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Effects of leaf litter species on macroinvertebrate community properties and mosquito yield in Neotropical tree hole microcosms

Abstract Detritus quality and quantity affect macroinvertebrate productivity and distribution in many freshwater ecosystems. This study experimentally investigated the effects of leaf litter from *Ceiba pentandra*, *Dipteryx panamensis*, *Ficus yoponensis*, and *Platypodium elegans* on macroinvertebrate species composition, richness, and abundance in artificial water-filled tree holes in a lowland moist forest of Panama. Species composition was similar among treatments, but species richness and longevity differed among litter types and were consistently highest with *Platypodium* litter. Similar patterns were observed in natural tree holes of the focal tree species. The mosquito *Culex mollis* was the most abundant species in the field experiment. Average conductivity and dissolved oxygen concentration differed among leaf species, but pH did not. Leaf toughness was positively correlated with mean macroinvertebrate abundance and cumulative species richness. A laboratory experiment measured *C. mollis* yield and pupation time in tree hole microcosms containing the four litter species. Cumulative mosquito mass and time to pupation differed among leaf litter species, with *Platypodium* litter supporting the greatest yield. Pupation was slowest on *Ceiba* litter. Grazing by mosquito larvae facilitated leaf decomposition in all treatments. Results suggest that differences in macroinvertebrate species richness and mosquito yield can be attributed to differences in nutritional quality among litter species.

Key words Detritus · Macroinvertebrate · Microcosm · Tropics

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Introduction

Detritus, especially decaying leaves and other plant matter, is an important resource for invertebrates in terrestrial (Anderson and Macfadyen 1976; Swift et al. 1979) and aquatic (Anderson and Sedell 1979; Webster and Benfield 1986) systems. Many freshwater macroinvertebrates directly consume leaf litter and/or its attached decomposer microfauna. Aquatic macroinvertebrate productivity and distribution are affected by detritus quality and quantity (Egglishaw 1964; Stout et al. 1985; Sweeney and Vannote 1986; Corkum 1992), which limit the growth of some taxa (Gee 1988; Richardson 1991; Dobson and Hildrew 1992).

The degradation rate of litter is influenced by physicochemical characteristics of the leaves, environmental conditions, and characteristics of decomposer microbes (Witkamp 1966; Kaushik and Hynes 1971; Swift et al. 1979; Webster and Benfield 1986). On land and in water, leaves tend to degrade at species-specific rates (Stout 1980; Webster and Benfield 1986; Cornejo et al. 1994; Cornelissen 1996), and the degradation rate of detritus and its nutritional quality are often correlated (Swift et al. 1979; Golladay et al. 1983). Thus, different species of leaves (and the microbes they support) may not be equal in nutritional value to the organisms that consume them (Sweeney and Vannote 1986; Canhoto and Graça 1995; Walker et al. 1997).

Lignin and nitrogen content influence the decomposition rates of leaves (Melillo et al. 1982; Taylor et al. 1989; Enríquez et al. 1993; Cortez et al. 1996; Ostrofsky 1997; but see Gillon et al. 1994 and Prescott 1995). Leaf toughness is a function of leaf fiber content (e.g., lignin, cellulose) and is negatively correlated to leaf nutrient content (% nitrogen) for many tropical plant species (Coley 1983). Typically, the relative mass of leaf material lost during decomposition is positively correlated with nitrogen content, and negatively correlated with lignin content (Swift et al. 1979; Webster and Benfield 1986) and toughness (Akanil and Middleton 1997).

Some aquatic macrodetritivores and microorganisms show feeding preferences when offered different leaf species in the laboratory (Bärlocher and Kendrick 1973; Friberg and Jacobsen 1994; Canhoto and Graça 1995). In natural settings, the species of litter present can influence aquatic macroinvertebrate community properties such as abundance, species composition, species richness, and trophic structure (Basaguren and Pozo 1994; Dobson 1994; Kok and Van der Velde 1994; Maloney and Lamberti 1995; Gee and Somerfield 1997).

Leaf litter is the nutrient base for food webs in most water-filled tree holes, phytotelmata formed by the collection of rainwater in branch crotches and other woody cavities (Kitching 1971). As in other aquatic systems, leaves in tree holes are degraded by leaching of soluble compounds into the water, and by the activity of microorganisms, especially bacteria and fungi (Fish and Carpenter 1982). Although some tree hole macroinvertebrates directly consume decaying litter (Carpenter 1982a; Paradise and Dunson 1997), most feed by grazing decomposer microbes from litter surfaces or filtering microbes suspended in the water column (Fish and Carpenter 1982; Walker and Merritt 1991). Tree holes are tractable study systems given their small size and relatively simple macroinvertebrate communities; they can be replicated for experiments with simple container microcosms that approximate the natural system, generally attracting the same species found in natural holes (Pimm and Kitching 1987; Fincke et al. 1997; Srivastava and Lawton 1998).

Leaf litter quality affects the growth of some tree hole macroinvertebrate species (Carpenter 1982b; Fish and Carpenter 1982; Lounibos et al. 1993; Walker et al. 1997), and faster-degrading leaves tend to support greater mosquito productivity (Fish and Carpenter 1982; but see Carpenter 1982b). In the forest of Barro Colorado Island (BCI), Panama, water-filled holes in some trees (e.g., free-standing *Ficus* spp.) are often dominated by leaf litter from the crown of the same tree. This pattern is especially evident in holes at midstory and canopy heights, but also occurs in understory holes (e.g., holes associated with tree buttresses or other convolutions that channel litterfall; personal observation). In addition, prior observations from natural tree holes on BCI (Yanoviak 1999) suggested a possible tree species effect on the diversity and composition of tree hole macroinvertebrates.

In low to moderately productive ecosystems, an increase in nutrient availability can support a larger number of individual consumers and coexisting species (reviewed by Rosenzweig 1995; Srivastava and Lawton 1998). This study was designed to test the hypothesis that different leaf litter species differing in quality (toughness) affect macroinvertebrate community structure (species composition, richness, and abundance), and mosquito yield and pupation time in artificial tree holes. I predicted that softer (higher-quality) leaves would degrade faster and support larger numbers of macroinvertebrate individuals and species in field-based con-tainer habitats. I also predicted that softer leaves would support greater mosquito yield and shorter mosquito pupation time in laboratory microcosms.

