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An acute trophic cascade among microorganisms in the tree hole ecosystem following removal of omnivorous mosquito larvae

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Abstract

Results of two field experiments showed that selective removal of omnivorous mosquito larvae (*Aedes triseriatus* (Say)) functioning as top predators in the food web of a temperate, tree hole ecosystem resulted rapidly in increased abundance of flagellate and then ciliate populations. Flagellate density increased from <1 per ml to >10³ per ml within 4 days of omnivore removal, followed shortly thereafter by an increase in ciliate density from <1 per ml to >10² per ml, after which flagellate density declined, and flagellate and ciliate densities stabilized. Rod-shaped bacteria increased slightly in density after removal of larval mosquitoes, then declined as protist density increased. Cocciform bacteria did not vary in density with these changes, thus the trophic cascade dampened at the remotest trophic level. Concomitant with the increase in protist densities, some bacteria formed elongated filaments >10 µm in length, likely an anti-predation, morphological response stimulated by suddenly intensified grazing as protozoan density rose. Results suggest that feeding by omnivorous mosquito larvae exhibited strong top-down effects on flagellate and ciliate populations, depressing them to below their equilibrium densities and nearly to extinction in tree hole ecosystems.

Keywords

Food web; Omnivory; Tree holes; Trophic cascade

Introduction

Community organization and dynamics are intimately associated with trophic relationships amongst community members, in arrangements known as food chains and webs (Pimm 2002, Thompson et al. 2007). Both theoretical analyses and empirical studies indicate that the intensity of predation by organisms at higher levels of trophic organization affects the abundance and production of organisms at successively lower levels, a phenomenon which when manifested in reciprocal patterns of abundance is termed a trophic cascade but more

generally is known as a “top-down” effect (Carpenter and Kitchell 1993, Strong 1992, Polis and Strong 1996, Polis et al. 2000). Omnivory has been defined ecologically as feeding at more than one trophic level in a recognizable food web (Pimm and Lawton 1978, Yodzis 1984). When exhibited by a top predator, omnivory is predicted to alter community structure depending upon the nature and complexity of the trophic relationships and the strength of the omnivory (McCann and Hastings 1997, Vadeboncoeur et al. 2005, Vandermeer 2006). The effects of top-level omnivory on dynamics of aquatic, microbial communities are poorly known, particularly given the typical situation of very high microbial densities. Further, protozoans, algae, and bacteria often exhibit marked anti-predator responses involving not only changes in numeric abundance predicted by trophic cascading effects, but also result in phenotypic changes (Jürgens 1994, Pernthaler 2005). Such phenotypic changes in microbial community composition, postulated to be due to differential ingestibility or digestibility of the microorganisms serving as prey (so-called functional compensation), have been observed in aquatic systems where intensive grazing pressure by filter feeding invertebrates or protozoans occurs (King et al. 1991, Simek et al. 1997, Sommer et al. 2003).

Predation-mediated, top-down effects on microbial communities have been observed in phytotelmata, a specialized type of aquatic ecosystem occurring in various living plant structures, such as pitcher plants and tree holes, whose invertebrate fauna typically includes mosquito larvae (Addicot 1974, Laird 1988, Washburn et al. 1988, Cochran-Stafira and von Ende 1998, Kneitel and Miller 2002, Walker et al. 1991). Phytotelmata are typically considered to be heterotrophic ecosystems where microbial decomposition of organic detritus provides the food resource base – in the form of microorganisms and particulate organic material – for larval mosquitoes and other insects (Fish and Carpenter 1982, Kitching 1987, Kaufman and Walker 2006). Larval mosquito feeding places strong pressure on microorganisms associated with detritus in such container habitats or microcosms meant to simulate them, reducing bacterial abundance (Kaufman et al. 1999, 2001), bacterial production (Kaufman et al. 2001, 2002), fungal biomass (Kaufman et al. 2002, Kaufman and Walker 2006), and bacterial and fungal community composition (Xu et al. 2008, Kaufman et al. 2008). The effects of larval mosquito filter-feeding on water column bacteria in the water column is less predictable, with both positive (Kaufman et al. 1999), negative (Walker et al. 1991), and neutral (Paradise and Dunson 1998, Kaufman et al. 2001, Kaufman et al. 2002) changes in bacterial abundance. Protozoans in the water column typically decrease in density in the presence of actively feeding mosquito larvae in phytotelmata (Paradise and Dunson 1998, Cochran-Stafira and von Ende 1998, Eisenberg et al. 2000, Kaufman et al. 2002), as well as increase rate of formation of digestion-resistant cysts (Washburn et al. 1988, Kaufman et al. 2002). In a laboratory study, Cochran-Stafira and von Ende (1998) showed that bacterial community structure was modified by the direct effects of grazing by protozoa and the pitcher plant mosquito (*Wyeomyia smithii* (Coquillett)), by the indirect effects of competitive interactions among three protozoans, and by the cascading effects of predation by mosquito larvae. Mosquito larvae are likely to be omnivorous top predators in phytotelmata, where all lower trophic levels are subject to their predation through indiscriminate (generalist) feeding upon all microbial groups (Merritt et al. 1992, Kitching 2000). Release of the lower trophic levels from omnipresent omnivory is predicted to cause

