystone predator, *W. smithii.* Keystone predators have been shown to mediate competitive interactions and control diversity at lower trophic levels (Paine 1966, 1974, Carpenter et al. 1987, Leibold 1991, Leibold 1996, Mills et al. 1993, Menge et al. 1994); and the ability of mosquito larvae to directly affect microbial

numbers and the overall structure of phytotelm communities is well known (Kurihara 1959, 1983, Maguire et al. 1968, Addicott 1974, Riviere 1985, Walker et al. 1991).

Manipulative experiments are a valuable technique for studying the effects of predation, competition, and other species interactions in aquatic food webs, but their interpretation is often hampered by logistical constraints. Replication of treatments is difficult or impractical, and thorough knowledge of all the taxa within each trophic level is usually impossible. An effective alternative is the reconstruction of a community from its component parts (Gilpin et al. 1986, Wilbur and Fauth 1990) and the manipulation of every species in that community in a laboratory setting. Drake (1991) and Lawler (1993) have provided examples of this approach in experimental aquatic microcosms comprised of (among others) a number of protistan species plus bacteria. In both studies, the experimental systems consisted of a series of microbial food webs that varied in the type and number of taxa present. These studies demonstrated the importance of community assembly mechanisms and historical effects in determining community structure, as well as the role of direct and indirect effects as mechanisms of community assembly. They also established a role for complex interactions in microbial communities. While these results provide important insights into species interactions and give support for major concepts in ecological theory, their conclusions are drawn from observations of a collection of arbitrarily selected organisms that were not necessarily representative of a specific natural community.

We used the reconstruction method to study the direct and indirect effects of predation on bacterial population density and species abundance patterns in the pitcher plant system. Based on data obtained from field censuses of *S. purpurea* pitcher communities (Cochran-Stafira 1993), we assembled communities in laboratory microcosms containing mosquito larvae, protozoa, and bacteria cultured from field isolates. We analyzed the response of the bacterial community to the direct effects of grazing by mosquito larvae and protozoa and the indirect effects of interactions among the pitcher food web members. We also tested for the ability of mosquito larvae, as predators of protozoa, to exert indirect top-down control of total bacterial cell density via cascading trophic effects. We hypothesized that changes in the numbers of bacterivorous protozoa would be reflected in complementary changes in bacterial cell density. We also predicted that, as the keystone species in the pitcher community, *Wyeomyia smithii* would have direct and indirect effects on bacterial population dynamics and community structure.

Study System

Sarracenia purpurea is a carnivorous plant typically found in bogs, fens, and other low-nutrient wetlands over a wide geographic range in North America (Lloyd 1942, Juniper et al. 1989). The leaves of these herbaceous perennials have evolved to form vase-shaped pitchers that collect rainwater into which prey insects fall and drown. In contrast to other members of the genus, *S. purpurea* probably does not produce its own digestive enzymes (Adams and Smith 1977, but see Stauffer 1987). Breakdown and digestion of prey is accomplished primarily by a variety of microbial and invertebrate inquilines living inside the pitchers (Addicott 1974, Bradshaw 1983, Istock et al. 1983, Cochran-Stafira 1993, Heard 1994), and the plant absorbs nitrogenous compounds and other micronutrients from the fluid (Bradshaw and Creelman 1984, Juniper et al. 1989).

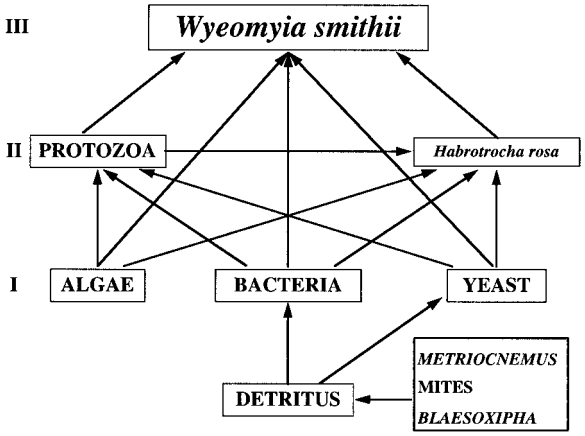

FIG. 1. Hypothesized organization of the food web in *Sarracenia purpurea* pitchers.

Pitcher inhabitants constitute a three trophic-level food web (Fig. 1). Since there is essentially no primary production within the pitcher (Cochran-Stafira 1993, Heard, 1994; T. Miller, *personal communication*), the detritus that forms as a result of the larval feeding activities of the pitcher plant midge *Metriocnemus knabi* Coq. (Chironomidae) and the pitcher plant flesh fly *Blaesoxipha fletcheri* Aldrich (Sarcophagidae) serves as the nutrient base for the food web. Bacteria, fungi, and mixotrophic algae utilize nutrients from this detritus and make up the basal trophic level (I in Fig. 1). Grazers such as rotifers and protozoans form the intermediate level (II), and feed on bacteria and small protists as well as fine detritus particles. The only rotifer found in the pitchers from our field site is the pitcher plant form of the bdelloid rotifer *Habrotrocha rosa* Donner (Bateman 1987, Cochran-Stafira 1993), although additional species have been reported from other locations (Addicott 1974; D. L. Cochran-Stafira, *unpublished manuscript*). The precise role of *H. rosa* in the pitcher community is unclear since it has been described as both a bacterivore (Plasota et al. 1980) and an omnivore that filter-feeds on small protozoa, algae, and bacteria (Bateman 1987). Larvae of the pitcher plant mosquito *Wyeomyia* generally have been

assumed to be the top predators (III), but are probably omnivorous and feed at all trophic levels. Several species of mites also commonly occur in pitchers (Fashing and O'Connor 1984), some of which may be bacterivores (R. Naczi, *personal communication*).

In this paper we describe interactions within food webs composed of *Wyeomyia* larvae, three bacterivorous protozoan species, and four bacterial taxa. The exact trophic position of *H. rosa* in the pitcher food web and its impact on community dynamics is discussed elsewhere (Cochran-Stafira 1993; D. L. Cochran-Stafira and C. N. von Ende, *unpublished manuscript*).

Methods

Collection, culture, and preparation of organisms

All taxa used to construct the experimental food webs were initially collected from *S. purpurea* pitchers at Cedarburg Bog, a large peatland in southeastern Wisconsin that is part of a Wisconsin Department of Natural Resources State Scientific Area operated by the University of Wisconsin–Milwaukee Field Station in Saukville, Wisconsin, USA (Ozaukee County, T11N, R21E). Organisms were maintained in continuous laboratory cultures (Cochran-Stafira 1993).

We collected diapausing fourth-instar *Wyeomyia smithii* larvae in November 1988, and established a culture by following the procedures of Istock et al. (1975). The culture was maintained at 25–27°C and 70–80% relative humidity with a 16-h photoperiod under two 40-watt, cool-white fluorescent bulbs. The larvae were fed with a 1% aqueous solution of Tetra Min E fish food. This was prepared by homogenizing the powdered food in distilled water for 30 s in a blender. The mixture was then allowed to settle for 10 min, after which the supernatant was collected and fed to the larvae. At the time of these experiments, *Wyeomyia* had been in culture for ~7 mo. Only late second- or early third-instar larvae of similar size (3–4 mm long) were used in the experimental microcosms. External bacterial contamination of the larvae was reduced by pretreatment with antibiotics 48 h prior to use. Larvae were transferred to an antibiotic–antimycotic solution (Sigma Chemicals, St. Louis, Missouri; penicillin, streptomycin, amphotericin B) for 24 h, followed by thorough rinsing and 24 h in sterile water. They were given a final rinse with fresh sterile water immediately before being added to experimental units.

Protozoa were isolated from pitcher fluid that had been filtered through 80 μm Nitex netting to remove debris and predators. A few drops of 1% aqueous Tetra Min E fish food solution (sterilized by autoclaving) were added to the filtrate to stimulate the bacterial growth that would serve as food for bacterivorous protozoa. After 3–7 d, a dense population of protozoa developed from which the ciliates *Colpoda* sp. and *Cyclidium* sp., both filter-feeding bacterivores, were isolated with a micropipet. Single cells were passed through several washes of sterile cerophyll medium (CM: Sonneborn's Paramecium medium; Nerad 1991) to reduce gross contamination by small flagellates and bacteria, and were then transferred to fresh medium. The kinetoplastid flagellate *Bodo* sp., a raptorial bacterivore, was isolated by serial dilution in sterile CM. These three protists were chosen because they were the only strictly heterotrophic species commonly found in field samples of pitchers (Cochran-Stafira 1993). In addition, they represented two distinct feeding mechanisms and provided a wide range of cell sizes: typical cell length was 50–60 μm for *Colpoda,* 25–30 μm for *Cyclidium,* and 8–10 μm for *Bodo.* Following isolation, all protozoa were maintained as monocultures in CM at room temperature under subdued light, and were fed with *Klebsiella pneumoniae* (ATCC 27889). Stock cultures were transferred to fresh CM every 3–4 d; 48-h log phase cultures were used in all experimental treatments. At the time of these experiments, the protozoans had been in continuous laboratory culture for 12 mo.

We selected four ''marker'' species of bacteria (designated A, B, C, D) and one yeast from a pool of microbial strains isolated from both pitcher contents and from the laboratory cultures of pitcher protozoa and *W. smithii.* These microorganisms were selected for their ability to grow on Tetra Min E–glucose medium (TMEG: 1% Tetra Min E, 0.1% glucose, distilled water; homogenized, filtered, and autoclaved) and for their distinct, easily identifiable colony morphologies and colors. All four bacterial isolates were small (<1–1.5 μm long), motile, gram negative, rods. One of the isolates (species B) was the *K. pneumoniae* used to feed the protozoan cultures. We did not attempt to identify the other three species since this was beyond the scope of this experiment and not essential for its interpretation. In addition, a pool of nonculturable contaminating microorganisms from the protozoa and *Wyeomyia* cultures was prepared by filtering the fluid from these cultures through a sterile 1-μm Nuclepore filter to remove the grazers and large debris particles. A standard inoculum was prepared by combining the four marker taxa with the contaminant pool to ensure uniform initial conditions for all experimental units. This inoculum obviously did not contain all the bacteria present in a natural pitcher community, but we assumed that if any changes in species abundances patterns were detected in this physiologically defined subset of the *S. purpurea* bacterial community, similar responses could be inferred for the natural assemblage.

Food web assembly

We constructed consumer food webs using all possible combinations of the presence and absence of *Colpoda, Cyclidium, Bodo,* and *Wyeomyia* larvae, along with a constant bacterial species pool in a ($2 \times 2 \times 2 \times 2$) factorial design with 3 replicates of each of the 16 treatment combinations (Fig. 2). From the large de-

	W+				W-			
	Co+		Co-		Co+		Co-	
	Cy+	Cy-	Cy+	Cy-	Cy+	Cy-	Cy+	Cy-
B+	1	2	3	4	5	6	7	8
B-	9	10	11	12	13	14	15	16

FIG. 2. Experimental design for the food web manipulations. Pluses and minuses refer to the presence or absence, respectively, of a particular grazer or predator species (Co = *Colpoda*; Cy = *Cyclidium*; B = *Bodo*; W = *Wyeomyia*). Each treatment cell has been numbered to designate which of the cells were extracted for analysis in each of the statistical subdesigns (see *Results* for details). There were three replicates of each of the 16 treatments.

sign we then extracted the appropriate treatment combinations for the analysis of all possible webs with one, two, three, and four consumer species.