Materials and methods

Experiments were conducted in the forest and laboratory buildings of the Smithsonian Tropical Research Institute on BCI (see Leigh et al. 1996 for a site description). Leaves of *Ceiba pentandra* (L.) (Bombacaceae), *Dipteryx panamensis* (Pitt.) (Fabaceae), *Ficus yoponensis* Desv. (Moraceae), and *Platypodium elegans* Vogel (Fabaceae) were used in all experiments. I chose these species because they are deciduous, relatively common on BCI (see Croat 1978), and frequently have tree holes in their trunks, buttresses, or branches. Fresh leaves were collected from tree crowns or recent branch falls, air dried in the laboratory for >10 days, and oven dried at 70°C for 48 h. Although drying leaves in this manner may affect their degradation rate (Taylor and Bärlocher 1996; Taylor 1998) and nutritive value (Bärlocher et al. 1978; Lounibos 1985), drying enabled standardization of initial litter weights for the experiments in this study. Fresh leaves tend to have a higher nutrient content and degrade faster than senescent leaves (Stout et al. 1985; Maloney and Lamberti 1995). I used fresh leaves because they are a substantial component of litterfall in tropical forests (Stout 1980) and fresh leaves often dominate the litter entering BCI tree holes during the wet season (personal observation).

Leaf toughness was used as an indirect measure of nutritional quality. Although toughness is a relatively ambiguous measure of leaf quality (Swift et al. 1979), it was the simplest way to quantify structural differences among the litter types used in the study. Drying can alter the fibrous structure of leaves (Goering and Van Soest 1970). To ensure that leaves used for toughness measurements were comparable to leaves used in experiments, I dried leaves of all species (as described above) and rehydrated them by total immersion in rainwater for 24 h before measuring toughness. Leaves were secured between two acrylic sheets through which an array of holes had been drilled. Five to eight leaves of each species were punched up to five times each (≥25 punches per species) with a commercial penetrometer (Chatillion). I avoided the midribs of the leaves in this procedure. The tip of the penetrometer rod was 3.0 mm in diameter, the entrance holes in the acrylic frame were 4.2 mm and the exit holes were 5.0 mm in diameter.

Field experiment

Five replicates of artificial tree holes (black plastic cups; 9 cm diameter × 12 cm height, ca 650 ml total volume), each containing one of the four experimental leaf species, were placed in the BCI forest in mid-August 1997. The 20 cups (4 leaf treatments × 5 replications) were tied to small trees (15–25 cm dbh) with 6-mm polypropylene rope (passed once around the tree and through two holes in the section of cup rim adjacent to the trunk). This design facilitated conduction of at least some stemflow to the cups. All cups were located about 1 m above the ground and >5 m apart.

Each cup initially contained 4.00 ± 0.01 g of dried leaf litter and a strip of balsa wood (0.15 × 4 × 12 cm) as an oviposition site for insect colonists (Novak and Peloquin 1981). Leaf fragment size was standardized (ca 15 cm²) among treatments as much as possible to control for leaf area effects (Cornejo et al. 1994; Maloney and Lamberti 1995). I added 350 ml rainwater and 50 ml filtered natural tree hole water pooled from five holes [in *Lacmellea panamensis* (Woods.) (Apocynaceae) and *Pterocarpus rohrii* Vahl (Fabaceae)] to each cup. Heavy rains filled all cups to capacity within 24 h of setup. I measured conductivity, dissolved oxygen (DO) and pH of the water with a Corning modular electronic meter immediately after the experiment was established, and again on days 2, 7, 21, and 49 to determine effects of litter species on general chemical characteristics of the water over time.

Each cup was emptied into a white pan in the field and the species composition and abundance of macroinvertebrates recorded once per week on weeks 1–5 and 7. Any extraneous macrodetritus (particles > 0.25 cm^2) present in a sample was removed. Except for occasional subsamples of larvae and pupae needed to confirm species identifications, I returned all macroinvertebrates to each cup after counting. At the end of the experiment, the remaining leaf material was collected, dried at 70°C for 48 h, and weighed to the nearest 0.001 g. In all treatments except *Ficus*, the remaining leaf fragments resembled the initial litter inputs (i.e., litter was not noticeably shredded by macroinvertebrates), and the leaf material could be recovered with forceps. *Ficus* leaves tended to soften rapidly and were skeletonized by scirtid beetle larvae, leaving behind primarily the venation and epidermis. I sieved the *Ficus* cup contents through a screen (1 mm mesh) in the field to collect these small fragments.

I used Sørensen's (1948) coefficient of similarity $[C_s = 2j(a + b)^{-1}$, where j = number of species common to two treatments, and a and b = the number of species in each treatment] to quantify overlap in species composition (all replicates pooled) among the four leaf litter types. Differences in macroinvertebrate species richness and abundance among treatments were analyzed with repeated-measures ANOVAs.

Laboratory experiment

A laboratory experiment was undertaken to determine differences in degradation rates of the four leaf species with and without *Culex mollis* mosquitoes, and differences in *C. mollis* yield and pupation time among the four litter species. I used *C. mollis* because it was an abundant early colonist in the field experiment (Table 1), it is relatively common in natural tree holes (Fincke et al. 1997), and its egg rafts are easy to recognize and collect in the field. Small plastic cups (8.5 cm height × 5 cm diameter) served as experimental microcosms.

The experimental design was a 4×2 factorial replicated five times, with leaf species and presence/absence of mosquitoes as main effects. Each of the 40 cups initially contained 150 ml filtered rainwater, 3 ml natural tree hole water, 333 ± 0.5 mg dry weight of one leaf species, and a strip of balsa wood ($1 \times 10 \times 0.15$ cm), and was allowed to stand for 2 days to allow fungal and bacterial growth. I added 100 neonate (< 12 h posthatch) *C. mollis* larvae to half of the cups over 5 days. Larvae added to a cup were pooled from at least two field-collected egg rafts (>20 rafts), and neonates originating from any single egg raft were divided among cups as much as possible. The litter and mosquito quantities used were within the range of densities observed in natural holes of the focal tree species. All cups were maintained at 23°C and rainwater was added weekly to compensate for evaporation.

Cups were checked daily and any newly formed mosquito pupae were collected to quantify differences in mosquito yield and pupation time among the four litter species. I used pupae as a measure of yield because they are easier to collect and handle than adults. Pupae accumulated from a cup were dried at 70°C for ≥24 h and weighed to the nearest 0.1 mg. The experiment was terminated 30 days after the first mosquito input. All leaf litter fragments and any *C. mollis* larvae remaining in the cups were collected, oven dried, and weighed.