large adjustments in trophic relationships, particularly when the trophic levels are relatively discrete (Kneitel and Miller 2002).

Because microorganisms are postulated to form a substantial portion of the larval mosquito diet and to contribute substantially to their growth (Kaufman et al. 2001, 2002, Kaufman and Walker 2006), the factors controlling available microbial biomass become important in understanding the limits to mosquito production. We have focused our attention on the larval habitat of the eastern tree hole mosquito, *Aedes triseriatus* (Say), as a model system for elucidating these relationships (Walker et al. 1991, Kaufman et al. 1999, 2001, 2002, Kaufman and Walker 2006, Xu et al. 2008, Kaufman et al. 2008). As a collector-gatherer, these larvae exhibit omnivorous behavior (Walker and Merritt 1991); gut content analysis revealed presence of bacteria, protozoa, and detritus in the food bolus (Walker et al. 1988). In the present study, we conducted a series of experiments in which *A. triseriatus* larvae were removed from natural habitats *in situ*, and the numeric and phenotypic responses of the protozoan and bacterial communities were assessed, to examine the effects of top-down omnivory on microbial community structure. A specific prediction tested here is that release of microbial populations forming prey for a top-level omnivore should adjust prey populations to new equilibrium densities (Kneitel and Miller 2002).

Materials and methods

Study sites

The study sites were Hudson Woods and Toumey Woods, located on the Michigan State University campus, (East Lansing, Michigan, USA). These woodlots (ca. 10 hectares each) consist of hardwoods, dominated by sugar maple (*Acer saccharum* Marsh) and American beech (*Fagus grandifolia* Ehrh.) trees, with fewer numbers of basswood (*Tilia americana* L.), and an understory of mainly sugar maple and beech saplings and herbaceous vegetation. A thriving population of *A. triseriatus* exists in both woods (Walker and Merritt 1988, Walker et al. 1991). A census of water-filled tree holes was conducted and tree holes containing *A. triseriatus* larvae were mapped. In Hudson woods, a network of 28 upright tires was established and *A. triseriatus* populations allowed to colonize them naturally. Prior to manipulation experiments conducted in June and July, when larvae are typically in the 3rd and 4th larval instars, mosquitoes were sampled following methods in Walker and Merritt (1988) and found to occur at densities greater than 300 per container.

Field experiment 1

The first experiment was a pilot study to estimate densities of bacteria and protozoa in tree hole water, and to evaluate the effects of addition of a bacterial based, commercial mosquito larvicide (Teknar HP-D) as a top predator removal manipulation. Teknar HP-D is a liquid suspension formulation, derived from fermentation of *Bacillus thuringiensis* var. *israelensis* serotype H-14. It contains by volume 1.6% active protein toxins and 98.4% inert ingredients, with a potency of 3000 international units/mg of product (Brar et al. 2006). Because the larvicidal proteins show specific toxicity for mosquito larvae after ingestion of very low doses of toxin, it provided a tool for selective removal of mosquito larvae *in situ* with minimal disturbance of the ecosystem. To confirm toxicity to *A. triseriatus* mosquito larvae

and to estimate the dose required to kill mosquito larvae in tree holes, we provisioned 1.0 l flasks with serial 10-fold dilutions of formulation (from 0.03 ppm to 10 ppm, three replicate flasks per dilution) and ten 3rd instar *A. triseriatus* larvae from a laboratory colony. Larvae were held overnight at room temperature, and checked for mortality and compared with controls containing no Teknar HP-D. Methods followed those described in Walker (1995). The estimated dilution at which 50% of larvae died (i.e., the LC₅₀), determined by probit analysis, was 0.1 ppm.

