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GRADUATE COLLEGE

# COMMUNITY ECOLOGY OF WATER-FILLED TREE HOLES IN PANAMA

A Dissertation

# SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

Doctor of Philosophy

By

STEPHEN P. YANOVIAK Norman, Oklahoma 1999

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# COMMUNITY ECOLOGY OF WATER-FILLED TREE HOLES IN PANAMA

## A Dissertation APPROVED FOR THE DEPARTMENT OF ZOOLOGY

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#### ABSTRACT

Most studies of aquatic community ecology have focused on large systems (e.g., lakes and streams) in temperate biomes. The primary goal of my dissertation research was to understand how abiotic factors, nutrient availability and predation affect macroorganism community structure in a small aquatic habitat. Water-filled tree holes are phytotelmata formed when rain collects in branch crotches and other wood-lined cavities, and are common in many neotropical forests. Unlike larger systems, the ratio of organism biomass to habitat volume is often great in tree holes, and their small size is amenable to experimental manipulation. In addition, it is possible to mimic this habitat with simple materials (i.e., plastic cups containing leaf litter and water). Natural and artificial tree holes in the forest of Barro Colorado Island (BCI), Panama served as focal systems for my doctoral research.

Chapter 1 provides a descriptive foundation for the experimental studies summarized in later chapters. Repeated surveys of natural and artificial water-filled tree holes on BCI (1994–1998) revealed 59 species of macroorganisms associated with this habitat. Over half are true flies (Insecta: Diptera), and half of the fly species are mosquitoes (Culicidae). Densities and occurrence frequencies of most taxa were similar between artificial and natural holes. A typical tree hole contains 5 or 6 macroorganism species at any time during the wet season, but up to 14 taxa occurred in some holes.

Chapter 2 addresses abiotic factors as determinants of species' distributions and community structure. Water-filled tree holes on BCI range in size from < 0.051

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to > 50 l and occur from ground level to the forest canopy. Total macroorganism abundance and species richness increased with hole volume. Species richness in both natural and artificial holes declined with increasing height above the ground, perhaps reflecting constraints imposed by harsher abiotic conditions in the canopy. Macroorganism abundance and species richness were not correlated with pH, conductivity or dissolved oxygen content of tree hole water (Appendix A).

Chapter 3 summarizes the general ecology of a new species, <u>Microvelia</u> <u>cavicola</u>, discovered in BCI tree holes. This species generally occurred in the same holes throughout the wet season and in successive years. Hole size and shape were the best predictors of its abundance. <u>Microvelia cavicola</u> reduced mosquito survival in laboratory experiments.

As in headwater streams and oligotrophic lakes, detritus forms the base of tree hole food webs. In Chapter 4, I show that leaves from different tree species (<u>Ceiba</u> <u>pentandra</u>, <u>Dipteryx panamensis</u>, <u>Ficus yoponensis</u>, and <u>Platypodium elegans</u>) decompose at different rates and degrade faster when grazers and detritivores are present. Pupation success was highest for mosquito larvae reared on <u>Platypodium</u> litter in the lab. Similar results were obtained in the field, where macroorganism species richness and persistence times were greatest in artificial holes containing <u>Platypodium</u> litter.

Chapter 5 presents results from a laboratory test of the trophic cascade hypothesis. Odonate larvae are the most common top predators in BCI tree holes, and mosquito larvae, which graze decomposer microbes from litter surfaces, are their

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principal prey. Litter decomposition rates were faster in the presence of mosquitoes, and odonates reduced mosquito survival. Odonates, however, had no indirect effect on litter decomposition. Small mosquito larvae apparently escaped predation and maintained a significant grazer effect on litter decay rates in the presence of odonates.

Chapter 6 summarizes the independent and combined effects of predation and leaf litter quantity on macroorganism communities in tree holes. Fewer species existed in holes containing large odonates vs. holes from which odonates were excluded. Species composition results and lab experiments (Appendices D and E) suggested that selective predation is responsible for this pattern. Standardized litter addition increased mean species richness in tree holes by attracting certain species and supporting a larger number of persistent species. Litter removal reduced mean richness, probably due to energy limitation and increased predator efficiency. Effects of litter manipulations disappeared 5 wks after treatments were terminated. In an artificial tree hole experiment, litter quantities affected macroorganism abundance and species richness, but only during the first three weeks of leaf decay. Addition of an odonate larva on Week 3 had no effect on abundance, but significantly reduced species richness relative to controls during the subsequent four weeks.

In combination, my results show that macroorganism community structure (especially species richness) in BCI tree holes is most strongly influenced by tree hole size and location, nutrient quality and quantity, and the presence of top predators. These factors operate at different time scales. In any given tree hole, species richness should be consistent over the long term (e.g., a decade) because a hole's size and

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location are relatively constant from year to year; the presence or absence of a top predator will influence species richness over a period of months; and the effects of fresh litter inputs are restricted to a period of weeks. Litter is likely to be most abundant, and top predators relatively sparse, in the early wet season. Thus, investigations of predation and nutrient availability framed in a seasonal context, or along gradients of hole size or disturbance frequency, are potentially interesting extensions of this work. **CHAPTER** 1

# THE MACROFAUNA OF WATER-FILLED TREE HOLES ON

BARRO COLORADO ISLAND, PANAMA

This chapter is formatted for submission to the journal Studies on Neotropical Fauna and Environment

#### ABSTRACT

The fauna of water-filled tree holes in neotropical forests is not well documented. I found 54 macroinvertebrate and 5 vertebrate taxa in artificial and natural tree holes censused over four wet seasons on Barro Colorado Island, Panama. Most of the species are in the insect order Diptera, occur as aquatic larvae in tree holes, and are detritivore/omnivores. Half (49%) of the collected species are considered specialists in this and similar container habitats, and three invertebrate taxa were previously unknown from tree holes. Successional patterns are weak in tree holes, but some taxa predictably colonize holes shortly after they are filled. The mosquito <u>Culex urichii</u> was more common and abundant in artificial than natural tree holes; occurrence frequencies and densities of most other taxa were similar among hole types.

#### INTRODUCTION

Tree holes are phytotelmata (i.e., plant-held waters, Varga 1928) formed by the collection of rainfall in rot holes or natural convolutions in the above-ground woody portions of trees (e.g., Kitching 1971). These are the most abundant standing water habitats in many tropical and temperate forests. A variety of macroorganisms use tree holes as breeding sites and many species breed exclusively in tree holes. Larvae of true flies (Diptera) are generally the most common and diverse inhabitants of this system. Because they are primary breeding sites for many disease vectors, especially mosquitoes (Diptera: Culicidae) and biting midges (Diptera:

Ceratopogonidae), tree holes are economically important habitats.

The macrofauna of water-filled tree holes is relatively well studied in the north temperate zone (e.g., Jenkins & Carpenter 1946, Snow 1949, Rohnert 1950, Kitching 1971, Copeland 1989, Pappas <u>et al</u> 1991, Tsuda <u>et al</u> 1994, Barrera 1996, Paradise 1997, Paradise & Dunson 1997, Sota 1998) and Australia (e.g., Kitching & Callaghan 1982, Kitching 1983, Jenkins & Kitching 1990). In Africa and the neotropics, most tree hole surveys have focused on mosquitoes and other potential disease vectors (e.g., Dunn 1927, Bates 1944, Galindo <u>et al</u> 1950, 1951, Galindo & Trapido 1955, Trapido <u>et al</u> 1955, Corbet 1964, Vitale 1977, Lounibos 1980, 1981, Lounibos & Munstermann 1981, Zavortink <u>et al</u> 1983, Lounibos <u>et al</u> 1987). Aside from the work of Snow (1949) in Guatemala and Fincke (e.g., 1998, 1999) in Panama, the non-mosquito fauna of neotropical tree holes is poorly known.

Water-filled tree holes are common habitats in lowland, seasonally moist forests of central Panama. They are filled each wet season (May-December), and typically dry up completely by late dry season (i.e., March; Fincke 1992a). Tree holes on Barro Colorado Island (BCI) range in volume from less than 0.05 l to more than 50 l (Fincke 1992a) and occur at all heights within the forest (CHAPTER 2). The goal of this study was to identify the common macroorganisms associated with water-filled tree holes on BCI and summarize their ecological roles within the tree hole ecosystem.

#### METHODS

I conducted this study in the lowland moist forest of BCI, Panama (see Leigh <u>et al</u> 1996 for a site description) and also sampled several tree holes on the mainland (e.g., Gigante Peninsula and along Pipeline Road in Parque Metropolitano). The same tree hole fauna occurred at all sites; hereafter, I refer only to tree holes of BCI.

A total of 206 natural water-filled tree holes and 153 artificial tree holes (black plastic cups or pans containing leaf litter, rain water, and an emergent section of tree bark or balsa wood) were sampled on BCI during the wet seasons of 1994–1997 (1995–1997 for natural holes). The size distributions of natural and artificial holes examined in this study are shown in Fig. 1. I accessed tree holes located > 2 m above the ground using various modifications of the single-rope climbing technique (Perry 1978). I censused most holes more than once in a year or in successive years; 657 censuses were from natural holes and 1684 were from artificial holes. Each hole was censused by removing its contents to a plastic pan and recording the abundance of all species (or morphospecies) present. Except for subsamples needed to confirm species identifications, I returned all contents to the holes. Additional methodological details are given in CHAPTER 2.

I determined the occurrence frequency (percent of holes occupied) for each species or higher taxon in natural holes. For holes censused more than once, I used data from the most species-rich census. I calculated the mean density (individuals per 0.5 1 where they occurred) for each species present in a subset of 100 natural holes  $\geq$  0.2 1 and  $\leq$  2.0 1. This range includes the mean (1.67 1 ± 0.34 SE) and median (0.46

1) volumes for the complete natural hole data set; holes outside this range were not used due to problems associated with extrapolation.

I calculated the percent occurrence and mean density (as above) of each taxon in artificial holes using data from 20 repeated censuses of the 40 containers used in a vertical stratification study (CHAPTER 2). Only census data from the small (0.65 l) containers were used for density calculations (n = 360 censuses divided among 18 holes). Artificial tree holes are most often used for experiments involving repeated sampling, and this method provided the best representation of the relative contribution of each taxon to the study. I did not use the entire artificial tree hole data set (1684 censuses) for these calculations because many of the 153 containers were in place for relatively short periods of time (i.e., creating a bias for early successional species), or were manipulated for experiments. Artificial holes used in the vertical stratification experiment remained in place for > 3 yrs and were not experimentally manipulated.

The damselfly <u>Megaloprepus coerulatus</u> is a common tree hole inhabitant, but was only found in understory holes (CHAPTER 2). I calculated the frequencies for this species using data only from natural and artificial holes in the understory.

Insect larvae and pupae collected in tree hole subsamples were returned to the insectary or lab for rearing and identification. Immature organisms were fixed and stored in 70% ethanol. Adults were killed by freezing and mounted on pins or points. Larvae of chironomid midges and other non-mosquito Diptera were mounted on microscope slides. Soft tissues of slide mounted specimens were macerated in warm 85% lactic acid for 15–30 min (Cumming 1992). The cleared specimens were then

rinsed in distilled water, dehydrated in ethanol (from 70 to 100% in three steps), and mounted in euparal. Invertebrates were identified to genus and higher taxonomic levels with keys of Usinger (1956), Pennak (1989), Stehr (1987, 1991), Merritt & Cummins (1996) and pertinent references in Hurlbert & Villalobos-Figueroa (1982). Mosquito species were determined with keys of Darsie (1993) and an unpublished key to the mosquitoes of Panama (Gorgas Mem. Lab., Ancon, Panama). Taxonomic assistance was provided by H. P. Brown (Coleoptera), J. Gelhaus (Diptera: Tipulidae), P. Gnaspini (Opiliones), D. Penney (Araneae), R. Pinto da Rocha (Opiliones), J. Polhemus (Hemiptera), N. Powers (Diptera: Culicidae), A. S. Rand (Vertebrata), P. Spangler (Coleoptera), F. C. Thompson (Diptera: Syrphidae), and N. Woodley (Diptera: Stratiomyidae). Vouchers were deposited at the Smithsonian Tropical Research Institute, Panama, the US National Museum, Washington DC, the Museu de Zoologia da Universidade de São Paulo, Brazil, and my private collection.

All taxa were classified based on their degree of association with the tree hole habitat following Rohnert's (1950) scheme as translated by Kitching (1971) and Fish (1983). In this system, "dendrolimnetoxene" refers to accidental species in tree holes; "dendrolimnetophile" refers to species that are facultative tree hole inhabitants (i.e., that occur in a variety of other aquatic or semi-aquatic settings); and "dendrolimnetobiont" refers to species that occur only in tree holes or similar phytotelmata. For simplicity, hereafter I refer to species in these groups as "accidental," "facultative," and "specialist" tree hole inhabitants. Where the assignment of a taxon to either the facultative or specialist class was uncertain, I used

its occurrence frequency in natural tree holes as a deciding factor. These decisions were based on the assumption that infrequent species in tree holes are using other breeding sites. Alternatively, some of these taxa may be tree hole specialists, but are rare on BCI.

The functional roles of tree hole taxa were determined from the literature (e.g., Pennak 1989, Merritt & Cummins 1996) and from observations of feeding behavior in the field and/or lab. For this study, "detritivores" consumed decaying leaf or woody material; "omnivores" filter fed in the water column or grazed indiscriminately on the surfaces of decaying litter; "predators" consumed other macroorganisms, sometimes conspecifics; and "scavengers" fed on dead macroorganisms trapped in the water surface film.

#### **RESULTS AND DISCUSSION**

I found 54 macroinvertebrate and 5 vertebrate taxa associated with water-filled tree holes on BCI (Table 1). More than half of the species (56%) are in the order Diptera. A typical water-filled hole on BCI contained 5 or 6 (mean = 5.3; range = 0 to 14) species of macroorganisms on any wet season day. Multiple factors influence diversity in individual BCI tree holes, including hole size and location (CHAPTER 2), nutrient quality (CHAPTER 4), nutrient quantity (CHAPTER 6), and the presence or absence of top predators (CHAPTER 6).

Based on the tree hole association classes of Rohnert (1950), 15% of the taxa I collected on BCI are accidental, 36% are facultative, and 49% are tree hole specialists

(Table 1). Some species (e.g., of Chironomidae and Syrphidae) were tentatively classed as specialists, but too little is known of their habitat specificity to be certain of this designation.

As in temperate forests (e.g., Kitching 1971, Fish & Carpenter 1982), tree holes on BCI are detritus-based systems. Most (61%) species found in BCI tree holes function ecologically as omnivores, detritivores, or both (Table 1). A few species (discussed below) primarily feed directly on decaying leaf material or wood. Omnivores and detritivores are the primary prey of predators in tree holes, although some predators also scavenge on dead or dying insects trapped in the surface film (CHAPTER 3; and O. M. Fincke, pers. obs.). Unlike temperate tree holes, obligate and facultative predators are common and diverse (36% of the non-accidental fauna; Table 1) in BCI tree holes.

Densities and frequencies of most taxa were similar between natural and artificial tree holes (Table 2). Density values must be interpreted with caution; although hole size and macroorganism abundance are correlated (APPENDIX A), the relationship is not linear for many taxa. For example, two or more odonates (including neonates) were often found in 0.5 l holes (Table 2), but their densities tend to be much lower in larger holes (cf. Fincke 1992a, 1998).

#### Notes on selected groups

The autecology of many tree hole inhabitants is poorly known. Below I summarize laboratory and field observations of the behavior and ecology of groups commonly encountered in tree holes.

#### Microfauna

The microorganism assemblages of water-filled tree holes have been largely ignored (but see Lackey 1940). In addition to the bacteria and fungi responsible for litter decomposition, protists, rotifers, microturbellarians, and ostracods commonly occur in tree holes on BCI. These microorganisms are considered accidental inhabitants of tree holes. All were rare or nonexistent in artificial holes, including holes that remained in place for > 3 yrs, suggesting that colonization is slow or requirements for their persistence were not met by the artificial systems.

Because my investigations focused on the macrofauna of tree holes, most of the protists and rotifers I encountered were living in association with larval dryopid beetles and larval odonates, especially mid-size pseudostigmatids (> 14 mm body length). Unidentified <u>Vorticella</u>-like protists and colonial rotifers successfully colonized virtually all exposed body surfaces of dryopid and odonate larvae (except the eyes and caudal lamellae of the latter). Accumulations of these groups were often sufficiently dense to make the insects appear as if infected with pathogenic fungi. At least one species of bdelloid rotifer (<u>Pleurotrocha</u> sp.?) also occurred in smaller numbers on odonate larvae. These solitary rotifers live in gelatinous tubes (2–3 mm in length) attached to the larger body surfaces of odonates, especially the abdomen.

Symbioses, especially parasitism and commensalism, occur between some genera of rotifers and freshwater macroinvertebrates (Pennak 1989). The lack of conspicuous accumulations of bdelloid rotifers on non-odonate insects (or on leaf litter) in natural and artificial tree holes suggests a commensalistic relationship with odonates. In an evolutionary context, several behavioral and life history factors that are collectively unique to odonate larvae may promote some specificity of sessile rotifers for this group. First, odonate larvae are highly mobile within tree holes. This mobility provides filter-feeding and potentially diffusion-limited microorganisms access to different types of resources within a hole. Second, mid- to large-size odonates are top predators and are unlikely to be eaten or have their body surfaces grazed by other organisms. Odonate larvae are, therefore, relatively safe hosts for epizoics and ectocommensals. Third, large surfaces of the odonate body are open for colonization. Odonate larvae use their forelegs to keep the eye surfaces clean, and frequently groom their legs and caudal lamellae with their mouthparts. However, they are unable to use their legs or mouthparts to effectively groom most of their large body sections (pers. obs.). Finally, odonates have a longer larval life span than most other organisms in tree holes (dryopids are the one exception). Although frequent molting of hosts during growth presents an obvious problem for ectocommensals, the relatively short life spans of rotifers (Pennak 1989) probably fit within the larval stadia of mid-size odonates in tree holes.

Two species of ostracods, tentatively identified as <u>Cypris</u> sp. (Cyprididae) and <u>Darwinula</u> sp. (Darwinulidae), commonly occur in BCI tree holes. <u>Cypris</u> were always

less abundant (< 50  $\Gamma^{-1}$ ) than <u>Darwinula</u> (often > 100  $\Gamma^{-1}$ ), and were typically seen swimming freely in the water column. <u>Darwinula</u> generally formed dense aggregations just below the water surface along the tree hole margins and on the edges of leaf litter. Both of these genera are also known from the oxygen-poor bottoms of lakes (Pennak 1989), which, in terms of physical and chemical habitat conditions, are probably very similar to tree holes (e.g., Fincke 1999; CHAPTER 2).

A microturbellarian tentatively identified as <u>Microstomum</u> sp. (Platyhelminthes: Turbellaria: Microstomidae) was often very abundant in BCI tree holes; its densities were occasionally high enough (> 20 ml<sup>-1</sup>) to cloud the tree hole water. At lower abundance, <u>Microstomum</u> formed yellowish aggregations on the edges of leaf litter and just below the water surface along the tree hole margin. These worms primarily feed on bacteria and other microorganisms (Pennak 1989).

#### **Arachnids**

I frequently found opilionids (Cynorta sp.) and the large ctenid spider Cupiennius coccineus resting on the interior walls of tree holes, where they presumably prey opportunistically on other invertebrates entering or leaving the holes. An opilionid was once observed to strike unsuccessfully at a mosquito that was approaching a tree hole. Ctenid spiders generally perch head-down on the hole wall with their first two pairs of legs on the water surface. Although not directly observed, it is likely that they feed on adult pseudostigmatid damselflies (Fincke 1992b), tipulids, syrphids and other relatively large insects visiting tree holes to oviposit. When disturbed, the spiders quickly crawl beneath the water surface and cling to detritus. They can remain submerged for > 5 min if necessary.

Spiders (Pholcidae) and various species of scorpions were occasionally found in BCI tree holes. Pholcids construct webs a few cm above the waterline and feed on mosquitoes and other small insects entering or exiting the hole. Scorpions use tree holes as daytime refugia or nesting sites, and are probably not important predators in this system. I most often found scorpions in dry crevices of slit-type holes (see Fincke 1992a for hole classification) and, like ctenid spiders, they quickly crawled into the water when disturbed.

#### **Odonates**

The dragonfly <u>Libellula</u> sp. is an accidental species in natural tree holes of BCI, although it sometimes colonized large artificial holes located in treefall gaps and was common in moats surrounding greenhouses. The only natural tree hole occurrence of <u>Libellula</u> sp. was in large, highly insolated holes of a recently fallen <u>Platypodium elegans</u> near Shannon Creek.

Larvae of the remaining five species of odonates (along with larvae of the mosquito <u>Toxorhynchites theobaldi</u> and <u>Dendrobates auratus</u> tadpoles), are the top predators in tree holes. The behavior and ecology of these odonate species have been studied extensively by Fincke (1992b, 1992c, 1994, 1998, 1999), and recent work has examined effects of tree hole odonates on mosquito abundance (Fincke <u>et al</u> 1997) and species richness (CHAPTER 6).

#### Hemiptera

<u>Microvelia cavicola</u> and <u>Paravelia myersi</u> are neustonic (i.e., surface-dwelling) predator/scavengers in tree holes. Laboratory experiments and field observations indicate that these species feed on mosquitoes emerging from holes as well as terrestrial insects that are trapped on the water surface. More specific information about <u>M. cavicola</u> is presented in CHAPTER 3. One unidentified species of water strider (Gerridae) was found in tree holes of a recently fallen <u>Platypodium elegans</u>, but did not persist and was probably an accidental colonist from pools in Shannon Creek.

#### Coleoptera

Larvae of scirtid beetles were the most abundant and conspicuous coleopterans in natural and artificial tree holes, and occurred at all heights in the forest (CHAPTER 2). Adults were rarely seen. Scirtid larvae eat leaf detritus, submerged decaying wood, and associated decomposer microbes. When food is abundant, larvae become adults in 3–5 wks.

I found adults of <u>Copelatus</u> sp. in natural and artificial tree holes on BCI, and at all heights in the forest. Typically, a single adult beetle would colonize an artificial hole within two weeks of setup, and a second adult (usually the opposite sex) would appear one or two weeks later. The consistency of this pattern suggests that adult <u>Copelatus</u> use pheromones to attract mates to suitable breeding habitats. Another

dytiscid beetle (<u>Laccophilus</u> sp.) sometimes occurred in large (6.65 l) artificial holes, especially those located in exposed sites such as tree fall gaps. They were never found in natural holes and are considered accidental colonists of container habitats.

This study is the first to report the occurrence of dryopid beetles in waterfilled tree holes (P. J. Spangler, pers comm.). Dryopid larvae are relatively common in natural tree holes on BCI (Table 2), where they feed on submerged decaying wood. Larvae also readily consumed decaying Ficus yoponensis leaf litter in the lab. These insects create vertical tunnels in the woody interior of natural holes as they feed, gradually increasing the size and physical heterogeneity of the habitat. Total volume is one factor affecting macroorganism diversity in BCI tree holes (CHAPTER 2), thus, dryopid larvae can be considered "ecosystem engineers" (sensu Jones <u>et al</u> 1994) in this system.

Field and laboratory observations indicated that dryopid larvae are the longest-lived macroorganisms in BCI tree holes, and are highly resistant to desiccation. Their growth was very slow even when food was provided <u>ad libitum</u> in the laboratory, and at least two complete wet seasons are probably required for development to adulthood in the field. Despite numerous attempts to create the appropriate conditions in rearing chambers, none of the larvae successfully pupated in the lab. The adults are unknown. I placed several final instar dryopid larvae in dry plastic cups in the lab to determine their ability to resist desiccation. The larvae became dormant and appeared dead within 48 h, but all resumed normal activity when rehydrated (even after 3 wks in

the air-conditioned environment). This suggests that larvae can survive the repeated or persistent drying that occurs in many holes during the dry season on BCI.

#### Mosquitoes (Diptera: Culicidae)

Mosquitoes of tropical tree holes are relatively well studied because of their role as vectors of human disease (see references in Introduction). The biology of <u>Toxorhynchites</u>, a top predator in tree holes, is especially well known (reviewed by Steffan & Evenhuis 1981), largely due to its potential use as a biological control agent. Bates (1949) and Clements (1992, 1999) thoroughly reviewed the biology of mosquitoes in general.

Galindo <u>et al</u> (1950, 1951, 1955) and Trapido <u>et al</u> (1955) surveyed container-breeding and other mosquitoes in Panama, and summarized the regional and local (e.g., within-forest vertical stratification) distributions of many species. A few of the tree hole species found in Panama by Galindo and coworkers were not encountered in this study. The absence of these species (e.g., <u>Sabethes cyaneus</u>) is primarily due to their oviposition preference for closed-top habitats with small lateral openings (Galindo <u>et al</u> 1951, 1955). These types of phytotelmata occur on BCI (e.g., hollow branches of <u>Pseudobombax septenatum</u> with 1-2 cm exterior openings), and female mosquitoes were often seen ovipositing in them. However, my investigations focused exclusively on tree holes with exposed water, which explains the absence of <u>Sabethes</u> spp. and some <u>Haemagogus</u> species from Table 1.

Limatus spp. and <u>Trichoprosopon digitatum</u> were rare in natural tree holes of BCI (Table 2), but colonized almost any recently-formed pool of water within 1 m of the ground. I generally found these species in water collected by fallen fruit husks (e.g. of <u>Tontelea ovalifolia</u>) and palm spathes, which are their typical breeding sites (e.g., Zavortink <u>et al</u> 1983). The few natural tree holes containing <u>Limatus</u> spp. and <u>T. digitatum</u> were either newly formed (see Succession below) or at ground level.