A two-way ANOVA was used to analyze differences in mean percent litter remaining among leaf treatments with and without mosquitoes. No mosquitoes survived beyond the third larval instar in one of the cups containing *Ceiba* leaves, so this replicate was excluded from the analysis of pupation time.

Natural tree holes

I compared the abundance, species richness, and composition of macroinvertebrates found in natural holes of the four focal tree species on BCI. Data were from multiple censuses of holes in living trees conducted from 1995 to 1997 on BCI (Yanoviak 1999). Natural tree holes collectively contained more taxa than the artificial tree holes of the field experiment. To standardize comparisons between the natural and artificial holes, I only used data for the 19 taxa that occurred in both types. The number of censuses differed among tree species, and mean macroinvertebrate species richness was correlated with sampling effort ($r = 0.97$, $P = 0.03$, $n = 4$), so ten censuses were randomly chosen from each tree species (all *Ceiba* censuses were used) for the analyses. Overlap in macroinvertebrate species composition among the four tree species (all samples pooled) was quantified with Sørensen's coefficient.

Data were tested for normality with normal probability plots and analyzed with one-way ANOVAs, except where noted above or in the Results. I used a posteriori Ryan-Einot-Gabriel-Welsch multiple-range tests (REGWQ option; SAS 1989) to compare means when ANOVA results were significant. Macroinvertebrate abundance and leaf toughness data were $\log(x + 1)$ transformed to correct variance heterogeneity (Sokal and Rohlf 1981), and proportional leaf mass data were arcsine square root transformed. Means presented in the Results are of untransformed data. Voucher specimens of collected macroinvertebrates were deposited at the Smithsonian Tropical Research Institute, Panama, and the US National Museum, Washington, D.C.

Results

Except for *Limatus assuleptus* and *Trichoprosopon digitatum*, all of the macroinvertebrates encountered in the field experiment commonly occur in natural holes on BCI. Most taxa colonized all treatments in approximately equal proportions (Table 1), and macroinvertebrate species composition was very similar among treatments (Table 2). Species turnover was generally consistent among litter types during the study. The abundance of *C. mollis* and the syrphid fly *Copestylum rafaelanum* declined over time, whereas scirtid beetles and the mosquitoes *Aedes terrens*, *Culex urichii* and *Haemagogus* spp. typically colonized later and increased in abundance over time (Fig. 1). Most other taxa (e.g., psychodid flies; Fig. 1) colonized cups irregularly during the middle weeks. This early successional pattern also occurs in newly formed natural tree holes on BCI (Yanoviak 1999).

The average number of macroinvertebrate species differed significantly among litter types in the field experiment ($F_{3,16} = 7.35$, $P < 0.003$; Fig. 2A). Average macroinvertebrate abundance also differed among treatments ($F_{3,16} = 10.87$, $P < 0.001$), but only for the first 4 weeks (Fig. 2B). *Platypodium* litter supported the greatest number of species (and individuals until week 5), whereas species richness and abundance were similar among the other litter types (Fig. 2).

Macroinvertebrate species colonizing the *Platypodium* treatment persisted for longer periods of time than in other treatments. The mean number of consecutive censuses that species occurred in the *Platypodium* treatment was greater than in the other treatments (Table 3). In terms of species frequencies, the average number of species present in a cup on two or more consecutive censuses was greatest in *Platypodium*, whereas the number of species not present on any consecutive census dates was similar among treatments

Table 1 Mean percent abundance of taxa occurring among different leaf types in the field experiment. Parentheses indicate the number of cups (out of five) containing a taxon within a treatment. Raw abundance data were pooled across sample dates for each cup and converted to proportions. Each cup was treated as a sample in the calculation of treatment means ($n = 5$). Some individuals were unavoidably counted more than once. Column totals are not exactly 100% due to rounding. *Asterisks* indicate that the taxon occurred in natural holes of the tree species

Taxon	Ceiba	Dipteryx	Ficus	Platypodium
Odonata: Pseudostigmatidae				
Mecistogaster spp.	0*	0.1 (1)*	0*	0.1 (1)*
Diptera: Ceratopogonidae				
Bezzia snowi	9.1 (2)	11.3 (3)*	0.1 (1)*	1.7 (3)*
Forcipomyia spp.	3.5 (3)	1.6 (2)	0.3 (1)*	3.7 (4)*
Diptera: Chironomidae				
Chironomus spp.	0.1 (1)*	0*	0*	0*
Diptera: Culicidae				
Aedes septemstriatus	0	0*	0	0.1 (1)*
A. terrens spp. complex	0.5 (2)*	2.4 (4)*	1.4 (3)*	1.1 (4)*
Anopheles eiseni	0.2 (1)*	0*	0.1 (2)*	0.1 (1)*
Culex conservator	0	0.2 (2)*	0.3 (1)	0*
C. corrigani	1.4 (2)	0*	0.4 (1)*	2.5 (2)*
C. mollis	36.9 (4)*	50.8 (5)*	53.2 (5)	51.3 (5)*
C. urichii	14.0 (5)	19.1 (5)*	19.1 (3)*	8.4 (5)*
Haemagogus spp.	0.3 (2)	0.3 (1)*	1.7 (3)*	0.7 (3)*
Limatus assuleptus	0	1.0 (4)	0.2 (2)	0
Orthopodomyia fascipes	0	0*	0*	3.8 (4)
Toxorhynchites theobaldi	0.3 (2)	0.2 (1)	0.1 (2)	0.1 (3)*
Trichoprosopon digitatum	13.3 (2)	2.0 (4)	1.1 (1)	1.8 (1)*
Diptera: Psychodidae				
Telmatoscopus spp.	0.3 (3)	0.5 (4)*	0.4 (3)*	3.2 (5)*
Diptera: Syrphidae				
Copestylum rafaelanum	14.8 (5)	6.3 (5)*	13.1 (5)*	19.0 (5)*
Diptera: Tipulidae				
Sigmatomera spp.	0.3 (1)*	1.2 (3)*	0.1 (2)*	0.2 (2)*
Coleoptera: Scirtidae				
Prionocyphon and *Scirtes* spp.	5.0 (2)*	3.1 (1)*	8.3 (4)*	2.3 (2)*
Count totals	1756	2126	2554	4759
Cumulative number of species	15	15	16	17

Table 2 Sørensen's similarity coefficients for macroinvertebrate assemblages occurring among the four leaf litter species in artificial holes of the field experiment and natural holes of the same tree species. Coefficients do not differ between artificial and natural holes (Wilcoxon two-sample test, $P = 0.13$)

	Dipteryx		Ficus		Platypodium	
	Artificial	Natural	Artificial	Natural	Artificial	Natural
Ceiba	0.800	0.609	0.903	0.571	0.875	0.560
Dipteryx	–	–	0.903	0.867	0.813	0.882
Ficus	–	–	–	–	0.848	0.813

(Table 3). Of 12 taxa that were rare (<1% total abundance) or absent in at least one other treatment, 10 were either present or more abundant (in absolute numbers) in the *Platypodium* treatment.