Tree holes and tires having larval populations >300 at the time of sampling and having at least 2 liters of water were chosen. There were 4 tree holes (2 rot holes, 2 pans) and 2 tires in the treated group, and 4 tree holes (2 rot holes, 2 pans) and 2 tires in the control group. Water samples (4.8 ml) were taken aseptically on May 1, 13 and 14 (i.e., days -13, -1, and 0 of the experiment relative to treatment day) and then 0.5 ml of Teknar HP-D (50 ppm per tree hole) was delivered by pipette into the treated groups on May 14. Water samples were again taken on May 15, 16, 17, 20, 22, 24, 28 and 30, i.e., days 1, 2, 3, 6, 8, 10, 12 and 14 post-treatment, in 1991. Bacterial and protozoan densities were estimated as described below.

Field experiment 2

The second experiment expanded on the results of the pilot study described above. There were 14 tree holes, all pans in American beech trees, divided into treated and control groups by random assignment. Water samples were taken from each source as described above, on three dates prior to treatment with Teknar HP-D on May 27, June 1 and 2, 1993 (i.e., days -6, -1, and 0 before treatment), and on 10 dates subsequent to treatment (June 3, 4, 7, 9, 11, 15, 17, 22, 24, and 29; i.e., days 1, 2, 5, 7, 9, 13, 15, 20, 22, and 27). The possible confounding effect of dead larvae in the experimental group serving as a microbial nutrient source was compensated by the addition of 300 heat-killed 4th instar larvae to each tree hole in the control group. Microorganisms were enumerated as described below.

Microbial densities

Microbial densities were enumerated using direct count methods with epifluorescence microscopy (Walker et. al. 1991). Water samples (4.8 ml) were drawn from the sources with clean, sterile pipettes at a depth of ca. 2 cm in the water column. Samples were placed in clean vials and stored in the dark at 5°C. Subsamples (0.25 to 1.0 ml) of the original sample were stained with diamidinophenyl indole or DAPI (final concentration, 2-4 µg/ml), and held for 30 minutes on ice in the dark. The sample was then transferred into a glass chimney secured by a clamp to a filter stem (17 mm inner diameter), and low vacuum pressure (30 cm Hg) was applied to draw the water through a 25 mm diameter 0.22 µm Sudan black-stained polycarbonate Nuclepore®, filter into a filtration flask, leaving particulate matter on the filter surface. The Nuclepore filter was backed by a 0.45 µm HA-type Mil-CK) lipore filter to provide for even distribution of microorganisms. The Nuclepore filter was removed from the holder, and placed on a slide prepared with a thin coating of Cargille type B immersion oil. The filter was then covered with an additional drop of oil and a cover slip. Slides were examined at 1000 × (oil immersion objective, for bacterial and protozoan enumeration) and 250 × (dry objective, for protozoan enumeration) on a Jenalumar A/D

epifluorescence microscope equipped with appropriate excitation and barrier filters. For bacterial enumerations, at least 15 different fields were examined until a minimum of 200 bacteria had been counted. Bacteria were categorized into cocciform and noncocciform groups (i.e., rods and filaments). In experiment 2, a group of very large, bacteria of an unbranched filament morphotype were observed and were also enumerated. Because protozoan numbers were relatively lower, and the area of each field relatively large, 25 fields were counted for protozoan enumeration. Protozoans were classified as a single group in experiment 1, but were enumerated as small flagellates (typically, nanoflagellates) or larger ciliates in experiment 2. These morphologies were easily recognizable with the methods described above. Counts of microorganisms were converted to estimated number per ml of original sample with a standard formula (Walker et al. 1991).

Data analysis

Data of estimated number of bacteria or protozoans per ml of original sample were transformed with $\log_{10}(x + 1)$. The data series were subjected to univariate, repeated measures analysis of variance following procedures described by von Ende (2001), where the main test of interest is the interaction of time and the main treatment effect. The Huyn-Feldt correction of degrees of freedom in F tests was used to reduce the effects of sphericity. Analyses were conducted using SAS version 8.02 (SAS 2001).

Results

Experiment 1

Observations showed that the treatment with Teknar HP-D was sufficient to eliminate all mosquito larvae from the treated tree holes in both experiments, as also documented by Xu et al. (2008) and Kaufman et al. (2008). Mosquitoes did not re-colonize treated sources during any of the experiments, but were consistently present in non-treated sources. Other common tree hole insects (larvae of scirtid beetles and ceratopogonid midges) were not eliminated by the treatment.