#### Non-mosquito Diptera

#### Ceratopogonidae

Ceratopogonid midge larvae (<u>Bezzia snowi</u>) frequently swim near the surface of the tree hole water and at the waterline, but I most often observed them partially embedded in the sediment at the bottom of holes. Although <u>Culicoides</u> spp. are common inhabitants of temperate tree holes (e.g., Snow 1949, Pappas <u>et al</u> 1991), they were rare in my collections.

Larvae of <u>B</u>. <u>snowi</u> and <u>Culicoides</u> spp. feed both singly and gregariously upon mosquito larvae and other small aquatic macroinvertebrates in tree holes. They rapidly recruited to struggling prey, and I occasionally observed as many as 12 <u>B</u>. <u>snowi</u> feeding on a single mosquito larva. <u>Bezzia snowi</u> larvae are also cannibalistic, more so at high densities. The quantitative effects of these secondary predators on the abundance and diversity of other organisms in BCI tree holes is unknown. However, young (< 5 mm body length) pseudostigmatid damselflies had low survivorship (i.e.,

were not present on two or more consecutive census dates) in artificial holes where  $\underline{B}$ . snowi were very abundant.

I did not observe the feeding behavior or examine the gut contents of <u>Dasyhelea</u> sp., but a species of <u>Dasyhelea</u> found in temperate tree holes is a detritivore/omnivore (Kitching 1971), and members of this genus presumably serve a similar function in BCI tree holes. All of the adult <u>Dasyhelea</u> reared in the lab were morphologically very similar and presumably represent a single species. More intensive collecting and rearing efforts may associate additional <u>Dasyhelea</u> species with BCI tree holes.

Unlike the other ceratopogonid genera, Forcipomyia are not truly aquatic in tree holes; the larvae live on the damp wood just above the water line, where they feed on microbes and detritus particles. Forcipomyia sp. colonized both artificial and natural holes, and occurred at all heights in the forest. All of the larvae I collected are morphologically very similar; adults were not reared. As with Dasyhelea, further collections may reveal more than one Forcipomyia species facultatively associated with tree holes on BCI.

#### Chaoboridae (Corethrellidae)

I found <u>Corethrella appendiculata</u> only in small natural holes, and they generally occupied the same individual holes in successive years. <u>Corethrella</u> <u>appendiculata</u> are predators of small invertebrates in tree holes, especially early-instar mosquito larvae (Petersen & Chapman 1969). Although <u>C. appendiculata</u> can

significantly reduce the abundance of <u>Aedes triseriatus</u> in tree holes of Florida (Lounibos 1983, 1985; but see Bradshaw & Holzapfel 1983), their effect on mosquito abundance and invertebrate community structure in tree holes of Panama is unknown.

#### Chironomidae

Larvae of <u>Chironomus</u> sp. live in small (1 cm) vertically orientated tubes on the bottom and interior walls of tree holes. The tubes are constructed of silken material with attached detritus. <u>Chironomus</u> larvae feed on decomposer microbes and fine particulate matter in tree holes. Their densities were occasionally  $\geq$  50 per liter in both natural and artificial holes.

#### Syrphidae

At least two species of syrphids develop in water-filled tree holes of BCI; <u>Copestylum rafaelanum</u> is the most common (Table 2). Syrphid larvae feed on detritus and decomposer microbes, and development to adulthood requires > 6 wks in the field. Adult female syrphids are apparently attracted to volatile compounds released when a hole is disturbed. On several occasions, up to three female <u>C</u>. <u>rafaelanum</u> recruited to a hole and began ovipositing as I was removing and examining the leaf litter. Adult females were never encountered at undisturbed holes. A female preparing to oviposit will perch head-downward on moist bark 5-25 cm above the tree hole water surface and probe the bark with her ovipositor. Eggs are inserted singly into separate bark crevices. Adults of a second syrphid species visited a large hole 17 m up in a <u>Dipteryx</u> <u>panamensis</u> tree while I was examining its contents. Attempts to collect these adults and to rear the few early-instar syrphid larvae I collected from the hole water were unsuccessful. This species was never found in artificial tree holes (Table 2).

#### Tipulidae

All three tipulid species occurred in natural and artificial tree holes, and were found in holes at all heights in the forest (CHAPTER 2). Based on adults reared in the lab, <u>Sigmatomera séguyi</u> was the most commonly encountered species (ca. 70% of collected individuals). The biology of <u>Sigmatomera</u> was described by R. C. Shannon (in Alexander 1930); here I augment his descriptions with observations made in the field and lab on BCI.

Adult female <u>Sigmatomera</u> spp. lay their eggs in the moist bark of tree holes. A female preparing to oviposit will perch 2–10 cm above the water surface in a shadow and out of plain view. During oviposition, the female curls her abdomen ventrad (more or less forming an S-shape) and, through a series of abdominal undulations, inserts her blade-like ovipositor into a bark crevice. All oviposition events that I observed occurred during the middle of the day, but only under very dark overcast conditions. When the weather is clear, oviposition probably occurs more often at night, or at dawn or dusk. On one occasion, an adult male was found resting on the tree trunk immediately above a hole. There was no evidence that it had recently emerged from that hole, suggesting that adults cue to tree holes to find mates.

Each <u>Sigmatomera</u> larva lives in a tube constructed of silken material secreted from glands associated with the mouthparts. Feces and debris are incorporated into the tube, making its outer diameter ca. 2x that of the larva. The tube is generally  $\geq$ 4x the length of the larva and often forms a U-shape with two openings at or above the water surface on the interior sides of the hole. Individuals starved for three or more days in laboratory rearing chambers (glass vials 2.5 cm in diameter and 5.5 cm in height containing ca. 12 ml water) constructed simple tubes with a single water surface access point and were inactive most of the time. Second instar larvae required 6-8 weeks to complete development in the lab, pupation occurred at an exposed end of the silk tube, and adults emerged in 5-7 days.

Sigmatomera larvae feed on decaying leaf material and the attached decomposer microfauna, but also prey on mosquito larvae (Alexander 1930, Snow 1949, Fish 1983) and presumably eat other insects browsing the surfaces of detritus in natural holes (Alexander 1930). In the lab, <u>Sigmatomera</u> larvae successfully developed to adulthood by feeding only on decaying <u>Ficus yoponensis</u> (Moraceae) leaves. When I added two fourth instar <u>Culex mollis</u> (Diptera: Culicidae) larvae to each of 12 rearing chambers, the activity of all 12 <u>Sigmatomera</u> larvae increased almost immediately. Although not quantified, this increase in activity appeared to be correlated with the number of times mosquito larvae grazed, bumped, or became temporarily trapped in the silk of the tipulid tubes. After 1–2 min, several <u>Sigmatomera</u> larvae extended the anterior 25–50% of their bodies into the water column. Passing mosquitoes were then subdued, either by contacting the tipulid

mouthparts or by becoming entangled in additional tipulid silk released in their immediate vicinity. The amount of silk surrounding the tipulid tubes approximately doubled within 24 h of mosquito input, and fresh tipulid silk appears to be more effective at prey capture than older silk (which the tipulid larvae often consumed; cf. Fish 1983). As noted by Shannon (in Alexander 1930), the glue-like silk is apparently a very attractive food source for mosquito larvae.

<u>Sigmatomera</u> larvae ate their prey singly and completely, but rarely fed on more than two mosquito larvae in 24 h. This feeding rate seems lower than that observed by Shannon, although he did not quantify short-term predation in his study. Additional work is needed to determine the effect of <u>Sigmatomera</u> on the abundance and diversity of other invertebrates in neotropical tree holes.

#### Vertebrates

The frogs <u>Agalychnis callidryas</u>, <u>Dendrobates auratus</u>, and <u>Physalaemus</u> <u>pustulosus</u> use water-filled tree holes as breeding sites. Like the mosquito <u>Toxorhynchites theobaldi</u> and odonates, <u>D</u>. <u>auratus</u> tadpoles are top predators in BCI tree holes. They tend to colonize holes in the early part of the wet season (Fincke 1999) and are generally found in the same holes year after year (pers. obs.). <u>Agalychnis callidryas</u> and <u>P</u>. <u>pustulosus</u> breed in a variety of aquatic habitats (Rand 1983, Scott 1983) and are less common than <u>D</u>. <u>auratus</u> in natural holes on BCI (Table 2). I found up to 8 <u>A</u>. <u>callidryas</u> tadpoles in large holes > 2 m above the ground, but they also occur in large understory holes in fallen trees (Fincke 1998). Adult <u>P</u>. <u>pustulosus</u> are poor vertical climbers (pers. obs.), and their egg masses and tadpoles occurred only in understory holes (e.g., < 2 m above ground). All three amphibian species also colonized artificial holes (Table 2), and I once found <u>D</u>. <u>auratus</u> tadpoles in the water collected by a fallen palm spathe.

Adult <u>Eleutherodactylus</u> spp. frogs and the gecko <u>Sphaerodactylus lineolatus</u> occasionally inhabited BCI tree holes. These species do not breed in tree holes; they use the habitat primarily as a source of shelter, and probably feed opportunistically on emerging or colonizing insects. <u>Sphaerodactylus lineolatus</u> was more common in artificial holes than natural holes (Table 2), and was most often encountered in the forest canopy, where it is also associated with vascular epiphytes (G. Zotz, pers. comm.). Male and female <u>S</u>. <u>lineolatus</u> were sometimes found in the same hole, and juveniles occurred with adults on two occasions. I never found more than one <u>Eleutherodactylus</u> in a hole. Like some of the arachnids, the frogs and geckos quickly crawled beneath the water surface and hid among the detritus when disturbed.

#### Miscellaneous taxa

<u>Dero</u> sp. (Annelida) are common in natural tree holes, where they sometimes form conspicuous, dense, reddish-brown aggregations on detritus and at the waterline. They feed on decaying litter and decomposer microbes. The lower frequency of <u>Dero</u> in artificial holes (Table 2) is probably due to their limited dispersal ability and the comparatively short existence of the containers. At least one other species of annelid (semi-aquatic Lumbriculidae) was present (but very rare) in BCI tree holes.
To my knowledge, this study is the first to report the presence of horsehair worms (Nematomorpha) in water-filled tree holes. Nematomorphs are internal parasitoids of relatively large-bodied insects, and adult worms become free living when their hosts contact water (Pennak 1989). The rarity of horsehair worms in tree holes (Table 2) suggests that they are not obligate parasitoids of facultative or specialist tree hole taxa. Many nematomorph species are not host- or habitat-specific (Pennak 1989), and their occurrence in this habitat is considered accidental, perhaps via infected terrestrial insects visiting tree holes in search of water. However, some nematomorphs attack mosquitoes and other aquatic insects (e.g., Poinar & Doelman 1974, Pennak 1989), and the possibility that certain tree hole taxa are occasionally infected cannot be completely dismissed.

I infrequently found land crabs (<u>Gecarcinus</u> sp.) inhabiting natural tree holes at or near ground level. <u>Gecarcinus</u> apparently use tree holes as a source of water (e.g., to hydrate their gills) and food. Where they occurred, the crabs were a significant source of disturbance in tree holes. They commonly redistributed the detritus in a hole (or removed it completely) over a period of days, probably in search of invertebrate prey. In addition, I once observed <u>Gecarcinus</u> actively foraging in the moist detritus of a dry tree hole. Other occasional visitors, such as coatis (<u>Nasua</u> <u>narica</u>) and spider monkeys (<u>Ateles geoffroyi</u>), similarly disturb wet and dry tree holes on BCI (Fincke 1992a; pers. obs.).

Adult dolichopodid flies and <u>Ectatomma</u> ants were the most common short-term opportunistic visitors of tree holes. Dolichopodid flies preyed upon

ceratopogonids and other small insect larvae swimming near the water surface. In both artificial and natural holes, the flies perched head-down 1–2 mm above the waterline and extracted prey by rapidly dipping their mouth parts through the surface film. Ectatomma ants (E. ruidum and E. tuberculatum) routinely included natural and artificial tree holes in their foraging territories; they were consistently (i.e., predictably) present at certain holes. I frequently observed <u>E</u>. tuberculatum workers striking (i.e., lunging forward with mandibles agape) at adult mosquitoes approaching natural tree holes, and <u>E</u>. ruidum workers were seen carrying second instar syrphid larvae away from artificial holes on two occasions.

## Succession

Most facultative and specialist tree hole species (Table 1) can be found in BCI tree holes at any time during the wet season, but several taxa colonize holes primarily during the first several weeks after filling. This is most evident in small holes that dry and refill frequently, and new holes created when large trees with fluted trunks (e.g., <u>Platypodium elegans</u> and <u>Quararibea astrolepis</u>) fall and collect rain water. The mosquitoes <u>Culex corrigani</u>, <u>C. mollis</u>, <u>C. urichii</u>, and sometimes <u>Limatus</u> spp. are generally the most abundant organisms in newly filled tree holes on BCI. If the new hole is within 1 m of the ground, the mosquito <u>Trichoprosopon digitatum</u> may also be present. Psychodidae, Chironomidae, Ceratopogonidae, and the syrphid <u>Copestylum rafaelanum</u> are additional early colonists. Whereas many of the species listed above may continue to colonize holes for several weeks or months, <u>Limatus</u> and <u>T. digitatum</u>

generally stop colonizing 2-4 wks after a hole fills, and the density of <u>C</u>. rafaelanum larvae declines from > 100 per liter to < 3 per liter.

Taxa appearing later in a hole (4 or more weeks post-filling) include pseudostigmatid damselflies, aeshnid dragonflies, dryopid beetles, the mosquito <u>Aedes</u> <u>terrens</u>, and scirtid beetles. Some of these taxa can survive periodic desiccation (e.g., Fincke 1994) and may pass the dry season by burrowing into the litter or sediment accumulated in the bottom of a hole. As a consequence, these "late" species sometimes appear in older holes (i.e., with substantial sediment accumulations) < 24h after they are filled.

## Artificial vs. natural tree holes

Although the fauna of natural and artificial tree holes was similar in overall composition and density, a few species differed with respect to their frequency of occurrence between hole types (Table 2). Some of these differences can be attributed to the size, location and physical characteristics of artificial holes relative to natural holes. For example, the absence of the frog <u>Agalychnis callidryas</u> from the artificial tree hole censuses (Table 2) was due to the relatively small size of containers used in the vertical stratification study; only 14% were > 3 l in volume (Fig. 1, CHAPTER 2). In addition, all of the artificial tree holes were secured to trees  $\geq$  1 m above ground level (CHAPTER 2), which prevented them from being visited by the crab <u>Gecarcinus</u> sp. or colonized by the frog <u>Physalaemus pustulosus</u>.

The greater frequency of <u>Culex urichii</u> (and to a much lesser extent, <u>C</u>. <u>secundus</u>) in artificial holes vs. natural holes (Table 2) was perhaps the most important faunistic difference between hole types. Unlike natural holes, <u>C</u>. <u>urichii</u> was often the most abundant mosquito species in artificial tree holes (Table 2). I partly attribute this difference, as well as differences in frequencies of <u>Haemagogus</u> spp., other <u>Culex</u> spp., and the hemipteran <u>Microvelia cavicola</u> to the greater exposure of artificial holes vs. natural holes. <u>Culex urichii</u> is a common inhabitant of water collected by fallen palm spathes (Bates 1949; pers obs.), and may prefer to oviposit in habitats with highly exposed surfaces. The opposite is probably true for at least some <u>Haemagogus</u> spp. (e.g., Galindo <u>et al</u> 1951, 1955) and <u>M</u>. <u>cavicola</u> (CHAPTER 3).

Artificial tree holes provide an excellent means of controlling multiple variables and increasing sample sizes for experimentation, but they are not perfect mimics of natural holes. Ecologically, the most important difference between artificial and natural holes is that artificial holes are not integral parts of trees. This has three main consequences: 1) artificial tree holes are younger than natural holes and thus lack biological history (e.g., accumulations of feces, refractory detritus, and sediments); 2) artificial tree holes collect less stemflow than natural holes; and 3) artificial tree hole water has no direct contact with living wood. The latter may nullify potential tree species effects on tree hole community structure by preventing the exchange of materials (e.g., tannins, sap, nitrogenous wastes) between the water and the tree. The results shown in Table 2 suggest that these differences do not affect

the composition of species that are attracted to artificial vs. natural holes. Lack of biological history, stemflow inputs and extensive tree contact may, however, influence chemical dynamics and the post-colonization longevity of some species in artificial holes (e.g., if critical nutrients become depleted or noxious wastes are not buffered). This must be considered when using artificial tree holes for experiments involving nutrient manipulations.

## Morphospecies descriptions and identification notes

I assigned several taxa to morphospecies based on distinguishing characteristics of lab-reared adults to provide a more complete overview of the diversity of organisms occurring in BCI tree holes. Below I summarize the physical characteristics (mainly color patterns and morphometrics) I used to separate morphospecies. These summaries are not intended as formal taxonomic descriptions. Parenthetical values are ranges for means. Measurements were made under a Wild<sup>®</sup> M5A dissecting scope fitted with an ocular micrometer.

In a few cases, taxa were identified to species level although not all of the individuals examined fit every characteristic listed the taxonomic keys or descriptions. Where potentially important or useful, I describe how my specimens differed from published descriptions.

## Coleoptera: Dryopidae

Until adults are collected, the genus of dryopid beetles occurring in BCI tree holes will remain unknown. The larvae are distinctive in that they are atypically large for dryopids (final instar 30–35 mm total length). The genus occurring in BCI tree holes is probably new to science (H. P. Brown and P. J. Spangler, pers. comm.).

## Coleoptera: Scirtidae

#### Prionocyphon sp.

All of the <u>Prionocyphon</u> in my collection fit a single morphotype, but more intensive collecting and adult rearing may reveal additional species occurring in BCI tree holes. Adult <u>Prionocyphon</u> sp. differed in body size (overall length = 2.9-4.8mm, n = 16, mean = 3.87 mm), but this variation was continuous. Other morphological characteristics of adults and larvae were similar among individuals.

#### Scirtes sp. 1

GENERAL: Body mostly black, highly reflective, with dense covering of short hairs. Hairs on epicranium, pronotum and elytra relatively robust and erect. Longest body hairs shorter than length of terminal antennal segment. Body length = 3.4 mm. HEAD: Mouthparts and antennae light brown. THORAX: Sternites dark brown with fine appressed hairs. LEGS: Fore and middle legs dark brown with fine, moderately appressed hairs. Hind femora black. ABDOMEN: Sternites dark brown to black with fine appressed hairs. MATERIAL EXAMINED: 1 male.

#### Scirtes sp. 2

GENERAL: Dorsal surfaces brown to black, distinctly reflecting indigo, with dense covering of long, fine, whitish hairs. Hairs on epicranium, pronotum and elytra erect. Longest body hairs longer than length of terminal antennal segment. Male and female color patterns differ slightly.

<u>MALE</u>: Body length 4.1 mm. HEAD: Mouthparts, antennae, and clypeus brown. Epicranium brown in anterior 1/4, black posteriorly except for small triangular brown patch behind each eye. THORAX: Anterior pronotal margins brown from level of posterior eye margin forward; pronotum otherwise black. Sternites brown with fine appressed hairs. LEGS: All legs brown with fine appressed hairs. ABDOMEN: Sternites brown with fine appressed hairs.

<u>FEMALE</u>: Body length 3.3 mm. HEAD: Mouthparts, antennae, and clypeus brown. Anterior margin of head brown from antennal insertions forward, epicranium black except for thin brown posterior eye margin. THORAX: Extreme anterior margin of pronotum brown; pronotum otherwise black. Sternites brown with fine appressed hairs. LEGS: All legs brown with fine appressed hairs. ABDOMEN: Sternites brown with fine appressed hairs.

MATERIAL EXAMINED: 1 male, 1 female.

## Diptera: Ceratopogonidae

## Bezzia snowi

Several of the male <u>Bezzia</u> specimens I examined did not perfectly match the description of <u>B</u>. <u>snowi</u> by Spinelli and Wirth (1991). However, the only conspicuous difference was the lack of ventral spines on the mid- and hind-femora. Some of these individuals were killed too quickly after emergence, and incomplete exoskeletal hardening may have been responsible for the lack of spines.

#### Diptera: Chironomidae

Taxonomic studies of neotropical Chironominae are few, and no keys to Panamanian species exist (B. Hayford and L. Ferrington, pers. comm.). Three adult Tanytarsini were reared from samples from two natural tree holes. All of these specimens are females and cannot be identified below the level of tribe. The rarity of this group in my collections suggests that they are accidental tree hole colonists. All of the <u>Chironomus</u> larvae and reared adults were morphologically very similar. Based on the structure of the larval mentum (size and arrangement of teeth), only one <u>Chironomus</u> species occurs in BCI tree holes.

#### Diptera: Psychodidae

Species of <u>Telmatoscopus</u> were not readily separable in the larval stage. <u>Telmatoscopus</u> sp. 1 was reared from artificial tree holes only, whereas the other two species were collected exclusively from natural holes. Psychodid larvae occurred in tree holes at all heights in the forest (CHAPTER 2), but reared adults originated only from understory holes.

#### Telmatoscopus sp. 1

GENERAL: Males and females are patterned similarly, but males are smaller. HEAD: Vertex with relatively long white hairs that appear black apically when viewed on their long axes. Antennae shorter than wing, verticils white. THORAX: Tergites with long white hairs as on head. WING: Hairs in basal 1/4 of dorsal surface long, white. Midpoints and apices of most longitudinal veins with distinct tufts of white hairs dorsally; vein R<sub>2</sub> dorsally with tuft of gold hairs in basal 1/3. Other wing hairs mostly brown, ranging from beige to black. Mean wing length: male = 2.6 mm (2.50-2.75); female = 3.2 mm (3.50-2.92). Average maximum wing width: male = 1.3 mm (1.25-1.33); female = 1.6 mm (1.75-1.33). ABDOMEN: Posterior margins of abdominal tergites with long white hairs immediately followed by black hairs, creating a series of transverse stripes. LEGS: Hairs and scales mainly brown. Apical margin of femur, tibia, and (at least) the first tarsal segment with white scales. Tibiae with white scales scattered along their length. MATERIAL EXAMINED: 5 males, 7 females.

#### Telmatoscopus sp. 2

GENERAL: Males and females are similar in size, but differ with respect to hair and scale color patterns.

MALE: HEAD: Vertex with relatively long white hairs that appear black apically when viewed on their long axes. Antennae shorter than wing, verticils light brown. THORAX: All tergites with long white hairs as on head; central brown patch (see female description) not present. WING: Hairs of dorsal wing surface comparatively long, mostly brown or black. Basal half and apices of many longitudinal veins with distinct tufts of white hairs. White tufts in basal halves of veins arranged to collectively form a white, dorsal, more or less diagonal, transverse wing band; R<sub>2</sub> and other veins lacking gold hairs. Wing membrane dark brown with occasional clear areas. Wing length: 2.1 mm. Maximum wing width: 0.9 mm. ABDOMEN: First three abdominal tergites with long white hairs dorsally; scattered white hairs also present near posterior margin of last tergite. All other hairs very long and brown. Genitalia with conspicuous white hair tuft. LEGS: Hairs and scales mainly brown. Apical margin of hind femur, tibia, and first two tarsal segments with ring of white scales. Basal margin of hind tibia and first tarsal segment also with ring of white scales. Leg segments without conspicuous white scales scattered along their lengths.

<u>FEMALE</u>: HEAD: Vertex with conspicuous long white hairs. THORAX: When viewed from above, dorsal hairs forming a large, central, dark brown patch that is surrounded by a distinct and nearly continuous lateral margin of white hair tufts. WING: Hairs of

dorsal wing surface comparatively short, mostly brown or black. Midpoints and apices of many longitudinal veins with distinct tufts of white hairs that are not arranged in a regular transverse pattern;  $R_2$  and other veins lacking gold hairs. Wing membrane uniformly light brown. Wing length: 2.0 mm. Maximum wing width: 1.0 mm. ABDOMEN: Hairs on abdominal tergites brown. LEGS: Hairs and scales mainly brown. Apical margin of mid and hind tibiae ringed with white scales. First (basal) tarsal segment of midleg ringed with white scales apically and basally. Leg segments without conspicuous white scales scattered along their lengths.

MATERIAL EXAMINED: 1 male, 1 female.

### <u>Telmatoscopus</u> sp. 3

HEAD: Vertex with relatively long light brown hairs that appear black apically when viewed on their long axes. Antennae longer than wing, verticils very dense, brown. THORAX: Tergites with long light brown and white hairs as on head. WING: Hairs brown. Wing length: 2.3 mm. Maximum wing width: 1.0 mm. ABDOMEN: Hairs long, white, as on thorax, without distinct pattern of arrangement. LEGS: Hairs and scales brown. Scales of tibiae and tarsi appearing bright beige in lateral view. MATERIAL EXAMINED: 1 male.

## Diptera: Tipulidae

Identifications of the <u>Sigmatomera</u> species listed in Table 1 were based on Alexander's key and adult descriptions (Alexander 1914, 1920, 1930). The taxonomy of this genus is currently being reviewed by J. Gelhaus and S. Podenas (J. Gelhaus, pers. comm.). Species of <u>Sigmatomera</u> were not separable in the larval stage, but observations in the lab suggested that last instar larvae of <u>S</u>. <u>occulta</u> are more orange in color relative to the brownish larvae of the other two species. Adult males and females are similar with respect to distinguishing characteristics, including size. <u>Sigmatomera amazonica</u> is generally smaller than the other two species.

All of my S. amazonica and S. occulta material closely fit Alexander's (1930) key and descriptions (Alexander 1914, 1920). However, some of my S. séguyi specimens differ from the formal description (Alexander 1930) in that wing crossvein m-cu is located slightly basad or in line with fork of M, rather than slightly distal to it. Although not mentioned by Alexander, the wing tips (distal 15%) on all of my S. séguyi are darkened with grayish-brown shading.