Experimental food webs were constructed in sterile 50-mL plastic screw-capped centrifuge tubes. These conical tubes roughly approximated the shape of *S. purpurea* pitchers and were sufficiently translucent to permit light penetration. Initial nutrient conditions were standardized by using the same batch of TMEG medium for all replicates. To ensure that the starting populations of grazers/predators in each replicate were of the same age and degree of conditioning to culture conditions, each protozoan inoculum was prepared from a single log phase stock culture, and *Wyeomyia* larvae from the same generation were selected. Since a single bacteria/yeast pool was prepared, each replicate received the same microbial inoculum.

Each tube was inoculated with 1 mL of a dilute suspension ($<10^5$ cells/mL) of the bacteria/yeast mixture. In each experiment, the null treatment (in which bacteria but no grazers were present) served both as a no-grazer control treatment and as a check for the efficient removal of any contaminating protozoa during preparation of the microbial inoculum. *Bodo* were introduced at an initial density of 10^4 cells/mL, which corresponded to flagellate densities observed in field samples (Cochran-Stafira 1993). Ciliates were rare in field collections, and their densities were highly variable. Initial ciliate densities were therefore arbitrarily based on those used by Turley et al. (1986): *Colpoda* and *Cyclidium* were each added at densities of 200 cells/mL when used alone or with *Bodo*, and at 100 cells/mL when both ciliates were used together, thereby maintaining a constant initial total density of ciliate grazers. Five *Wyeomyia* larvae were added to the appropriate tubes for a final density of 1 larva per 5 mL of TMEG media. This density was low compared to natural larval densities in pitchers (Addicott 1974, Cochran-Stafira 1993); however, the low density was necessary to permit coexistence of the larvae with their protozoan prey species at least for the short duration (4 d) of the experiment. Sterile TMEG medium was added to each replicate to yield a total volume of 25 mL per tube. Temperature, light, and humidity regimes were the same as those used for *Wyeomyia* culture.

Data collection and transformation

We aseptically removed two 1-mL samples for protozoa counts as well as bacterial total counts and species abundances at 24-h intervals for 96 h. Protozoan samples were preserved for counting with 1 drop/mL saturated $HgCl_2$ plus 1 drop/mL 0.04% bromophenol blue (Pace and Orcutt 1981). The undiluted protozoan samples were counted using a hemocytometer or Whipple grid with either a Sedgewick-Rafter cell or Palmer cell depending on protozoan density. Plate counts, rather than direct counts, were used to determine bacterial densities because we were interested in the abundances of individual bacterial species, not merely total bacterial population size. Direct microscopic counting techniques would not distinguish among taxa. Bacterial samples were serially diluted in sterile dilution buffer (Franson 1985), and triplicate 1.0-mL samples of the 10^{-4}, 10^{-5}, and 10^{-6} dilutions were spread on plate count agar (Difco). Plate count agar was chosen because each of the four marker species grew rapidly and produced easily distinguishable colonies on this medium. The plates were incubated at 27°C for 4 d. We selected the 10^{-5} dilution as ''optimal'' for counting samples from all of the experimental treatments; below 10^{-5} the dominant species overgrew the plate, and above 10^{-5} rare species were often missed. Individual taxa were distinguished by colony morphology and color. The yeast species used in the original inoculum did not grow in the artificial pitchers, and few bacterial contaminants (generally <1 colony per plate) were detected on the spread plates. We therefore restricted our analyses of the bacterial community to the four marker bacteria in the original inoculum.

Natural pitcher communities are highly dynamic and undergo rapid changes in structure over short time intervals (D. L. Cochran-Stafira, *unpublished data*). Pilot experiments showed that protozoan interactions and bacterial species responses to the grazer assemblage varied significantly over time, and predation effects were most obvious between 48 and 72 h, often becoming nonsignificant by 96 h as some prey species went extinct (Cochran-Stafira 1993). Therefore, only data from the 72-h samples will be discussed here.

The absence of a bacterial colony type on countable plates did not necessarily indicate that the species was not present in the experimental unit. Because we counted only the 10^{-5} dilutions, it was possible that species occurring at low densities might have been missed. Therefore, all zero bacterial counts were recorded as 1×10^4, one order of magnitude below the minimum counting sensitivity. For protozoa, failure to count at least one cell in the 1-mL sample was judged to be an indication that the organism was no longer present and

a value of zero was assigned. It was not possible to take additional samples to confirm these extinctions because this would have significantly reduced the volume in the tubes over the 4-d sampling period. Bacteria and protozoa counts were $\log_{10}$ and $\log_{10}(X + 1)$ transformed respectively to reduce variance heterogeneity.

Statistical analyses

Bacterial abundances.—This experiment involved sampling four bacterial species from the same experimental unit (centrifuge tube). Because the abundances of the bacterial species were unlikely to be independent, changes in bacterial community structure were analyzed by profile analysis of variance (PANOVA; Harris 1985, Simms and Burdick 1988, von Ende 1993) using PROC ANOVA (SAS 1989) with bacterial species as the within-subjects factor. Multivariate *F* statistics for Wilks' lambda, Pillai's Trace, the Hotelling-Lawley Trace, and Roy's Greatest Root were equal in these analyses. If a significant multivariate response was detected using PANOVA, follow-up univariate tests on each bacterial species were used to determine which bacterial taxa were affected. We used profile analyses of specific contrast statements (PROC GLM; SAS 1989) for a priori comparisons among the bacterial communities in particular treatments.

Significant main effects and interaction terms have been interpreted as evidence for direct and indirect effects, respectively, in analysis of variance of factorial designs (Wilbur and Fauth 1990, Worthen and Moore 1991). This approach, however, must be used with caution when dealing with microbial systems where there is a very strong link between the organisms themselves and the abiotic nature of their environment. Each member of the microbial community is capable of altering the physico-chemical characteristics of its aqueous environment via the release of metabolic by-products and extracellular enzymes (Gill 1972). In addition, organisms at higher trophic levels can modify the abiotic environment in ways that may be significant at the scale of basal-level microbiota such as phytoplankton or bacteria (McQueen et al. 1992). As a result, direct and indirect effects in such systems tend to be confounded, and their detection and quantification can be difficult.

In this experiment, the resource base for bacteria in replicates containing *Wyeomyia* larvae was not the same as in those without mosquito larvae due to the accumulation of fecal pellets and other wastes. Consequently, interpretation of main effects and interactions in the full four-way model was not possible since predation effects were confounded with changes in resource levels and water quality that were not consistent across all treatment combinations. In order to isolate direct and indirect predation effects, we subdivided the analysis into a series of smaller ''statistical'' experiments by extracting the appropriate treatment combinations from the large design (Fig. 2) that focused on ''simple'' effects and simple interactions (Maxwell and Delaney 1990, Wade 1992). This is similar to the approach used in other studies in which the effects of different combinations of associate species on the focal species were independently analyzed (Vandermeer 1969, Neill 1974, Miller 1994).

A simple effect is the difference observed between two treatment means when the treatments differ by only the presence and absence of the factor in question (Wade 1992). We can use the example of a three trophic-level food web containing *Colpoda, Wyeomyia,* and bacteria to illustrate how simple effects differ from the main effects in a conventional ANOVA. In the analysis of the two-way model, detection of a main effect of *Colpoda* requires calculating a mean square by summing over both levels (presence and absence) of the *Wyeomyia* treatment (Keppel 1982). Since the environmental characteristics of replicates with *Wyeomyia* are not the same as those found in replicates without *Wyeomyia,* the main effect of *Colpoda* is not a valid measure of the effect of this ciliate on the bacterial assemblage. In contrast, the simple effect of *Colpoda* is calculated at a single level of the *Wyeomyia* factor (i.e., absent), and eliminates the problem of inherent differences between the control and experimental groups. Since *Colpoda* might also affect the abiotic environment, the simple effect of *Wyeomyia* would best represent the impact of this species on the bacterial assemblage.

A simple interaction is the observed difference in a factor's simple effect when measured in the presence or absence of some other factor (Wade 1992). In the two-factor example, the simple interaction between *Wyeomyia* and *Colpoda* is equivalent to the *Wyeomyia* $\times$ *Colpoda* interaction term from the two-way ANOVA. In larger designs where there are more than two factors, and therefore more than one possible interaction, the highest order statistical interaction term represents the simple interaction among all treatment factors. In a design that has *Colpoda, Cyclidium,* and *Wyeomyia* as factors, the three-way *Colpoda* $\times$ *Cyclidium* $\times$ *Wyeomyia* interaction represents the simple interaction among these three factors; lower order interactions and main effects are invalid.

The direct effects of *Colpoda, Cyclidium, Bodo,* and *Wyeomyia* on bacterial community structure were determined from the analysis of simple effects in PANOVA of single factor designs containing only the grazer species under consideration. Indirect effects were analyzed by examining simple interactions from PANOVA of each subdesign containing two or more consumer species. For example, to analyze the effects of the interaction among *Colpoda, Bodo,* and *Wyeomyia,* a factorial design having these three consumer species as factors was extracted, and only the three-way *Colpoda* $\times$ *Bodo* $\times$ *Wyeomyia* interaction was interpreted; lower order interaction terms and main effects were ignored. For each category of food webs analyzed, we used a sequential Bonferroni procedure with all follow-

TABLE 1. Summary of the responses of the four-species (ABCD) bacterial community in single grazer species food webs.

Multivariate tests				Univariate tests				
					Species			
Source	*P*	Source	*P*	Source	A	B	C	D
a) Species (S)	0.0001*	c) (Co vs. Cy) × S	0.0125*	e) C vs. Co	*	NS	NS	*
Grazers × S	0.0001*	(Co vs. B) × S	0.0001*	C vs. Cy	*	NS	NS	NS
		(Cy vs. B) × S	0.0032*	C vs. B	NS	NS	NS	*
b) (C vs. Co) × S	0.0001*			C vs. W	NS	*	NS	*
(C vs. Cy) × S	0.0001*	d) (Co vs. W) × S	0.0001*					
(C vs. B) × S	0.0020*	(Cy vs. W) × S	0.0001*	f) Co vs. Cy	*	NS	NS	*
(C vs. W) × S	0.0001*	(B vs. W) × S	0.0001*	Co vs. B	*	NS	*	*
				Cy vs. B	*	NS	NS	NS
				g) Co vs. W	*	*	*	NS
				Cy vs. W	*	*	NS	*
				B vs. W	NS	*	NS	*

Notes: (a) The five treatments (C = Control; Co = *Colpoda*; Cy = *Cyclidium*; B = *Bodo*; W = *Wyeomyia*) were considered as levels of a single ''Grazer'' factor in a one-way profile analysis of variance using bacterial species as the within-subjects factor. (b–d) Contrasts between each grazer treatment and the control as well as all other pairwise contrasts between grazer treatments were examined to determine whether changes in bacterial community structure were grazer-specific. (e–g) Univariate ANOVAs were again used to determine which bacterial taxa were responsible for the differences between treatments.
* Significant at an experiment-wise error rate of 0.05 according to a sequential Bonferroni procedure; NS = not significant.

up univariate tests and analyses of contrast statements to preserve a 0.05 experiment-wise type I error rate (Day and Quinn 1989, Rice 1989).