Densities of protozoans prior to treatment were very low but increased by 3 orders of magnitude in the treated containers within 4 days of larval removal (Fig. 1). Protozoan densities remained at elevated levels in treated containers at 14 days post-predator removal, while those in unmanipulated tree holes remained at low levels. At the start of the experiment, bacterial numbers were similar between experimental and control groups. Bacterial densities did not respond to treatment, indicating that predator removal did not affect them, but also that addition of ca. 50 ppm of Teknar HP-D did not add appreciably to bacterial densities and was therefore not a confounding treatment effect.

Repeated-measures analysis of variance showed that protozoan density was significantly affected by removal of larval *A. triseriatus* ($F_{1,10} = 56.7$; $P < 0.0001$), by day of sampling ($F_{10,100} = 8.96$; $P < 0.0001$), and by interaction of day and the main treatment ($F_{10,100} = 11.15$; $P < 0.0001$). However, there was no significant effect of removal of mosquitoes on cocciform bacteria ($F_{1,10} = 0.01$; $df = 1, 10$; $P = 0.91$), nor a significant interaction of day of sampling and the main treatment ($F_{10,100} = 0.93$; $P = 0.47$), nor did bacterial densities vary

significantly among sampling days ($F_{10,00} = 1.44$; $P = 0.22$). There was no significant effect of removal of larval *A. triseriatus* on noncocciform bacteria ($F_{1,10} = 0.64$; $P = 0.44$), nor a significant interaction of day of sampling and the main treatment ($F_{10,100} = 1.31$; $df = 10, 100$; $P = 0.28$), although noncocciform bacterial densities varied significantly among sampling days ($F_{10,100} = 3.0$; $df = 10, 100$; $P = 0.04$).

Experiment 2

Results of enumerations of microbial populations are shown in Fig. 1, and representative examples of these groups are shown in Fig. 2. Ciliate, flagellate, and long, unbranched filaments were at very low densities prior to larval mosquito removal, and remained low in the control tree holes after the treatment date. In tree holes from which mosquito larvae had been removed, however, densities of all three groups increased markedly following top predator removal (Fig. 1). All three enumeration responses were significantly affected by predator removal, by sampling day, and by the interaction of the predator removal and sampling day (Table 1). Cocciform bacterial density increased significantly over time, but was not affected by predator removal, or by the interaction of sampling day and the main treatment (Fig. 1, Table 1). Non-cocciform bacterial density (excluding long, unbranched filaments) increased in number for a short period after the removal date in treated sources, but not in non-treated sources and it was a marginally, statistically significant trend ($P = 0.07$). However, there was variation among sampling days in noncocciform density as well as a significant interaction of the main treatment and sampling day (Table 1), indicating that this group of bacteria responded to removal of larval mosquitoes with a temporary increase in density.

Discussion

The experimental approach presented here was facilitated by the ready availability of water-filled tree holes densely occupied by *A. triseriatus* larvae, allowing replication and multiple-day sampling of natural habitats with minimal disturbance. Tree holes are also amenable to simulation with structured microcosms placed in laboratory and field settings. Previous research has identified heterotrophic microorganisms and the senescent leaf detritus pool as key food resources for these mosquitoes, forming the basis for production of mosquitoes (Walker et al. 1997, Kaufman and Walker 2006). Accordingly, studies focused on trophic dynamics of the microbial compartments of the tree hole ecosystem become important in explaining patterns of variation in mosquito production from tree holes, where competition for food limits larval mosquito growth (Carpenter 1983, Merritt et al. 1992).

The microbial community in water samples from tree holes was readily enumerated with epifluorescence microscopy and direct count methodology as described here, and the heterotrophic nature of the ecosystem was confirmed with no observations of algae nor any pigmented protozoans in any samples. Following other studies in larger aquatic systems, we observed that very small flagellates and much larger ciliates were observable in the water column of tree holes (Fig. 3), primarily when larval mosquito feeding pressure had been alleviated through direct removal by killing. When mosquito larvae were present, these protozoans were rare or absent. Three categories of bacteria were observed: very small

cocciforms, small noncocciforms (typically, small rods), and elongated, unbranched filaments typically exceeding well over 10 μ in length. The latter were only quantified by the direct count method in experiment 2, when categories of protozoans and bacteria were enumerated more finely, and were only observed when mosquito larvae had been removed and when protozoan densities had increased. The trophic structure in the water column of the tree holes under study, therefore, consisted of a food web with an omnivorous species as a top predator; intermediate predators (ciliates and flagellates) serving as prey for mosquito larvae, predators on bacteria, and with flagellates as prey for ciliates; and bacteria of three different and easily distinguishable morphotypes, all non-predaceous but serving as prey for higher trophic levels (Fig. 3). Kneitel and Miller (2002) identified a similarly structured food web where larvae of the mosquito *Wyeomyia smithii* were omnivorous predators in water-filled pitcher plants, based upon observations of responses of the microbial community to experimental removal. Large populations of larvae could be expected to clarify the water column of microorganisms and fine particulates owing to the collector-gatherer omnivory of *A. triseriatus* larvae (Walker and Merritt 1991).