## CONCLUSION

Water-filled tree holes on BCI (and other forested sites in central Panama) are occupied or visited by a diverse macrofauna that is dominated by detritivorous and omnivorous larval Diptera. Aside from Snow's (1949) study, this is the most comprehensive investigation of organisms using tree hole habitats in the neotropics. More intensive collections of larvae and adults are needed to complete the species list for some groups (e.g., Coleoptera: Scirtidae, Diptera: Ceratopogonidae). Detailed ecological information remains sparse for many tree hole species, especially secondary and facultative predators.

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Table 1. The macrofauna associated with water-filled tree holes on BCI based on 1994–1997 wet season samples from natural and artificial holes. Families and genera are listed alphabetically within higher taxa. Life stage = portion of life cycle that most commonly occurs in tree holes. Functions: O = omnivore, D = detritivore, P = predator, S = scavenger.

Taxon	Life Stage	Function	Association
NEMATOMORPHA			
Gordioidea			
Chordodidae	adult	n/a	accidental
ANNELIDA			
Oligochaeta			
Naididae			
Dero sp.	all	0	facultative

Taxon	Life Stage	Function	Association
ARTHROPODA			
Arachnida			
Scorpiones	all	Р	accidental
Araneae			
Ctenidae			
Cupiennius coccineus	all	Р	accidental
Pholcidae	all	Р	accidental
Opiliones			
Cosmetidae			
Cynorta sp.	all	Р	accidental
Crustacea			
Decapoda			
Gecarcinidae			
Gecarcinus sp.	adult	P, S	accidental

Taxon	Life Stage	Function	Association
Insecta			
Odonata			
Aeshnidae			
Gynacantha membranalis	larva	Ρ	specialist
Triacanthagyna dentata	larva	Р	specialist
Libellulidae			
Libellula sp.	larva	Р	facultative
Pseudostigmatidae			
Mecistogaster linearis	larva	Р	specialist
Mecistogaster ornata	larva	Р	specialist
Megaloprepus coerulatus	larva	Р	specialist
Hemiptera			
Veliidae			
Microvelia cavicola	all	P, S	specialist
Paravelia myersi	all	P, S	specialist

Taxon	Life Stage	Function	Association
Coleoptera			
Dryopidae			
Genus 1	larva	D	specialist
Dytiscidae			
Copelatus sp.	adult	Р	facultative
Laccophilus sp.	all	Р	accidental
Scirtidae (Helodidae)			
Prionocyphon spp.	larva	D	specialist
Scirtes sp. 1	larva	D	specialist
Scirtes sp. 2	larva	D	speciaiist
Diptera			
Ceratopogonidae			
Bezzia snowi	larva	Р	specialist
Culicoides spp.	larva	Р	facultative
Dasyhelea sp.	larva	<b>D</b> , O	specialist
Forcipomyia sp.	larva	0	facultative

Taxon	Life Stage	Function	Association
Chaoboridae			
Corethrella appendiculata	larva	Р	specialist
Chironomidae			
Chironomus sp.	larva	0	specialist?
Tanytarsini	larva	0	facultative
Culicidae			
Aedes septemstriatus	larva	0	facultative
Aedes terrens spp. complex	larva	Ο	specialist
Anopheles eiseni	larva	Ο	facultative
Culex allostigma	larva	O, P	facultative
Culex conservator	larva	Ο	specialist
Culex corrigani	larva	Ο	specialist
Culex mollis	larva	Ο	specialist
Culex secundus	larva	Ο	facultative
Culex urichii	larva	Ο	facultative
Haemagogus equinus	larva	Ο	specialist
Haemagogus lucifer	larva	Ο	specialist

.

Taxon	Life Stage	Function	Association
Haemagogus leucotaeniatus	larva	0	specialist
Limatus assuleptus	larva	Ο	facultative
Limatus durhamii	larva	Ο	facultative
Orthopodomyia fascipes	larva	Ο	specialist
Toxorhynchites theobaldi	larva	Р	specialist
Trichoprosopon digitatum	larva	O, P	facultative
Psychodidae			
<u>Telmatoscopus</u> sp. 1	larva	Ο	facultative
<u>Telmatoscopus</u> sp. 2	larva	Ο	facultative
<u>Telmatoscopus</u> sp. 3	larva	Ο	facultative
Stratiomyidae			
Zuercheria beguaerti	larva	D, O	facultative
Syrphidae			
Copestylum rafaelanum	larva	D, 0	specialist?
Species 1	larva	D, 0	specialist?

Taxon	Life Stage	Function	Association	
Tipulidae				
Sigmatomera amazonica	larva	D, P	specialist	
Sigmatomera occulta	larva	D, P	specialist	
Sigmatomera séguyi	larva	D, P	specialist	
VERTEBRATA				
Anura				
Dendrobatidae				
Dendrobates auratus	tadpole	Р	facultative	
Hylidae				
Agalychnis callidryas	tadpole	Ο	facultative	
Leptodactylidae				
Eleutherodactylus spp.	adult	adult P		
Physalaemus pustulosus	egg/tadpole	Ο	facultative	
Sauria				
Gekkonidae				
Sphaerodactylus lineolatus	adult	Р	accidental	

Table 2. Occurrence frequency and density of the macrofauna associated with water-filled tree holes on BCI based on 1994–1997 wet season samples from natural (Nat) and artificial (Art) holes. Frequencies: Nat = % of 206 natural holes in which a taxon was found, Art = % of 800 artificial hole censuses in which a taxon was found. Frequencies for <u>Megaloprepus coerulatus</u> are based on understory censuses only. \* = taxon occurred in some artificial holes, but not the subset used for frequency determination. Density = mean (SD) number of individuals per 0.5 liter where they occurred in 40 artificial and 100 natural holes. <sup>a</sup> = maximum number of individuals per hole. n/a = not present in the subset of holes used for density calculations. Density values for many taxa (e.g., odonates) cannot be extrapolated to larger holes (cf. Fincke 1998).

	Frequ	iency	Den	sity
Taxon	Nat	Art	Nat	Art
NEMATOMORPHA	1.5	0.3	2ª	2ª
ANNELIDA				
Dero sp.	24.8	1.9	16.5 (24.5)	13.3 (8.0)
ARTHROPODA				
Araneae				
Cupiennius coccineus	1.0	5.6	1ª	1ª
Opiliones				
<u>Cynorta</u> sp.	7.3	3.4	2ª	2ª

	Freq	uency	Der	isity
Taxon	Nat	Art	Nat	Art
Crustacea				
Gecarcinus sp.	0.5	0	1ª	0
Insecta				
Odonata				
Aeshnidae	13.1	16.6	1.4 (1.2)	n/a
Libellulidae				
<u>Libellula</u> sp.	0.8	0*	n/a	n/a
Pseudostigmatidae				
Mecistogaster spp.	37.4	37.5	1.4 (1.0)	1.3 (0.9)
Megaloprepus coerulatus	33.0	25.0	1.9 (1.2)	1.5 (0.7)
Hemiptera				
Microvelia cavicola	42.2	2.0	5.2 (5.8)	1.0
Paravelia myersi	4.4	1.3	0.9 (0. <b>9</b> )	2.0
Coleoptera				
Dryopidae	14.6	0. <b>9</b>	3.2 (2.5)	1.0
Dytiscidae				
Copelatus sp.	2.4	13.3	1.4 (0. <b>9</b> )	1.2 (0.5)
Scirtidae (Helodidae)	45.1	<b>42</b> .1	8.9 (11.7)	6.6 (5.3)

	Frequency		Density		
Taxon	Nat	Art	Nat	Art	
Diptera					
Ceratopogonidae					
Bezzia, Culicoides, Dasyhelea	33.0	20.4	11.7 (19.1)	10.9 (12.7)	
Forcipomyia sp.	8.3	7.4	9.8 (21.8)	9.7 (15.6)	
Chaoboridae					
Corethrella appendiculata	12.1	0.6	2.7 (1.6)	2.5 (0.7)	
Chironomidae	25.7	14.4	12.9 (9.0)	6.5 (6.3)	
Culicidae					
Aedes septemstriatus	5.3	0.4	3.1	1.7 (1.2)	
Aedes terrens spp. complex	51.0	71.3	6.7 (7.9)	10.6 (11.2)	
Anopheles eiseni	31.1	15.0	3.8 (4.7)	2.4 (2.2)	
Culex allostigma	1.0	2.3	5.0	2.2 (0.8)	
Culex conservator	19.9	3.1	4.7 (5.2)	3.7 (1.8)	
Culex corrigani	20.4	9.8	12.3 (14.6)	13.6 (17.2)	
Culex mollis	21.8	7.9	15.3 (29.4)	14.4 (10.1)	
Culex secundus & urichii	16.0	84.8	4.4 (4.5)	14.6 (22.1)	
Haemagogus equinus & lucifer	37. <b>9</b>	7.4	5.4 (6.4)	4.3 (4.5)	

	Freq	uency	Density		
Taxon	Nat	Art	Nat	Art	
Haemagogus leucotaeniatus	6.3	2.4	3.9 (2.1)	3.0 (1.4)	
Limatus assuleptus & durhamii	1.0	1.6	n/a	14.1 (12.8)	
Orthopodomyia fascipes	3.4	5.6	7.1 (2.9)	12.2 (18.3)	
Toxorhynchites theobaldi	8.3	1.5	2.0 (1.6)	1.0	
Trichoprosopon digitatum	1.0	3.4	2.1	2.3 (1.5)	
Psychodidae					
Telmatoscopus spp.	15.5	4.8	6.0 (7.4)	3.6 (2.8)	
Stratiomyidae					
Zuercheria bequaerti	<b>5</b> .3	0.6	1.4 (1.6)	n/a	
Syrphidae					
Copestylum rafaelanum	5.8	7.0	1.4 (0.8)	5.3 (6.1)	
Species 1	0.5	0	n/a	n/a	
Tipulidae					
Sigmatomera spp.	42.2	36.3	3.4 (3.3)	1.5 (1.0)	

	Freq	uency	Density		
Taxon	Nat	Art	Nat	Art	
VERTEBRATA					
Anura					
Agalychnis callidryas	2.9	0*	1.0	n/a	
Dendrobates auratus	6.3	3.9	1.8 (2.4)	1.6 (0.5)	
Eleutherodactylus spp.	1.9	3.5	1ª	1ª	
Physalaemus pustulosus	1.5	0*	11.4	n/a	
Sauria					
Sphaerodactylus lineolatus	0.5	2.6	1ª	2ª	



Fig. 1. Size (volume) distributions of natural and artificial tree holes examined in this study. n = 201 natural holes (5 of 206 holes not measured) and 153 artificial holes.

**CHAPTER 2** 

# COMMUNITY STRUCTURE IN WATER-FILLED TREE HOLES OF PANAMA: EFFECTS OF HOLE HEIGHT AND SIZE

This chapter is formatted for publication in the journal

<u>Selbyana</u>

## ABSTRACT

Abiotic conditions differ from canopy to understory in rain forests. Many taxa are more diverse or abundant in the canopy and/or have vertically stratified species distributions. I compared community parameters (species richness, abundance and composition) of tree hole macroorganisms at different heights (canopy, midstory, understory) in the forest of Barro Colorado Island, Panama. Data from multiple censuses of 40 artificial tree holes over 3 wet seasons (1995-97) revealed a small but significant decrease in the average number of species present with increasing height above the ground. Species richness and abundance were greater in larger holes. Similar patterns were observed in 206 natural tree holes. Of seven top predator species, one was not found in holes > 7 m above the ground; four other species occurred only in understory (ca. 1 m) holes. Chemical properties of tree hole water did not differ with height, but canopy tree holes dried out more frequently and were thermally less stable than midstory and understory holes. Harsh thermal conditions and higher disturbance frequency may be responsible for the decline in species richness with height.

## INTRODUCTION

Data from insecticide fogging and other collection methods (reviewed by Erwin 1995 and Basset et al. 1997) suggest that rain forest canopies harbor the bulk of global biodiversity and are more species-rich than lower forest levels (e.g., Erwin

1982, Sutton et al. 1983, Kitching et al. 1993). Forest canopies contain a variety of patchy microhabitats, such as litter packs (e.g., Longino & Nadkarni 1990, Nadkarni & Longino 1990), tank bromeliads (e.g., Pittendrigh 1948) and tree holes (e.g., Galindo et al. 1951) with partly or wholly concealed faunas that cannot be effectively sampled by fogging (Adis 1990, Stork & Hammond 1997). The biotic diversity of these habitats in the canopy and along vertical gradients within tropical forests is not well known, partly because data collection tends to be especially labor- and time-intensive (Tobin 1995).

Many organisms, including epiphytes (e.g., Pittendrich 1948, Hosokawa et al. 1964, Benzing 1995), invertebrates (e.g., Fichter 1939, Adams 1941, Wilson 1959, Longino & Nadkarni 1990, Hammond et al. 1997, Brühl et al. 1998), lizards (Reagan 1995), birds (Pearson 1971, Terborgh 1980) and mammals (Harrison 1962) have vertically stratified distributions in temperate and tropical forests. Among tropical insects, height-specific distributions are best known for sylvan mosquito species (e.g., Dunn 1927, Bates 1944, Causey & Dos Santos 1949, Mattingly 1949, Galindo et al. 1950, 1951, 1955, Trapido et al. 1955, Corbet 1964).

The forest canopy is drier, windier, and receives more light than lower forest levels (e.g., Allee 1926, Hosokawa et al. 1964, Chazdon & Fetcher 1984, Nadkarni 1994, Parker 1995). Differences in physiological tolerances among species with respect to one or more of these factors are generally thought to cause stratified distributions (see above references). Predation pressure (e.g., Corbet 1964, Papageorgis 1975), resource availability (e.g., Terborgh 1980, Basset 1992) and
distributions of preferred hosts (e.g., Galindo et al. 1950, Dunn et al. 1968) may also influence the vertical ranges of some species.

Tree holes are phytotelmata created by collections of rain water in tree cavities, and are important breeding sites for several neotropical pest species and disease vectors. As a result, most tree hole research has focused on the biology of economically important groups, especially mosquitoes. Community-level studies of Central American tree holes are few (e.g., Snow 1949), although tree hole predator assemblages in Panama have been well studied (e.g., Fincke 1992a, 1994, 1998, 1999). In understory tree holes, coexistence of major predators depends on asymmetrical interspecific competition and seasonal drying; abiotic factors other than hole volume are not predictive of species occupancy (Fincke 1998, 1999). Little is known of the abiotic and biotic factors affecting diversity in tropical tree holes at canopy and midstory heights.

Water-filled tree holes are useful for community ecology studies given their relatively small size, clearly defined boundaries and characteristic animal assemblages (e.g., Kitching 1971, 1983, Lounibos 1983, Nielsen 1990). Additionally, species inhabiting natural tree holes also colonize artificial container habitats (e.g., Pimm & Kitching 1987, Fincke et al. 1997, Srivastava & Lawton 1998, CHAPTER 1), thus providing a means to control multiple factors in ecological experiments.

The primary objective of this study was to determine how communities of macroorganisms in tree holes differ in terms of species richness, abundance and composition with vertical position in the forest. Two secondary objectives were to

assess the importance of tree hole size (volume) as a determinant of community structure, and to determine how abiotic conditions such as water chemistry and temperature vary with height above the ground.

### MATERIALS AND METHODS

I conducted this study in the lowland moist forest of BCI, Panama (see Leigh et al. 1996 for a site description). Water-filled tree holes are common and widely distributed in the BCI forest during the wet season (May-December), and they typically dry up completely by late dry season (i.e., March; Fincke 1992a). BCI tree holes range in volume from < 0.05 1 to > 50 1 (Fincke 1992a) and occur at all heights within the forest (pers. obs.). In this study, midstory and canopy tree holes were accessed using various modifications of the single-rope climbing technique (Perry 1978).

## Natural tree holes

I censused the macrofauna of 206 different natural tree holes on BCI during three wet seasons (May through December 1995, May through August 1996, and July through December 1997). Of these, 22 were at midstory height (10-20 m) and 30 were in the canopy (> 20 m). Height classes were based on the arbitrary vegetation divisions of Hubbell and Foster (1990). Although sample sizes were low at midstory and canopy heights, I censused these holes with greater frequency (up to 5 times per year) than understory holes (generally once or twice each season). Midstory and

canopy holes were found by: 1) searching for overflow stains on tree trunks or branches, 2) visually scanning nearby tree crowns while climbing for this and other projects, and 3) observing monkeys drinking from holes during their movements through the canopy.

When censusing holes, I first examined the contents with a flashlight and recorded all of the macroorganisms seen. I then removed the hole contents to a white plastic pan, using a siphon (2 cm inside diam) to empty large holes and a turkey baster to drain small holes. Most of the detritus and sediments remaining in a hole after water removal were hand-collected to a depth of ca. 3 cm (depending on hole morphology), and each hole was washed up to three times to dislodge animals clinging to its interior walls. I inspected the empty hole with a flashlight to spot elusive invertebrates such as odonate larvae (Fincke 1994). I collected subsamples of taxa that could not be positively identified in the field and returned the water, organisms, and detritus to the hole.

Height above the ground, maximum volume, and host tree species were recorded for the majority of holes censused. I estimated the volume of very large holes from their linear dimensions and shape. Volume was used as a measure of hole size because it is relatively easy to quantify in the field and is correlated with hole surface area (e.g., Fincke 1994).

## Artificial tree holes

In May 1995, I placed two different sizes of artificial tree holes (black plastic containers; small =  $0.65 \, \text{l}$ , medium =  $1.5 \, \text{l}$ ) in canopy trees of primary forest at three different heights ("understory":  $1.0-1.3 \, \text{m}$ ; "midstory":  $10-16 \, \text{m}$ ; and "canopy":  $21-35 \, \text{m}$ ). The factorial array of 6 artificial tree holes (2 sizes x 3 height classes) was replicated in each of 6 trees (<u>Ceiba pentandra [1]</u>, <u>Dipteryx panamensis</u> [1], <u>Hymenaea courbaril [1]</u>, <u>Pseudobombax septenatum [3]</u>), for a total of 18 small and 18 medium containers evenly distributed among the three heights. In addition, I placed one large (6.65 l) pan-shaped container at canopy height in each of the 6 trees.

I secured all artificial tree holes to tree trunks or branches with 6 mm polypropylene rope and soft wire bent to form hooks. Most (90%) of the artificial tree holes remained in place for the entire 3 yr span of this study. An alternative method—suspending containers from tree branches with a rope and pulley system (e.g., Loor & DeFoliart 1970)—proved inefficient because data were frequently lost due to disturbance from canopy mammals (pers. obs.) or spillage during collection.

I added leaf litter from the forest floor to each container at the time of setup and monthly thereafter during the 1995 wet season. Natural litterfall was allowed to accumulate in the holes. The initial quantity of litter placed in each container was ca. 33% of the total container volume (uncompressed litter measured in a large graduated cylinder), and within the range of litter densities observed in natural holes (pers. obs.). Subsequent litter inputs were  $\leq 10\%$  of total container volume. I added litter to containers in 1996 and 1997 only when their contents had been removed or

otherwise significantly disturbed between censuses. Each container was filled to capacity with rain water or filtered lake water, and a piece of tree bark was inserted to serve as an oviposition site for insect colonists.

I recorded the species composition and abundance of macroorganisms (animals with largest body dimension  $\geq 1.0$  mm) occurring in all artificial tree holes 20 times: every 14 d from late May to early December 1995 ( $\underline{n} = 13$  censuses); once monthly from May to August 1996 ( $\underline{n} = 3$  censuses); and once monthly from July to October 1997 ( $\underline{n} = 4$  censuses). For each census, the contents of a container were emptied into a white plastic pan and, after allowing the detritus to settle, the individuals of each species were counted. I then returned all of the material to the container. The time required to complete a census varied with container size and macroorganism abundance, but I spent no more than 60 min counting the contents of a single container. I censused all containers on a given tree on the same day.

Two of the large artificial tree holes fell from the canopy within 4 months of placement; the sections of rope securing them were cut by <u>Azteca</u> spp. ants. I subsequently inspected all other containers and replaced the rope with wire where necessary. All of the large containers were repeatedly disturbed and occasionally drained by monkeys (pers. obs.). I continued to refill and census these artificial holes because they provided useful qualitative species composition and species distribution information, but I decided <u>a priori</u> to exclude this size class from statistical analyses.

#### Abiotic factors

I measured pH in 194 natural holes (once each) and in all of the artificial tree holes on 8 different census dates using fresh broad- and narrow-range Hydrion® colorimetric test paper or a Corning® modular electronic meter, depending on availability. There was no difference among values obtained with both methods (paired t-test: t = 0.88, df = 14, P = 0.39). Conductivity and dissolved oxygen (DO) were measured in 86 natural holes and 36 of the 40 remaining artificial holes (once each) in 1997 with the electronic meter. Measurements were made at a depth of 4 cm or less (10 cm for conductivity) immediately prior to censusing the hole.

I measured diel variation in artificial tree hole water temperature along the vertical gradient by submerging a Taylor® minimum/maximum thermometer in each of the three medium volume containers on a tree for 24 h. I simultaneously measured min/max air temperatures with a second thermometer secured to the tree next to each medium container. This procedure was repeated twice for each tree; the complete data set spans three months of the 1995 wet season.

I conducted an experiment to determine: 1) if exposed (e.g., canopy) artificial holes dry out more frequently than understory holes, and 2) the minimum initial water volume that prevents an artificial hole from completely drying during the wet seasondry season transition. Plastic containers (= small artificial tree holes described above), each containing 100, 200, 300, 400 or 500 ml tap water were placed in forest understory. Four holes drilled in the side of each container prevented filling beyond experimental volumes, and container rims were trimmed so that they exceeded the

maximum water level by ca. 3 cm. A second group of five containers was placed in the lab clearing in direct sunlight, where temperature and humidity are similar to canopy conditions (S. Paton, personal communication). The presence or absence of water in each cup was recorded daily for 175 days beginning mid-August 1997.

Data were tested for normality with normal probability plots prior to analysis. The log(x+1) transformation was used to correct variance heterogeneity when necessary (Sokal & Rohlf 1981). All means presented in results are  $\pm$  SE and were calculated from untransformed data. Means were compared using a posteriori Ryan-Einot-Gabriel-Welsch multiple range tests (REGWQ option of GLM procedure; SAS 1989) when ANOVA results were significant.

#### RESULTS

#### Community parameters

Mean macroorganism species richness in natural tree holes was lower in the canopy than at midstory and understory heights (ANOVA:  $F_{2.74} = 4.07$ , P = 0.02; REGWQ test), but macroorganism abundance did not differ among height classes ( $F_{2.72} = 0.12$ , P = 0.88; TABLE 1). The number of species and individuals in a hole were correlated with hole size (richness:  $\underline{r} = 0.45$ ,  $\underline{n} = 201$ , P < 0.01; abundance:  $\underline{r} = 0.43$ ,  $\underline{n} = 185$ , P < 0.01). Larger holes generally contained more predator species, and tadpoles of <u>Agalychnis callidryas</u> and <u>Physalaemus pustulosus</u> only occurred in holes > 3 1. Effects of hole size on species richness and abundance did

not differ among height classes (ANCOVA; richness:  $F_{2,74} = 1.40$ , P = 0.25; abundance:  $F_{2,72} = 1.31$ , P = 0.28).

Average species richness in artificial tree holes differed among height classes (repeated-measures ANOVA;  $F_{2,30} = 3.78$ , P = 0.03), and was lower in midstory and canopy than in the understory (REGWQ test on overall means; FIGURE 1). Macroorganism abundance did not differ among heights ( $F_{2,30} = 0.92$ , P = 0.41; FIGURE 1). Medium volume artificial holes contained more species ( $F_{1,30} = 4.99$ , P = 0.03) and individuals ( $F_{1,30} = 26.6$ , P < 0.01) than small holes (FIGURE 2). There were no interactions among main effects of height and volume for species richness and abundance in artificial tree holes (2-way repeated-measures ANOVA; richness:  $F_{2,30}$ = 0.13, P = 0.87; abundance:  $F_{2,30} = 0.42$ , P = 0.66), and time\*treament effects were not significant (P > 0.25 for all tests).

About 60 species of macroorganisms (mostly aquatic insects) are associated with water-filled tree holes on BCI (CHAPTER 1). Of these, larvae and pupae of two mosquito species, <u>Haemagogus lucifer</u> (TABLE 2) and <u>Trichoprosopon digitatum</u>, were found only in understory tree holes. These distributions generally agree with the findings of Galindo et al. (1950, 1951). Larvae of the giant damselfly <u>Megaloprepus</u> <u>coerulatus</u> were not found in artificial or natural holes above 7 m. Artificial holes lacking <u>M</u>. <u>coerulatus</u> typically were occupied by other top predator species, and there was a negative association between the presence of <u>M</u>. <u>coerulatus</u> and <u>Mecistogaster</u> spp. in natural holes (G = 5.14, df = 1, P < 0.025). The phantom midge <u>Corethrella appendiculata</u> (TABLE 2) and the frog <u>P</u>. <u>pustulosus</u> only occurred in

understory holes. Mean species richness did not differ between similar size holes with and without <u>C</u>. <u>appendiculata</u> (t = 1.70, df = 35, P = 0.10). All other species were represented by at least one individual at all heights.

<u>Trichoprosopon digitatum</u> occurred in artificial holes only during the first census date and was very rare in natural tree holes (2 of 206 holes). Species of <u>Haemagogus</u> could not be differentiated based on larval and pupal characters in the field. However, adults reared from subsamples indicated that <u>H</u>. <u>lucifer</u> was replaced by <u>H</u>. <u>equinus</u> at higher forest levels. Subsample rearings also indicated that these two species do not co-occur in understory holes, but more consistent and thorough sampling is required before drawing conclusions about their distributions. The frog <u>P</u>. <u>pustulosus</u> did not colonize the artificial tree holes used in this study and only occurred in three natural holes.