Protozoan abundances.—Changes in protozoan densities due to interspecific competition and/or predation by *Wyeomyia* were analyzed by ANOVA on the cell densities per milliliter for each species in each subdesign. We used a conservative Bonferroni-adjusted alpha for each analysis.

RESULTS

Direct effects of grazing: do grazers cause changes in bacterial community structure?

To test the direct, simple effects of each grazer on bacterial community structure, we extracted the treatments representing the control (no grazer) and each of the *Colpoda*, *Cyclidium*, *Bodo,* and *Wyeomyia* single grazer treatments from the full design (Fig. 2: treatments 8, 12, 14, 15, 16). These five treatments were considered to be levels of a single ''Grazer'' factor in a one-way profile analysis of variance. The four bacterial species responded differently to grazing by the different grazer taxa, as shown by the significant bacterial Species effect and the significant overall Species × Grazers interaction (Table 1a). Each of the four grazer species produced a pattern of bacterial abundances that differed significantly from the ungrazed control (Table 1b) and from the patterns produced by the other grazer taxa (Table 1c, d; Fig. 3a–e). *Klebsiella pneumoniae* (species B) decreased significantly only in the presence of *Wyeomyia* and was not affected by any of the protozoa (Table 1e–g). Species A decreased in the presence of *Colpoda* and *Cyclidium* but was not affected by *Bodo* or *Wyeomyia.* Although the univariate tests showed no significant response of species C to any of the four consumer species, *Colpoda* slightly reduced its mean density, while the other three consumers caused the mean density of this bacterium to increase slightly. The density of species D was decreased by *Colpoda* and *Wyeomyia,* and was increased by *Bodo.*

Indirect effects in multi-species webs: does Wyeomyia *predation alter the effects of protozoan grazing on bacterial community structure?*

According to the keystone predator and trophic cascade models, reductions in protozoan cell densities due to *Wyeomyia* predation should result in a change or reduction in the impact of protozoan grazing on the bacterial community. If *Wyeomyia* larvae feed mainly on protozoa when available, we might predict that, by reducing the grazer population, mosquito larvae would cause the bacterial community to resemble its ungrazed state. However, since the larvae are omnivorous filter feeders, consuming both protozoa and bacteria, it was also possible that the bacterial community would either resemble one grazed only by *Wyeomyia* larvae, or would be intermediate between the protozoa- and *Wyeomyia*-grazed communities. To test these predictions, three (2 × 2) factorial designs were extracted representing food webs containing combinations of *Colpoda* and *Wyeomyia* (Fig. 2: treatments 10, 12, 14, 16), *Cyclidium* and *Wyeomyia* (Fig 2: treatments 11, 12, 15, 16), and *Bodo* and *Wyeomyia* (Fig 2: treatments 4, 8, 12, 16).

Significant interactions between *Cyclidium* and *Wyeomyia,* and *Bodo* and *Wyeomyia* (Table 2a), had indirect effects on the bacterial assemblage that primarily involved species C (Table 2b). Alone, each of these three consumers caused a slight, though statistically nonsignificant, increase in the density of C over that seen in the control (Fig. 3c–e), but when either

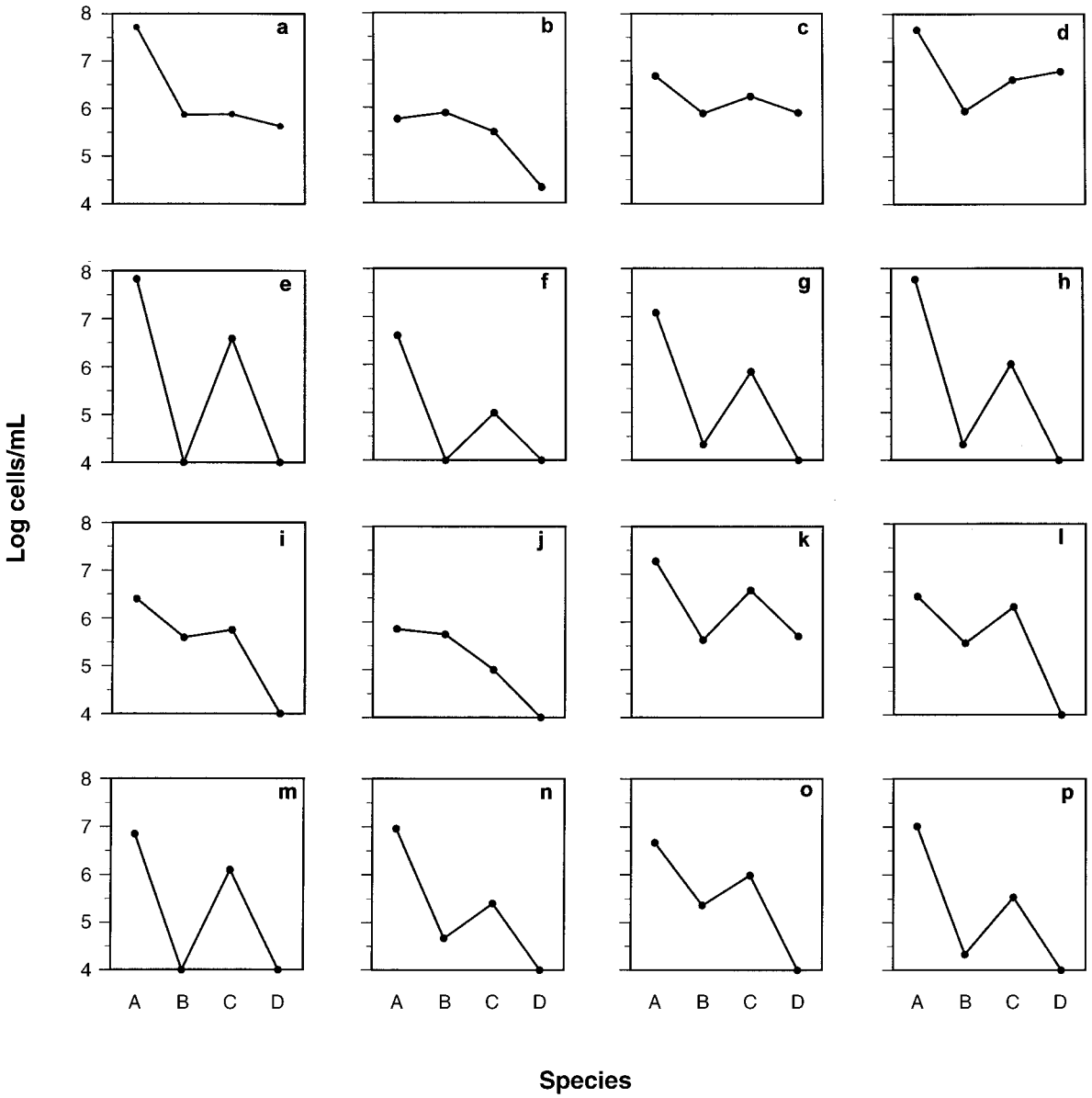

FIG. 3. Responses of the four-species bacterial community to grazing by protozoa and *Wyeomyia* in each of the 16 food web constructs: (a) control; (b) *Colpoda*; (c) *Cyclidium*; (d) *Bodo*; (e) *Wyeomyia*; (f) *Colpoda* + *Wyeomyia*; (g) *Cyclidium* + *Wyeomyia*; (h) *Bodo* + *Wyeomyia*; (i) *Colpoda* + *Bodo*; (j) *Colpoda* + *Cyclidium*; (k) *Cyclidium* + *Bodo*; (l) *Colpoda* + *Cyclidium* + *Bodo*; (m) *Colpoda* + *Bodo* + *Wyeomyia*; (n) *Colpoda* + *Cyclidium* + *Wyeomyia*; (o) *Cyclidium* + *Bodo* + *Wyeomyia*; (p) *Colpoda* + *Cyclidium* + *Bodo* + *Wyeomyia*. Bacterial species B was *Klebsiella pneumoniae.*

Cyclidium or *Bodo* was combined with *Wyeomyia* (Fig. 3g, h), C decreased significantly. While *Bodo* caused species D to increase above the control level, *Wyeomyia* caused a drastic reduction in the density of this bacterium that was not reversed when *Bodo* was included in the food web. Although the multivariate response of the bacterial assemblage to the interactions between *Colpoda* and *Wyeomyia* was only marginally significant, species A and D did show significant univariate responses (Table 2a, b). In the presence of mosquito larvae, the decrease in the density of bacterial species A due to grazing by *Colpoda* was significantly reversed (Table 2b, Fig. 3b, f). *Colpoda* and *Wyeomyia* decreased D both alone and in combination.

The bacterial communities of each of these two-consumer food webs differed significantly from those of the control (Table 2c, e) and from each other (Table 2d, f). Each grazer–predator combination produced a different bacterial community in terms of species abundances with most of the differences attributable to species A (Table 2f, Fig. 3f–h). The overall species abundance profiles from food webs containing *Wyeomyia*

TABLE 2. Summary of the responses of the four-species (ABCD) bacterial community in food webs containing one protozoan species plus *Wyeomyia* (see Table 1 for treatment abbreviations).

Multivariate tests		Univariate tests				
			Species			
Source	*P*	Source	A	B	C	D
a) Co × W × S	0.050	b) Co × W	*	NS	NS	*
Cy × W × S	0.001*	Cy × W	NS	NS	*	NS
B × W × S	0.002*	B × W	NS	NS	*	*
c) (C vs. CoW) × S	0.0085*	e) C vs. CoW	*	*	NS	*
(C vs. CyW) × S	0.0003*	C vs. CyW	*	*	NS	*
(C vs. BW) × S	0.0001*	C vs. BW	NS	*	NS	*
d) (CoW vs. CyW) × S	0.0115*	f) CoW vs. CyW	*	NS	NS	NS
(CoW vs. BW) × S	0.0001*	CoW vs. BW	*	NS	NS	NS
(CyW vs. BW) × S	0.0017*	CyW vs. BW	*	NS	NS	NS

Notes: (a) Each subdesign was analyzed as a two-factor profile analysis; only the two-way interaction terms were interpreted. (b) Univariate ANOVAs were used to evaluate the responses of each bacterial species to the grazer–predator interaction. (c, d) The bacterial species abundance pattern of each food web was compared to the control and to each of the other webs using profile analysis of the appropriate contrast statements followed by univariate tests (e, f) to determine which bacterial taxa were responsible for the differences.

* Significant at an experiment-wise error rate of 0.05 according to a sequential Bonferroni procedure; NS = not significant.

(Fig. 3e–h) tended to resemble each other in striking contrast to the control and the experimental systems containing only a single protozoan species (Fig. 3a–d). The data support our hypothesis that omnivory by *Wyeomyia* larvae produced a bacterial community that was intermediate between that produced by mosquitoes or protozoa alone, but the effects of the interactions between the larvae and protozoan grazers were not uniform across all bacterial taxa. The strong effect of *Wyeomyia* on the bacterial community also tends to support its hypothesized role as a keystone species in this system.