Use of a bacterial larvicide formulation as an experimental tool for selectively removing *A. triseriatus* larvae apparently worked as intended. The introduction of this bacterial-based larvicide did not introduce vegetative bacterial cells of a sufficient density to elevate noncocciform bacterial densities as revealed in experiment 1 (Fig. 1). Elsewhere, we have shown that introduction of a similar commercial formulation allowed selective removal of larvae, followed by measurable changes in bacterial diversity in both the water column and on leaf surfaces, based upon 16S rDNA sequence library analyses (Xu et al. 2008, Kaufman et al. 2008). Interestingly, no 16S rDNA clones matching *Bacillus thuringiensis* were found in the tree holes or microcosms assigned to the experimental manipulation in either of those studies. From those observations, we have confidence that our use of the bacterial toxins did not cause confounding experimental perturbations that would interfere with data collection or interpretation of results.

The dramatic response of the microbial community to release from larval feeding pressure shows that the food web in the water column of tree hole ecosystems conforms to a top-down, omnivory trophic system (Vadeboncoeur et al. 2005). Both of our experiments therefore revealed a strong trophic cascade in this ecosystem. Particularly striking was the rapid development of high densities of flagellate and ciliate populations after larval removal; flagellate densities increased first, rapidly followed by ciliate densities, after which flagellate densities declined but did not return to the low (and nearly absent) densities observed prior to removal of mosquito larvae. These changes occurred in less than a week and over at least 2 orders of magnitude. In some individual tree holes, flagellate densities varied over a period of a few days by 4 orders of magnitude, with as many as 4,000 flagellates per ml of water recorded on certain sampling days in certain tree holes. Although we have not estimated bio volume of microbes here, these responses suggest that the basal resources of the protozoans were sufficient to allow high production under conditions where their densities were not kept in check by larval mosquito feeding. Other research in tree holes (Washburn et al. 1988) indicates that larval mosquito feeding pressure stimulates cyst formation of protozoa, a phenomenon also observed in microcosms in field settings and provisioned with natural

tree hole microflora (Kaufman et al. 2002). Of interest is that protozoans would be a source of sterols and long chain, polyunsaturated fatty acids, both essential to the diet of mosquito larvae (Dadd 1980, Kaufman et al. 1999). That larvae would feed so intensively as to depress protozoan densities to orders of magnitude below their predator-free equilibrium densities suggests that growth rates of these microbes are insufficient to keep pace with larval feeding rate, thereby limiting availability of key nutrients to larvae. Eisenberg et al. (2000) suggested that refugia for protozoans allowed co-existence with heavy larval feeding pressure, although it was not investigated here. Given their rapid resurgence numerically post-predator removal, such refugia must surely exist in the tree holes we studied. Additionally, cyst formation would provide a means for maintaining populations within the system. However, cysts were not enumerated in this study.

In contrast with ciliate and flagellate populations, bacterial densities were less responsive to predator removal effects; indeed, cocciform bacteria did not respond to experimental predator removal under any conditions (Fig. 1, 2) and the response of noncocciform morphotypes was only evident in the second experiment and was not extreme, occurring within a range of 10^6 /ml cell density (Fig. 2, Table 1). A useful interpretation of repeated measures analysis of variance with a single experimental treatment is to examine the interaction of main treatment and sampling day (von Ende 2001); indeed, a significant interaction term for noncocciform bacteria (i.e., small rods) was observed in experiment 2 ($P = 0.002$; Table 1), suggesting that this bacterial group responded with a real but temporary increase in density immediately following larval removal. These results indicate that the trophic cascade dampens at these deeper trophic levels, compared to the ciliate and flagellate trophic levels which are more proximate to the highest, top predator trophic level. Elsewhere, we have shown in microcosm experiments that water column bacterial densities and rates of production did not vary with larval presence or absence in laboratory microcosms, although leaf surface-associated bacteria did respond to this treatment (Kaufman et al. 2002).