#### Abiotic factors

Average DO content of water in natural tree holes increased with height (FIGURE 3), but conductivity and pH were similar among height classes (ANOVA; conductivity:  $F_{2,83} = 1.98$ , P = 0.14; pH:  $F_{2,191} = 2.14$ , P = 0.12; TABLE 1). Chemical parameters were not correlated with natural tree hole volume (APPENDIX A). The DO content of artificial tree hole water was lower in the understory than in midstory or canopy (FIGURE 4). Conductivity did not differ among heights or sizes of artificial tree holes (2-way ANOVA; height:  $F_{2,28} = 0.39$ , P = 0.68; size:  $F_{1,28} = 0.01$ , P = 0.96; height\*size:  $F_{2,28} = 0.51$ , P = 0.60), nor did average pH (2-way repeated-measures ANOVA; height:  $F_{2,30} = 0.87$ , P = 0.43; size:  $F_{1,30} = 0.15$ , P = 0.71; height\*size:  $F_{2,30} = 0.43$ , P = 0.66; time\*treatment: P > 0.25 for all tests).

Minimum artificial tree hole water temperatures were similar to air temperatures and did not differ among height classes (2-way ANOVA; air/water:  $F_{1,30}$ = 0.20, P = 0.66; height:  $F_{2,30}$  = 0.10, P = 0.90; air/water\*height:  $F_{2,30}$  = 0.31, P = 0.73), but maximum temperatures were lower in the understory than in midstory and canopy (FIGURE 5). Natural tree hole temperatures were not measured in the canopy with sufficient consistency for statistical analysis, but water temperatures of both natural and artificial holes in the canopy often exceeded 40°C on cloudless days. The water temperature of natural tree holes in the understory was consistently lower (24.7°C ± 0.14) than the surrounding air temperature (26.7°C ± 0.22) during daylight hours (paired t-test: t = 11.1, df = 37, P < 0.0001).

Small exposed holes were dry more often than large understory holes in the drying experiment (FIGURE 6), and the average maximum number of consecutive dry days was greater for containers in the clearing  $(14.0 \pm 1.2)$  than in the understory  $(6.0 \pm 2.7; t = 2.7, df = 6, P = 0.018)$ . Although not quantified, natural tree holes in the canopy dried out faster and with greater frequency than understory holes of similar volume. Of the artificial tree holes used in the vertical stratification portion of this study, three midstory containers and five canopy containers were completely dry on one or more census dates. None of the understory containers dried out during the wet season. The number of species in a container on a dry census date  $(1.6 \pm 0.73)$ 

was less than the number observed on the previous wet census date (4.9  $\pm$  0.83; paired t-test: t = 6.2, df = 7, P < 0.001).

#### DISCUSSION

Results of this study show that species richness and abundance tend to decline with the height of a hole above the forest floor. Galindo et al. (1950, 1951, 1955) and Trapido et al. (1955) conducted similar vertical stratification studies in Panama. Although they did not sample natural tree holes in the canopy or record distributions of non-mosquito taxa, their results for open-top containers in lowland forest show larger cumulative numbers of mosquito species in the understory than in midstory or canopy (Galindo et al. 1951:107-108). Kitching (1971) and Copeland (1989) also surveyed insects of tree holes > 2 m above the ground as part of their temperate zone investigations. Kitching observed larger densities of selected taxa in tree holes above 2 m than in holes closer to the ground, and suggested differences in nutrient quality and relative organic matter content as mechanisms for this pattern. Copeland did not address the distribution of tree hole insects with respect to vertical location.

## Drying and temperature disturbance

Frequent drying and high temperatures in canopy holes relative to holes at lower levels are probable causes for the decline in species richness with height. The drying experiment showed (not surprisingly) that the dehydration frequency of tree holes is greater in exposed sites than in the understory. These results are conservative

because natural tree holes in both canopy and understory dried out more often than the cups used in the experiment. I attribute this difference to several factors, including: 1) wicking effects of wood lining natural holes, 2) windier and brighter wet season conditions of the forest canopy vs. the lab clearing (S. Paton, personal communication), 3) absorption of tree hole water by host trees (Lacmellea panamensis, <u>Macrocnemum glabrescens</u> and other species send adventitious roots into their tree holes), and 4) frequent drinking of tree hole water by arboreal and terrestrial mammals. Regardless of differences in drying dynamics between containers and natural holes, the experimental results provide an estimate of relative drying frequency in the canopy vs. understory.

Habitat drying is a catastrophic disturbance for many aquatic organisms and significantly affects tree hole community structure (Bradshaw & Holzapfel 1983, 1988, Lounibos 1985). Although some species of tree hole invertebrates have life history or other adaptations enabling them to survive periodic dehydration (e.g., Lounibos 1985, Bradshaw & Holzapfel 1988, Fincke 1994, Juliano & Stoffregen 1994, Sota et al. 1994), the net effect of drying is reduced species richness of aquatic organisms (see RESULTS). Tree hole drying may also reduce faunal diversity indirectly through secondary predation and minimal colonization. Ants, birds, mammals, and the crab <u>Gecarcinus</u> sp. commonly forage for insect larvae in moist or recently dry tree holes (Fincke 1992b, CHAPTER 1), thereby depressing the abundance and diversity of organisms present in the hole when it is refilled. In addition, desiccation kills eggs of some tree hole mosquitoes (Bates 1949, Galindo et al. 1955),

and potential colonists may completely avoid or deposit fewer eggs in sites lacking water (e.g., Frank et al. 1976), especially if wet holes are available nearby (e.g., at lower levels in the forest).

Prolonged or frequent dry periods can influence nutrient availability in small aquatic habitats (Bärlocher et al. 1978, Aspbury & Juliano 1998). Allochthonous detritus (mainly leaf litter) is the energy base for food webs in water-filled tree holes (Kitching 1971) and is generally degraded by the activities of bacteria and fungi, which are consumed by grazing and filter-feeding macroinvertebrates (Fish & Carpenter 1982, Walker & Merritt 1991). Larvae of several insect species also feed directly on decaying plant material in tree holes (e.g., Paradise & Dunson 1997a, CHAPTER 1). The species richness of macroinvertebrates in tree holes is influenced by leaf litter characteristics (CHAPTER 4), and drying may affect litter quality by altering decay processes (e.g., Cornejo et al. 1994, Taylor & Bärlocher 1996, Taylor 1998) or nutrient content (e.g., Bärlocher et al. 1978). Litter drying reduces the productivity of decomposer microbes (Clein & Schimel 1994) and the macroinvertebrates that consume them (e.g., Aspbury & Juliano 1998) when the litter is subsequently immersed in rain water. Thus, the repeated dehydration that occurs in canopy tree holes may depress diversity by altering the quality of resources available to the community when wetter conditions return.

Temperature is a potentially limiting ecological factor in aquatic environments (e.g., Nebeker & Lemke 1968, Sweeney & Vannote 1986, Corkum & Hanes 1992). Temperatures observed in canopy tree holes exceeded the thermal tolerances reported

for many mosquito species (reviewed by Bates 1949). Extreme temperatures also reduce egg hatching success in aquatic insects (Sweeney 1984), and some diurnallyactive tree hole mosquitoes deposit fewer eggs at higher environmental temperatures (Bates 1947). Although these direct and indirect effects were not quantified in this study, it is likely that high temperatures in canopy tree holes contributed to lower macrofauna diversity through differential mortality and colonization success among species.

The nutritional quality of leaf litter is also influenced by thermal conditions. Some microbial processes (i.e., enzyme activity; Sinsabaugh et al. 1981) associated with litter decomposition are temperature-sensitive (reviewed by Webster & Benfield 1986). If extremely high or variable temperatures reduce microbial productivity or alter microbial species compositions, secondary consumers (e.g., mosquitoes) may become food-limited and locally extinct, ultimately depressing diversity.

#### Species distributions

Only five species had vertically restricted distributions, making the regional pool of tree hole-associated taxa larger in the understory than at other levels in the forest. This difference had no direct effect on mean species richness in tree holes at different heights. Two of the understory species (<u>T</u>. <u>digitatum</u> and <u>P</u>. <u>pustulosus</u>) were rare, and removing them from the analysis did not change the outcome. These species were uncommon because they are not obligate tree hole inhabitants. Water-filled fruit husks (e.g., of <u>Tontelea ovalifolia</u>) and fallen palm spathes are preferred

oviposition sites for <u>T</u>. <u>digitatum</u> (Zavortink et al. 1983); <u>P</u>. <u>pustulosus</u> breeds in a variety of habitats (Rand 1983), and is relatively infrequent in BCI tree holes (e.g., Fincke 1999, CHAPTER 1). <u>Haemagogus lucifer</u> (an obligate container breeder) appeared to be replaced by the congener <u>H</u>. <u>equinus</u> in canopy and midstory tree holes.

<u>Megaloprepus coerulatus</u> is one of 7 top predator species found in BCI tree holes (Fincke 1999). Top predators tend to reduce the number of co-occurring prey species (CHAPTER 6), and secondary predators like <u>C</u>. <u>appendiculata</u> may have similar effects on diversity in tree holes. Intraguild predation stabilizes the number of top predator species within a hole, and coexistence of predators is mediated by hole volume (e.g., Fincke 1994, 1999). While <u>M</u>. <u>coerulatus</u> and <u>C</u>. <u>appendiculata</u> contribute to the larger species pool of potential tree hole colonists in the understory, they are also likely to reduce species richness in individual holes through prey consumption and exclusion of other predators through intraguild predation. Thus, the negative effects of these species on local diversity in tree holes probably balances their contribution to the regional species pool in the understory.

#### Physicochemical factors

The positive correlations of species richness and abundance with hole volume shown in this study are typical of phytotelmata (e.g., Smart 1938, Frank et al. 1977, Bradshaw & Holzapfel 1988, Sota 1996, Fincke 1999). Larger containers are larger targets for colonization, and some colonists adjust the number of eggs they deposit

based on water volume (Frank et al. 1976, Fincke 1992b). Larger phytotelmata tend to collect more nutrients in the form of leaf litter (e.g., Sota 1996). This results in a correlation between the size of the energy base and the size of the habitat, and leads to greater species diversity and abundance in larger holes (Wright 1983, Kitching & Pimm 1985, Srivastava & Lawton 1998). In addition, top-down effects of individual predators on diversity and abundance should increase in strength with decreasing volume both among holes and within a drying hole (e.g., Fincke 1994). The probability that a hole will dry out is inversely related to its volume (e.g., Sota et al. 1994), and drying frequency affects the abundance and diversity of species present (discussed above).

Conductivity and pH had no significant influence on community properties of BCI tree holes, and did not vary predictably with hole size or height above the ground (APPENDIX A). This result was expected; larvae of most tree hole species are tolerant of a broad range of chemical conditions (Bates 1949, Snow 1949, Petersen & Chapman 1969, Fincke 1999; but see Paradise & Dunson 1997b). The increase in DO concentration with increasing height is attributed to algal growth in the artificial tree holes and windier conditions in the midstory and canopy. Filamentous algae also occurred in some natural tree holes of the canopy. DO is typically very low in tree holes, and most macroinvertebrate larvae in tree holes breathe atmospheric oxygen through siphons and other morphological adaptations.

#### CONCLUSION

Water-filled tree holes on BCI do not follow the pattern of relatively high arthropod diversity in tropical forest canopies shown by fogging and related studies. The species pool of potential tree hole colonists was slightly larger in the understory, and the diversity of macroorganisms in individual tree holes generally decreased with the height of a hole above the forest floor. Higher disturbance frequency (i.e., frequent drying and extreme water temperatures) in the forest canopy is the most parsimonious explanation for the latter pattern, but this conclusion awaits further experimental evidence. Many avenues of investigation remain open with respect to tree hole community structure, and studies addressing the indirect effects of drying and predator distributions on community properties should provide interesting results.

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TABLE 1. Means ( $\pm$  SE) of community parameters and abiotic factors in natural tree holes at different height classes. Not all variables were measured in all holes; ranges in brackets, samples sizes in parentheses.

	Understory	Midstory	Canopy
Richness	5.9 ± 0.17	4.3 ± 0.74	3.1 ± 0.34
	[1 - 14]	[0 - 10]	[0 – 6]
	(154)	(22)	(30)
Abundance	31.7 ± 2.36	30.3 ± 8.16	41.4 ± 14.2
	[1 - 153]	[0 – 147]	[0 - 373]
	(150)	(18)	(28)
Volume (l)	1.08 ± 0.23	5.81 ± 2.3	1.77 ± 0.78
	[0.02 - 29.8]	[0.02 - 45.8]	[0.013 - 12.8]
	(154)	(22)	(25)
<b>Conductivity</b> ( $\mu$ S)	286 ± 23.5	462 ± 194	324 ± 34.4
	[55 – 951]	[117 – 1982]	[146 - 540]
	(65)	(9)	(12)
pH	5.96 ± 0.06	6.34 ± 0.17	6.06 ± 0.12
	[3.4 - 8.0]	[5.0 – 7.5]	[5.0 – 7.0]
	(150)	(20)	(24)

TABLE 2. Percent occurrence of selected taxa in natural (Nat) and artificial (Art) tree holes among the three forest height classes. Values are cumulative frequencies for natural holes and means across 3 yrs for artificial holes. Data from multiple censuses of a single hole were pooled. Sample sizes in parentheses.

	Under	story	Mids	story	Car	юру
	Nat	Art	Nat	Art	Nat	Art
Taxon	(154)	(12)	(22)	(12)	(30)	(16)
Annelida: Oligochaeta	<u>.                                    </u>				<u> </u>	
Dero sp.	28	42	14	8	10	0
Odonata: Aeshnidae	10	36	36	22	7	21
Odonata: Pseudostigmatidae						
Mecistogaster spp.	40	71	50	86	13	69
Megaloprepus coerulatus	32	24	0	0	0	0
Diptera: Ceratopogonidae						
Bezzia snowi	36	44	18	78	7	56
Forcipomyia sp.	10	25	5	36	10	40
Diptera: Chaoboridae						
Corethrella appendiculata	14	8	0	0	0	0

# TABLE 2. Continued.

	Understory		Midstory		Canopy	
	Nat	Art	Nat	Art	Nat	Art
Taxon	(154)	(12)	(22)	(12)	(30)	(16)
Diptera: Chironomidae						
Chironomus sp.	29	61	9	39	3	33
Diptera: Culicidae						
Aedes terrens spp. complex	53	94	45	81	20	87
Anopheles eiseni	33	53	36	53	3	42
Culex conservator	22	8	14	25	7	13
<u>C</u> . <u>corrigani</u>	22	33	23	25	3	19
<u>C</u> . mollis	12	22	9	1 <b>9</b>	3	4
<u>C</u> . <u>urichii</u>	12	<del>9</del> 7	14	94	30	96
Haemagogus (H.) spp.	27	42	33	58	34	56
Toxorhynchites theobaldi	5	6	5	3	3	8
Diptera: Psychodidae						
Telmatoscopus spp.	7	8	14	6	3	6
Diptera: Stratiomyidae						
Zuercheria bequaerti	3	3	27	3	3	4

## TABLE 2. Continued.

	Understory		Midstory		Canopy	
	Nat	Art	Nat	Art	Nat	Art
Taxon	(154)	(12)	(22)	(12)	(30)	(16)
Diptera: Syrphidae						
Copestylum rafaelanum	5	1 <b>9</b>	5	16	13	38
Diptera: Tipulidae						
Sigmatomera spp.	40	72	59	78	20	62
Hemiptera: Veliidae						
Microvelia cavicola	35	14	50	14	3	6
Paravelia myersi	3	0	14	8	3	8
Coleoptera: Dryopidae	12	0	36	11	7	2
Coleoptera: Scirtidae						
Prionocyphon sp.						
and Scirtes spp.	43	72	50	83	33	62
Anura: Dendrobatidae						
Dendrobates auratus	5	14	5	8	13	10

#### FIGURE LEGENDS

FIGURE 1. Mean ( $\pm$  SE) macroorganism species richness (A) and abundance (B) in artificial tree holes at three heights. The sharp increase in abundance on census 17 was caused by excessive nutrient inputs—fruits of <u>Gustavia superba</u> fell into understory holes 2 wks prior to that census.

FIGURE 2. Mean ( $\pm$  SE) macroorganism species richness (A) and abundance (B) in different sizes of artificial tree holes.

FIGURE 3. Mean (+ SE) dissolved oxygen content of natural tree holes in different height classes. ANOVA:  $F_{2,83} = 6.22$ , P < 0.01. Horizontal lines connect means that are not significantly different. Understory <u>n</u> = 65, midstory <u>n</u> = 9, canopy <u>n</u> = 12.

FIGURE 4. Mean (+ SE) dissolved oxygen content of small (open bars) and medium (shaded bars) artificial tree holes in different height classes. Two-way ANOVA; height:  $F_{2,28} = 5.51$ , P = 0.01; size:  $F_{1,28} = 0.01$ , P = 0.94; height\*size:  $F_{2,28} = 0.75$ , P = 0.48. Horizontal lines connect means that are not significantly different. FIGURE 5. Mean (+ SE) maximum air (open bars) and water (shaded bars) temperatures in different height classes of artificial tree holes. Two-way ANOVA; height:  $F_{2,30} = 27.8$ , P < 0.001; air/water:  $F_{1,30} = 2.49$ , P = 0.13; height\*air/water:  $F_{2,30} = 0.07$ , P = 0.93. Horizontal lines connect means that are not significantly different.

FIGURE 6. Number of days that containers of different volume and exposure level (understory = open bars, clearing = shaded bars) were completely dry out of 175 observations.  $X^2 = 35.14$ , df = 5, P < 0.0001.












FIGURE 4











**CHAPTER 3** 

# DISTRIBUTION AND ABUNDANCE OF MICROVELIA CAVICOLA

## POLHEMUS (HETEROPTERA: VELIIDAE) ON

### BARRO COLORADO ISLAND, PANAMA

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#### ABSTRACT

Data and observations from 1995, 1996 and 1997 wet season surveys of natural and artificial tree holes suggest that hole morphology and size are important predictors of the distribution and abundance of <u>Microvelia cavicola</u> Polhemus. The bugs occurred in 42% of the natural holes sampled and < 3% of artificial hole censuses. The distribution of <u>M. cavicola</u> was not associated with holes in particular tree species or with hole height above the ground. Abundance increased with hole size and decreased slightly with hole height. Analyses of presence/absence data showed significant within- and between-year microhabitat fidelity in this species. <u>Microvelia cavicola</u> reduced the emergence success of mosquitoes in a laboratory experiment, but their quantitative effect on mosquitoes in under natural conditions is unknown. The foraging behavior of <u>M. cavicola</u> is similar to that described for other <u>Microvelia</u> species.

#### INTRODUCTION

Tree holes are phytotelmata (i.e., plant-held waters) formed by the collection of rainfall in rot holes or other cavities in the woody portions of trees (e.g., Kitching 1971). A variety of macroorganisms use tree holes as breeding sites, and more than 50 invertebrate species are directly or indirectly associated with water-filled tree holes in Panama (CHAPTER 1). The behavior and ecology of some of these taxa, such as odonates (e.g., Fincke 1992a, 1992b, 1994, 1998) and mosquitoes (e.g., Galindo et al. 1950, 1951, 1955) are well documented. Aside from Snow's (1949) work in

Guatemala, relatively little is known about the biology of the many other invertebrates that use this habitat.

Numerous biotic factors, including predation (e.g., Bradshaw & Holzapfel 1983, CHAPTER 6), competition (e.g., Fincke 1992b, Juliano 1998), and nutrient availability (e.g., Srivastava & Lawton 1998, CHAPTERS 4 and 6), may affect the presence or absence of invertebrate species in water-filled tree holes and similar habitats. In Panama, abiotic characteristics of tree holes, such as size, shape, disturbance frequency, and height above the ground, additionally influence the distributions of some organisms (e.g., Galindo et al. 1951, Fincke 1998, 1999, CHAPTER 2).

Two genera of neustonic true bugs (Heteroptera: Veliidae), <u>Microvelia</u> Westwood and <u>Paravelia</u> Breddin, live and breed in neotropical phytotelmata (e.g., Drake & Hussey 1954, Polhemus & Polhemus 1991), including water-filled tree holes. The goals of this study were to: 1) describe the abundance, local distribution and behavior of a new <u>Microvelia</u> species, <u>M. cavicola</u> (Polhemus, in review), discovered in water-filled tree holes of Panama; and 2) determine how the distribution of <u>M. cavicola</u> is related to factors such as tree species, hole volume, and hole height above the ground.

#### METHODS

This study was conducted in the lowland moist forest of Barro Colorado Island (BCI), Panama (see Leigh et al. 1996 and Leigh 1999 for site description). Tree holes, the only known natural habitat of <u>M</u>. <u>cavicola</u>, are common and broadly distributed in the BCI forest. Most holes contain water throughout the wet season (May through December) and many (especially small or exposed holes) dry completely by March (Fincke 1992b).

I collected all individuals of <u>M</u>. <u>cavicola</u> used in this study with an aspirator from the tree hole water surface and interior walls. Live specimens were transported to the laboratory for identification and behavioral observations. Only a portion of the population within a hole was removed on a collection date. Voucher specimens were killed in 80% ethanol, mounted on points, and deposited as allotypes (Polhemus, in review).

#### <u>Field</u>

I recorded the abundance of <u>M</u>. <u>cavicola</u> in artificial and natural tree holes on BCI during three consecutive wet seasons: May-December 1995, May-August 1996, and July-December 1997. I censused the same artificial holes (0.65 l and 1.5 l black plastic containers filled with leaf litter, rain water, and an emergent piece of tree bark) every year as part of a vertical stratification study (CHAPTER 2). Natural holes were censused in a variety of tree species, but most sampled holes were in <u>Dipteryx</u> <u>panamensis</u> (Pitt.) (20%), two free-standing <u>Ficus</u> species (<u>F</u>. <u>insipida</u> Willd. and <u>F</u>. <u>voponensis</u> Desv.; 16%), <u>Gustavia superba</u> (H.B.K.) (8%), or <u>Platypodium elegans</u> Vogel (28%). I determined volumes of most of the natural holes by removing water with a turkey baster into a graduated cylinder. Volumes of the largest holes were estimated from dimensions of appropriately shaped polygons. Tree hole height and volume were not correlated (Pearson  $\mathbf{r} = 0.164$ , P = 0.13, n = 87). Volume was used as a measure of overall hole size for analyses because volume and surface area are correlated in natural holes (e.g., Fincke 1994). Additional methodological details and summaries of tree hole characteristics are presented elsewhere (CHAPTER 2).

Host-tree specificity was analyzed with a chi-square test using <u>M</u>. <u>cavicola</u> presence/absence data for natural holes in the tree species listed above (Ficus spp. pooled for analysis). I only used data from the first occurrence of <u>M</u>. <u>cavicola</u> in each hole to prevent pseudoreplication (Hurlbert 1984) and avoid possible effects of disturbance caused by hole sampling.

During 1996 field surveys, it appeared that a hole containing <u>M</u>. <u>cavicola</u> on the first sample date was more likely to contain this species on later sample dates than holes from which it was initially absent. Two separate chi-square analyses were used to address this site fidelity question. The first chi-square tested short term site fidelity with the null hypothesis of no association between presence of the bugs in the first sample of a hole and any of the four subsequent samples (pooled) for the 1996 natural hole data. In the same manner, the second chi-square tested for long term site fidelity among 25 holes sampled in both 1995 and 1996. The expected value for each cell in the contingency tables was the product of presence/absence probabilities determined

from the total number of holes sampled in each census period. Data from 1997 censuses were excluded because some holes were frequently disturbed or manipulated for experiments.

#### Laboratory

I maintained several subpopulations of <u>M</u>. <u>cavicola</u> in clear glass or plastic dishes in the laboratory ( $24 \pm 0.5$  °C; irregular lighting conditions) for observations of feeding behavior and adult longevity. Each culture dish (ca. 10 cm diam., 1.5 cm deep) was filled to a depth of 0.5 cm with rain water and contained  $\leq$  30 bugs. Cork disks (10 mm diam, 2 mm thick) served as a perching sites and the dishes were kept covered except during observations. I supplied live food (one dealated tortricid moth per dish) weekly. I removed prey remains after 24 hr, and replaced the dish water every 7-10 d to prevent fungal growth. Lab populations of <u>M</u>. <u>cavicola</u> were maintained for up to 60 d before all cultures were terminated.

I conducted an experiment to determine the effects of <u>M</u>. <u>cavicola</u> on mosquito emergence success. I placed 10 adult <u>M</u>. <u>cavicola</u> starved  $\geq 3$  d in a plastic cup (8.5 cm ht., 5.5 cm diam.) containing 50 ml rain water, a stick perch and four pupae of the tree hole mosquito <u>Culex urichii</u> (Coquillett). Control cups lacked <u>M</u>. <u>cavicola</u>. I replicated the experiment seven times, and used new predators and prey in each replicate. Cups were individually covered with netting (0.5 mm mesh) to prevent escape of veliids and adult mosquitoes. I checked each cup at least twice daily and terminated the experiment when all mosquitoes emerged or were dead (ca. 2 d). I

analyzed the number of mosquitoes surviving to adulthood in each cup with a t-test assuming unequal variance.