The indirect effects stemming from interactions between mosquito larvae and the ciliates *Colpoda* and *Cyclidium* can be explained by the significant decrease of these two taxa due to predation (Table 3a, Fig. 4a, b). However, *Wyeomyia* predation had no effect on *Bodo* (Table 3a, Fig. 4c), suggesting that the indirect effects produced by the interaction of these two species were not trophic-linkage effects, and may be more related to nutrient recycling or perhaps changes in the feeding behavior of *Bodo.*

Do interactions between pairs of protozoa alter their individual effects on bacterial community structure?

Previous experiments had shown that there were highly significant interactions among the protozoa of the pitcher food web (Cochran-Stafira 1993). We next tested the hypothesis that these interactions would also exert indirect effects on the bacterial community. Three (2 × 2) factorial subdesigns were extracted consisting of all presence/absence combinations of *Colpoda* and

TABLE 3. Summary of the responses of each protozoan species to the presence of other protozoa and/or *Wyeomyia* in each food web design (see Table 1 for treatment abbreviations). Changes in protozoan cell densities per milliliter were analyzed by ANOVA on the cell densities of each species in their respective food web subdesigns (a–e).

		Response variables		
Food web type	Source	*Colpoda*	*Cyclidium*	*Bodo*
a) 1 protozoa + W	W	0.0010*	0.0072*	0.9887
b) 2 protozoa	Co	NA	0.0007*	0.0001*
	Cy	0.1140	NA	0.7484
	B	0.1835	0.0047*	NA
c) 2 protozoa + W	Co × W	NA	0.1490	0.0001*
	Cy × W	0.6636	NA	0.9657
	B × W	0.6395	0.0005*	NA
d) 3 protozoa	Co × Cy	NA	NA	0.7400
	Co × B	NA	0.0009*	NA
	Cy × B	0.8630	NA	NA
e) 3 protozoa + W	Co × Cy × W	NA	NA	0.9723
	Co × B × W	NA	0.0756	NA
	Cy × B × W	0.2005	NA	NA

* Significant at an experiment-wise error rate of 0.05 according to a simple Bonferroni procedure; NA = not applicable.

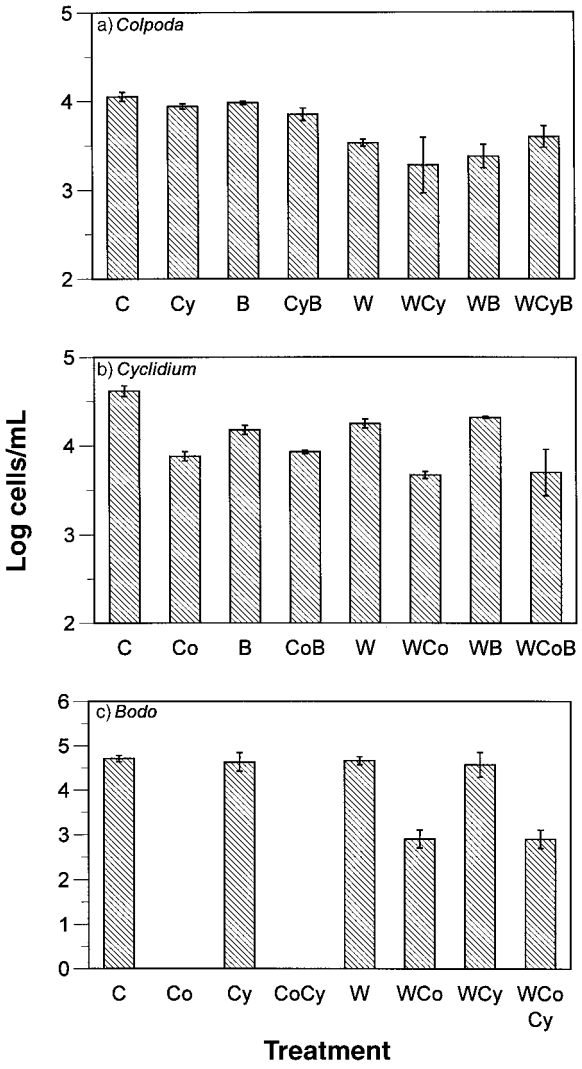

FIG. 4. Responses of (a) *Colpoda*, (b) *Cyclidium*, and (c) *Bodo* to the presence of *Wyeomyia* and/or the other two protozoan species (C = control; Co = *Colpoda*; Cy = *Cyclidium*; B = *Bodo*; W = *Wyeomyia*). Data are means ± 1 SE.

Bodo (Fig. 2: treatments 6, 8, 14, and 16), *Cyclidium* and *Bodo* (Fig. 2: treatments 7, 8, 15, and 16), and *Colpoda* and *Cyclidium* (Fig. 2: treatments 13–16). We predicted two possible outcomes: (1) interactions among protozoa could produce changes in grazing intensity that would result in a simply numerical response in which overall bacterial cell densities would be altered without any changes in relative abundances; and (2) the increase in diversity of grazing types might produce differential changes in bacterial species abundances due to differences in prey selection by individual protozoa species, or to indirect effects stemming from competition or other interactions between pairs of grazers.

There was strong evidence for indirect effects in these food webs. The multivariate bacterial responses to the interactions between *Colpoda* and *Bodo*, and *Colpoda* and *Cyclidium* were highly significant (Table 4a); the *Colpoda* × *Bodo* interaction affected bacterial species A and D, while the *Colpoda* × *Cyclidium* interaction affected only species A (Table 4b). The negative impact of *Colpoda* on species A was reversed in the presence of *Bodo* (Fig. 3 b vs. i), and the increase in species D caused by *Bodo* was reversed by *Colpoda* such that their combined effect was even greater than the effect of the ciliate alone (Fig. 3 d vs. i). When both *Colpoda* and *Cyclidium* were present, the bacterial community profile was more similar to that produced by *Colpoda* alone, with only minimal evidence of the effects of *Cyclidium* (Fig. 3 b vs. j). The *Cyclidium* × *Bodo* interaction produced only a marginally significant multivariate response; however, the univariate tests showed that the density of species A was significantly higher when both grazers were present than when *Cyclidium* was present alone (Fig. 3 c vs. k). Furthermore, the increase in species D produced by *Bodo* was reversed in the presence of *Cyclidium* (Fig. 3 d vs. k). All pairwise contrasts between the bacterial communities of the three webs were significant (Table 4c) indicating that the bacterial community in each of the two-grazer webs was different (Fig. 3i–k) with the major differences due to species A, C, and D. None of the pairwise protozoan interactions showed a statistically significant effect on species B (Table 4b, d).

Indirect effects in each of these two-consumer food webs were produced by strong competitive interactions between pairs of protozoans. There was a clear dominance hierarchy among the three protists. *Colpoda* was the most dominant of the three species, and was unaffected by *Cyclidium* and *Bodo* (Table 3b, Fig. 4a). *Bodo* ranked second among the competitors; its density was significantly decreased by *Colpoda*, but was unaffected by *Cyclidium* (Table 3b, Fig. 4c). Finally, *Cyclidium* density was decreased by *Colpoda* and to a lesser extent by *Bodo* (Table 3b, Fig. 4b). These results clearly support the hypothesis that competitive interactions among the protozoan grazers produce indirect effects on the bacterial assemblage.

Does predation by Wyeomyia *affect the interactions between protozoan species pairs?*

As a keystone predator, *Wyeomyia* might alter the competitive relationships between protozoan species pairs and thus produce additional trophic-linkage indirect effects that could affect the bacterial assemblage. However, as we have argued, *Wyeomyia* is an omnivore, and as such might create complex three-way interactions that might also give rise to indirect effects. We therefore focused on the three-way interactions in the analysis of three (2 × 2 × 2) factorial designs consisting of combinations of *Colpoda*, *Cyclidium*, and *Wyeomyia* (Fig. 2: treatments 9–16), *Colpoda*, *Bodo*, and *Wyeomyia* (Fig. 2: treatments 2, 4, 6, 8, 10, 12,

TABLE 4. Summary of the responses of the four-species (ABCD) bacterial community in food webs containing two protozoan species (see Table 1 for treatment abbreviations).

Multivariate tests		Univariate tests				
			Species			
Source	*P*	Source	A	B	C	D
a) Co × B × S	0.0007*	b) Co × B	*	NS	NS	*
Cy × B × S	0.05	Cy × B	*	NS	NS	*
Co × Cy × S	0.0009*	Co × Cy	*	NS	NS	NS
c) (CoB vs. CyB) × S	0.0038*	d) CoB vs. CyB	*	NS	*	*
(CyB vs. CoCy) × S	0.0006*	CyB vs. CoCy	*	NS	*	*
(CoB vs. CoCy) × S	0.0025*	CoB vs. CoCy	*	NS	*	NS

Notes: (a) Each subdesign was analyzed by a two-factor profile analysis of variance; only the interaction terms were interpreted. (b) Univariate ANOVAs were used to evaluate the responses of each bacterial species to the two-grazer interaction. (c) The bacterial species abundance pattern of each food web was compared to each of the other two-grazer webs using profile analysis of the appropriate contrast statements followed by univariate tests (d) to determine which bacterial taxa were responsible for the differences.

* Significant at an experiment-wise error rate of 0.05 according to a sequential Bonferroni procedure; NS = not significant.

14, 16), and *Cyclidium, Bodo,* and *Wyeomyia* (Fig. 2: treatments 3, 4, 7, 8, 11, 12, 15, 16).

Significant interactions that had indirect effects on the entire bacterial assemblage were found in the food web containing *Wyeomyia, Colpoda,* and *Bodo,* and in the *Wyeomyia, Cyclidium,* and *Bodo* web (Table 5a). The *Wyeomyia* × *Colpoda* × *Bodo* interaction significantly affected bacterial species C and D (Table 5b, Fig. 3m). Based on the large decrease in species B and D and the slight increase in C, we attribute these effects mainly to omnivory by *Wyeomyia.* Species A, C, and D were significantly modified by the *Wyeomyia* × *Cyclidium* × *Bodo* interaction (Table 5b, Fig. 3o). In this case, however, *Wyeomyia* did not seem to be the dominant factor behind the changes in bacterial species abundances; the most striking differences between the *Cyclidium–Bodo* web and the *Cyclidium–Bodo–Wyeomyia* web were the decreases in species A and D (Fig. 3 o vs. k). Furthermore, the presence of the two protozoans prevented *Wyeomyia* from drastically reducing species B. The *Wyeomyia* × *Colpoda* × *Cyclidium* interaction was not significant for the multivariate response; only species C was significantly affected (Table 5a and b, Fig. 3n). Each of the three combinations of protozoa and mosquito larvae produced a different bacterial species abundance pattern (Table 5c, Fig. 3m–o), and the differences in each case were due mainly to changes in the density of a single bacterial taxon (Table 5d). In general, bacterial communities in these three food webs tended to share a similar species profile with each other and with the other food webs that contained mosquito larvae (Fig. 3 m–o and e–h).

Wyeomyia altered two of the interactions between protozoan species pairs. The most striking result was the facilitation of coexistence between *Colpoda* and *Bodo.* In food webs containing *Colpoda,* whether alone or combined with *Cyclidium, Bodo* always disappeared (Table 3, Figure 4c); however, when mosquito larvae were included in the web, their predation reduced the density of *Colpoda* allowing the small flagellate to coexist, albeit at densities well below those of the control. While *Wyeomyia* and *Bodo* each caused decreases in *Cyclidium* density, the amount of decrease was very slightly reduced when both mosquito larvae and the

TABLE 5. Summary of the responses of the four-species (ABCD) bacterial community in food webs containing two protozoan species plus *Wyeomyia* (see Table 1 for treatment abbreviations).