Data from natural holes in the four tree species showed patterns similar to the artificial holes of the field experiment. For the subset of 19 species found in both the natural and artificial holes, abundance did not differ among tree species, but holes in *Platypodium* were the most species rich (Table 4). Holes in different tree species also differed with respect to mean height and volume (Table 4), which can influence the number of macroinvertebrate species present (Yanoviak 1999) and possibly confound tree species effects. Patterns of macroinvertebrate species overlap among tree species were similar to
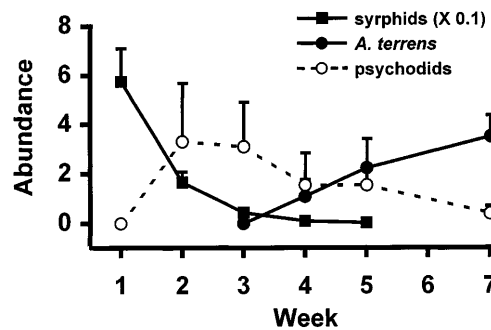

Fig. 1 Mean (+SE) abundance of the syrphid fly *Copestylum rafaelanum* (× 0.1 for clarity), the mosquito *Aedes terrens*, and psychodid flies over the course of the field experiment ($n = 20$ for each mean)

Table 3 Means (SE in parentheses) of longevity variables for macroinvertebrates occurring in the field experiment. *Persistence* number of consecutive samples a species was present in a cup, *Consecutive* number of species present on ≥2 consecutive censuses, *Irregular* number of species not present on consecutive censuses. χ^2 values are from Kruskal-Wallis tests; $df = 3$, *$P < 0.05$

	Ceiba	Dipteryx	Ficus	Platypodium	χ^2
Persistence	2.0 (0.52) ($n = 15$)	2.6 (0.47) ($n = 15$)	2.0 (0.54) ($n = 16$)	3.8 (0.33) ($n = 17$)	8.46*
Consecutive	4.2 (0.20) ($n = 5$)	5.8 (0.37) ($n = 5$)	3.8 (0.37) ($n = 5$)	7.8 (0.37) ($n = 5$)	15.75*
Irregular	3.4 (0.68) ($n = 5$)	4.2 (0.37) ($n = 5$)	3.2 (0.37) ($n = 5$)	2.8 (0.58) ($n = 5$)	3.55

Table 4 Mean (SE in parentheses) macroinvertebrate species richness and abundance, and mean (SE in parentheses) height and volume for natural holes in the focal tree species sampled on Barro Colorado Island from 1995 to 1997. *Holes* number of individual holes censused, *Censuses* total number of censuses per tree species. χ^2 values are from Kruskal-Wallis tests with $df = 3$ and $n = 10$ censuses for each mean; **$P < 0.01$
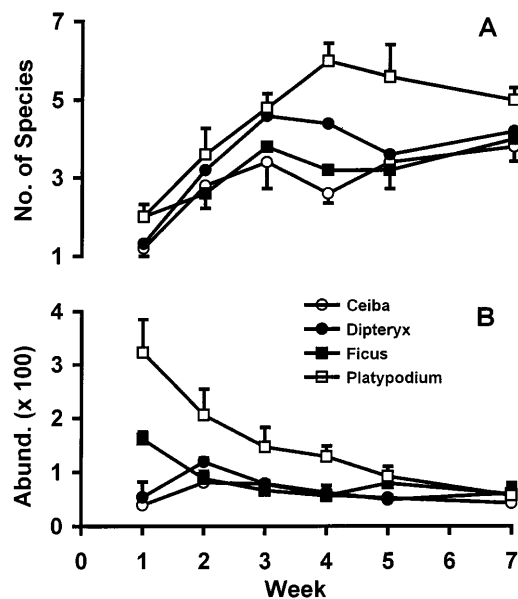
	Ceiba	Dipteryx	Ficus	Platypodium	χ^2
Richness	2.4 (0.50)	4.5 (0.67)	3.6 (0.60)	6.6 (0.85)	14.95**
Abundance	34.7 (9.4)	27.5 (14.2)	67.3 (38.6)	75.6 (36.0)	1.10
Height (m)	26.2 (3.70)	10.8 (3.92)	7.8 (1.88)	2.3 (2.10)	18.74**
Volume (l)	8.91 (1.30)	1.50 (0.18)	0.29 (0.07)	0.42 (0.07)	23.83**
Holes	5	17	12	19	
Censuses	10	22	17	33	

Fig. 2 Mean (+SE) species richness (**A**) and abundance (**B**) of macroinvertebrates censused in the field experiment ($n = 5$ for each mean). There was a significant time × treatment interaction for abundance ($P = 0.007$), but not for richness ($P = 0.35$)

Fig. 3 Mean (+SE) force required to punch through the four leaf species with a penetrometer. $n = 25$ for *Ceiba* and *Platypodium*, 26 for *Dipteryx*, and 36 for *Ficus*. The *horizontal line* connects means that are not significantly different

the field experiment, and average overlap values did not differ between natural and artificial holes (Table 2).