#### RESULTS

<u>Microvelia cavicola</u> occurred in 87 (42%) of the 206 different natural holes sampled over the three years. Their presence or absence showed no association with holes in particular species of trees ( $\chi^2 = 5.35$ , df = 3, P > 0.10). Half (53%) of the natural holes in which <u>M</u>. <u>cavicola</u> was found contained 3 or more individuals, and their mean (± SE) abundance was 10.5 ± 1.5 individuals per hole. The average density of <u>M</u>. <u>cavicola</u> (individuals per cm<sup>2</sup> surface area; ± 1 SE) was greater in highly shaded holes (0.13 ± 0.03; n = 29) than in more exposed holes (0.03 ± 0.01; n = 25, t = 2.66, P = 0.01). The most <u>M</u>. <u>cavicola</u> recorded in a census (70) occurred in a large, shaded, pan-shaped hole in the base of a mature <u>Pseudobombax</u> <u>septenatum</u> (Jacq.).

Adults and immatures of <u>M</u>. <u>cavicola</u> were found at all heights and in all size classes of holes censused except those < 100 ml in volume (Fig. 1). They occurred in 10 (1.9%) of the 520 artificial hole censuses in 1995, 3 (2.5%) of 120 censuses in 1996, and 3 (1.9%) of 160 censuses in 1997. Their abundance never exceeded 2 individuals per artificial hole.

Natural holes containing <u>M</u>. <u>cavicola</u> were larger than holes lacking the bugs (t = 5.86, P < 0.0001). Abundance of <u>M</u>. <u>cavicola</u> in natural holes increased with hole volume and showed a weak negative relationship with hole height (Fig. 1, Table 1).

However, exclusion of data from holes > 17 m above the ground (n = 4) nullified the significant slope of the height-abundance relationship (t = -1.70, P > 0.05).

Of the 43 natural holes repeatedly sampled in 1996, 15 (35%) contained one or more bugs on the first sample date and 22 (51%) never contained <u>M. cavicola</u>. The association between their presence in a hole on the first sample date and any subsequent sample date was significantly different from random ( $\chi^2 = 10.95$ , df = 1, P < 0.001). Of the 25 natural holes censused in both 1995 and 1996, 11 contained <u>Microvelia</u> in 1995. Nine (82%) of these 11 again contained <u>M. cavicola</u> in 1996. This association was also significantly different from random ( $\chi^2 = 15.62$ , df = 1, P < 0.001).

Mosquito emergence success was reduced by <u>M</u>. <u>cavicola</u> in the laboratory-based predation experiment (Fig. 2). The density of veliids used in the experiment (0.42 cm<sup>-2</sup> surface area) was greater than the mean ( $\pm$  SE) density for small natural holes (area < 200 cm<sup>2</sup>; 0.14 ± 0.04, n = 15), but was still less than the maximum density (0.46 cm<sup>-2</sup>) of <u>M</u>. <u>cavicola</u> observed in the field. This artificially high predator abundance was balanced by the experimental prey density (0.08 ml<sup>-1</sup>), which was greater than the mean ( $\pm$  SE) mosquito density (0.02 ± 0.006; n = 15, including larvae and pupae) observed in small natural holes.

Examination of mosquito remains indicated that most were attacked shortly after exposure of the thorax during emergence. On several occasions, I observed  $\underline{M}$ . <u>cavicola</u> probing the thoracic horns of mosquito pupae visiting the surface, and in every case the pupae responded by immediately descending into the water column.

Six pupae in the predator treatment (none in controls) were found dead with no evidence of an emergence attempt (i.e., death by drowning).

In field and lab feeding trials, <u>M</u>. <u>cavicola</u> rapidly recruited to prey struggling in the surface film. Prey were quickly subdued and their activity typically stopped within 1 min after arrival of the first bug. In the lab, more than 20 <u>M</u>. <u>cavicola</u> were observed to feed simultaneously on a single moth by initially aggregating side to side and then climbing over one another as foraging space became limited. The last few individuals to arrive at a prey item were often denied access by interference from those already feeding. Although aggressive interactions among individuals were frequent in the lab and the field, there was no evidence of cannibalism, even during prolonged periods (i.e., > 10 d) of food limitation. Only one of the > 200 <u>M</u>. <u>cavicola</u> kept in captivity died during the 60 d study period.

#### DISCUSSION

Hole morphology, exposure and size appear to be important factors limiting the distribution of <u>M</u>. <u>cavicola</u> in the BCI forest. Their greatest density occurred in slit-type natural holes (see Fincke 1992b for classification) or bowl-type holes with steep sides and a shaded or concealed water surface. The low occurrence (and abundance) of veliids in artificial tree holes was probably because the water surface in the containers was more exposed than in natural holes. Where <u>M</u>. <u>cavicola</u> did occur, its abundance increased with hole size (Fig. 1). Mosquitoes, which are potential prey for veliids (Frick 1949; this study), also tend to increase in abundance with hole

volume (e.g., Lounibos 1983). Although larger holes are often more exposed, the greater (in absolute terms) availability of prey and spatial resources probably facilitates the coexistence of large numbers of <u>M</u>. <u>cavicola</u>, which interact aggressively when food is scarce.

The distribution of <u>M</u>. <u>cavicola</u> in the BCI forest differs markedly from that of the Old World veliid <u>Cylicovelia kenyana</u> Polhemus & Copeland, the only other tropical tree hole species for which local occurrence and abundance data have been collected. Polhemus and Copeland (1996) found a significant positive association between hole height and the presence of <u>C</u>. <u>kenyana</u>, but only sampled holes to a maximum height of 6.4 m. My results showed a weak negative relationship between tree hole height and <u>Microvelia</u> abundance on BCI. <u>Cylicovelia kenyana</u> also differs from <u>M</u>. <u>cavicola</u> in that it does not occur in shaded tree holes and its presence or absence is independent of hole volume (Polhemus & Copeland 1996).

There are at least two explanations for the short-term and long-term site fidelity exhibited by <u>M</u>. <u>cavicola</u> in BCI tree holes. First, the bugs may be highly microsite-selective both within and between years such that consistency in their occurrence reflects non-random annual recolonization of "preferred" holes. A second possibility is that current <u>M</u>. <u>cavicola</u> distributions represent many years of population persistence in certain holes, i.e., holes that are sufficiently large or sheltered to resist complete drying. The local distribution of <u>M</u>. <u>cavicola</u> is probably limited by both availability of suitable tree holes and habitat disturbance frequency, but detailed

investigations of dispersal, colonization and possible dry season diapause are needed to determine the degree to which each of these (or other) explanations are applicable.

The feeding behavior of <u>M</u>. <u>cavicola</u> is similar to that described for other members of the genus (e.g., Bueno 1910, 1917, Frick 1949, Travers 1993). Adult mosquitoes and other mobile insects visiting tree holes (i.e., to oviposit) are not typically eaten by <u>M</u>. <u>cavicola</u> (pers. obs.); the most common sources of food appear to be dead and dying invertebrates that become trapped in the water surface film (e.g., Bueno 1910, 1917), and insects in the process of emerging from the water. The rapid and direct recruitment of <u>M</u>. <u>cavicola</u> to struggling prey suggests that they locate food by detecting water surface vibrations (e.g, Travers 1993). Although not observed in this study, Hoffmann (1924) noted that <u>Microvelia</u> can also capture prey occurring just beneath the water surface. This sort of predation may partly explain the drowning deaths of mosquito pupae in the lab experiment. The effect of <u>M</u>. <u>cavicola</u> on mosquito survivorship under completely natural circumstances is not known, but may be important when abundance of the bugs in a hole is high relative to mosquito abundance.

Growth rates of <u>M</u>. <u>cavicola</u> in the lab were similar to those described for temperate (Bueno 1910, 1917) and tropical (Frick 1949) congeners (pers. obs.). The longevity of individuals in the lab suggests that  $\geq 2$  generations are produced each wet season and that adults are relatively long-lived.

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Table 1. Multiple regression output for effects of tree hole volume and height on <u>Microvelia cavicola</u> abundance. n = 87 natural holes containing 1 or more individuals. Data were log-transformed before analysis to correct variance heterogeneity (Sokal & Rohlf 1981). Model:  $F_{2,84} = 20.02$ , P = 0.0001,  $R^2 = 0.323$ 

Variable	Estimate	SE	t <sub>obs</sub>	P
y-intercept	-1.324	0.334	-3.97	0.0002
Height	-0.221	0.074	-2.97	0.0039
Volume	0.656	0.109	6.00	0.001

#### FIGURE CAPTIONS

Fig. 1. Effect of tree hole height (filled circles) and volume (open circles) on <u>Microvelia cavicola</u> abundance in the 87 natural holes where it occurred. Mean ( $\pm$ SE) height = 2.6  $\pm$  0.5 m and volume = 1992  $\pm$  425 ml.

Fig. 2. Mean (+ SE) number of mosquitoes successfully emerging from cups containing <u>M</u>. <u>cavicola</u> and control cups lacking <u>M</u>. <u>cavicola</u>. t = 8.82, df = 10, P < 0.001







**CHAPTER 4** 

# EFFECTS OF LEAF LITTER SPECIES ON MACROINVERTEBRATE COMMUNITY PROPERTIES AND MOSQUITO YIELD IN NEOTROPICAL TREE HOLE MICROCOSMS

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#### ABSTRACT

Detritus quality and quantity affect macroinvertebrate productivity and distribution in many freshwater ecosystems. I 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 <u>Culex</u> 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  $\underline{C}$ . 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 <u>Platypodium</u> 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.

#### INTRODUCTION

Detritus, especially decaying leaves and other plant matter, is an important resource for invertebrates in terrestrial (e.g., Anderson and Macfadyen 1976; Swift et al. 1979) and aquatic (e.g., Minshall 1967; Anderson and Sedell 1979; Webster and Benfield 1986; Bowen 1987) systems, and forms a critical link in the large-scale cycling of energy and nutrients (e.g., Olson 1963; Haines and Foster 1977; Merritt et al. 1984; Vitousek 1984). In freshwater ecosystems, leaf litter and its attached decomposer microfauna is consumed by a variety of macroinvertebrates (e.g., Kaushik and Hynes 1968) and can limit the growth of some taxa (e.g., Gee 1988; Richardson 1991; Dobson and Hildrew 1992). Detritus quality and quantity affect the productivity and distribution of many benthic invertebrates (e.g., Egglishaw 1964; Cummins et al. 1973; Ward and Cummins 1979; Wallace et al. 1982; Stout et al. 1985; Sweeney and Vannote 1986).

The degradation rate of litter is influenced by physicochemical characteristics of the leaves, environmental conditions, and characteristics of decomposer microbes (e.g., Witkamp 1963; 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 (e.g., Petersen and Cummins 1974; Short et al. 1980; Stout 1980; Benfield and Webster 1985; Webster and Benfield 1986; Cornejo et al. 1994; Cornelissen 1996), and the degradation rate of detritus and its nutritional quality are often correlated (e.g., Swift et al. 1979; Golladay et al. 1983; but see Prescott 1995). Thus, different

species of leaves (and the microbes they support) may not be equal in nutritional value to the organisms that consume them (e.g., Otto 1974; Sweeney and Vannote 1986; Canhoto and Graça 1995; Walker et al. 1997).

Lignin content, nitrogen content, and combinations of these factors influence litter decomposition rates (e.g., Suberkropp et al. 1976; Fogel and Cromack 1977; Melillo et al. 1982; Taylor et al. 1989; Enríquez et al. 1993; Cotrufo et al. 1994; Cortez et al. 1996; but see Gillon et al. 1994 and Prescott 1995). Leaf toughness is a function of leaf fiber content (lignin, cellulose, etc.), 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 (e.g., Akanil and Middleton 1997).

Some aquatic macrodetritivores and microorganisms show feeding preferences when offered different leaf species in the laboratory (e.g., Wallace et al. 1970; Kaushik and Hynes 1971; Bärlocher and Kendrick 1973; Friberg and Jacobsen 1994; Canhoto and Graça 1995), and in aquatic systems such as streams (Basaguren and Pozo 1994; Dobson 1994) and ponds (Kok and Van der Velde 1994), macroinvertebrate abundance, species composition, species richness, and trophic structure are influenced by the species of litter present. Gee and Somerfield (1997) also observed differences in invertebrate community structure among litter species in mangroves during the early stages of leaf decomposition.

Leaf litter is the nutrient and energy (sensu Wright 1983) base for food webs in most water-filled tree holes, phytotelmata formed by the collection of rain water 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 (e.g., Carpenter 1982a; Paradise and Dunson 1997), most feed by grazing decomposer microbes from litter surfaces or filtering microbes suspended in the water column (e.g., Fish and Carpenter 1982; Walker and Merritt 1991).

Water-filled tree holes occur in many temperate deciduous and tropical forests. 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 (Fincke et al. 1997; CHAPTER 1).

Leaf litter quality affects the growth of some tree hole macroinvertebrate species (e.g., 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 <u>Ficus</u> 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; pers. obs.). In addition, observations from natural tree holes on BCI (e.g., CHAPTER 2) 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] and Srivastava and Lawton [1998]). I designed this study 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 container habitats. I also predicted that softer leaves would support greater mosquito yield and shorter mosquito pupation time in laboratory microcosms.

#### METHODS

I conducted experiments 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 <u>Ceiba pentandra</u> (L.) (Bombacaceae), <u>Dipteryx panamensis</u> (Pitt.) (Fabaceae), <u>Ficus yoponensis</u> Desv. (Moraceae), and <u>Platypodium elegans</u> 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 (pers. obs.). Fresh leaves were collected

from tree crowns or recent branch falls, air dried in the laboratory for > 10 d, and oven dried at 70°C for 48 h. Although drying leaves in this manner may affect their degradation rate (e.g., Gessner and Schwoerbel 1989; Taylor and Bärlocher 1996; Taylor 1998) and nutritive value (e.g., Bärlocher et al. 1978; Lounibos 1985), drying enabled the standardization of initial litter weights for the experiments in this study. Fresh leaves tend to have 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 (e.g., Stout 1980) and fresh leaves often dominate the litter entering BCI tree holes during the wet season (pers. obs.).

I used leaf toughness 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 this 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 rain water for 24 hours 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 ( $n \ge 25$  punches per species) with a commercial penetrometer (Chatillion Co.). 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. I compared mean toughness  $(g \cdot mm^{-2} required to penetrate the leaf)$  among the four species with a one-way ANOVA.

#### Field Experiment

Five replicates of artificial tree holes (black plastic cups; 9 cm diameter x 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 x 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 ca. 1 m above the ground and > 5 m apart.

Each cup initially contained 4.00 g ( $\pm$  0.01 g) of leaf litter and a strip of balsa wood (0.15 cm x 4 cm x 12 cm) as an oviposition site for insect colonists (Novak and Peloquin 1981). I standardized the size of leaf fragments ( $\approx$  15 cm<sup>2</sup>) among treatments as much as possible to control for leaf area effects (Cornejo et al. 1994; Maloney and Lamberti 1995). I added 350 ml rain water and 50 ml filtered natural tree hole water pooled from 5 holes [in Lacmellea panamensis (Woods.) (Apocynaceae) and Pterocarpus rohrii Vahl (Fabaceae)] to each cup. Heavy rains and associated stemflow filled all cups to capacity within 24 h of setup. I measured conductivity, dissolved oxygen (DO) and pH of the water 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.

I emptied each cup into a white pan in the field and recorded the species composition and abundance of macroinvertebrates once per week on weeks 1–5 and 7. Any extraneous macrodetritus (particles > 0.25 cm<sup>2</sup>) 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 I was able to recover the leaf material with forceps. Ficus leaves tended to soften rapidly and were skeletonized by scirtid beetle larvae, leaving primarily the venation and leaf epidermis behind. 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  $[C_s = 2j \cdot (a + b)^{-1}]$ , 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 among the four leaf litter types. Differences in the mean number of macroinvertebrate species and their mean abundance among treatments were analyzed with repeated-measures ANOVAs. I used a one-way ANOVA and an a posteriori Ryan-Einot-Gabriel-Welsch multiple range test

(REGWQ option; SAS 1989) to analyze differences in the fraction of initial leaf mass remaining at the end of the experiment among leaf species.

#### Laboratory Experiment

I conducted a lab experiment to determine differences in degradation rates of the four leaf species with and without <u>Culex mollis</u> mosquitoes, and differences in <u>C</u>. <u>mollis</u> yield and pupation time among the four litter species. I used <u>C</u>. <u>mollis</u> 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 x 5 cm diameter) served as tree hole microcosms.

The experimental design was a 4 x 2 factorial replicated 5 times, with leaf species and presence/absence of mosquitoes as main effects. Each of the 40 cups initially contained 150 ml filtered rain water, 3 ml natural tree hole water, 333 mg ( $\pm$  0.5 mg) dry weight of one leaf species, a strip of balsa wood (1 cm x 10 cm x 0.15 cm), and was allowed to stand for two days to allow fungal and bacterial growth. The litter quantity used was one half of the litter density in the field experiment, but also within the range of densities observed in natural holes. I added 100 neonate (< 12 h post hatch) <u>C</u>. mollis larvae to half of the cups over 5 days. This quantity of larvae was based on the average density of <u>C</u>. mollis present during the first census of the field experiment (excluding cups lacking this species) and is within the range of <u>C</u>. mollis densities seen in natural holes (pers. obs.). Larvae added to a cup were pooled

from at least two field-collected egg rafts (n > 20 rafts), and neonates originating from any single egg raft were divided among cups as much as possible. All cups were maintained at 23  $\pm$  0.5°C under irregular lighting conditions. Rain water was added weekly to compensate for evaporation.

I checked each cup daily and collected any newly formed mosquito pupae 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  $\geq$  24 h and weighed to the nearest 0.1 mg. I terminated the experiment 30 d after the first mosquito input. All leaf litter fragments and any <u>C</u>. mollis larvae remaining in the cups were collected, oven dried and weighed.

A 2-way ANOVA was used to analyze differences in mean percent litter remaining among leaf treatments with and without mosquitoes. I used one-way ANOVAs and REGWQ tests to compare mean total mosquito yield (dry mass of pupae plus larvae), pupation time (number of days to the appearance of the first pupa), and individual pupa mass (obtained by division) among litter species. No mosquitoes successfully pupated in one of the cups containing <u>Ceiba</u> leaves, so I excluded that replicate from the analysis of development 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 (CHAPTER 2). 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 (Pearson r = 0.97, P = 0.03, n = 4), so I used 10 censuses randomly chosen from each tree species (all <u>Ceiba</u> censuses were used) for the analyses. I quantified overlap in macroinvertebrate species composition among the four tree species (all samples pooled) with Sørensen's coefficient.

All data were tested for normality with normal probability plots prior to analysis. 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 graphical results are of untransformed data.

#### RESULTS

All of the species observed in this study also occur in natural tree holes on BCI. Most macroinvertebrate taxa colonized all treatments in approximately equal proportions (Table 1), and species composition was very similar among litter treatments (Table 2). Two mosquito species occurred only in the <u>Platypodium</u> treatment, and chironomid midges occurred only in the <u>Ceiba</u> treatment (Table 1).
Local extinctions and species turnover were generally consistent among treatments during the course of the study. Examples include the gradual disappearance of  $\underline{C}$ . <u>mollis</u> and the syrphid <u>Copestylum raphaelanum</u> (Fig. 1). Some taxa (e.g., psychodid flies) first appeared in the middle censuses but did not persist (Fig. 1), and several other taxa (e.g., the mosquitoes <u>Aedes terrens</u>, <u>Culex urichii</u>, and <u>Haemagogus</u> spp.) colonized cups during the latter portion of the field experiment or gradually increased in abundance from weeks 1-7 (Fig. 1). This early successional pattern also occurs in newly-formed natural tree holes on BCI (CHAPTER 1).

The predators <u>Bezzia snowi</u> and <u>Toxorhynchites theobaldi</u>, and the facultative (often cannibalistic) predator <u>Trichoprosopon digitatum</u>, appeared to differ in abundance among litter types in the field experiment (Table 1). However, their mean log-transformed abundance (all species combined and pooled by time within a cup) did not differ among treatments (ANOVA:  $F_{3,16} = 0.15$ , P = 0.93), nor did their mean relative abundance ( $F_{3,16} = 0.76$ , P = 0.53).

The average number of species in field censuses differed significantly among litter types ( $F_{3,16} = 7.35$ , P < 0.003; Fig. 2a), as did average macroinvertebrate abundance ( $F_{3,16} = 10.87$ , P < 0.001; Fig. 2b). There was a significant time\*treatment interaction for abundance (P = 0.007), but not for richness (P = 0.35). <u>Platypodium</u> litter consistently supported the greatest number of species and individuals, whereas these community parameters were similar among the other litter types (Fig. 2). Macroinvertebrate species colonizing the <u>Platypodium</u> treatment persisted for longer periods of time than in other treatments. The mean number of consecutive censuses that species occurred in the <u>Platypodium</u> 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 <u>Platypodium</u>, whereas the number of species not present on any consecutive census dates was similar among treatments (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 <u>Platypodium</u> 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 <u>Platypodium</u> 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 (CHAPTER 2) and possibly confound tree species effects. Patterns of macroinvertebrate species overlap among tree species were similar to 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. <u>Platypodium</u> and <u>Ficus</u> leaves were tougher than <u>Dipteryx</u> and <u>Ceiba</u> 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). Dissolved oxygen 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 lab experiment corroborated the field results; <u>Ficus</u> and <u>Platypodium</u> had lower mean proportions of litter mass remaining than <u>Ceiba</u> and <u>Dipteryx</u> ( $F_{3,32} = 880$ , 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 <u>Ficus</u> and <u>Platypodium</u> treatments than in <u>Ceiba</u> and <u>Dipteryx</u> ( $F_{3,16} = 51.22$ , 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 <u>Dipteryx</u> litter had longer average development 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. These results and published studies suggest at least two ways in which differences in leaf characteristics influence the community structure of macroinvertebrates in tree holes. First, chemicals leached into the water or volatilized from water containing different types of leaves may attract different suites of colonists. Second, differences in nutrient content or herbivore defenses (e.g., Grime et al. 1996) among litter species may affect the abundance of decomposer microbes, which are the principal food of many tree hole macroinvertebrate species. Low quality litter may therefore cause secondary extinctions (and ultimately support fewer macroinvertebrate species) due to nutrient/energy limitation (e.g., Connell and Orias 1964; Wright 1983).

Tree holes on BCI and elsewhere are primarily colonized by macroinvertebrates that are specialists in these and similar container habitats, so the high degree of similarity in macroinvertebrate species composition among the four litter types (Tables 1 and 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.

Mosquitoes are typically the most abundant and species-rich inhabitants of neotropical tree holes, and unequal mosquito distribution among the litter species

(Table 1) may have contributed to differences in macroinvertebrate abundance and species richness between treatments (Fig. 2). The most probable mechanism for this unequal species distribution is selective colonization, possibly reflecting ovipositional preferences based on chemical characteristics of litter types. Mosquitoes use a variety of physical and chemical cues when selecting aquatic oviposition sites (reviewed by Bentley and Day 1989), and compounds dissolved in and volatilized from tree hole water are particularly important attractants and deterrents for some container-breeding species (e.g., Petersen and Chapman 1969; Lounibos 1978; Bentley et al. 1979, 1982; Kramer and Mulla 1979), including some congeners of this study. Orthopodomyia fascipes, for example, may have been attracted to an oviposition cue or combination of cues unique to the water containing Platypodium litter (Table 1). Although I did not conduct detailed chemical analyses, differences in water quality and odor between treatments were apparent. Water containing Ficus and Platypodium leaves was cloudy and pungent, whereas Ceiba and Dipteryx water was comparatively clear and moderately scented. Physical habitat characteristics were standardized by the use of artificial tree holes, further suggesting that most variation in species composition due to differential colonization can be attributed to chemical differences among treatments.

The pseudostigmatid damselflies observed in this experiment only occurred on the last two census dates, and I consider their patchy distribution among litter species (Table 1) an artifact given the short duration of the experiment relative to their hatching time. Pseudostigmatid eggs require from 12 to 196 days from oviposition to hatch (Fincke 1998; 1999), and it is probable that most cups were colonized by

damselflies that did not hatch in time to be counted. In addition, tree hole survey data indicate that pseudostigmatids are generalists with respect to physical and chemical characteristics of holes (Fincke 1999), and it is likely that damselflies would have appeared in all treatments had the experiment run longer.

Species loss and turnover in the field experiment suggest that secondary extinctions account for some differences in species richness and abundance among leaf litter treatments. Predators in tree holes can maintain prey abundance below carrying capacity (e.g., Bradshaw and Holzapfel 1983; Fincke et al. 1997) and, unlike predators in more open systems (e.g., Paine 1966), predators in tree holes (CHAPTER 6) and other phytotelmata (e.g., Addicott 1974) tend to reduce prey species richness. The lack of difference in mean predator abundance among treatments (see Results) and similarity in overall mean abundance between <u>Ceiba</u> and other litter types (Fig. 2b) suggest that predator effects on prey abundance were evenly distributed among treatments.

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 (e.g., 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 (e.g., Carpenter 1983; Hard et al. 1989; Léonard and Juliano 1995), and higher quality litter presumably prevents or delays secondary extinctions that would otherwise result from resource depletion. The greater longevity of species (Table 3) and the greater abundance of rare species in the <u>Platypodium</u> treatment support the conclusion that <u>Platypodium</u> 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 species used differ in nutritional quality during the early stages of decomposition. Ficus and Platypodium had the fastest degradation rate (Figs. 4 and 6), the greatest toughness (Fig. 3), and leached the most material into the water (as indicated by higher conductivity; Fig 5a). Ficus and Platypodium also supported the greatest mosquito yield in the laboratory experiment (Fig. 7), and the largest species richness and abundance in the field experiment occurred in Platypodium litter (Fig. 2). Although average species richness in the Ficus treatment of the field experiment 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) during the first week of the study. The convergence of mean macroinvertebrate species richness and abundance among treatments during the last weeks of the experiment (Fig. 2) suggests that soluble nutrients became limiting and that the remaining litter material was entering the second phase of decomposition, where more resistant components of the leaves (e.g., lignin) are slowly degraded (e.g., Carpenter 1982a; Gillon et al.