Multivariate tests		Univariate tests				
			Species			
Source	*P*	Source	A	B	C	D
a) W × Co × B × S	0.0003*	b) W × Co × B	NS	NS	*	*
W × Cy × B × S	0.0008*	W × Cy × B	*	NS	*	*
W × Co × Cy × S	0.0960	W × Co × Cy	NS	NS	*	NS
c) (WCoB vs. WCyB) × S	0.0295*	d) WCoB vs. WCyB	NS	*	NS	NS
(WCyB vs. WCoCy) × S	0.0051*	WCyB vs. WCoCy	NS	NS	*	NS
(WCoB vs. WCoCy) × S	0.0015*	WCoB vs. WCoCy	NS	NS	*	NS

Notes: (a) Each design was analyzed by three-way profile analysis of variance; only the three-way interaction term was interpreted. (b) Univariate ANOVAs were used to evaluate the responses of each bacterial species to the grazer–predator interaction. (c) The bacterial species abundance patterns of the three different food webs were compared using profile analysis of the appropriate contrast statements followed by univariate tests (d) to determine which bacterial taxa were responsible for the differences.

* Significant at an experiment-wise error rate of 0.05 according to a sequential Bonferroni procedure; NS = not significant.

TABLE 6. Summary of the responses of the four-species (ABCD) bacterial community in food webs containing three protozoan species and three protozoan species plus *Wyeomyia* (see Table 1 for treatment abbreviations).

Multivariate tests		Univariate tests				
			Species			
Source	*P*	Source	A	B	C	D
a) Co × Cy × B × S	0.0003*	b) Co × Cy × B	*	NS	*	*
c) W × Co × Cy × B × S	0.0008*	d) W × Co × Cy × B	NS	NS	*	*

Notes: (a) The effect of all three protozoa on the bacterial community was determined by examining the three-way interaction term from the three-factor profile analysis of bacterial species densities. (b) Univariate ANOVAs were used to evaluate the responses of each bacterial species to interactions among the three protozoans. (c) The effect of *Wyeomyia* on the community was determined by analyzing the four-way interaction term from the four-factor profile analysis of bacterial species densities followed by univariate tests (d) to determine which bacterial taxa were affected.

* Significant at an experiment-wise error rate of 0.05 according to a sequential Bonferroni procedure; NS = not significant.

flagellate were present (Table 3c, Fig. 4b). Because of the small scale of the effect, however, this interaction may not be biologically meaningful.

These changes produced by *Wyeomyia* in both the protozoan and bacterial assemblages further support our contention that *Wyeomyia* serves as the keystone species and exerts a dominant role in structuring the protozoan and bacterial communities.

What are the effects of three-way protozoan interactions?

This analysis looked at a factorial design in which all three species of protozoa were used together (Fig. 2: treatments 5, 6, 7, 8, 13–16). Since the analyses of all the smaller webs showed that bacterial community structure was dependent on the species composition of each food web design, we predicted that there would be a significant three-way interaction indicating that the combination of all three protozoan grazers produced a bacterial community distinct from those produced in the smaller designs.

There was a significant three-way multivariate interaction (Table 6a), and results from the univariate tests showed that bacterial species A, C, and D were primarily responsible for the change in bacterial community structure (Table 6b, Fig. 3l). Compared to webs containing only *Colpoda,* there were increases in the densities of species A and C. Species D decreased substantially, probably due to grazing by *Colpoda.* Species B was not significantly affected by the interaction among the three grazers. This is consistent with the pattern seen in all food webs that lacked mosquito larvae.

Only one minor but statistically significant difference was observed among the three protozoans in this food web. *Cyclidium* density was slightly lower in the three-species combination than when it was grown with *Bodo* alone, but slightly higher than when it was grown with *Colpoda* alone (Table 3d, Fig. 4b).

What is the effect of Wyeomyia *in webs containing three protozoan species?*

This experiment made use of the full (2 × 2 × 2 × 2) factorial design (Fig. 2: treatments 1–16), which included the three protozoan taxa plus *Wyeomyia* larvae. There was a significant four-way multivariate interaction (Table 6c), and bacterial species C and D were primarily responsible for the changes in community structure (Fig. 3p). Once again, *Wyeomyia* appeared to be the most important species in this system in terms of impact on the bacterial community. The overall bacterial species abundance profile for the complete four-species food web resembled those produced in other food web constructs containing mosquito larvae, with only minor differences due to interactions among protozoan taxa.

There were no significant three-way (*Wyeomyia* × Protozoa × Protozoa) interactions for *Colpoda, Cyclidium,* or *Bodo* (Table 3e). Predation by mosquito larvae remained the primary factor affecting *Colpoda* densities (Fig. 4a). *Cyclidium* densities decreased in response to competition with *Colpoda* and *Bodo,* as well as predation by mosquitoes. *Wyeomyia* did not affect the outcome of the competition with *Colpoda,* but did reduce the effect of *Bodo* to a slight degree (Fig. 4b). Competition with *Colpoda* continued to have a strong negative effect on *Bodo* that was reversed by *Wyeomyia* predation on the ciliate (Fig. 4c).

Do predation effects cascade through the food web to affect bacterial cell density?

We asked whether we could detect the cascading effects of predation on bacterial cell density in a trophic-species web composed of a top predator (*Wyeomyia*), intermediate level grazers (three species of bacterivorous ''Protozoa''), and a basal level assemblage (''Bacteria''). We tested the trophic cascade hypothesis by analyzing the effects of the presence/absence of *Wyeomyia* larvae and protozoa on total bacterial cell density or biomass per milliliter in a (2 × 2) factorial subdesign. Because the four bacteria used in this experiment were essentially the same size, changes in bacterial abundance could also be interpreted as changes in biomass. Protozoan biomass was measured as the total biovolume per milliliter estimated from the mean cell dimensions for each species (Wetzel and Likens 1991). The effects of *Wyeomyia* on total protozoan

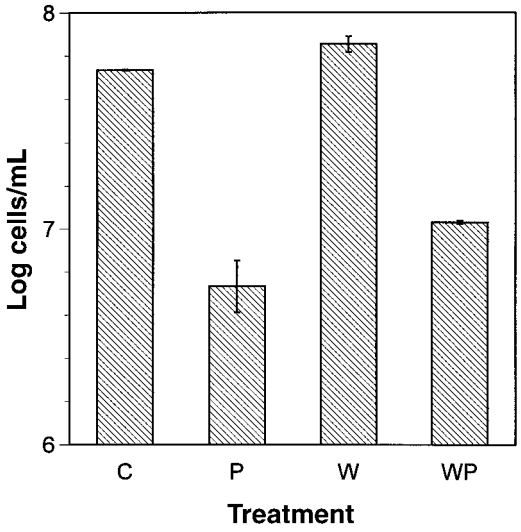

FIG. 5. Response of total bacterial population size (cells per milliliter) to cascading trophic effects of *Wyeomyia* predation on protozoa (C = control; W = *Wyeomyia*; P = protozoa). Data are means ± 1 SE.

density and biomass were analyzed using *t* tests on the $\log_{10}$ transformed data. Changes in bacterial cell density were analyzed by two-factor ANOVA on the $\log_{10}$ transformed total counts. An a priori contrast was used to determine if the mean bacterial count in the Protozoa treatments differed significantly from treatments containing both Protozoa and *Wyeomyia.*

Both Protozoa and *Wyeomyia* had significant main effects on bacterial density (Fig. 5). Protozoan grazing decreased mean bacterial cell density per milliliter by >90% (df = 1, 8, $F = 212.01$, $P < 0.0001$), while *Wyeomyia* produced a significant increase (df = 1, 8, $F = 10.97$, $P = 0.01$), possibly due to the regeneration of nutrients in fecal pellets. There was no significant interaction between *Wyeomyia* and protozoa (df = 1, 8, $F = 2.02$, $P = 0.19$), but in the presence of both predators and grazers, mean bacterial counts were nearly double those in the protozoa only treatments (Fig. 5). This increase was significant (Bonferroni $P = 0.01$), and primarily associated with species A, which more than tripled (Fig. 3 p vs. l).

The classic trophic cascade model would require that the increase in bacterial abundance (biomass) in treatments containing both predators and grazers be due to a decline in grazer biomass due to predation by mosquito larvae. This was not the case; predation by *Wyeomyia* larvae did not significantly lower mean protozoan density per milliliter (df = 4, $t = 1.076$, $P = 0.3425$) or biomass per milliliter (df = 4, $t = 1.757$, $P = 0.1538$). This result was unexpected since field censuses of pitcher communities have shown strong negative effects of *Wyeomyia* larvae on protozoa (Addicott 1974, Cochran-Stafira 1993). To explain the apparent absence of a significant predator effect we examined the counts for each protozoan species independently in each of the two treatments. Mosquito predation reduced mean *Colpoda* density by 41.1% and *Cyclidium* density by 20% (Fig. 4a and b). In the absence of *Wyeomyia, Bodo* was eliminated in all replicates, but when mosquitoes were present *Bodo* persisted at densities of just under 1000 cells/mL (Fig. 4c). This partial recovery of *Bodo* populations combined with the decrease in population densities of the two ciliates explains the failure of predation to reduce overall protozoan numbers and biomass. The trophic cascade in this system can thus be explained in terms of the species-specific direct effects of mosquito predation on the protozoan assemblage, and the consequent indirect effects on individual bacterial species. *Wyeomyia* predation reduced the density of *Colpoda* thus allowing bacterial species A to increase sufficiently to significantly reverse the negative impact of the protozoan community on bacterial density and biomass.

DISCUSSION

Direct and indirect effects

We have shown that both direct and indirect effects are important in determining the species abundance patterns of both protozoans and bacteria in laboratory reconstructions of the detritus-based food web found in the pitcher fluid of *Sarracenia purpurea.* Our data clearly demonstrate that predation and competitive interactions among species at higher trophic levels, produce shifts in the species abundance profiles of the basal-level bacterial assemblage that can have significant effects on total bacterial population densities.

Bacterial community structure was significantly altered through the direct effects of grazing by omnivorous *Wyeomyia smithii* larvae and each of the three species of bacterivorous protozoa, *Colpoda, Cyclidium,* and *Bodo.* Each grazer produced a distinctly different bacterial community in terms of bacterial species abundance profiles. These species-specific responses to protozoan grazing may have been due to selective feeding on different bacterial taxa. A number of studies have shown that grazing by protozoa can alter the morphological and/or size distribution patterns of aquatic bacterial assemblages (Epstein and Shiaris 1992, Balčiūnas and Lawler 1995). The mechanism most frequently proposed for these changes is size selective grazing, although other factors such as toxicity (e.g., *Chromobacterium violaceum*) can contribute to edibility (Curds and Vandyke 1966, Berk et al. 1976, Mitchell et al. 1988, Verity 1991). Since the four marker species in this study were all small rods of approximately the same size and degree of motility, edibility was not based solely on morphological characteristics. It is therefore unlikely that the observed changes in bacterial species relative abundances were simply due to the removal of certain shape or size classes by size selective grazers; some chemical cues may have been involved as well. Since *Wyeomyia* larvae are orders of

magnitude larger than protozoa and bacteria, they probably do not filter-feed selectively on items as small as bacteria. The strikingly consistent bacterial profiles observed in food webs containing mosquito larvae (i.e., strong reductions of species B and D) may have been linked to differential growth rates among the bacteria or to competitive interactions among bacterial taxa that arose in response to nutrient input by the larvae.