The four leaf species used in this study differed with respect to their physicochemical properties. *Platypodium* and *Ficus* leaves were tougher than *Dipteryx* and *Ceiba* leaves ($F_{3,108} = 51.40$, $P < 0.001$; Fig. 3), but degraded faster (measured as percent leaf mass remaining at the end of the experiment; $F_{3,16} = 273$, $P < 0.001$; Fig. 4). Average leaf toughness was positively correlated with average macroinvertebrate abundance (pooled across time; $r = 0.96$, $P = 0.035$, $n = 4$) and cumulative
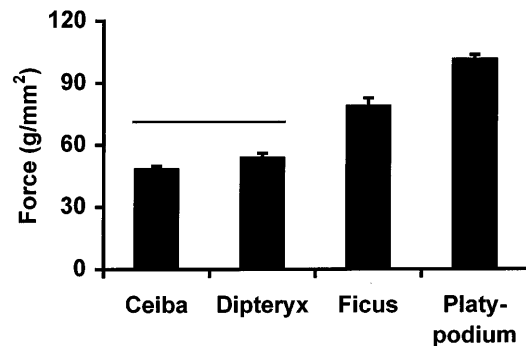
species richness among treatments ($r = 0.99$, $P = 0.006$, $n = 4$). Water conductivity (influenced by material leached from litter) was greatest in the *Ficus* and *Platypodium* treatments ($F_{3,16} = 27.9$, $P < 0.001$; Fig. 5A). DO also differed significantly among leaf types ($F_{3,16} = 7.18$, $P = 0.003$, Fig. 5B), but this difference is attributed to a single measurement day, and the time × treatment interaction was highly significant ($P = 0.0001$). There was no difference in mean pH among treatments ($F_{3,16} = 2.26$, $P = 0.12$), and pH gradually increased over the course of the experiment (time effect: $F_{3,16} = 42.2$, $P < 0.001$; Fig. 5C).

Rates of leaf litter degradation in the laboratory experiment corroborated the field results: *Ficus* and *Platypodium* had lower mean proportions of litter mass remaining than *Ceiba* and *Dipteryx* ($F_{3,32} = 880$, $P < 0.001$; Fig. 6). All litter types degraded faster in the presence of mosquitoes ($F_{1,32} = 152$, $P < 0.001$; Fig. 6). Mosquito yield (cumulative total mass) was greater in *Ficus* and *Platypodium* treatments than in *Ceiba* and
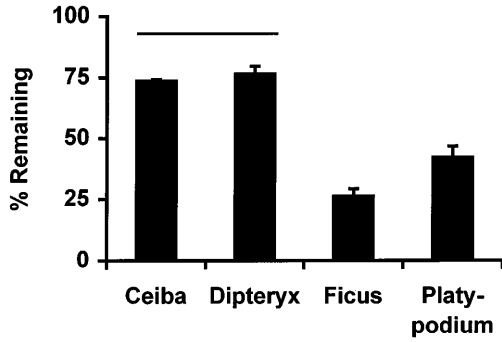
Fig. 4 Mean (+1 SD) percent of initial litter mass remaining in the four treatments at the end of the field experiment. $n = 5$ for each mean. The *horizontal line* connects means that are not significantly different
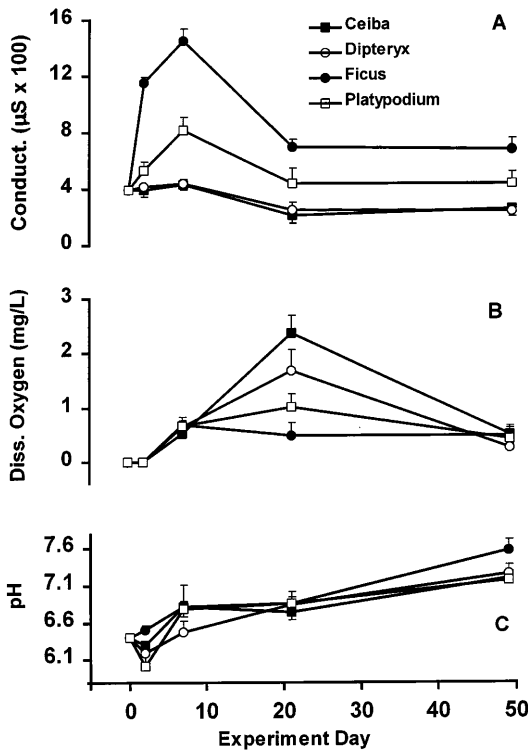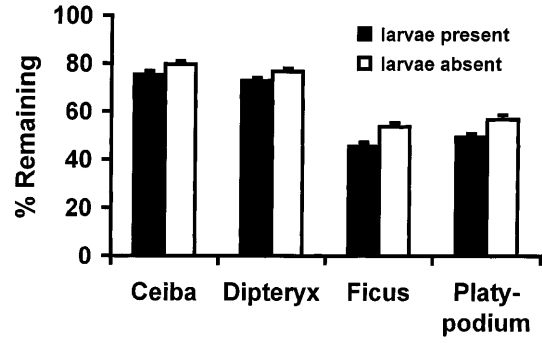

Fig. 6 Mean (+1 SD) percent of initial litter mass remaining in the four treatments at the end of the laboratory experiment ($n = 5$ for each mean). All means are significantly different among leaf species within a treatment and between treatments

Fig. 5 Mean ($\pm$SE) conductivity (A), dissolved oxygen (B), and pH (C) of the water in the four treatments of the field experiment ($n = 5$ for each mean)
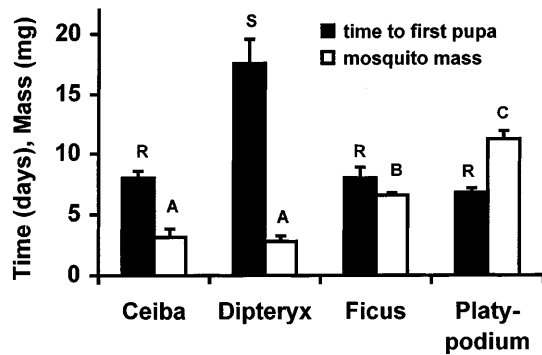

Fig. 7 Mean (+SE) number of days to appearance of first pupa and total mosquito mass from the four litter species in the laboratory experiment. The ordinate scale is the same for both variables. Means marked with the same letters are not significantly different within development time (R, S) and mass (A, B, C)

Dipteryx ($F_{3,16} = 51.22$, $P < 0.001$), but average individual pupa mass did not differ among litter species ($F_{3,16} = 0.39$, $P = 0.76$). Mosquitoes reared on *Dipteryx* litter had longer average pupation time (days to the appearance of the first pupa) than mosquitoes in the three other treatments ($F_{3,15} = 23.0$, $P < 0.001$; Fig. 7).

Discussion

Although the experimental microcosms used in this study were highly simplified representatives of natural tree holes, the results suggest that the composition of leaf litter in tree holes and similar container habitats can influence macroinvertebrate community properties and mosquito development. Given that physical habitat characteristics were standardized by using artificial holes, most variation in community parameters can be attributed to chemical or nutritional differences among leaf species. Chemicals leached into the water from different types of leaves may attract different species or different numbers of colonizing species during community assembly. Alternatively, differences in nutrient content among litter types may cause local extinctions in an established macroinvertebrate community due to energy limitation (Connell and Orias 1964; Wright 1983).