1994). In combination, these laboratory and field results indicate that differences in nutritional quality among leaf types during the first few weeks of decay are largely responsible for differences in species richness and abundance among treatments.

My 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 tend to be carbon-rich and provide a colonization substrate for microorganisms that may use external nutrient sources for growth (e.g., Suberkropp 1995). Microbial assemblages show some specificity for different types and species of detritus (e.g., Swift et al. 1979; Cornejo et al. 1994; Lodge 1997), and differ in their ability to degrade leaf fiber (e.g., 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 margins) 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 macroinvertebrate community parameters, 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 secondary extinctions. All measured DO and pH values were within the range observed for natural tree holes on BCI (Fincke 1999; CHAPTER 2).

The proportions of litter mass remaining among the four litter species in the laboratory experiment were consistently lower in the presence of <u>C</u>. <u>mollis</u> (Fig. 6). Carpenter (1982a) and Fish and Carpenter (1982) observed similar facilitation effects of the tree hole mosquito <u>Aedes triseriatus</u> on leaf litter decomposition in microcosm experiments. <u>Culex mollis</u> feeds primarily by grazing leaf surfaces (pers. obs.), 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 (e.g., Lopez et al. 1977; Carpenter 1982a) or by excreting nutrients that are limiting to the decomposers

(e.g., 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 <u>C</u>. <u>mollis</u> pupa was similar in all treatments of the lab experiment except <u>Dipteryx</u> (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. 6) and results from the field experiment (Figs. 2, 3, and 4) show that <u>Ceiba</u> and <u>Dipteryx</u> leaves are approximately equal in nutritional value to macroinvertebrates, therefore, slow growth of mosquitoes on <u>Dipteryx</u> litter seems anomalous. Differences in the abundance or composition of the microbial decomposer fauna, or growth-inhibiting anti-herbivore compounds in the <u>Dipteryx</u> 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 (species composition, abundance and diversity) in tree holes, at least during the early stages of leaf decay.

Many questions stemming from this work remain to be answered: Are decomposer microbes in tree holes taxonomically or physiologically different from those of other systems? How do the leaf species used in this study differ with respect to concentrations of nitrogen and other nutrients? Do anti-herbivore compounds in leaves retard mosquito growth? Is succession in tree hole macroinvertebrate assemblages driven by changes in the litter resource over time? Tree holes offer unique biotic and abiotic conditions for litter decomposition, and answers to these and related questions will provide a basis for comparison with other systems.

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Table 1. Mean percent abundance of taxa occurring among different leaf types in the field experiment. Parentheses indicate the number of cups (out of 5) 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. \* = 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 sp.	3.5 (3)	1.6 (2)	0.3 (1)*	3.7 (4)*	
Diptera: Chironomidae					
Chironomus sp.	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*	

Taxon	Ceiba	Dipteryx	Ficus	Platypodium	
<u>C. corrigani</u>	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	5.0 (2)*	3.1 (1)*	8.3 (4)*	2.3 (2)*	
Count Totals	1756	2126	2554	4759	
Cumulative No. 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 (Art) and natural holes of the same tree species (Nat). Coefficients do not differ between artificial and natural holes (Wilcoxon 2-sample test; P = 0.13).

	Dipteryx		Ficus		Platypodium		
	Art	Nat	Art	Nat	Art	Nat	_
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. Means (SE) of longevity variables for macroinvertebrates occurring in the field experiment. Persist = number of consecutive samples a species was present in a cup, Consec = number of species present on  $\geq 2$  consecutive censuses, and Irreg = number of species not present on consecutive censuses. Persist n = number of species; n = 5 for all other means. X<sup>2</sup> values are from Kruskal-Wallis tests; df = 3. \*\* = P < 0.05

	Ceiba	Dipteryx	Ficus	Platypodium	X <sup>2</sup>
Persist	2.0 (0.52)	2.6 (0.47)	2.0 (0.54)	3.8 (0.33)	8.46**
n	15	15	16	17	
Consec	4.2 (0.20)	5.8 (0.37)	3.8 (0.37)	7.8 (0.37)	15.75**
Irreg	3.4 (0.68)	4.2 (0.37)	3.2 (0.37)	2.8 (0.58)	3.55

Table 4. Mean (SE) macroinvertebrate species richness and abundance, and mean (SE) height and volume for natural holes in the focal tree species sampled on BCI from 1995 to 1997. Holes = number of individual holes censused. Censuses = total number of censuses per tree species.  $X^2$  values are from Kruskal-Wallis tests with df = 3 and n = 10 censuses for each mean. \*\* = P < 0.01

	Ceiba	Dipteryx	Ficus	Platypodium	X <sup>2</sup>
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	

## FIGURE CAPTIONS

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

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 <u>Ceiba</u> and <u>Platypodium</u>, 26 for <u>Dipteryx</u>, and 36 for <u>Ficus</u>. The horizontal line connects means that are not significantly different.

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

Fig. 6. Mean (+1 SD) percent of initial litter mass remaining with (shaded bars) and without (open bars) mosquitoes 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. 7. Mean (+ SE) number of days to appearance of first pupa (shaded bars) and total mosquito mass (open bars) 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).









# FIGURE 3











## FIGURE 6






CHAPTER 5

# TROPHIC INTERACTIONS IN NEOTROPICAL TREE HOLE MICROCOSMS

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#### ABSTRACT

Populations of aquatic organisms are thought to be either resource or predator limited, depending on their trophic position. I investigated the effects of a top predator, <u>Mecistogaster</u> spp. (Odonata: Pseudostigmatidae), on survivorship of the grazer <u>Culex mollis</u> (Diptera: Culicidae) and decomposition rates of leaf litter in tree hole microcosms. In a factorial experiment using 200 ml cups, significantly less litter mass remained when grazers (51%) and grazers plus predators (51%) were present, than in controls (57%). Predators alone and with grazers had little effect on litter decomposition rate, suggesting that trophic cascades are infrequent in tree holes. Selective predation on larger grazer size classes created a refuge for younger mosquitoes, which maintained the significant grazer effect. The number of mosquitoes surviving to pupation was lower in the presence of odonates, but time to the appearance of the first pupa and pupal mass of did not differ among treatments. Although mosquito larvae facilitated decomposition of litter in tree holes and may have become food limited, their survival was ultimately checked by predation.

#### INTRODUCTION

Hairston et al. (1960; "HSS") and Fretwell (1977, 1987) hypothesized that populations at different trophic levels are regulated by either resources or predation, and that the relative importance of these processes depends upon the number of trophic levels in the community and the trophic position of the focal population. These models are the basis for trophic cascade hypotheses, in which removal of a top

predator is predicted to increase the abundance of intermediate consumers, thereby indirectly affecting the biomass of organisms two or more links down the food chain. An alternative "top-down" model by Menge and Sutherland (1976; "MS") proposed that most lower trophic levels are predator-limited due to omnivory (predators feeding at multiple trophic levels) in food webs. Like HSS-Fretwell, the MS model states that the importance of predation to community structure is dependent upon the number of trophic levels in the system.

Despite criticism of the realism and applicability of the HSS-Fretwell model (Murdoch 1966, Ehrlich and Birch 1967), and trophic levels in general (e.g., Polis and Strong 1996), trophic cascades and other indirect effects of consumers on community structure occur in large aquatic systems (e.g., Carpenter et al. 1985, Power 1990, Vanni and Findlay 1990, Brönmark et al. 1992) and some terrestrial systems (e.g., Spiller and Schoener 1994, Carter and Rypstra 1995, Dial and Roughgarden 1995; also see review by Wootton 1994).

Strong (1992) suggested that the most robust empirical support for trophic cascade hypotheses comes from aquatic ecosystems because they tend to have lower diversity (i.e., simpler food webs) than terrestrial communities. Polis and Strong (1996) further argued that only certain aquatic systems (e.g., mesotrophic lakes) are prone to trophic cascades. Few detailed investigations of trophic interactions have been conducted in other aquatic settings. For example, the trophic ecology of detritus-based and small aquatic communities (e.g., temporary pools and phytotelmata) remains largely unstudied (Fretwell 1977, M. E. Power, pers. comm.).

Water-filled tree holes are phytotelmata formed when rain collects in branch crotches and other wood-lined cavities, and are common aquatic habitats in temperate hardwood and tropical forests (e.g., Kitching 1971, Fish 1983). Unlike large aquatic systems such as lakes and streams, tree holes are a limiting resource for many of the organisms that colonize them (e.g., Fincke 1994), and the generally high ratio of macroorganism biomass to habitat volume promotes strong interspecific interactions. Small size and relatively simple communities make tree holes experimentally tractable, and they can be reproduced as laboratory microcosms without compromising the scale of the natural system (Beyers and Odum 1993, Lawton 1995).

Detritus, especially leaf litter, is an important nutrient source in many aquatic systems (e.g., Kaushik and Hynes 1968, Cummins et al. 1973, Anderson et al. 1978, Bowen 1987), and is the base of food webs in most water-filled tree holes (Kitching 1971). Leaf litter in tree holes is primarily consumed by bacteria and fungi (Fish and Carpenter 1982), and mosquito larvae are typically the most common grazers on this microfauna, although other arthropods also consume decomposers and the litter itself (e.g., Paradise and Dunson 1997, CHAPTER 1). In the lowland moist forest of Barro Colorado Island, Panama (BCI), larvae of <u>Aedes, Culex</u> and <u>Haemagogus</u> mosquitoes are the principal prey of top predators in tree holes, which include odonate larvae, <u>Dendrobates</u> tadpoles, and <u>Toxorhynchites</u> mosquito larvae (Fincke et al. 1997, Fincke 1999).

Microcosm studies combining leaf litter and larvae of the temperate tree hole mosquito <u>Aedes triseriatus</u> (Say) showed that consumption of the decomposer

microfauna by mosquito larvae enhances litter degradation rates (Carpenter 1982, Fish and Carpenter 1982). Similar facilitation effects of grazers occur in other aquatic systems (e.g., Hargrave 1970, Barsdate et al. 1974, Harrison 1977, Lopez et al. 1977). Several mechanisms for facilitation have been suggested: 1) grazers fragment or directly consume leaf material in addition to the decomposer fauna (Fenchel 1970, Harrison 1977, Carpenter 1982); 2) continuous grazing pressure stimulates decomposer metabolism (Lopez et al. 1977, Carpenter 1982), or favors more efficient decomposer taxa (Harrison 1977, Lopez et al. 1977); and 3) one or more inorganic compounds excreted by grazers facilitates growth of the decomposers (Hargrave 1970, Lopez et al. 1977, Walker et al. 1997).

Unlike temperate tree holes, tropical tree holes tend to support diverse predator assemblages (e.g., Kitching 1990, Fincke 1998). Predators significantly reduce mosquito survivorship in tree holes (Fincke et al. 1997, Nannini and Juliano 1998), and can influence mosquito life history and fitness characteristics such as development time and adult mass (Lounibos et al. 1993, Grill and Juliano 1996). These strong predator effects, in combination with mosquito effects on litter decomposition, suggest that tree hole communities are prone to trophic cascades.

The purpose of this study was to determine the independent and interactive effects of a top predator and grazers on litter decomposition rates in tree hole microcosms on BCI. I hypothesized that the consumption rate of leaf litter by decomposers would differ in simple food webs of different length (i.e., in the presence or absence of grazing mosquitoes, and with and without a predaceous

odonate). The following predictions were tested: 1) grazing by larvae of the tree hole mosquito <u>Culex mollis</u> Dyar and Knab enhances leaf litter degradation; 2) predation by odonates reduces the survivorship (= successful pupation) and time to pupation of mosquitoes; and 3) the presence of an odonate indirectly reduces the degradation rate of leaf litter.

#### METHODS

Experiments were conducted in the laboratory building of the Smithsonian Tropical Research Institute on BCI during January and February 1998. Tree hole microcosms consisted of plastic cups (8.5 cm height x 5 cm diameter) containing 125 ml filtered rain water. Tree hole water was collected and pooled from 5 natural holes on BCI, and 3 ml was added to each cup as a microfauna inoculum. An emergent strip of milled balsa wood (1 cm x 10 cm x 0.15 cm) served as a predator perch site and was included in all treatments. I added 200  $\pm$  0.5 mg dried ( $\geq$  10 d in air, then 48 h at 70°C) Platypodium elegans Vogel leaf litter as a nutrient source. The bulk of the litter in each cup consisted of whole leaflets (each  $\approx 15 \text{ cm}^2$ ) collected from recent branch falls, but every cup also contained one or more leaf fragments (each < 1.0cm<sup>2</sup>) to standardize the initial litter mass. Leaflets of <u>P</u>. elegans were used because they are small and are often found in natural tree holes (pers. obs.). Platypodium elegans is deciduous and common on BCI (Croat 1978), and frequently has one or more tree holes in its trunk or branches (pers. obs.). Cup contents were allowed to stand for 48 hours prior to the start of the experiment to permit establishment of

bacterial and fungal assemblages. All cups were maintained at  $23 \pm 0.5$  °C and rain water was periodically added to compensate for evaporation.

The experimental design consisted of a 2 x 2 factorial replicated 6 times, with presence/absence of predator and grazers as main effects. Treatment 1 contained odonates (predators) only, Treatment 2 contained mosquito larvae (grazers) only, and Treatment 3 contained both predators and grazers. The 6 control cups lacked grazers and predators. Each cup in Treatments 1 and 3 received one medium size (15-18 mm) larva of the pseudostigmatid damselfly Mecistogaster sp. The two species of Mecistogaster that occur on BCI (M. linearis and M. ornata) are difficult to distinguish as larvae and were not differentiated in this experiment. However, both species have similar foraging behavior and maximum growth rates (Fincke 1992a). Pseudostigmatids are the most common top predators in BCI tree holes and generally have a longer larval stage than other large predators In the system (Fincke 1992b, 1998, 1999). All 12 Mecistogaster larvae were obtained from different natural tree holes and starved for > 24 h before the start of the experiment. I biased the odonate larvae used in Treatment 3 toward smaller initial sizes (15-17 mm) because maximal growth and foraging rates of Mecistogaster tend to occur around that body length (Fincke 1992a, Fincke et al. 1997).

I used larvae of the tree hole mosquito <u>Culex mollis</u> as grazers in the experiment. This species is relatively common in natural tree holes on BCI (Fincke et al. 1997, CHAPTER 1), and its egg rafts are easy to recognize and collect. At the start of the experiment Treatments 2 and 3 each received 25 neonate (< 12 h post hatch)

<u>C. mollis</u> larvae every other day to a total of 100 larvae per cup. Mosquitoes added to a given treatment on a given date were reared from at least two different fieldcollected egg rafts (n > 16 rafts). Leaf litter and odonate densities used in the experiment were within the ranges observed for natural tree holes on BCI (pers. obs.). Although total mosquito density was biased toward the maximum found in small (< 500 ml) holes, the gradual addition of neonates over several days approximated mosquito recruitment patterns in natural and artificial holes augmented with fresh litter.

For convenience in collection and handling, I operationally defined mosquito survivorship as successful transformation to the pupa stage. I collected <u>C</u>. mollis pupae from the cups daily and stored them in a drying oven (70°C) until the end of the experiment, when all pupae accumulated within a replication were weighed to the nearest 0.1 mg. Fresh weights ( $\pm$  0.1 mg) and body lengths ( $\pm$  0.25 mm, excluding caudal lamellae) of <u>Mecistogaster</u> larvae were recorded at the start and end of the experiment. Leaf litter remaining in the cups at the end of the experiment was collected with forceps, rinsed in distilled water to remove grazer feces or fungal accumulations, dried (70°C, 48 h), and weighed to the nearest 0.1 mg. I terminated the experiment 31 days after the first mosquito input, when all larvae in Treatment 3 (predator plus grazers) pupated or were consumed. The few mosquito larvae remaining in Treatment 2 were collected, dried and weighed.

I compared the fraction of initial leaf litter mass remaining across treatments with a two-way ANOVA and an a posteriori Ryan-Einot-Gabriel-Welsch multiple

range test (SAS 1989). Proportional data were arcsine-square root transformed prior to analyses (Sokal and Rohlf 1981). I analyzed differences in odonate size between the start and end of the experiment with a paired t-test. Linear regressions showed that odonate fresh weight and body length were highly correlated both at the start ( $F_{1,9}$ = 761,  $r^2$  = 0.99, P < 0.0001) and end ( $F_{1,9}$  = 253,  $r^2$  = 0.97, P < 0.0001) of the experiment, therefore, only the change in mean fresh weight was analyzed. One odonate larva in Treatment 3 molted during the experiment and was excluded from predator size comparisons. Means for the total number of <u>C</u>. mollis pupae developed, total pupal mass, individual pupa mass (obtained by division), and days to appearance of the first pupa were compared across grazer and grazer plus predator treatments with ANOVA and Bonferroni adjustment. Homogeneity of variance was determined with F-tests prior to all analyses.

#### RESULTS

The amount of leaf litter remaining at the end of the experiment was greater in the absence of mosquitoes ( $F_{1,20} = 15.22$ , P < 0.001), but there was no significant effect of the predator ( $F_{1,20} = 0.47$ , P = 0.50) or grazer\*predator interaction effect ( $F_{1,20} = 0.11$ , P = 0.74; Fig. 1). Fewer than 20 of the initial 100 mosquitoes used in each grazer replicate successfully pupated (Fig. 2), but the average cumulative number of pupae and average cumulative pupal biomass were significantly greater in the absence of predators (Table 1). The mean number of days to appearance of the first pupa and the mean individual pupa mass did not differ between the two

treatments (Table 1). The foraging behavior of mosquito larvae surviving to the last week of the experiment differed dramatically from larvae that successfully pupated in the second week. Older larvae spent more time actively browsing leaf material and browsed a larger leaf area. These behavioral shifts were not anticipated and therefore not quantified.

The mass of odonates in Treatment 1 (predators only), did not differ between the start and end of the experiment (n = 6, t = 0.074, P = 0.94), whereas odonate fresh mass in Treatment 3 (grazers plus predators) increased (n = 5, t = 5.98, P = 0.001; Fig. 3). Detached and free-floating fungal fragments drifting within striking distance of the odonates were not consumed (pers. obs.).

Treatments containing mosquitoes had comparatively clear water and no apparent microbial biofilm present on the leaf litter. In contrast, the water in the control cups and predator-only treatment was cloudy (presumed due to abundant bacteria) throughout the experiment and the leaves were covered with a conspicuous accumulation of decomposers (fungal strands extending up to 10 mm above the leaf surfaces), especially during the latter 3 weeks.

## DISCUSSION

The HSS-Fretwell model predicts that the lowest trophic level is food limited in odd-link food webs and grazer limited in even-link food webs. The combination of species used in this study did not result in a true 3 trophic level system due to selective feeding by the odonates (= predator inefficiency; Fretwell 1977, 1987).

<u>Mecistogaster</u> depressed survivorship of <u>C</u>. <u>mollis</u> (Table 1), but these odonates (and the other common pseudostigmatid on BCI, <u>Megaloprepus coerulatus</u> [Drury]) are size-selective predators, preferentially feeding upon third and fourth instar mosquito larvae when prey are abundant (APPENDIX D). This size-based refugium allows younger mosquito larvae to graze on and limit the growth of decomposers despite the presence of a predator. The lack of a predator effect and the similarity in remaining litter biomass between the two treatments containing mosquitoes (Fig. 1) support this conclusion.

Although no cascade effect occurred, my results fit the predictions of the HSS-Fretwell model more closely than the Menge and Sutherland (1976) model and are similar to those of Power (1990), who found that one of the top predators in her system did not control the dominant grazers due to the presence of refugia (Power et al. 1992). Other predators occurring in BCI tree holes, such as larvae of the aeshnids <u>Gynacantha membranalis</u> Karsch and <u>Triacanthagyna dentata</u> Geijskes feed more voraciously and less selectively than pseudostigmatids (APPENDICES D and E). A duplicate experiment conducted with one of these species may yield a cascade effect.

Results of this study support the prediction that grazing by mosquito larvae facilitates the degradation of leaf litter in tree hole microcosms (Fig. 1). Carpenter (1982) and Fish and Carpenter (1982) obtained similar results in their tree hole microcosm experiments. Direct consumption of leaf material by <u>C</u>. <u>mollis</u> is one possible, but unsupported, explanation for this outcome. Larvae of some tree hole mosquito species consume litter and fine detritus particles in addition to decomposer

microfauna (e.g., Carpenter 1982, Walker et al. 1988), and such omnivory generally confounds the trophic level approach (Polis 1991, Lodge et al. 1994, Polis and Strong 1996). However, based on observations of <u>C</u>. mollis foraging behavior and the lack of physical damage to the leaves, there was no evidence that the larvae consumed a measurable portion of the litter material in this experiment.

My results cannot address the possibility that grazing by mosquito larvae enhanced microbial metabolism (e.g., Hargrave 1970, Lopez et al. 1977, Carpenter 1982), but there is evidence that feeding by <u>C</u>. mollis larvae facilitated litter decomposition by altering the composition of the decomposer fauna (e.g., Harrison 1977, Lopez et al. 1977). Specifically, the minimal fungal development and higher water clarity in treatments containing <u>C</u>. mollis imply that the larvae control the accumulation of fungal hyphae. Slow but continued growth of <u>C</u>. mollis larvae in the relatively clear water suggests that alternate prey (i.e., bacteria) were still available.

Using electron microscopy, Fish and Carpenter (1982) observed a dramatic reduction in the density of fungal hyphae and bacteria on leaf surfaces exposed to mosquito larvae. Perhaps grazing pressure alters the successional state of decomposer assemblages in tree holes by promoting the existence of fast-growing bacteria over slower-growing fungi. Grazing affects producer species composition and enhances diversity in some aquatic and terrestrial systems (e.g., Paine and Vadas 1969, Collins et al. 1998), and more diverse assemblages may utilize resources more effectively (Tilman et al. 1996). In tree holes, frequent turnover and greater diversity in the

bacterial assemblage due to mosquito grazing may ultimately result in more efficient and complete consumption of the litter resource.

Another possible mechanism for grazer-facilitated litter degradation in this experiment is the enhancement of bacterial and fungal growth by nutrients derived from mosquito excretory products. Decomposing litter, especially litter composed of fresh leaves (as in this study), contains an abundance of organic and inorganic nutrients (e.g., Walker et al. 1997), but a substantial portion of those nutrients are only readily available during the early stages of decomposition (Carpenter 1982). Critical inorganic nutrients (e.g., N and P) leaked or excreted from consumers can enhance productivity of lower trophic levels in aquatic systems (Lopez et al. 1977, Durbin et al. 1979, Vanni and Findlay 1990; but see Barsdate et al. 1974 and Harrison 1977) and may stimulate growth of microdetritivores in tree holes (Walker et al. 1997). Dissolved nutrients were not quantified in this study, but excretory products of grazers could have had such an effect.

Although not significantly different from controls, the intermediate quantity of litter disappearing from the predator-only treatment (Fig. 1) suggests that the presence of odonates alone can facilitate litter decay. Swimming and crawling activity of the predator potentially enhances litter degradation by: 1) stirring the water and increasing the growth of diffusion-limited decomposers (Lopez et al. 1977), and 2) sloughing older bacterial and fungal accumulations from leaf surfaces, thereby creating space for additional decomposer colonization. Also, wastes excreted by the odonates may

facilitate growth of decomposers by providing critical inorganic nutrients in the manner discussed for grazers above.

Because odonates reduce mosquito abundance in artificial and natural holes (Fincke et al. 1997), the negative effect of <u>Mecistogaster</u> on <u>C</u>. <u>mollis</u> survivorship (Table 1) was expected. Odonates occasionally eat mosquito pupae (APPENDIX D), so survivorship operationally defined as adult emergence rather than successful pupation may have produced an even stronger result. Grill and Juliano (1996) found that predation by <u>Toxorhynchites rutilus</u> mosquito larvae led to shortened development time and increased adult mass in the tree hole mosquito <u>Aedes triseriatus</u>, perhaps by reducing density-dependent inhibitory effects. Surrogates of these variables did not differ among treatments in this study (Table 1), probably because of low mosquito survivorship even when predators were absent.

I attribute low mosquito survivorship in the absence of predators to nutrient limitation and possibly density dependent factors; both have strong effects on other tree hole mosquito species (e.g., Moore and Fisher 1969, Fish and Carpenter 1982, Carpenter 1983, Hard et al. 1989, Léonard and Juliano 1995). Evidence for food limitation in this study includes slow appearance of first pupae (Fig. 2) compared to ca. 5 d for  $\underline{C}$ . mollis reared on ad libitum yeast or fish food in the same laboratory (pers. obs.), and the relative clarity of the water in both treatments containing mosquitoes. In addition, differences in the foraging behavior of mosquito larvae surviving to the last week of the experiment relative to those that successfully pupated in the second week may be a reflection of starvation stress.

Although predators can significantly reduce mosquito abundance in tree holes (Fincke et al. 1997) and alter community parameters such as species richness (CHAPTER 6), such "top-down" effects may be subordinate to the effects of nutrient quality and quantity on community structure. My results suggest that this is especially true in the presence of selective predators.

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Table 1. Comparison of mean  $\pm$  S.E. mosquito variables among Treatments 2 (grazers only) and 3 (grazers plus predator). n = 6 for each mean. Bonferroni adjusted  $\alpha = 0.0125$ .

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Variable	Grazers only	Grazers + Predator	F <sub>1,10</sub>	Р
Days to first pupa	9.17 ± 1.72	9.33 ± 0.82	0.05	0.83
Cumulative number of pupae	10.5 ± 2.95	3.33 ± 1.75	26.19	< 0.0005
Cumulative Pupal mass (mg)	3.28 ± 0.67	0.88 ± 0.65	39.85	< 0.0001
Individual pupal mass (mg)	0.323 ± 0.068	0.251 ± 0.097	2.18	0.17

#### FIGURE CAPTIONS

Fig. 1. Mean (+ SE) proportions of litter mass remaining at the end of the experiment. Horizontal lines connect means that are not significantly different. Ctrl = control, Pr = predator only, Gr = grazers only, Gr+Pr = grazers plus predators.