Interactions among the protozoan grazers and between the protozoans and mosquito larvae had indirect effects on the bacteria. Competitive interactions among *Colpoda, Cyclidium,* and *Bodo* produced changes in the bacterial species profiles, and each combination of protozoans gave rise to a different pattern of bacterial species abundances. *W. smithii* interacted with protozoa to produce changes in bacterial community structure.

Wootton (1993, 1994) has defined two categories of indirect effects: interaction chains and interaction modifications. Interaction chains are defined as indirect effects that result from a series of direct interactions between species pairs. Interaction chains do not affect the mathematical functions describing the interactions among species; instead, the outcome of the interaction is altered. For example, predation by the sea star *Pisaster* keeps populations of the competitive dominant *Mytilus* at low levels, thereby reducing competition for space and increasing local species diversity (Paine 1966). We identified two interaction chains in our microcosms: (1) *Wyeomyia* predation on *Colpoda* modified the competition between *Colpoda* and *Bodo* allowing the two protozoans to coexist (Fig. 4c); (2) *Wyeomyia* predation on *Colpoda* reduced the impact of grazing by the ciliate on bacterial species A (Fig. 3 f vs. b).

Interaction modifications are defined as indirect effects in which one species alters the nature of the interaction between two other species. The results of these interactions are difficult to predict since they are emergent properties of multispecies assemblages, and are not derived from pairwise interactions. For example, the impact of planktivorous fish on zooplankton populations may be altered if a perceived risk of predation alters their feeding behavior (Wootton 1994). There was evidence for interaction modifications among the components of our microcosms. Both *Wyeomyia* predation and competition with *Bodo* had negative impacts on *Cyclidium* densities in pairwise comparisons. However, when both mosquito larvae and flagellates were present, *Cyclidium* density increased 38% over the *Bodo* only treatment, and 18% over the *Wyeomyia* treatment (Fig. 3b). The mechanism behind this indirect effect is not clear; there are no obvious shifts in the relative abundances of the four bacterial species that might suggest an increase in a preferred food species for the ciliate. It is possible that nutrient input via mosquito feces and/or increases in nonculturable bacteria were responsible for the slight but significant increase in *Cyclidium.* Another interaction modification involved *Colpoda, Bodo,* and bacterial species D; *Colpoda* caused species D to decrease, while *Bodo* caused D to increase. When both grazers were present we expected D to be at a density intermediate to the two single-grazer treatments. However, there was a further decrease in cells per milliliter, suggesting that the competitive interaction between the two grazers somehow intensified the negative effect of the dominant competitor, *Colpoda,* on this bacterium. This may have again been due to a shift in the abundances of nonculturable bacteria which, stimulated by a change in resource levels, negatively affected species D.

Precisely defining the nature of the direct and indirect effects of predation in this food web is difficult. While the ultimate causes are certainly based on the presence of the predators and grazers (top-down regulation), the proximal causes are less clear because top-down (consumer driven) and bottom-up (resource driven) controls tended to be confounded (McQueen et al. 1992). Even the so-called ''direct effects'' of grazing probably included some aspects of indirect effects via nutrient recycling. Mosquito larvae added a considerable amount of recycled nutrients to the system in the form of feces and molted exoskeletons. Similarly, the protozoan grazers may have altered the abiotic environment within the microcosms. What we have termed the direct effects of individual grazer taxa, may in fact, be the result of a combination of direct grazing as well as the indirect effects of grazer-mediated changes in the competitive interactions among bacterial species, resulting in overall changes in species abundances. These observations are consistent with those relating to zooplankton control of phytoplankton community structure directly by selective grazing and indirectly through nutrient recycling (Carpenter and Kitchell 1984, Carpenter et al. 1985, Vanni 1987, Elser 1992).

Because of the variability in community constituents among *S. purpurea* pitchers (Cochran-Stafira 1993, Harvey and Miller, 1996), we cannot claim to fully understand all the possible interactions among pitcher inquilines. Furthermore, we have not been able to include rotifers, other insect larvae, or mites in our analyses since their relationships to the microbial component are unclear and currently under investigation (D. L. Cochran-Stafira, *unpublished data*). However, by using the community reconstruction approach we have been able to examine the complex suite of interactions among a representative subset of species from pitcher communities at Cedarburg Bog. Experimental manipulation of this relatively simple food web allowed us to dissect the interactions among its four consumer taxa, and analyze their effects on the bacterial assemblage. As a result, we can now begin to describe the pitcher food web, including the bacterial component, in terms of a complex interaction web (Fig. 6) that involves direct predatory and competitive interactions, interaction chains, and interaction modifications, the effects of which permeate throughout the community.

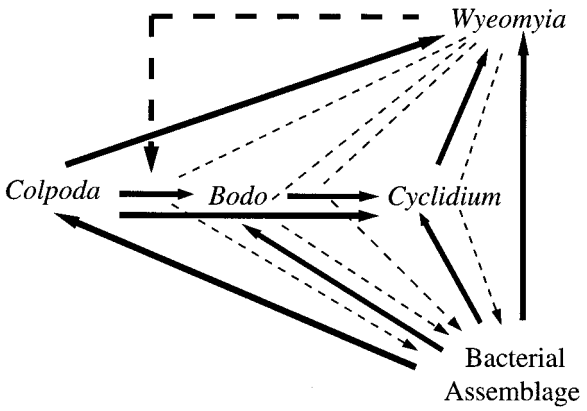

FIG. 6. Interaction web for the *Sarracenia purpurea* inquilines used in the laboratory microcosms. Solid arrows are direct interactions. Dashed arrows represent indirect effects and are drawn with the arrow head indicating the target organism(s); the tail of the arrow indicates the species that is responsible for producing the indirect effect. Horizontal arrows designate competitive interactions; upward-pointing vertical or diagonal arrows designate predation or indirect effects mediated by predation. Heavy lines represent strong interactions; thin lines represent less intense interactions. Individual bacterial taxa are not represented here since they may vary among pitchers.

Was there a trophic cascade?

Detection of a trophic cascade in the pitcher system is significant, since a number of previous attempts to verify cascading trophic effects in microbial food webs has been unsuccessful; typically, the cascade has truncated before the basal bacterial level (Pace and Funke 1991, Pace 1993). The trophic cascade in our microcosms was mediated not by the overall reduction in intermediate-level grazers, but by changes in the species composition and relative abundance patterns of the protozoan assemblage. In addition, grazing by omnivorous *Wyeomyia smithii* larvae did not reduce total numbers of bacteria. Rather, bacterial densities were slightly elevated in the presence of mosquito larvae possibly due to the regeneration of nutrients in fecal pellets or the release of additional organic nutrients from damaged bacterial cells. Our experimental results contrast with studies of regulation of bacterial populations in tree holes (Walker et al. 1991) and beech pans (Walker and Merritt 1988) in which filter-feeding by *Aedes triseriatus* significantly reduced bacterial densities and held populations below carrying capacities in these container habitats. The discrepancy may be due to the low larval densities in our microcosms. Field studies suggest that at the high larval densities usually seen in unmanipulated pitchers, *Wyeomyia* may be similarly capable of holding bacterial populations in *Sarracenia* pitchers well below carrying capacity (Cochran-Stafira 1993). Of the three protozoan species, *Colpoda* and *Cyclidium* were responsible for most of the change in bacterial total abundance; although *Bodo* caused increases in species C and D, these were offset by small decreases in A and B so that the net effect of grazing by *Bodo* was no significant decrease in total bacterial bacterial density. This may have been due to nutrient mobilization by flagellate grazing, which stimulated the population growth rate of the bacteria thus allowing them to keep pace with the rate of predation.

The cascade was apparently produced as a result of the differential feeding of the bacterivores. This supports arguments that the heterogeneity within trophic levels can influence the effects of consumers on basal-level communities (Vanni 1987, Leibold 1989, Leibold and Wilbur 1992, Richardson and Threlkeld 1993). Data from field censuses (Cochran-Stafira 1993) and field manipulations (D. L. Cochran-Stafira, *unpublished data*) also support the trophic cascade hypothesis. Field densities of *Wyeomyia* larvae in pitchers are high, and as a result, protozoa other than small heterotrophic flagellates and mixotrophic chrysophytes are very rare; however, bacterial densities are high and show relatively little variation.

Does Wyeomyia smithii *function as a keystone predator?*

The data support our original hypothesis that *Wyeomyia smithii* can function as a keystone predator (sensu Paine 1969) in the pitcher community. At the densities used in this experiment, predation modified competitive interactions among the three protozoan species and fostered coexistence. This is in marked contrast to the earlier study by Addicott (1974), in which he was unable to demonstrate any increase in protozoan species diversity as a result of *Wyeomyia* predation. Instead, he concluded that protozoan species richness declined monotonically with increasing larval density while species evenness increased.

There are a number of possible explanations for the discrepancy between the two studies. First, Addicott ran his experiments in natural pitchers in the field, while our manipulations were done in artificial ''test tube'' pitchers in the laboratory using a subset of the complete pitcher community. As we have shown, bacterivory by omnivorous mosquito larvae combined with nutrient recycling from their fecal wastes significantly altered bacterial species abundances in our experimental communities. It is possible that in natural pitcher communities with a more diverse bacterial assemblage, the direct and indirect effects of *Wyeomyia* on the bacterial assemblage might feed back through the food web to higher trophic levels and modify competitive interactions among the protozoan species (Stone and Weisburd 1992). We have some evidence for this from field and laboratory experiments in natural pitchers in which we manipulated trophic structure (D. L. Cochran-Stafira, *unpublished data*). The bacterial species abundance patterns responded to each change in trophic structure as they did in the test tube experiments, and *Wyeomyia* still appeared to have the greatest impact on bacterial community structure. The com-

petitive interactions among protozoan taxa, however, were somewhat variable suggesting that the relative importance of *Wyeomyia* predation may be community-specific; i.e., dependent on community species composition.

Second, keystone predators, as originally defined by Paine (1969), promote community diversity by lowering the numbers of the competitive dominant, thus permitting the persistence of species that are inferior competitors but resistant to predation (Leibold, *in press*). Addicott attributed the pitcher community's lack of response to *Wyeomyia* predation to an absence of strong interactions among protozoan prey community at his study sites. In contrast, we found strong competitive interactions among *Colpoda, Cyclidium,* and *Bodo,* which were altered by larval predation. In the absence of the predator, the three protozoans formed a competitive hierarchy with *Colpoda* as the dominant species followed by *Bodo,* and *Cyclidium. Wyeomyia* predation promoted species diversity in our experimental community primarily by alleviating the intense competition between *Colpoda* and *Bodo,* thus permitting coexistence among all three protozoans. Again, differences in the relative importance of predation in the two studies might be due to the differences in community species composition. Pitcher communities at our field site generally had low protozoan diversity, and the three strongly interacting taxa used in this experiment represented the most commonly occurring species. Higher natural species richness at Addicott's field sites might have reduced the intensity or significance of the competitive interactions making the community less susceptible to the effects of predation.