Compounds dissolved in tree hole water can attract and deter some container-breeding insect species (Petersen and Chapman 1969; Lounibos 1978; Bentley et al. 1979, 1982), including congeners of this study. However, the high overlap in macroinvertebrate species composition among treatments (Table 2) indicates that selective colonization during community assembly was not a primary cause of differences in species richness among litter types. Tree holes on BCI and elsewhere are gen-

erally colonized by macroinvertebrates that are specialists in these and similar container habitats, so high similarity in macroinvertebrate species composition among treatments and among natural holes in the focal tree species (Tables 1, 2) was expected. The presence of several species in natural holes that were absent from artificial holes (Table 1) suggests that even greater overlap in species composition might have occurred had the experiment run longer. For example, eggs of pseudostigmatid damselflies require 12–196 days from oviposition to hatch (Fincke 1998, 1999), and it is probable that most cups contained damselfly eggs that did not hatch within the experiment time span.

The results of this study provide stronger support for nutrient limitation (i.e., local extinctions) than differential colonization as a mechanism for differences in macroinvertebrate species richness and abundance among treatments. Greater nutrient availability generally leads to greater consumer species richness within a habitat (Rosenzweig 1995), as shown for temperate artificial tree hole communities by Srivastava and Lawton (1998). One hypothesized mechanism for positive productivity-diversity relationships is that greater resource availability allows the persistence of rare species (Connell and Orias 1964; Wright 1983; Rosenzweig 1995). Leaf litter can be a limiting resource for tree hole macroinvertebrates that feed on the decomposer microfauna (Carpenter 1983; Hard et al. 1989; Léonard and Juliano 1995), and higher-quality litter presumably prevents or delays extinctions that would otherwise result from local resource depletion. The greater longevity of species (Table 3) and the greater abundance of rare species in the *Platypodium* treatment support the conclusion that *Platypodium* litter is a higher-quality nutrient source than the other litter types used.

The results of this study also provide correlative evidence that the leaf types differed in nutritional quality during the early stages of decomposition. *Ficus* and *Platypodium* had the fastest degradation rates (Figs. 4, 6), the greatest toughness (Fig. 3), and leached the most material into the water (as indicated by higher conductivity; Fig. 5A). Mosquito yield was greatest on *Ficus* and *Platypodium* litter in the laboratory experiment (Fig. 7), and *Platypodium* supported the largest species richness and abundance in the field experiment (Fig. 2). Although average species richness in the *Ficus* treatment was similar to *Ceiba* and *Dipteryx* treatments (Fig. 2A), *Ficus* litter supported a larger number of macroinvertebrates (Fig. 2B) and slightly higher species richness (Fig. 2A) at week 1. The convergence of mean macroinvertebrate species richness and abundance among treatments during the last weeks of the experiment (Fig. 2) suggests that the remaining litter material was entering the second phase of decomposition, when more resistant components of the leaves (e.g., lignin) are slowly degraded (Carpenter 1982a; Gillon et al. 1994).

The prediction that softer leaves would degrade faster was not supported. I attribute this discrepancy to the secondary role of leaf fiber as a colonization substrate for microorganisms, possible host specificity among decomposer microbes, and the comparatively closed nature of the tree hole habitat. Lignin and other leaf fibers tend to slow decomposition by interfering with enzyme activity (Melillo et al. 1982; Webster and Benfield 1986), but also provide a colonization substrate for microorganisms that may use external nutrient sources for growth (Suberkropp 1995). Microbial assemblages show some specificity for different types and species of detritus (Swift et al. 1979; Cornejo et al. 1994; Lodge 1997), and differ in their ability to degrade leaf fiber (Suberkropp and Klug 1980; Chamier 1985). Although microbes were not examined in this project, microbial densities and growth differed among litter species in a similar tree hole microcosm study (Fish and Carpenter 1982).

An important difference between tree holes and larger aquatic systems is that soluble nutrients derived from leaf litter (plus contributions from tree sap and wood) tend to accumulate in tree hole water and are potentially available to microbes attached to leaf fibers. Heavy rains and stemflow cause some dilution and nutrient export from tree holes (Walker and Merritt 1988; Walker et al. 1991), but the effect is small compared to the losses that occur in flowing water. Conductivity results (Fig. 5A) suggest that material leached from leaves is retained in the system for several weeks. Thus, in confined standing-water habitats, leaves with greater fiber content may provide more substrate for specialized microbial assemblages and ultimately degrade faster, especially if exogenous nutrients are abundant.

Whereas differences in conductivity (Fig. 5A) resulted from differences in leaching among litter types and may have indirectly influenced the macroinvertebrate communities, the difference in DO among treatments (Fig. 5B) and variation in pH over the course of the field experiment (Fig. 5C) probably had little or no effect on community parameters. Most tree hole macroinvertebrates obtain atmospheric oxygen at the water surface and are not limited by DO content. The increase in pH over the course of the experiment was not extreme and I do not consider it to be a potential cause of extinctions. All measured DO and pH values were within the range observed for natural tree holes on BCI (Fincke 1999; Yanoviak 1999).

The proportion of litter mass remaining in the laboratory experiment was consistently lower in the presence of *C. mollis* (Fig. 6). Carpenter (1982a) and Fish and Carpenter (1982) observed similar facilitation effects of the tree hole mosquito *A. triseriatus* on leaf litter decomposition in microcosm experiments. *C. mollis* feeds primarily by grazing leaf surfaces (personal observation), and there was no evidence that these larvae shredded or directly consumed any of the leaf material in this study. The presence of grazers can enhance litter degradation by stimulating microbial metabolism through continuous cropping (Lopez et al. 1977; Carpenter 1982a) or by excreting nutrients that are limiting to the decomposers (Hargrave 1970; Walker et al. 1997). One or both of these mechanisms may be responsible for

the difference in decomposition rates with and without mosquitoes in this study.