Fig. 2. Cumulative number of mosquito pupae developed (all replicates combined) in the grazer-only (solid line) and grazer plus predator (dashed line) treatments over the course of the experiment.

Fig. 3. Mean (+ SE) fresh mass of odonates at the start (shaded bars) and end (open bars) of the experiment in predator-only (Pr) and grazer plus predator (Gr+Pr) treatments. n = 6 for Pr, and n = 5 for Pr+Gr. \* = P < 0.05





FIGURE 2







**CHAPTER 6** 

# PREDATION, NUTRIENTS, AND COMMUNITY STRUCTURE IN WATER-FILLED TREE HOLES OF PANAMA

This chapter is formatted for submission to the journal

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#### ABSTRACT

Predation and nutrient availability influence community structure in many aquatic ecosystems. I conducted predator (odonate) and nutrient (leaf litter) manipulation experiments to determine their independent effects on macroorganism species richness and abundance in water-filled tree holes of Panama. I additionally tested for interactive effects of these factors in artificial tree holes. Large odonates reduced species richness in natural and artificial tree holes and reduced macroorganism abundance when individual hole volume was standardized. In natural tree holes, standardized leaf litter addition and removal increased and decreased (respectively) species richness by ca. 1 species relative to controls. Macroorganism abundance was greater in litter addition holes than in litter removal holes, and abundance increased with litter quantity in artificial tree holes. There was no interactive effect of predation and nutrients in artificial holes, partly due to the time frame of the experiment.

#### INTRODUCTION

Predation and nutrient availability influence the structure of ecological communities in a variety of systems (e.g., Sih et al. 1985; Rosenzweig 1995). In relatively large aquatic settings such as lakes and streams, predators often have strong direct and indirect effects on prey assemblages (e.g., Brooks and Dodson 1965; Zaret 1980; Flecker 1984; Kerfoot and Sih 1987; Blois-Heulin et al. 1990; Diehl 1992; Sih

et al. 1992; McPeek and Peckarsky 1998), and can indirectly influence populations at lower (e.g., basal) trophic levels (e.g., Power 1990; Brönmark et al. 1992; Carpenter and Kitchell 1993; Malmqvist 1993). Changes in inorganic nutrient (e.g., nitrogen and phosphorus) quantities in lakes and streams influence primary productivity (e.g., Schindler and Fee 1974; Proulx et al. 1996; Spencer and Ellis 1998), which is reflected in consumer populations or assemblages (e.g., Hershey et al. 1988; Hart and Robinson 1990; Peterson et al. 1993; Perrin and Richardson 1997). Leaf litter quality and quantity may similarly regulate detritivore populations and detritus-based assemblages in large aquatic systems (e.g., Gee 1988; Richardson 1991; Dobson 1994; Kok and Van der Velde 1994).

The effects of predation and nutrients on communities of small aquatic habitats are less studied. However, predators can influence species richness and/or the abundance of some species (often other predators) in pitcher plants (Addicott 1974), bromeliads (e.g., Lounibos et al. 1987), <u>Heliconia</u> sp. bracts (e.g., Naeem 1988), bamboo stumps (Sota and Mogi 1996), tree holes (e.g., Bradshaw and Holzapfel 1983; Fincke et al. 1997; Nannini and Juliano 1998), cattle tanks (e.g., Murdoch et al. 1984), automobile tires (e.g., Focks et al. 1980), and temporary pools (e.g., Blaustein and Margalit 1994; Schneider and Frost 1996). Predation tends to reduce species richness in these systems (but see Louton et al. 1996 and Lounibos et al. 1997). This contradicts the results of Paine's (1966) classical study, in which predators enhanced diversity in intertidal communities. Part of the reduction in species richness and abundance in small systems can be attributed to the physical

constraints of these habitats (e.g., Addicott 1974); predators can patrol the entire habitat with relatively high efficiency, thus few temporal or spatial refugia exist and local extinctions are likely (but see Lounibos et al. 1997).

Plant detritus (e.g., leaf litter) forms the base of food webs in most phytotelmata (e.g., Kitching 1971; Naeem 1990; Sota 1996). As in larger systems, litter in phytotelmata is primarily degraded by leaching and microorganisms, especially bacteria and fungi (Fish and Carpenter 1982). Macroorganisms consume decaying litter directly (e.g., Paradise and Dunson 1997; CHAPTER 1), or indirectly by grazing and filtering decomposer microbes from litter surfaces and water column, respectively (e.g., Fish and Carpenter 1982; Walker and Merritt 1991). Growth rates and other life history characteristics of some tree hole macroinvertebrate species are influenced by litter quality and quantity (e.g., Carpenter 1982a; Fish and Carpenter 1982; Hard et al. 1989; Lounibos et al. 1993; Léonard and Juliano 1995; Walker et al. 1997; Nannini and Juliano 1998). Naeem (1990) found that detritus quantity has complex effects on community structure in <u>Heliconia</u> bracts, and Jenkins et al. (1992) showed that litter quantity influences species richness and food chain lengths in artificial tree holes.

Water-filled tree holes are relatively common aquatic habitats in tropical moist forests (see Kitching 1971 and Snow 1949 for thorough descriptions of this system). In Panama, ca. 60 different species of macroorganisms are associated with tree holes. This list includes 7 top predators; larvae of pseudostigmatid damselflies are the most common (Fincke 1999; CHAPTER 1). Qualitative surveys and results of a separate study (Fincke et al. 1997) suggested that top predators depress the abundance and

species richness of other organisms in tree holes. Fincke et al. (1997) additionally showed that predator effects on mosquito abundance are lessened when nutrient levels are high in artificial tree holes.

I designed this study to test the hypothesis that macroorganism community structure (i.e., species richness, composition, and abundance) in water-filled tree holes of Panama is influenced by the presence or absence of top predators (odonates) and the availability of nutrients (leaf litter). Based on the results from other studies of small aquatic habitats and prior observations in tree holes of Panama, I predicted that macroorganism species richness and abundance would be lower in holes containing odonate larvae than in holes without odonates. Because the number of consumer species and individuals in a habitat is often linked to nutrient availability (reviewed by Srivastava and Lawton 1998 and Rosenzweig 1995), I predicted that addition of leaf litter to tree holes will increase macroorganism species richness and abundance relative to controls, and that removal of litter would have the opposite effect. Finally, I predicted that effects of nutrient availability and predation are interactive (i.e., odonate effects are respectively masked or enhanced when leaf litter abundance is high or low).

#### METHODS

This study was conducted in the lowland moist forest of Barro Colorado Island (BCI), Panama (see Leigh et al. 1996 for a site description). The fauna and abiotic characteristics of BCI tree holes are described elsewhere (CHAPTERS 1 and 2). All

tree holes used in this study were located in the understory (max. height 1.5 m) and  $\leq$  3 l in volume. I measured pH, height above the ground, total volume, and water surface area in each hole at the start of experiments. Hydrion® narrow- and broad-range colorimetric strips were used for all pH measurements.

I censused the macrofauna of each hole by removing its contents with a turkey baster or siphon into a white pan. Macroorganisms were identified and counted, and subsamples were collected when species could not be determined in the field. After each census, the organisms, litter, and water were returned to the hole and the pan was rinsed. Additional methodological details for natural and artificial tree hole sampling are given in CHAPTER 2.

#### **Predation**

I conducted a predator exclusion experiment to determine the effects of odonates on species richness and abundance of other macroorganisms in tree holes. In December 1995, I censused the macrofauna of 40 readily accessible natural tree holes in the forest understory. I removed odonates and other top predators from 20 of the holes, and placed a canopy of chicken wire (2–3 cm mesh) over the hole openings to prevent recolonization by odonates. Earlier experiments using artificial tree holes showed that chicken wire or netting deters oviposition by odonates, but permits colonization by other tree hole inhabitants (Fincke et al. 1997; Fincke 1998). I distributed variation in hole size between covered and uncovered holes as much as possible. Hereafter, I refer to uncovered holes as the <u>control</u> treatment and covered holes as the odonate <u>exclusion</u> treatment. All 40 holes were left untouched until the start of the following wet season.

In mid-May 1996, I surveyed all holes for the presence of odonates and other top predators, and removed any odonate larvae found in predator exclusion holes (5 of 20 holes). Six of the holes (3 control and 3 exclusion) were dry or otherwise disturbed, so I removed them from the experiment. I censused the remaining 34 holes every 14 d from 10 June until 29 July, 1996 (n = 4 censuses per hole), and I measured pH in each hole during censuses 1, 2 and 4. Any leaf litter trapped by the chicken wire covering an exclusion hole was collected and placed inside the hole after each census.

## <u>Nutrients</u>

In mid-August 1997, I divided 36 natural tree holes equally among three litter treatments: a control group, a litter removal group, and a litter addition group. I censused all holes 2 d before the start of experimental manipulations (Week 0). I assigned holes to treatment groups based in part on general shape, tree species, and initial macroorganism species richness to distribute potential sources of variance among groups as much as possible.

I removed all macrodetritus (> 1 cm<sup>2</sup>) from the 12 litter removal holes at the start of the experiment and at least once each week thereafter until Week 6. Care was taken to keep disturbance to a minimum during litter removal. Litter addition holes received 0.014 g leaf litter per cm<sup>2</sup> water surface area on Week 0, and then 0.007

g·cm<sup>-2</sup> on Weeks 2 and 4. I based the litter addition quantities on the average oven dry weight of litter collected over a 30 d period in artificial tree holes (650 ml cups, 71 cm<sup>2</sup> opening) tied to trees 1.0 m above ground level (mean = 0.007 g·cm<sup>-2</sup> surface area, SD = 0.33). Litter added to holes consisted of approximately equal proportions of leaves or leaflets from <u>Ceiba pentandra</u>, <u>Dipteryx panamensis</u>, <u>Ficus yoponensis</u> and <u>Platypodium elegans</u>. I collected the leaf material from tree crowns or recent branch falls as described in CHAPTER 4. All litter was air dried ≥ 7 d and then oven dried (70°C, ≥ 48 hrs) before weighing.

I recorded the number of macroorganism species present and their abundance in each hole on Weeks 2–6. I censused all holes again on Week 10 (i.e., after  $\geq 5$ wks without litter addition or removal) to determine if the macrofauna had recovered from manipulations. One of the control holes dried up during the experiment and was not included in analyses.

# Predator-Nutrient Interaction

On 1 October 1997, I secured 30 artificial tree holes (650 ml black plastic cups) to trees separated by 5-10 m in the forest understory. I evenly divided the cups into three nutrient availability treatments based on the quantity of initial litter inputs: high =  $4.00 \text{ g} (\pm 0.001)$ , medium =  $0.40 \text{ g} (\pm 0.001)$ , and low =  $0.04 \text{ g} (\pm 0.0005)$ . The composition, origin, and treatment of litter was the same as described for the nutrient availability experiment. I filled the cups with filtered (0.25 mm mesh) rain

water and left them undisturbed for 3 wks to allow development of macroorganism assemblages.

At the end of the third week (hereafter Day 0), I censused the contents of all artificial tree holes and added one mid-size <u>Megaloprepus coerulatus</u> (Odonata: Pseudostigmatidae; 10–18 mm body length excluding caudal lamellae) to half of the cups in the experiment. The initial mean body length of odonates did not differ among treatments ( $F_{2,12} = 1.32$ , P = 0.30). I censused the macrofauna of all cups on Days 4, 8, 16, and 32. The odonates were not counted in censuses. I checked the experiment every 3 d for the presence of <u>M</u>. <u>coerulatus</u> larvae and removed any macrodetritus (> 0.25 cm<sup>2</sup>) foreign to the experiment. I collected the fraction of experimental detritus remaining in the cups on Day 32. Some litter fragments were lost from two cups that were disturbed between Days 16 and 32, so I excluded these data from the analysis of remaining litter mass.

I measured the body length of the <u>M</u>. <u>coerulatus</u> larvae on Days 8, 16 and 32. One <u>M</u>. <u>coerulatus</u> larva was missing from the high litter treatment on Day 8, and one was missing from the medium treatment on Day 16. I replaced the missing individuals with new mid-size larvae, but excluded these replicates from the analysis of predator growth. <u>Toxorhynchites theobaldi</u>, another top predator in tree holes (e.g., Fincke 1999), occasionally colonized the non-predator holes. I included this species in census data, but consistently removed it when it occurred to reduce confounding of odonate predator effects. I analyzed species richness and abundance data from Day 4–32
censuses with a 2 x 3 factorial repeated-measures ANOVA using presence or absence of <u>M</u>. <u>coerulatus</u> and litter quantity as main effects.

I tested all data for normality and homogeneity of variance with normal probability plots and F-tests prior to analysis. I used Ryan-Einot-Gabriel-Welsch multiple range tests (REGWQ option; SAS 1989) to compare means when ANOVA results were significant. All G-tests had df = 1 unless otherwise noted, and G values were adjusted with Williams' correction (Sokal and Rohlf 1981). Proportional data were arcsine square-root transformed and the log-transformation was used to correct variance heterogeneity when necessary (Sokal and Rohlf 1981). Means shown in the results were calculated from untransformed data.

# RESULTS

Tree hole physicochemical properties, macroorganism species richness, and macroorganism abundance did not differ between controls and treatments at the start of the predator exclusion (Table 1) and litter addition/removal (Table 2) experiments. Average pH was similar between control and predator exclusion holes during the 1996 censuses (repeated-measures ANOVA:  $F_{1,32} = 0.98$ , P = 0.33; time\*treatment P = 0.095).

#### Predator Exclusion

I predicted that macroorganism species richness and abundance would be lower in tree holes containing odonates than in holes without these top predators. The timing of the experiment (early wet season) was problematic because odonates had not colonized 5 of the 17 control holes by the second census date. Moreover, many of the holes that were colonized contained only small odonate larvae (< 14 mm in body length), which have relatively slow feeding and growth rates (Fincke 1992a; Fincke et al. 1997). These problems were resolved by the third census date, when all control holes contained at least one large (> 19 mm) odonate larva or  $\ge 2$  mid-size (14–18 mm) larvae.

Analyses of data from the third and fourth censuses showed that the abundance of macroorganisms and the abundance of mosquitoes alone were unaffected by the presence of odonates (repeated-measures ANOVAs:  $F_{1,32} < 1.70$ , P > 0.20; time\*treatment P > 0.11 for both tests; Fig. 1a), but odonates reduced species richness by 1.12 taxa overall ( $F_{1,32} = 5.65$ , P = 0.024; time\*treatment P = 0.13; Fig. 1b). The size of a hole affects the number of organisms present (CHAPTER 1 and APPENDIX A), and, although not statistically different, control holes tended to be larger than exclusion holes (Table 1). When data from larger holes (i.e., > 500 ml) were converted to the number of macroorganisms per 500 ml, macroorganism abundance was significantly lower in the presence of odonates ( $F_{1,32} = 6.80$ , P = 0.014; time\*treatment P = 0.12).

Of 13 potential prey taxa (i.e., relatively common species with larvae living in the water), the presence of the mosquito <u>Culex urichii</u> and the ceratopogonid midge <u>Bezzia snowi</u> were negatively associated with the presence of large odonate larvae in control holes (data pooled from all censuses; G > 4.72, P < 0.05 in both tests). Similar (but non-significant) patterns were shown by larvae of the psychodid fly <u>Telmatoscopus</u> spp. and scirtid beetles (G < 3.7, P < 0.10 in both tests). These four taxa were found marginally more often in predator exclusion holes than control holes (Kruskal-Wallis test on the number of censuses these taxa were present in each treatment;  $X^2 = 3.14$ , P = 0.076). The presence of <u>Aedes terrens</u>, the most common mosquito species in natural tree holes on BCI (Fincke et al. 1997; CHAPTER 1), was not associated with the presence or absence of odonates (G = 0.23, P > 0.50). This species also did not differ in occurrence frequency between treatments (Kruskal-Wallis  $X^2 = 2.22$ , P = 0.14).

In summary, large odonates reduced the number of macroorganism species present in natural tree holes, and reduced total macroorganism abundance when hole sizes were standardized. The occurrence of four prey species showed a tendency for negative association with the presence of odonates, whereas the most common potential prey species did not.

#### <u>Nutrients</u>

I predicted that addition and removal of leaf litter would respectively increase and decrease macroorganism species richness and abundance relative to control holes. Differences in macroorganism abundance among all three treatments were marginally non-significant during Weeks 2–6 (repeated-measures ANOVA:  $F_{2,32} = 2.82$ , P = 0.074; Fig. 2a). However, litter addition holes contained more macroorganisms than litter removal holes ( $F_{1,22} = 7.12$ , P = 0.014). Addition of leaf litter to tree holes increased the overall average species richness by 1.04 species over controls, whereas litter removal reduced richness by 1.08 species (Fig. 2b). These differences were significant among treatments during Weeks 2–6 ( $F_{2,32} = 5.00$ , P = 0.013). There were no time\*treatment interactions for abundance or richness (P > 0.23 for all tests). Five weeks after litter manipulations were terminated (Week 10), average macroorganism abundance and number of species did not differ among treatments (abundance:  $F_{2,33} = 0.13$ , P = 0.88; richness:  $F_{2,33} = 0.08$ , P = 0.92; Fig. 2).

The cumulative number of species found in each treatment was similar (litter addition = 26, litter removal = 30, control = 27), and the differences in richness among treatments during Weeks 2-6 could not be attributed to the appearance or local extinction of a single species. All 35 holes used in the experiment contained one or more top predator taxa. The odonates <u>Megaloprepus coerulatus</u>, <u>Mecistogaster spp.</u>, and Aeshnidae occupied 60%, 68.6%, and 20% of the holes, respectively. <u>Dendrobates auratus</u> tadpoles and larvae of the mosquito <u>Toxorhynchites theobaldi</u> occupied 5.7% and 28.6% of the holes, respectively. Tadpoles of <u>D</u>. <u>auratus</u> were found only in litter removal holes, but all other top predators occurred in all treatments and were evenly distributed among treatments (G < 2.3, df = 2, P > 0.10 in all tests; aeshnids and <u>T</u>. <u>theobaldi</u> were pooled to meet test assumptions).

I used species composition data to determine if differences in richness among treatments was due to selective colonization and local extinction of certain species. Of the 30 taxa found in this experiment, 12 occurred in > 5 holes of each treatment. For these 12, the mean proportion of holes in litter addition treatment that were colonized only during Weeks 2–6 did not differ from controls (t = 1.24, df = 22, P = 0.23), nor did the mean proportion of holes that were occupied on Week 0 but not on subsequent weeks (i.e., local extinction) in the litter removal treatment (t = 0.10, df = 22, P = 0.92). However, this subset of taxa included odonates, veliid bugs and tipulid flies, which are relatively long-lived and can survive extended periods of food limitation (CHAPTERS 1 and 3).

I condensed the list of focal taxa to six relatively short-lived species (i.e., those most likely to respond to short-term changes in nutrient availability): <u>Aedes terrens</u>, <u>Anopheles eiseni</u>, <u>Bezzia snowi</u>, <u>Culex corrigani</u>, <u>Dero sp.</u>, and <u>Haemagogus (H.)</u> spp. Where they occurred, these taxa colonized a larger proportion of litter addition holes (68%) than control holes (41%) only after the start of manipulations (t = 3.62, df = 10, P = 0.005). All of these species also disappeared from  $\geq$  50% of the same litter addition holes after manipulations were terminated (i.e., between Weeks 6 and 10). The opposite pattern did not occur in the litter removal treatment; the proportion of litter week 0 did not differ from controls (t = 0.82, df = 7, P = 0.44; test assumed unequal variance).

The mean ( $\pm$  SE) persistence time (number of consecutive censuses that a species was present in a hole) did not differ between litter removal holes and litter addition holes (Kruskal-Wallis  $X^2 = 0.17$ , P = 0.68). However, the average number of species present for  $\geq 2$  consecutive censuses was greater in litter addition holes (6.75  $\pm$  0.75) than in litter exclusion holes (4.33  $\pm$  0.47; Kruskal-Wallis  $X^2 = 4.84$ , P = 0.027).

In summary, the addition and removal of leaf litter respectively increased and decreased macroorganism species richness relative to controls. Macroorganism abundance and the number of persistent species were greater in litter addition holes than litter removal holes. Six taxa generally colonized litter addition holes only after manipulations were initiated, then became locally extinct in most of those holes during the five weeks after manipulations were terminated. Local extinctions in litter removal holes were inconsistent among taxa.

# Predator-Nutrient Interaction

I predicted that predation and nutrient availability would have interactive effects on species richness and abundance within the physically standardized confines of artificial tree holes. Twenty-two macroorganism species were encountered in the experiment (all censuses combined). Larvae of the mosquito <u>Culex urichii</u> occurred in more cups and on more census dates than any other species. Initial (Day 0) macroorganism species richness and abundance were similar in holes that received odonates and those that did not ( $F_{1,28} < 1.07$  and P > 0.31 for both tests; Fig. 3).

After three weeks of colonization time (i.e., on Day 0), mean macroorganism abundance (Fig. 4a) and species richness (Fig. 4b) were greatest in the high litter quantity treatment ( $F_{2,27} > 4.17$ , P < 0.026 in both tests). Abundance and richness did not differ between low and medium litter quantity treatments.

Predation and nutrient availability did not interact to shape macroorganism community structure in the artificial holes; interactions were non-significant for both abundance ( $F_{2,24} = 0.01$ , P = 0.99) and species richness ( $F_{2,24} = 0.78$ , P = 0.47) during the Day 4–32 censuses (time\*predator\*litter P > 0.23 in both tests). The overall mean number of taxa was greater by 0.59 species in cups lacking odonates ( $F_{1,24} = 9.91$ , P = 0.004; Fig. 3b); data from Days 16 and 32 caused this effect (time\*predator P = 0.008). Total macroorganism abundance and the abundance of mosquitoes alone did not differ between predator and non-predator treatments ( $F_{1,24} < 0.24$ , P > 0.63, time\*predator P > 0.35 in both tests; Fig. 3a).

Of the 18 potential prey species in this experiment, only larvae of the mosquito <u>Toxorhynchites theobaldi</u> were more common (i.e., occurred in more cups) where odonates were absent (G = 4.76, P < 0.05). In low and medium litter quantity treatments combined, larvae of scirtid beetles and the mosquito <u>Culex urichii</u> were collectively more likely to decline in abundance when predators were present, and either increase or remain relatively constant in abundance when predators were absent ( $X^2 = 5.57$ , P = 0.018, df = 1, n = 26 cups containing <u>C. urichii</u>, scirtids, or both). Declines in abundance of these taxa were not associated with the presence or absence of odonates in the high litter treatment (Fisher's Exact two-tailed P = 0.56, n = 13).

Macroorganism abundance was greater in the high litter quantity treatment  $(F_{2,24} = 10.06, P = 0.0007; Fig. 4a)$ , but means did not differ among treatments on Days 16 and 32. The dytiscid beetle <u>Copelatus</u> sp. occurred only in the medium litter treatment; the syrphid fly <u>Copestylum rafaelanum</u>, the tipulid fly <u>Sigmatomera</u> spp., the psychodid fly <u>Telmatoscopus</u> spp., and the mosquitoes <u>Culex mollis</u> and <u>Trichoprosopon digitatum</u> occurred only in the high litter treatment. <u>Copestylum rafaelanum</u> and <u>Culex mollis</u> were found only on Day 0 and Day 4 censuses, whereas <u>Sigmatomera</u> spp. occurred only on Day 16 and Day 32 censuses. Despite the unequal distributions of these taxa among treatments and census dates, litter quantity had no effect on mean species richness ( $F_{2,24} = 0.26$ , P = 0.78; Fig. 4b). There were no time\*treatment interactions for abundance or richness (P > 0.10 in both tests).

Persistence time (the number of consecutive censuses that a species was present in a cup) was lower in the high litter treatment than in the other treatments (Kruskal-Wallis  $X^2 = 6.20$ , P = 0.045), but the number of species present in a cup on  $\ge 2$  consecutive census dates did not differ among litter quantities (Kruskal-Wallis  $X^2$ = 3.49, P = 0.17). The 5 taxa that occurred only in the high litter treatment (listed above) were more likely to be present in a cup on only one census date than on two or more consecutive census dates (G = 4.87, P < 0.05).

Although initial predator size was similar among litter treatments, the average length of <u>M</u>. <u>coerulatus</u> larvae increased with litter quantity over time ( $F_{2,10} = 9.03$ , P = 0.006; time\*litter P = 0.03) and mean lengths differed among all treatments on Day 32 (Fig. 5). Predation and nutrient quantity had an interactive effect on the

proportion of litter remaining at the end of the experiment ( $F_{2,22} = 4.03$ , P = 0.032); a larger fraction of litter remained when predators were present, but only when initial litter quantities were at low and medium levels (Fig. 6). The proportion of litter remaining in cups with predators was correlated with the log of initial litter mass (Pearson r = 0.618, n = 14, P = 0.019). The same was true in cups without predators (r = 0.898, n = 14, P = 0.0001).

### DISCUSSION

Despite considerable work on phytotelm communities, my study is the first to show that predation and nutrient availability influence the number of species present in water-filled tree holes (but see the artificial tree hole study by Srivastava and Lawton 1998). Specifically, both natural and artificial tree holes contained fewer macroorganism species in the presence of top predators, and respectively contained more or fewer species when litter was added or removed. Because tree holes on BCI typically contain only 5 or 6 species on any wet season day, the loss or addition of a single species reflects a substantial proportional change in community structure.