A third important difference between the two studies was the amount of available nutrients. Addicott supplemented the natural food levels in his experimental pitchers with additional insect prey at the start of each experiment so that his communities had relatively high nutrient levels. Our artificial pitchers received a much lower initial input of resources in the form of fine particulate organic matter (fish food) and dissolved glucose. In his discussion of species diversity patterns along productivity gradients, Leibold (1996) suggests that keystone predators promote increased species diversity in systems with intermediate levels of productivity but not in those with high or low productivity. Low productivity communities do not support many predators, have low diversity, and are dominated by good competitors that are highly edible. Communities at high productivities support larger numbers of predators, also have low diversity, and are dominated by poorer competitors that are highly inedible. Communities at intermediate productivities are the most diverse because competitive dominants are kept at low levels by moderate numbers of predators feeding on the edible taxa. Thus, the differences in the effects of *Wyeomyia* on community diversity in these two experiments might be due to the differences in their initial nutrient levels.

Recent reviews of the keystone species concept (Mills et al. 1993, Power et al. 1996) have attempted to clarify its meaning and provide an operational definition for the term. The most general definition of a keystone species is one ''whose impact on its community or ecosystem is large, and disproportionately large relative to its abundance'' (Power et al. 1996). Thus, despite the apparent community-specific nature of its ability to control protozoan species diversity, we feel that we are justified in conferring ''keystone'' status on the larvae of *Wyeomyia smithii.* Their omnivorous feeding habits may in part be responsible for their dominance of pitcher community dynamics. By feeding on detritus they provide a means of rapid nutrient recycling for the system that may feed back up through the food web to alter competitive interactions and species abundance patterns among grazers and bacteria. As such, they may be an important link in a network of facilitation interactions (Heard 1994) that extends throughout the pitcher food web. As predators, mosquito larvae modify competitive interactions among protozoan grazers and generate indirect effects on total bacterial abundances and community structure. At normal field densities, mosquito larvae quickly eliminated ciliates from pitcher communities (Cochran-Stafira 1993) and kept protozoan diversity low, while at low densities in this experiment, larval predation modified competitive interactions and promoted protozoan species diversity. These predation effects cascaded down to the basal-level bacterial assemblage. The larvae were also important grazers on the bacterial assemblage, and there was a striking similarity in bacterial species profiles in webs containing *Wyeomyia* (Fig. 3). Thus the larvae were responsible for the overall architecture of the food web and the subsequent interactions among grazers and the basal-level bacterial community.

Integrating microbial communities into food webs

This experiment represents one of the first attempts to examine the response of a basal-level bacterial community to predation and its direct and indirect effects in a fully resolved aquatic microbial food web. In contrast to other studies that have dealt exclusively with the effects of predation on bacterial size distributions (Turley et al. 1986) or biomass and productivity (Pace and Funke 1991, Pace 1993), we included both trophic-species and species level phenomena. Our results support the hypothesis that top-down effects can play a major role in the regulation of heterotrophic microbial communities in freshwater ecosystems; however, detection of these predation-mediated effects is dependent on the level of food web resolution. When the pitcher food web was resolved at the trophic-species level (predator–grazers–bacteria), there was no evidence of a significant *Wyeomyia*–Protozoa interaction, although total bacterial density did rebound in response

to the presence of the predator (Fig. 5). However, when the food web was analyzed at the species level of resolution, there was strong evidence for compensatory shifts in bacterial species abundances as a result of both the direct effects of predation by individual grazer taxa and indirect effects stemming from interactions among the grazers and from the cascading effects of *Wyeomyia* predation on protozoan grazers. The bacterial responses to predation in this community raise an interesting question of species ''functional redundancy'' in the pitcher system. Data from field censuses show a high degree of species variability from pitcher to pitcher (Cochran-Stafira 1993, Harvey and Miller 1996). Since the microbial community is presumed to be responsible for the digestion of prey insects in *S. purpurea* pitchers, this variability may not affect the performance of the microbial community as the basal-level component of this detritus-based ecosystem. The bottom-up effects of these changes in bacterial community species composition on the rest of the pitcher food web are currently under investigation (D. L. Cochran-Stafira, *unpublished data*).

By studying this detritus-based food web at the species level of resolution, we have demonstrated that interactions among components of microbial communities and between microbes and higher trophic levels are just as complex as those involving plants, herbivores, and their animal predators. It is therefore appropriate to consider the microbes as integral parts of the entire ecosystem, not merely as decomposers or food resources, but as fully interacting members of the community.

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Literature Cited

Adams, R. M., II, and G. W. Smith. 1977. An S. E. M. survey of the five carnivorous pitcher plant genera. American Journal of Botany **64**:265–272.

Addicott, J. F. 1974. Predation and prey community structure: an experimental study of the effect of mosquito larvae on the protozoan communities of pitcher plants. Ecology **55**:475–492.

Azam, F., T. Fenchel, J. G. Field, J. S. Grey, L. A. Meyer-Reil, and F. Thiungstad. 1983. The ecological role of water-column microbes in the sea. Marine Ecology Progress Series **10**:257–263.

Balčiūnas, D., and S. P. Lawler. 1995. Effects of basal resources, predation, and alternative prey in microcosm food chains. Ecology **76**:1327–1336.

Bateman, L. E. 1987. A bdelloid rotifer living as an inquiline in leaves of the pitcher plant, *Sarracenia purpurea*. Hydrobiologia **147**:129–133.

Berk, S. G., R. R. Colwell, and E. B. Small. 1976. A study of feeding responses to bacterial prey by estuarine ciliates. Transactions of the American Microscopical Society **95**: 514–520.

Bradshaw, W. E. 1983. Interaction between the mosquito *Wyeomyia smithii*, and the midge *Metriocnemus knabi*, and their carnivorous host *Sarracenia purpurea*. Pages 161–189 *in* J. H. Frank and L. P. Lounibos, editors. Phytotelmata: terrestrial plants as hosts for aquatic insect communities. Plexus, Medford, New Jersey, USA.

Bradshaw, W. E., and R. A. Creelman. 1984. Mutualism between the carnivorous purple pitcher plant and its inhabitants. American Midland Naturalist **112**:224–227.

Carpenter, S. R. 1988. Complex interactions in lake communities. Springer-Verlag, New York, New York, USA.

Carpenter, S. R., and J. F. Kitchell. 1984. Plankton community structure and limnetic primary production. American Naturalist **124**:159–172.

Carpenter, S. R., J. F. Kitchell, and J. R. Hodgson. 1985. Cascading trophic interactions and lake productivity. BioScience **35**:634–639.

Carpenter, S. R., J. F. Kitchell, J. R. Hodgson, P. A. Cochran, J. J. Elser, M. M. Elser, D. M. Lodge, D. Kretchmer, X. He, and C. N. von Ende. 1987. Regulation of lake primary productivity by food web structure. Ecology **68**:1863–1876.

Christian, R. R. 1994. Aggregation and disaggregation of microbial food webs. Microbial Ecology **28**:327–329.

Cochran-Stafira, D. L. 1993. Food web interactions and bacterial community structure in *Sarracenia purpurea* pitchers. Dissertation. Northern Illinois University, DeKalb, Illinois, USA.

Curds, C. R., and J. M. Vandyke. 1966. The feeding habits and growth rates of some fresh-water ciliates found in activated-sludge plants. Journal of Applied Ecology **3**:127–137.

Day, R. W., and G. P. Quinn. 1989. Comparisons of treatments after an analysis of variance in ecology. Ecological Monographs **59**:433–463.

Drake, J. A. 1991. Community-assembly mechanics and the structure of an experimental species ensemble. American Naturalist **137**:1–26.

Elser, J. J. 1992. Phytoplankton dynamics and the role of grazers in Castle Lake California. Ecology **73**:887–902.

Elser, J. J., and S. R. Carpenter. 1988. Predation-driven dynamics of zooplankton and phytoplankton in a whole-lake experiment. Oecologia **76**:148–154.

Elser, J. J., M. M. Elser, N. A. MacKay, and S. R. Carpenter. 1988. Zooplankton-mediated transitions between N- and P-limited algal growth. Limnology and Oceanography **33**: 1–14.

Epstein, S. S., and M. P. Shiaris. 1992. Size-selective grazing of coastal bacterioplankton by natural assemblages of pigmented flagellates, colorless flagellates, and ciliates. Microbial Ecology **23**:211–225.

Fashing, N. J., and B. M. O'Connor. 1984. *Sarraceniopus*—a new genus for histiostomatid mites inhabiting the pitchers of the Sarraceniaceae (Astigmata: Histiostomatidae). International Journal of Acarology **10**:217–227.

Federle, T. W., R. J. Livingston, D. A. Meeter, and D. C. White. 1983. Modifications of estuarine sedimentary microbiota by exclusion of epibenthic predators. Journal of Experimental Marine Biology and Ecology **73**:81–94.

Fenchel, T. 1982. Ecology of heterotrophic microflagellates.