The number of days to the appearance of the first *C. mollis* pupa was similar in all treatments of the laboratory experiment except *Dipteryx* (Fig. 7), indicating that nutrients were sufficiently abundant in most treatments to support mosquito growth during the first week. Cumulative mosquito yield in the laboratory (Fig. 7) and results from the field experiment (Figs. 2, 3, and 4) show that *Ceiba* and *Dipteryx* leaves are approximately equal in nutritional value for macroinvertebrates; slow growth of mosquitoes on *Dipteryx* litter therefore seems anomalous. Differences in the abundance or composition of the microbial decomposer fauna, or growth-inhibiting anti-herbivore compounds in the *Dipteryx* leaves may explain this delayed development.

Observations prior to this study suggested a possible tree species effect on macroinvertebrate diversity in water-filled tree holes on BCI. Data from natural tree holes support these observations, but should be interpreted with caution; multiple factors unrelated to the tree species can influence macroinvertebrate diversity in tree holes. By using artificial tree holes as an experimental system, I controlled some of these potentially confounding factors and showed that leaf litter quality may contribute to variation in macroinvertebrate community structure in tree holes, at least during the early stages of leaf decay.

References

Akanil N, Middleton B (1997) Leaf litter decomposition along the Porsuk River, Eskisehir, Turkey. Can J Bot 75: 1394–1397

Anderson JM, Macfadyen A (eds) (1976) The role of terrestrial and aquatic organisms in decomposition processes. Blackwell, London

Anderson NH, Sedell JR (1979) Detritus processing by macroinvertebrates in stream ecosystems. Annu Rev Entomol 24: 351–377

Bärlocher F, Kendrick B (1973) Fungi and food preferences of *Gammarus pseudolimnaeus*. Arch Hydrobiol 72: 501–516

Bärlocher F, Mackay RJ, Wiggins GB (1978) Detritus processing in a temporary vernal pool in southern Ontario. Arch Hydrobiol 81: 269–295

Basaguren A, Pozo J (1994) Leaf litter processing of alder and eucalyptus in the Agüera stream system (northern Spain). II. Macroinvertebrates associated. Arch Hydrobiol 132: 57–68

Bentley MD, McDaniel IN, Yatagai M, Lee H, Maynard R (1979) *p*-Cresol: an oviposition attractant of *Aedes triseriatus*. Environ Entomol 8: 206–209

Bentley MD, McDaniel IN, Davis EE (1982) Studies of 4-methylcyclohexanol: an *Aedes triseriatus* (Diptera: Culicidae) oviposition attractant. J Med Entomol 19: 589–592

Canhoto C, Graça MAS (1995) Food value of introduced eucalypt leaves for a Mediterranean stream detritivore: *Tipula lateralis*. Freshwater Biol 34: 209–214

Carpenter SR (1982a) Comparisons of equations for decay of leaf litter in tree-hole ecosystems. Oikos 39: 17–22

Carpenter SR (1982b) Stemflow chemistry: effects on population dynamics of detritivorous mosquitoes in tree-hole ecosystems. Oecologia 53: 1–6

Carpenter SR (1983) Resource limitation of larval treehole mosquitoes subsisting on beech detritus. Ecology 64: 219–223

Chamier A-C (1985) Cell-wall-degrading enzymes of aquatic hyphomycetes: a review. Bot J Linn Soc 91: 67–81

Coley PD (1983) Herbivory and defensive characteristics of tree species in a lowland tropical forest. Ecol Monogr 53: 209–233

Connell JH, Orias E (1964) The ecological regulation of species diversity. Am Nat 98: 399–414

Corkum LD (1992) Relationships between density of macroinvertebrates and detritus in rivers. Arch Hydrobiol 125: 149–166

Cornejo FH, Varela A, Wright SJ (1994) Tropical forest litter decomposition under seasonal drought: nutrient release, fungi and bacteria. Oikos 70: 183–190

Cornelissen JHC (1996) An experimental comparison of leaf decomposition rates in a wide range of temperate plant species and types. J Ecol 84: 573–582

Cortez J, Demard JM, Bottner P, Monrozier JJ (1996) Decomposition of Mediterranean leaf litters: a microcosm experiment investigating relationships between decomposition rates and litter quality. Soil Biol Biochem 28: 443–452

Croat TB (1978) Flora of Barro Colorado Island. Stanford University Press, Stanford

Dobson M (1994) Microhabitat as a determinant of diversity: stream invertebrates colonizing leaf packs. Freshwater Biol 32: 565–572

Dobson M, Hildrew AG (1992) A test of resource limitation among shredding detritivores in low order streams in southern England. J Anim Ecol 61: 69–77

Egglishaw HJ (1964) The distributional relationship between the bottom fauna and plant detritus in streams. J Anim Ecol 33: 463–476

Enríquez S, Duarte CM, Sand-Jensen K (1993) Patterns in decomposition rates among photosynthetic organisms: the importance of detritus C:N:P content. Oecologia 94: 457–471

Fincke OM (1998) The population ecology of *Megaloprepus coerulatus* and its effect on species assemblages in water-filled tree holes. In: Dempster JP, McLean IFG (eds) Insect populations in theory and in practice. Kluwer, Dordrecht, pp 391–416

Fincke OM (1999) Organization of predator assemblages in Neotropical tree holes: effects of abiotic factors and priority. Ecol Entomol 24: 13–23

Fincke OM, Yanoviak SP, Hanschu RD (1997) Predation by odonates depresses mosquito abundance in water-filled tree holes in Panama. Oecologia 112: 244–253

Fish D, Carpenter SR (1982) Leaf litter and larval mosquito dynamics in tree-hole ecosystems. Ecology 63: 283–288

Friberg N, Jacobsen D (1994) Feeding plasticity of two detritivore-shredders. Freshwater Biol 32: 133–142

Gee JHR (1988) Population dynamics and morphometrics of *Gammarus pulex* L.: evidence of seasonal food limitation in a freshwater detritivore. Freshwater Biol 19: 333–343

Gee JM, Somerfield PJ (1997) Do mangrove diversity and leaf litter decay promote meiofaunal diversity? J Exp Mar Biol Ecol 218: 13–33

Gillon D, Joffre R, Ibrahima A (1994) Initial litter properties and decay rate: a microcosm experiment of Mediterranean species. Can J Bot 72: 946–954

Goering HK, Van Soest PJ (1970) Forage fiber analyses (apparatus, reagents, procedures, and some applications). Handbook 379. US Department of Agriculture, Washington, DC

Golladay SW, Webster JR, Benfield EF (1983) Factors affecting food utilization by a leaf shredding aquatic insect: leaf species and conditioning time. Holarctic Ecol 6: 157–162