# **Predation**

The greater species richness in predator exclusion holes may be a conservative result. Some leaf litter potentially falling into the odonate exclusion holes was probably deflected by the chicken wire covering. As shown by the results of the litter

manipulation and predator-nutrient interaction experiments, greater litter quantities support higher species richness in tree holes.

Results of this and related studies suggest that selective predation, not reduced abundance (e.g., Addicott 1974), contributed to reduced species richness in tree holes containing odonates. In laboratory trials, medium and large odonates preferentially fed on larger mosquitoes when several sizes of the same prey species were available (APPENDIX D). Most odonates also preferred <u>Culex mollis</u> larvae over <u>Aedes terrens</u> and other <u>Culex</u> spp. when similar-sized individuals of each species were presented simultaneously (APPENDIX E). Other unreplicated lab experiments indicated that odonates preferentially feed on all <u>Culex</u> species (except possibly <u>C</u>. <u>conservator</u>) over <u>A. terrens</u> (pers. obs.).

Data from the field experiments described above provide correlative evidence for selective predation by odonates. The four potential prey species that were negatively associated with large odonates (or nearly so) in natural tree holes are among the largest common prey available (final instars > 5 mm body length). <u>Toxorhynchites theobaldi</u>, the only species that occurred significantly less often when odonates were present in the artificial tree hole experiment, is also one of the largest non-odonate invertebrates in this system (cf. Bradshaw and Holzapfel 1983). In addition, <u>T</u>. theobaldi is a potential competitor (i.e., for mosquito prey) with odonates, and its greater frequency in the absence of odonates probably reflects the absence of intraguild predation and priority effects (Fincke 1999). The lack of association between the presence of <u>A</u>. terrens and odonates, and the similar

occurrence frequency of <u>A</u>. <u>terrens</u> between control and odonate exclusion holes, suggest that this species is not preferred prey in natural tree holes.

Although odonates selectively feed on large prey, they will also take smaller organisms on occasion (APPENDIX D), and probably pursue a broad range of prey types when food is limiting. Like other predacious aquatic insects (e.g., Formanowicz 1982), odonates appear to switch between sit-and-wait and active foraging tactics depending on their degree of starvation (pers. obs.). Some tree hole mosquitoes alter their behavior in the presence of a predator (Grill and Juliano 1996), but most species must frequently move between the water surface and submerged detritus to obtain oxygen and food, respectively. This activity makes them especially vulnerable to attack by sit-and-wait predators. My results and observations suggest that selective predation and sparse prey refugia combine to promote local prey extinctions in tree holes.

An alternative explanation for reduced richness in the presence of odonates is that some prey species avoid holes containing the predators (i.e., by detecting chemical or other cues). Although the presence of some prey species was negatively associated with the presence of odonates, the pattern was too weak to support an explanation based on differential colonization. Moreover, odonates are the most common top predators in tree holes (Fincke 1999), and most potential prey species are specialists in this habitat (CHAPTER 1). Any species that depends on tree holes as a reproductive resource throughout the wet season would likely become extinct (on a regional scale) in an attempt to avoid odonates.

#### <u>Nutrients</u>

There are at least two possible explanations for increased species richness in litter addition holes. First, the greater energy supply may have led to greater abundance and persistence of rare species (e.g., Srivastava and Lawton 1998). Second, some species may be differentially attracted to chemical or other cues (Bentley and Day 1989) associated with the early stages of leaf decay. These mechanisms are not mutually exclusive, and results of the litter addition/removal experiment offer some support for each. Six taxa colonized holes proportionally more often after litter additions and then disappeared from most of the same holes after litter manipulations were terminated. Because these species were not unique to litter addition holes, differential colonization of holes containing fresh litter inputs is a more probable explanation for their appearance and subsequent local extinctions than is energy limitation. The lack of difference in species' persistence times indicates that species turnover was similar among treatments, but the larger number of persistent species in litter addition holes suggests that the added energy enabled a larger number of potentially competing species to coexist.

The loss of species from litter removal holes is less easily explained because species composition did not change in a consistent manner. However, top predators occurred in all holes used in this experiment, and the lower species richness may have resulted from additive effects of nutrient limitation and enhanced predator efficiency. Leaf litter is functionally important both as food and as a source of structural

heterogeneity in many aquatic habitats (e.g., Richardson 1992). In spatially limited systems such as tree holes, the role of leaf litter as a source of physical complexity may be of relatively great importance. Litter potentially restricts the movements of odonates and can serve as temporary refugia for prey. Thus, litter removal should increase predator-prey encounter rates. The potential result is a stochastic pattern of local extinction, with some species becoming extinct due to nutrient limitation and others disappearing due to predation. The relative importance of each of these factors is presumably sensitive to hole-specific conditions (e.g., nutrient inputs from stemflow; Carpenter 1982a) and characteristics of prey species (e.g., size-structure), which cannot be addressed with data from this study.

## Predator-Nutrient Interaction

Although there was no interaction between predation and nutrient quantity in the artificial tree hole experiment, predator effects matched the patterns observed in natural holes: macroorganism species richness was greater in the absence of odonates, but abundance was not (Fig. 3). The presence of <u>T</u>. theobaldi did not confound the absence of odonate predators (i.e., richness and abundance results were unchanged when the three cups containing <u>T</u>. theobaldi on every census date were removed from the analysis).

Effects of litter quantity on community structure were restricted to the first few censuses (i.e., 5 wks after the experiment was set up). This result indicates that the major effects of litter resources on community structure occur only during the early

stages of leaf degradation, when most soluble compounds are leached into the water (e.g., Carpenter 1982b; Gillon et al. 1994). Results of the litter manipulation experiment in natural holes (Fig. 2; Week 10) also support this conclusion.

The degradation rate of a leaf is associated with physical characteristics such as permeability and thickness (e.g., Gillon et al. 1994), and the correlation of percent litter remaining with initial litter mass in the artificial tree holes is attributed to the greater relative surface area of leaf fragments in the low and medium litter treatments. The interaction between predation and resource quantity effects on litter degradation rates (Fig. 6) suggests that predators reduce the ability of grazers and detritivores to facilitate the degradation of litter (e.g., Carpenter 1982b; Fish and Carpenter 1982) when resources are limiting (but see CHAPTER 5). The greater frequency with which <u>Culex urichii</u> and scirtid beetles showed a tendency to decline in abundance in low and medium litter cups containing predators is a likely mechanism for the interaction. Both taxa are potential prey for odonates and function as grazing omnivores or detritivores in tree holes (CHAPTER 1).

The lower average persistence time of species in the high litter quantity treatment of the artificial tree hole experiment is partly due to the time frame of data collection. Holes were allowed to stand undisturbed for 3 wks before Day 0. As a result, early successional species (e.g., <u>Culex mollis</u> and <u>Copestylum rafaelanum</u>; CHAPTER 1) were progressing toward local extinction during the first two censuses, and other taxa (e.g., <u>Sigmatomera spp.</u>) were just beginning to colonize the cups. All

of these species occurred only in the high litter treatment, and their turnover reduced average persistence times relative to the other treatments.

The lack of a significant predator effect on mosquito abundance and the lack of a predator\*nutrient interaction in this study contrast the findings of Fincke et al. (1997). Differences between results of these studies are partly attributed to the type of data gathered in each, and to the relatively short duration of my artificial tree hole experiment. Odonates can reduce the abundance of certain size classes or stages (e.g., pupae) of mosquitoes without significantly affecting total abundance (Fincke et al. 1997). Such an effect may have occurred in this study, but macroorganism abundance was not quantified by size or life stage.

Some taxa in the artificial tree hole study (<u>C</u>. <u>urichii</u> and scirtid beetles) indicated a trend toward development of a predator-nutrient interaction, and odonates grew faster in the high litter quantity treatment (Fig. 5; cf. Fincke et al. 1997). However, total macroorganism abundance in my experiment had barely stabilized by the last two censuses (Fig. 3a). Fincke and coworkers censused artificial tree holes over a much longer period of time (e.g., up to 10 wks after mosquito abundance became relatively stable) and included fallen fruit as a nutrient source. Thus, the comparatively short experimental time span and lack of prolonged nutrient availability precluded a predator-nutrient interaction in this study.

#### CONCLUSION

Although the mechanisms were elusive due to inconsistencies in species composition effects, my results show that predation and nutrient availability influence community structure in water-filled tree holes. My results also suggest that these factors operate at different time scales. Leaf litter inputs affect community parameters over a period of weeks, whereas predation may act over many months (i.e., as long as large odonates are present). On BCI, fresh litter tends to be most abundant in the early wet season (e.g., Foster 1996), when hole occupancy by odonates is relatively low (Fincke 1992b). Thus, examination of predation and nutrient effects on community structure in a seasonal context would be an appropriate extension of this work.

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Table 1. Means (SD) of physicochemical properties, macroorganism species richness, and macroorganism abundance measured at the start of the predator exclusion experiment (December 1995). Area = estimated water surface area. Control = holes not covered with chicken wire, Exclusion = holes covered with chicken wire to prevent odonate colonization. n = 17 for each mean, df = 32 for all t-tests.

	Control	Exclusion	t <sub>obs</sub>	Р
Height (cm)	55 (42)	64 (44)	1.18	0.25
Volume (ml)	1075 (922)	583 (433)	0.60	0.55
[range]	[20 - 3000]	[17 – 1600]		
Area (cm²)	111 (83)	62 (51)	1.66	0.11
рН	6.06 (0.95)	6.32 (0.63)	0.94	0.36
Richness	4.8 (2.4)	4.9 (2.0)	0.15	0.89
Abundance	48.7 (45.6)	29.7 (26.1)	0.56	0.58

Table 2. Means (SD) of physical characteristics, macroorganism species richness, and macroorganism abundance measured at the start of the nutrient addition/removal experiment (Week 0).

	Litter Added	Litter Removed	Control	F <sub>2,33</sub>	P
Height (cm)	62 (42)	51 (45)	0.41 (0.35)	0.77	0.47
Volume (ml)	801 (533)	833 (865)	662 (472)	0.21	0.82
[range]	[200 - 2000]	[200 - 3000]	[250 – 2000]		
Area (cm <sup>2</sup> )	138 (12 <b>2</b> )	110 (84)	134 (110)	0.11	0.89
Richness	4.6 (2.1)	5.2 (2.8)	5.6 (1.8)	0.70	0.51
Abundance	20.8 (19.0)	23.1 (18.4)	45.5 (47.0)	2.19	0.13

#### FIGURE CAPTIONS

Fig. 1. Mean ( $\pm$  SE) abundance (A) and species richness (B) of non-odonate macroorganisms in control holes (odonates present) and odonate exclusion holes. n = 17 for each mean.

Fig. 2. Mean ( $\pm$  SE) macroorganism abundance (A) and species richness (B) in natural tree holes to which leaf litter was added on Weeks 0, 2, and 4 (Add), removed at least weekly until Week 6 (Rem), or unmanipulated (Ctrl). n = 11 for control (Ctrl) means, n = 12 for all others.

Fig. 3. Mean ( $\pm$  SE) macroorganism abundance (A) and species richness (B) in artificial tree holes with (Predator) and without (No Predator) the odonate <u>Megaloprepus coerulatus</u>. n = 15 for each mean.

Fig. 4. Mean ( $\pm$  SE) macroorganism abundance (A) and species richness (B) in artificial tree holes containing different initial quantities of litter (High = 4.0 g, Medium = 0.4 g, Low = 0.04 g). n = 10 for each mean.

Fig. 5. Mean ( $\pm$  SE) body length (mm) of larval <u>Megaloprepus coerulatus</u> added (on Day 0) to artificial tree holes containing different initial quantities of litter (High = 4.0 g, Medium = 0.4 g, Low = 0.04 g). n = 4 for High and Medium treatment means, n = 5 for Low treatment means.

Fig. 6. Mean (+ SE) percent litter mass remaining in artificial tree holes at the end of the predator-nutrient interaction experiment. High, Medium and Low refer to different initial quantities of litter in holes to which the predator <u>Megaloprepus</u> <u>coerulatus</u> was (dark bars) or was not (open bars) added. n = 4 for the "High-No Predator" and "Low-Predator" means. n = 5 for all other means.





















# FIGURE 6



APPENDICES

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## APPENDIX A

# CORRELATION OF COMMUNITY PARAMETERS WITH ABIOTIC FACTORS

Table A1. Pearson correlation coefficients for community parameters and abiotic factors in 206 natural tree holes. Average values were used for holes sampled multiple times. Sample sizes (in parentheses) differ because not all variables were measured in all holes. Richness mean = 4.9, n = 206, range = 0-14. Abund. = abundance, DO = dissolved oxygen, Cond. = conductivity. \*\* = P < 0.01. Methodological details are given in CHAPTER 2.

	Abund.	Height (m)	Volume (ml)	pH	DO (mg·l <sup>-1</sup> )	Cond. (µS)
Richness	0.71** (196)	-0.20** (190)	0.45** (201)	0.07 (182)	-0.06 (68)	-0.12 (68)
Abund.		-0.05 (181)	0.43** (185)	0.04 (176)	0.12 (68)	-0.20 (68)
Height			-0.02 (188)	0.08 (177)	0.18 (65)	-0.02 (65)
Volume			—	0.09 (180)	-0.13 (67)	0.10 (67)
Mean	35.2	3.9	1689	6.00	1.26	314
Range	0-373	0.06-34.0	13-45800	3.4-8.0	0.0-4.9	55-1982
n	196	190	201	182	68	68
#### APPENDIX B

### BODY LENGTH-BIOMASS RELATIONSHIPS AMONG ODONATES

Table B1 summarizes relationships between dry mass, fresh mass, and body length for three odonate taxa that commonly occur in tree holes of Barro Colorado Island, Panama. Representative curves are shown in Figs. B1 and B2. Individuals were not differentiated based on sex. "<u>Mecistogaster</u> spp." included <u>M. linearis</u> and <u>M. ornata</u>. "Aeshnids" included <u>Gynacantha membranalis</u> and <u>Triacanthagyna</u> <u>dentata</u>.

I measured body length of pseudostigmatids (Megaloprepus coerulatus and Mecistogaster spp.) from the anterior edge of the labrum to the posterior edge of the last abdominal sternite (i.e., not including caudal lamellae) using a ruler or calipers. I measured aeshnids from the anterior labral margin to the tip of the caudal cerci. Body lengths were approximated to the nearest 0.25 mm. I measured fresh and dry mass to the nearest 0.0001 g on an A&D<sup>•</sup> ER-180A balance. Fresh mass was determined from live larvae that were carefully blotted dry on a cotton cloth before weighing. I dried freshly killed larvae in an oven at 70°C for  $\geq$  24 hr (depending on body size) to determine dry mass.

Table B1. Biomass-body size relationships for tree hole odonates. Range = body lengths (mm) of the smallest and largest individuals measured within a taxon. n = number of individuals measured. dm = dry mass (g), fm = fresh mass (g), bl = body length (mm).

			Dry mass vs. Body le	s vs. Body length	
Taxon	Range	<u>n</u>	Equation	<b>r</b> <sup>2</sup>	
M. coerulatus	6.5 - 29.0	17	$dm = 3.0x10^{-6} \cdot bl^{2.93}$	0.990	
Mecistogaster spp.	8.5 – 27.25	18	$dm = 2.0 \times 10^{-6} \cdot bl^{3.09}$	0.984	
Aeshnidae	4.25 - 37.0	23	$dm = 5.0x10^{-5} \cdot bl^{2.68}$	0.995	
			Dry mass vs. Fresh mass		
Taxon		n	Equation	r <sup>2</sup>	
<u>M</u> . <u>coerulatus</u>	6.5 - 29.0	17	$dm = 0.195 \cdot fm - 0.003$	0.982	
Mecistogaster spp.	8.5 - 27.25	18	$dm = 0.213 \cdot fm - 0.003$	0.9 <b>69</b>	
Aeshnidae	4.25 - 35.0	12	$dm = 0.187 \cdot fm - 0.006$	0.933	



Fig. B1. Megaloprepus coerulatus dry mass vs. body length.



Fig. B2. Megaloprepus coerulatus dry mass vs. fresh mass

## APPENDIX C

## **BIOMASS OF SELECTED MOSQUITO SPECIES**

I reared adult mosquitoes from field-collected larvae in the laboratory or insectary. Larvae were fed ad libitum flake fish food and active dry yeast. Adults were killed by freezing, separated by species and sex, and dried at 70°C for  $\ge 24$  h. I weighed 2–5 mosquitoes (depending on size) as a group and estimated the mass of an individual by dividing group weight by group size. I treated the weight estimate from each group as a separate data point for determination of means and for analyses. Weights were measured to the nearest 0.01 mg on an A&D°ER-180A balance. Mean male mass and mean female mass were compared within species using t-tests assuming equal variance. Results are summarized in Table C1.

Table C1. Mean (SD) dry mass (mg) of adult mosquitoes. n = number of groups of mosquitoes weighed. \*\* = P < 0.02.

	MALES		FEMALES		
Species		Mean	_ <u>n</u>	Mean	t_
Aedes terrens spp. complex	6	0.337 (0.072)	8	0.496 (0.056)	4.67**
<u>Culex</u> corrigani	4	0.217 (0.055)	5	0.275 (0.058)	1.52
Culex mollis	8	0.316 (0.054)	9	0.443 (0.104)	3.20**
<u>Culex urichii</u>	4	0.576 (0.047)	1	0.650	_
<u>Haemagogus</u> ( <u>H</u> .) spp.	5	0.376 (0.070)	5	0.593 (0.063)	5.18**
Haemagogus leucotaeniatus	2	0.508 (0.200)	3	0.658 (0.080)	1.24
Limatus assuleptus	5	0.235 (0.056)	7	0.327 (0.048)	3.07**
Orthopodomyia fascipes	3	1.033 (0.252)	9	1.050 (0.238)	0.10
Trichoprosopon digitatum	1	1.300	1	1.300	_

#### APPENDIX D

## SIZE-SELECTIVE PREDATION BY TOP PREDATORS IN TREE HOLES

I conducted several replicated laboratory experiments to determine if larval damselflies (Pseudostigmatidae: <u>Mecistogaster</u> spp. and <u>Megaloprepus coerulatus</u>), larval dragonflies (Aeshnidae: <u>Gynacantha membranalis</u> and <u>Triacanthagyna dentata</u>), and <u>Dendrobates auratus</u> tadpoles preferentially feed on specific size classes of prey. Species of <u>Mecistogaster</u> and genera of Aeshnidae were pooled for experiments.

Each predator was starved  $\geq 36$  hr and placed in an artificial tree hole (black plastic cup, 650 ml max. volume) with ca. 200 ml tap water and 25 <u>Culex mollis</u> (Diptera: Culicidae) prey. A small amount of active dry yeast was added to each cup as food for prey (except in <u>D</u>. <u>auratus</u> trials), and a strip of balsa wood (0.1 cm x 1.5 cm x 10 cm) was included as a predator perch site. In each trial, prey were evenly divided among 5 size/life stage classes (4 larval instars plus pupae). Prey body length (excluding respiratory siphon) ranges were determined from measurements of  $\geq 5$ individuals in each life stage. Odonates were grouped into size classes based on head capsule width. An ocular micrometer was used for all measurements. New predators and prey were used in each trial. Controls lacked predators.

I recorded the number of prey individuals consumed (or dead in controls) in each size class after 12 hrs. Means for each predator were analyzed with Ryan-Einot-Gabriel-Welsch multiple range tests and Kruskal-Wallis ( $X^2$  approximation) tests. Results are shown in Table D1.

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Table D1. Mean (SE) number of prey consumed (or dead in controls) in each size class. Predator head capsule widths (mm): A = 2.0-3.3, B = 3.3-4.3, C = 4.3-5.3, D = 6.1-7.3. Body length ranges (mm) for each prey life stage: I = 1.5-1.8, II = 2.4-2.6, III = 3.7-4.1, IV = 5.1-5.6, and P = pupae (3.4-4.5 mm<sup>2</sup> in lateral aspect). Within a row, means followed by the same letter do not differ. n = number of replicates. \*\* = P < 0.01.

Predator	<u>n</u>	I	п	111	IV	P	<u>X</u> <sup>2</sup>
Control	10	0.1 <b>a</b> (0.10)	0.2a (0.13)	0.2a (0.13)	0.1a (0.10)	0.0a (0.0)	2.60
Meg. coerulatus A	12	0.5a (0.23)	0.4a (0.23)	2.2b (0.27)	0.8a (0.43)	0.6a (0.23)	19.8**
Meg. coerulatus B	10	0.4a (0.22)	1.5a (0.54)	4.0b (0.39)	4.1b (0.28)	1.8a (0.49)	28.0**
Meg. coerulatus C	10	0.3a (0.21)	0.7ab (0.37)	1.7b (0.37)	3.7c (0.50)	2.3bc (0.65)	21.5**
Mecistogaster B	10	0.1a (0.10)	1.0ab (0.30)	1.7b (0.33)	2.2b (0.47)	1.5ab (0.41)	18.0**
Mecistogaster C	10	0.1a (0.10)	0.8ab (0.33)	1.6b (0.37)	1.5b (0.37)	1.1ab (0.49)	14.0**
Aeshnidae B	10	0.1a (0.10)	0.8ab (0.33)	2.2b (0.51)	2.1b (0.41)	1.4ab (0.48)	16.6**
Aeshnidae D	11	0.7a (0.40)	1.8ab (0.47)	3.3bc (0.43)	3.8c (0.47)	3.5c (0.61)	21.7**
<u>D</u> . <u>auratus</u>	9	3.7a (0.44)	4.2a (0.36)	4.3a (0.17)	2.0b (0.37)	1.2b (0.28)	26.9**

## APPENDIX E

# SPECIES-SELECTIVE PREDATION BY TOP PREDATORS IN TREE HOLES

I conducted several replicated laboratory experiments to determine if larval damselflies (Pseudostigmatidae: <u>Mecistogaster</u> spp. and <u>Megaloprepus coerulatus</u>), larval dragonflies (Aeshnidae: <u>Gynacantha membranalis</u> and <u>Triacanthagyna dentata</u>), and <u>Dendrobates auratus</u> tadpoles preferentially feed on certain species of prey. Species of <u>Mecistogaster</u> and genera of Aeshnidae were not differentiated for experiments.

Each predator was starved  $\geq$  36 hr and placed in an artificial tree hole (black plastic cup, 650 ml max. volume) with ca. 200 ml tap water and 5 similar-sized larvae of each of two or three mosquito species (i.e., second and third instars of larger species were matched with fourth instar larvae of the smallest species). A small amount of active dry yeast was added to each cup as food for prey (except in <u>D</u>. <u>auratus</u> trials), and a strip of balsa wood (0.1 cm x 1.5 cm x 10 cm) was included as a predator perch site. Ranges of head capsule widths (mm) of odonates used in experiments were: <u>Megaloprepus coerulatus</u>, 3.3–5.3; <u>Mecistogaster</u> spp., 4.5–5.0; and Aeshnidae, 5.0–7.0. <u>Dendrobates auratus</u> tadpoles were 13–16 mm in body length (tail excluded). New predators and prey were used in each trial, and control trials lacked predators. Head capsule widths were measured with an ocular micrometer.

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I recorded the number of prey individuals of each species remaining after 12 hrs. Prey preferences were determined using the selection index ( $\alpha$ ) of Manly et al. (1972) as described by Scott and Murdoch (1983):

$$\alpha_{i} = \ln(R_{i}/N_{i}) / [\Sigma \ln(Rj/Nj)]$$

In this equation,  $N_i$  and  $R_i$  are initial and final abundances of species *i*, respectively, and the summation includes all prey species. Alpha ranges between 0 and 1 (for no preference,  $\alpha = 0.5$  when two prey species are offered and 0.33 when three prey species are offered). Alpha values approximate a normal distribution (Manly 1974), and were analyzed with ANOVAs or t-tests. Results are presented in Tables E1 and E2.

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- Manly, B. F. J., P. Miller, and L. M. Cook. 1972. Analysis of a selective predation experiment. Am. Nat. 106: 719-736.
- Scott, M. A. and W. W. Murdoch. 1983. Selective predation by the backswimmer, <u>Notonecta</u>. Limnol. Oceanogr. 28: 352-366.

Table E1. Mean (SE)  $\alpha$ -values obtained from selective predation experiments in which three prey species were presented. n = 10 feeding trials for each mean. \*\* = P < 0.01. Within a row, means followed by the same letter do not differ (based on Ryan-Einot-Gabriel-Welsch multiple range tests). There was no mortality in control trials.

		Prey Species		_
Predator	Culex urichii	Culex corrigani	Culex mollis	F <sub>2,27</sub>
Control	0	0	0	-
Meg. <u>coerulatus</u>	0.27a (0.10)	0.35a (0.11)	0.38a (0.09)	0.33
Mecistogaster spp.	0.13a (0.04)	0.21a (0.05)	0.67b (0.08)	24.5**
Aeshnidae	0.27a (0.09)	0.16a (0.06)	0.56b (0.09)	6.94**

Table E2. Mean (SE)  $\alpha$ -values obtained from selective predation experiments in which two prey species were presented. n = number of feeding trials for each predator species. \*\* = P < 0.01. There was no mortality in control trials.

	_	Prey S			
Predator	<u>n</u>	Culex mollis	Aedes terrens	t <sub>obs</sub>	
Control	7	0	0	_	
Meg. <u>coerulatus</u>	7	0.76 (0.10)	0.24 (0.10)	3.56**	
Mecistogaster spp.	9	0.80 (0.09)	0.20 (0.09)	7.07**	
Aeshnidae	6	0.51 (0.13)	0.49 (0.13)	0.15	
<u>D</u> . <u>auratus</u>	5	0.95 (0.05)	0.05 (0.05)	13.5**	







IMAGE EVALUATION TEST TARGET (QA-3)









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