IV. Quantitative occurrence and importance as bacterial consumers. Marine Ecology Progress Series **9**:35–42.
Franson, M. H. 1985. Standard methods for the examination of water and waste water. American Public Health Association, Washington, D.C., USA.
Gifford, D. J. 1991. The protozoan–metazoan link in pelagic ecosystems. Journal of Protozoology **38**:81–86.
Gill, D. E. 1972. Density dependence and population regulation in laboratory cultures of *Paramecium.* Ecology **53**: 701–708.
Gilpin, M. E., M. P. Carpenter, and M. J. Pomerantz. 1986. Pages 23–40 *in* J. Diamond and T. J. Case, editors. Community ecology. Harper and Row, New York, New York, USA.
Harris R. J. 1985. A primer of multivariate statistics. Second edition. Academic Press, Orlando, Florida, USA.
Harvey, E., and T. E. Miller. 1996. Variance in composisiton of inquiline communities in leaves of *Sarracenia purpurea* L. on multiple spatial scales. Oecologia **108**:562–566.
Heard, S. B. 1994. Pitcher-plant midges and mosquitoes: a processing chain commensalism. Ecology **75**:1647–1660.
Hessen, D. O., and J. P. Nilssen. 1986. From phytoplankton to detritus and bacteria: effects of short-term nutrient and fish perturbations in a eutrophic lake. Archiv für Hydrobiologie **105**:273–284.
Hrbacek, J., M. Dvorakova, V. Korinek, and L. Prochazkova. 1961. Demonstration of the effect of the fish stock on the species composition of zooplankton and the intensity of metabolism of the whole plankton assemblage. Verhandlungen–Internationale Vereinigung für theoretische und angewandte Limnologie **14**:192–195.
Istock, C. A., K. Tanner, and H. Zimmer. 1983. Habitat selection by the pitcher-plant mosquito, *Wyeomyia smithii*: behavioral and genetic aspects. Pages 191–204 *in* J. H. Frank and L. P. Lounibos, editors. Phytotelmata: terrestrial plants as hosts for aquatic insect communities. Plexus, Medford, New Jersey, USA.
Istock, C. A., J. Zisfein, and K. J. Vavra. 1975. Ecology and evolution of the pitcher-plant mosquito. 2. The substructure of fitness. Evolution **30**:535–547.
Juniper, B. E., R. J. Robins, and D. M. Joel. 1989. The carnivorous plants. Academic Press, London, UK.
Jurgens, K., and G. Stolpe. 1995. Seasonal dynamics of crustacean zooplankton, heterotrophic nanoflagellates and bacteria in a shallow, eutrophic lake. Freshwater Biology **33**: 27–38.
Keppel, G. 1982. Design and analysis: a researcher's handbook. Second edition. Prentice-Hall, Englewood Cliffs, New Jersey, USA.
Kitching, R. L. 1971. An ecological study of water-filled treeholes and their position in the woodland ecosystem. Journal of Animal Ecology **40**:281–302.
Kurihara, Y. 1959. Synecological analysis of the biotic community in microcosm. VIII. Studies on the limiting factor in determining distribution of mosquito larvae in the polluted water of bamboo containers, with special reference to relation of larvae to bacteria. Japanese Journal of Zoology **12**:391–400.
———. 1983. The succession of aquatic dipterous larvae inhabiting bamboo phytotelmata. Pages 55–77 *in* J. H. Frank and L. P. Lounibos, editors. Phytotelmata: terrestrial plants as hosts for aquatic insect communities. Plexus Publishing, Medford, New Jersey, USA.
Lawler, S. P. 1993. Direct and indirect effects in microcosm communities of protists. Oecologia **93**:184–190.
Leibold, M. A. 1989. Resource edibility and the effects of predation and productivity on the outcome of trophic interactions. American Naturalist **134**:922–949.
———. 1991. Trophic interaction and habitat segregation between competing *Daphnia.* Oecologia **86**:510–520.
———. 1996. A graphical model of keystone predators in food webs: trophic regulation of abundance, incidence, and diversity patterns in communities. American Naturalist **147**:784–812.
———. *In press.* Biodiversity and nutrient enrichment in pond plankton communities. Evolutionary Ecology.
Leibold, M. A., and H. M. Wilbur. 1992. Interactions between food-web structure and nutrients on pond organisms. Nature **26**:341–343.
Lloyd, F. E. 1942. The carnivorous plants. Chronica Botanica, 9. Ronald Press, New York, New York, USA.
Maguire, B., Jr. 1971. Phytotelmata: biota and community structure determination in plant-held waters. Annual Review of Ecology and Systematics **2**:439–464.
Maguire, B., Jr., D. Belk, and G. Wells. 1968. Control of community structure by mosquito larvae. Ecology **49**:207–210.
Maxwell, S. E., and H. D. Delaney. 1990. Designing experiments and analyzing data: a model comparison perspective. Wadsworth, Belmont, California, USA.
McQueen, D. J., R. France, and C. Kraft. 1992. Confounded impacts of planktivorous fish on freshwater biomanipulations. Archiv für Hydrobiologie **125**:1–24.
Menge, B. A., E. L. Berlow, C. A. Blanchette, S. A. Navarrete, and S. B. Yamada. 1994. The keystone species concept: variation in interaction strength in a rocky intertidal habitat. Ecological Monographs **64**:249–286.
Miller, T. E. 1994. Direct and indirect species interactions in an early old-field plant community. American Naturalist **143**:1007–1025.
Mills, L. S., M. E. Soule, and D. F. Doak. 1993. The keystone-species concept in ecology and conservation. BioScience **43**:219–224.
Mitchell, G. C., J. H. Baker, and M. A. Sleigh. 1988. Feeding of a freshwater flagellate, *Bodo saltans,* on diverse bacteria. Journal of Protozoology **35**:219–222.
Naeem, S. 1988. Resource heterogeneity fosters coexistence of a mite and a midge in pitcher plants. Ecological Monographs **58**:215–227.
———. 1990. Resource heterogeneity and community structure: a case study in *Heliconia inbricata* phytotelmata. Oecologia **84**:29–38.
Neill, W. E. 1974. The community matrix and interdependence of the competition coefficients. American Naturalist **108**:399–408.
Nerad, T. A. 1991. Catalog of protists. 17th edition. American Type Culture Collection, Rockville, Maryland, USA.
Pace, M. L. 1993. Heterotrophic microbial processes. Pages 252–277 *in* S. R. Carpenter and J. F. Kitchell. The trophic cascade in lakes. Cambridge University Press, Cambridge, UK.
Pace, M. L., and E. Funke. 1991. Regulation of microbial communities by nutrients and herbivores. Ecology **72**:904–914.
Pace, M. L., and J. D. Orcutt, Jr. 1981. The relative importance of protozoans, rotifers, and crustaceans in a freshwater zooplankton community. Limnology and Oceanography **26**:822–830.
Paine, R. T. 1966. Food web complexity and species diversity. American Naturalist **100**:65–75.
———. 1969. A note on trophic complexity and community stability. American Naturalist **103**:91–93.
———. 1974. Intertidal community structure: experimental studies on the relationship between a dominant competitor and its principal predator. Oecologia (Berlin) **15**:93–120.
Plasota, K., M. Plasota, and W. J. H. Kunicki-Goldfinger. 1980. Methods for obtaining an axenic culture of *Habrotrocha rosa* Donner 1949. Hydrobiologia **73**:37–38.
Pomeroy, L. R., and W. J. Wiebe. 1988. Energetics of microbial food webs. Hydrobiologia **159**:7–18.

Porter, K. G., M. L. Pace, and J. F. Battey. 1979. Ciliate protozoans as links in freshwater planktonic food chains. Nature **277**:563–565.

Porter, K. G., H. Paerl, R. Hodson, M. Pace, J. Priscu, B. Riemann, D. Scavia, and J. Stockner. 1988. Microbial interactions in lake food webs. Pages 209–227 *in* S. R. Carpenter, editor. Complex interactions in lake communities. Springer-Verlag, New York, New York, USA.

Porter, K. G., E. B. Sherr, B. F. Sherr, M. Pace, and R. W. Sanders. 1985. Protozoa in planktonic food webs. Journal of Protozoology **32**:409–415.

Power, M. E., D. Tilman, J. A. Estes, B. A. Menge, W. J. Bond, L. S. Mills, G. Daily, J. C. Castilla, J. Lubchenko, and R. T. Paine. 1996. Challenges in the quest for keystones. Trends in Ecology and Evolution **46**:609–620.

Rashit, E., and M. Bazin. 1987. Environmental fluctuations, productivity, and species diversity: an experimental study. Microbial Ecology **14**:101–112.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution **43**:223–225.

Richardson, W. B., and S. T. Threlkeld. 1993. Complex interactions of multiple aquatic consumers: an experimental mesocosm manipulation. Canadian Journal of Fisheries and Aquatic Sciences **50**:29–42.

Riemann, B. 1985. Potential importance of fish predation and zooplankton grazing on natural populations of freshwater bacteria. Applied and Environmental Microbiology **50**:187–193.

Riviere, F. 1985. Effects of two predators on community composition and biological control of *Aedes aegypti* and *Aedes polynesiensis.* Pages 121–135 *in* J. R. Rey and J. H. Frank, editors. Ecology of mosquitoes. Florida Medical Entomology Laboratory, Vero Beach, Florida, USA.

SAS Institute 1989. SAS/STAT user's guide, version 6. Fourth edition. Volumes 1 and 2. SAS Institute, Cary, North Carolina, USA.

Seifert, R. P., and F. H. Seifert. 1976. A community matrix analysis of *Heliconia* insect communities. American Naturalist **110**:461–483.

Sherr, E. B., and B. F. Sherr. 1991. Planktonic microbes: tiny cells at the base of the ocean's food webs. Trends in Ecology and Evolution **6**:50–54.

Sherr, B. F., E. B. Sherr, and C. S. Hopkinson. 1988. Trophic interactions within pelagic microbial communities: indications of feedback regulation of carbon flow. Hydrobiologia **159**:19–26.

Simms, E. L., and D. S. Burdick. 1988. Profile analysis of variance as a tool for analyzing correlated responses in experimental ecology. Biometrical Journal **30**:229–242.

Sinclair, J. L., and M. Alexander. 1989. Effect of protozoan predation on relative abundance of fast- and slow-growing bacteria. Canadian Journal of Microbiology **35**:578–582.

Stauffer, R. E. 1987. Cytochemical tests of acid phosphatase secretion by carnivorous plants. Proceedings of the Rochester Academy of Sciences **15**:181–185.

Stone, L., and R. S. J. Weisburd. 1992. Positive feedback in aquatic ecosystems. Trends in Ecology and Evolution **7**: 263–267.

Turley, C. M., R. C. Newell, and D. B. Robins. 1986. Survival strategies of two small marine ciliates and their role in regulating bacterial community structure under experimental conditions. Marine Ecology Progress Series **33**:59–70.

Vadas, R. L. 1989. Food web patterns in ecosystems: a reply to Fretwell and Oksanen. Oikos **56**:339–343.

Vandermeer, J. H. 1969. The competitive structure of communities: an experimental approach with protozoa. Ecology **50**:362–371.

Vanni, M. J. 1987. Effects of nutrients and zooplankton size on the structure of a phytoplankton community. Ecology **68**:624–635.

Verity, P. G. 1991. Feeding in planktonic protozoans: evidence for non-random acquisition of prey. Journal of Protozoology **38**:69–76.

von Ende, C. N. 1993. Repeated measures analysis: growth and other time-dependent measures. Pages 113–137 *in* S. Scheiner and J. Gurevitch, editors. The design and analysis of ecological experiments. Chapman and Hall, New York, New York, USA.

Wade, M. J. 1992. Sewall Wright: Gene interaction and the shifting balance theory. Pages 35–62 *in* J. Antonovics and D. Futuyma, editors. Oxford surveys of evolutionary biology VI. Oxford University Press, New York, New York, USA.

Walker E. D., D. L. Lawson, R. W. Merritt, W. T. Morgan, and M. J. Klug. 1991. Nutrient dynamics, bacterial populations, and mosquito productivity in tree hole ecosystems and microcosms. Ecology **72**:1529–1546.

Walker, E. D., and R. W. Merritt. 1988. The significance of leaf detritus to mosquito (Diptera: Culicidae) productivity from treeholes. Environmental Entomology **17**:199–206.

Wetzel, R. G. 1995. Death, detritus, and energy flow in aquatic ecosystems. Freshwater Biology **33**:83–89.

Wetzel, R. G., and G. E. Likens. 1991. Limnological analysis. Second edition. Springer-Verlag, New York, New York, USA.

White, D. C., and R. H. Findlay. 1988. Biochemical markers for measurement of predation effects on the biomass, community structure, nutritional status, and metabolic activity of microbial biofilms. Hydrobiologia **159**:119–132.

Wilbur, H. M., and J. E. Fauth. 1990. Experimental aquatic food webs: interactions between two predators and two prey. American Naturalist **135**:176–204.

Wootton, J. T. 1993. Indirect effects and habitat use in an intertidal community: interaction chains and interaction modifications. American Naturalist **141**:51–70.

———. 1994. The nature and consequences of indirect effects in ecological communities. Annual Review of Ecology and Systematics **25**:443–466.

Worthen, W. B., and J. L. Moore. 1991. Higher-order interactions and indirect effects: a resolution using laboratory *Drosophila* communities. American Naturalist **138**:1092–1104.