Hard JJ, Bradshaw WE, Malarkey DJ (1989) Resource- and density-dependent development in tree-hole mosquitoes. Oikos 54: 137–144

Hargrave BT (1970) The effect of a deposit-feeding amphipod on the metabolism of benthic microflora. Limnol Oceanogr 15: 21–30

Kaushik NK, Hynes HBN (1971) The fate of the dead leaves that fall into streams. Arch Hydrobiol 68: 465–515

Kitching RL (1971) An ecological study of water-filled tree-holes and their position in the woodland ecosystem. J Anim Ecol 40: 281–302

Kok CJ, Van der Velde G (1994) Decomposition and macroinvertebrate colonization of aquatic and terrestrial leaf material in alkaline and acid still water. Freshwater Biol 31: 65–75

Leigh EG Jr, Rand AS, Windsor DM (eds) (1996) The ecology of a tropical forest, 2nd edn. Smithsonian Institution, Washington, DC

Léonard PM, Juliano SA (1995) Effect of leaf litter and density on fitness and population performance of the hole mosquito *Aedes triseriatus*. Ecol Entomol 20: 125–136

Lodge DJ (1997) Factors related to diversity of decomposer fungi in tropical forests. Biodivers Conserv 6: 681–688

Lopez GR, Levinton JS, Slobodkin LB (1977) The effect of grazing by the detritivore *Orchestia grillus* on *Spartina* litter and its associated microbial community. Oecologia 30: 111–127

Lounibos LP (1978) Mosquito breeding and oviposition stimulant in fruit husks. Ecol Entomol 3: 299–304

Lounibos LP (1985) Interactions influencing production of treehole mosquitoes in south Florida. In: Lounibos LP, Rey JR, Frank JH (eds) Ecology of mosquitoes: proceedings of a workshop. Florida Med Entomol Lab, Vero Beach, pp 65–77

Lounibos LP, Nishimura N, Escher RL (1993) Fitness of a treehole mosquito: influences of food type and predation. Oikos 66: 114–118

Maloney DC, Lamberti GA (1995) Rapid decomposition of summer-input leaves in a northern Michigan stream. Am Midl Nat 133: 184–195

Melillo JM, Aber JD, Muratore JF (1982) Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. Ecology 63: 621–626

Novak RJ, Peloquin JJ (1981) A substrate modification for the oviposition trap used for detecting the presence of *Aedes triseriatus*. Mosq News 41: 180–181

Ostrofsky ML (1997) Relationship between chemical characteristics of autumn-shed leaves and aquatic processing rates. J North Am Benthol Soc 16: 750–759

Paradise CJ, Dunson WA (1997) Insect species interactions and resource effects in treeholes: are helodid beetles bottom-up facilitators of midge populations? Oecologia 109: 303–312

Petersen JJ, Chapman HC (1969) Chemical factors of water in tree holes and related breeding of mosquitoes. Mosq News 29: 29–36

Pimm SL, Kitching RL (1987) The determinants of food chain lengths. Oikos 50: 302–307

Prescott CE (1995) Does nitrogen availability control rates of litter decomposition in forests? Plant Soil 168/169: 83–88

Richardson JS (1991) Seasonal food limitation of detritivores in a montane stream: an experimental test. Ecology 72: 873–887

Rosenzweig ML (1995) Species diversity in space and time. Cambridge University Press, Cambridge, UK

SAS (1989) SAS/STAT user's guide, vol 2, GLM-VARCOMP. SAS Institute, Cary, NC

Sokal RR, Rohlf FJ (1981) Biometry. Freeman, New York

Sørensen T (1948) A method of establishing groups of equal amplitude in plant sociology based on similarity of species content. K Dan Vidensk Selsk Biol Skrift 5: 1–34

Srivastava DS, Lawton JH (1998) Why more productive sites have more species: experimental test of theory using tree-hole communities. Am Nat 152: 510–529

Stout J (1980) Leaf decomposition rates in Costa Rican lowland tropical rainforest streams. Biotropica 12: 264–272

Stout RJ, Taft WH, Merritt RW (1985) Patterns of macroinvertebrate colonization on fresh and senescent alder leaves in two Michigan streams. Freshwater Biol 15: 573–580

Suberkropp K (1995) The influence of nutrients on fungal growth, productivity, and sporulation during leaf breakdown in streams. Can J Bot 73: S1361–S1369

Suberkropp K, Klug MJ (1980) The maceration of deciduous leaf litter by aquatic hyphomycetes. Can J Bot 58: 1025–1031

Sweeney BW, Vannote RL (1986) Growth and production of a stream stonefly: influences of diet and temperature. Ecology 67: 1396–1410

Swift MJ, Heal OW, Anderson JM (1979) Decomposition in terrestrial ecosystems. University of California Press, Berkeley

Taylor BR (1998) Air-drying depresses rates of leaf litter decomposition. Soil Biol Biochem 30: 403–412

Taylor BR, Bärlocher F (1996) Variable effects of air-drying on leaching losses from tree leaf litter. Hydrobiologia 325: 173–182

Taylor BR, Parkinson D, Parsons WFJ (1989) Nitrogen and lignin content as predictors of litter decay rates: a microcosm test. Ecology 70: 97–104

Walker ED, Merritt RW (1988) The significance of leaf detritus to mosquito (Diptera: Culicidae) productivity from treeholes. Environ Entomol 17: 199–206

Walker ED, Merritt RW (1991) Behavior of larval *Aedes triseriatus* (Diptera: Culicidae). J Med Entomol 28: 581–589

Walker ED, Lawson DL, Merritt RW, Morgan WT, Klug MJ (1991) Nutrient dynamics, bacterial populations, and mosquito productivity in tree hole ecosystems and microcosms. Ecology 72: 1529–1546

Walker ED, Kaufman MG, Ayres MP, Riedel MH, Merritt RW (1997) Effects of variation in quality of leaf detritus on growth of the eastern tree-hole mosquito, *Aedes triseriatus* (Diptera: Culicidae). Can J Zool 75: 707–718

Webster JR, Benfield EF (1986) Vascular plant breakdown in freshwater ecosystems. Annu Rev Ecol Syst 17: 567–594

Witkamp M (1966) Decomposition of leaf litter in relation to environment, microflora, and microbial respiration. Ecology 47: 194–201

Wright DH (1983) Species-energy theory: an extension of species-area theory. Oikos 41: 496–506

Yanoviak SP (1999) Community ecology of water-filled tree holes in Panama. PhD thesis, University of Oklahoma, Norman