The appearance of long, unbranched filaments after predator removal in experiment 2, combined with rarity of these bacterial morphotypes in unmanipulated tree holes, suggests that some noncocciform bacteria responded to the bloom of flagellates and ciliates with antipredation morphological changes. Such phenotypic responses have been observed in lake ecosystems, primarily in response to increased protozoan predation that is, in turn, mediated by planktonic metazoans such as cladocerans (Hahn et al. 1999, Jürgens et al. 1999), where elongated bacterial cells are thought to be resistant to predation by flagellates. Indeed, such elongated bacteria can dominate bacterial biomass in lake ecosystems (Pernthaler et al. 2004). However, Wu et al. (2004) have challenged this notion by observing successful predation of the long filaments by relatively small flagellates. By contrast, Corno and Jürgens (2006) observed that bacteria of 7 μ m in length were vulnerable to predation by the bacterivorous flagellate *Ochromonas* sp., whereas larger filaments were resistant to predation and became the dominant form in microcosms with these predators. Although the elongated, filamentous bacteria we observed were not identified phylogenetically, taxa identified from 16S rDNA sequence libraries from tree holes (Xu et al. 2008, Kaufman et al.

2008) include bacteria that exhibit this phenotype, such as *Comamonas* and some Bacteroidetes (Hahn et al. 1999).

Our observation of the very rapid development of bacterial biomass in the form of elongated, unbranched filaments suggests that basal (“bottom up”) resources are not limiting for heterotrophic bacteria in tree holes and that the primary control of trophic structure is from the top down, predatory forces mediated by omnivorous mosquito larvae and (when larvae are absent) protozoans. Such a conclusion is supported by Corno and Jürgens (2006), in which study experimentally-induced carbon starvation limited the ability of bacterial cells to elongate to filaments and kept them in a predation-vulnerable size range. Elsewhere, we have suggested that nutrient limitation in tree holes is mainly due to ready availability of nitrogen, when amended to tree hole water in microcosms, increased microbial biomass and mosquito production (Kaufman and Walker 2006). Carbon (as glucose) stimulated bacterial production in the water column but not sufficiently to compensate for the heavy grazing effects of mosquito larvae in microcosms (Kaufman et al. 2002).

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Figure 1.

Mean density of protozoans and bacteria in habitats of larvae of the eastern tree hole mosquito, *Aedes triseriatus*. In experiment 1 (left column of panels), data from direct counts of protozoans, noncocciform bacteria, and cocciform bacteria are presented. In experiment 2 (right column of panels), protozoans were enumerated into ciliate and flagellate groupings, and noncocciform bacteria were enumerated into small rods and unbranched, elongated filaments. Day 0 is the day when larvae were selectively removed from some containers (open squares) but not others (dark triangles), $n = 6$ per mean in experiment 1 and $n = 7$ per mean in experiment 2.

Figure 2.

Photomicrographs of DAPI-exposed water samples examined with epifluorescence microscopy on 0.22 μ Nuclepore filters. **A:** A bloom of flagellates with evident cocciform and noncocciform bacteria. **B:** A ciliate, smaller flagellates, and bacteria for perspective. **C:** Long, unbranched filaments, a bacterial morphotype observed in experiment 2 after removal of mosquito larvae and subsequent increase in density of flagellates and ciliates.

Figure 3.

Schematic of the food web in the water column of tree hole habitats of the eastern tree hole mosquito, *Aedes triseriatus*, as delineated by the experiments and methods described in the text.

Table 1
Results of repeated measures analysis of variance on $\log_{10}(x+1)$ -transformed microbial densities in Experiment 2.

Response variable	Main treatment effect			Sampling day			Interaction of main treatment and sampling day		
	F-statistic	df	P value	F-statistic	df	P value	F-statistic	df	P value
Ciliates	151.75	1,12	0.0001	14.49	12, 144	0.0001	10.74	12, 144	0.0001
Flagellates	8.73	1,12	0.01	5.0	12, 144	0.0001	2.55	12, 144	0.02
Unbranched filaments	34.78	1,12	0.0001	13.99	12, 144	0.0001	5.62	12, 144	0.0001
Noncocciform Bacteria	3.9	1,12	0.07	4.21	12, 144	0.0002	3.28	12, 144	0.002
Cocciform bacteria	0.00	1,12	0.92	4.7	12, 144	0.0001	0.3	12, 144	0.8