

METHODS IN ECOLOGY

Insect Sampling in Forest Ecosystems

EDITED BY

SIMON R. LEATHER

Department of Biological Sciences

Imperial College of Science, Technology and Medicine

Silwood Park

Ascot

UK

SERIES EDITORS

J.H. LAWTON CBE, FRS

Natural Environment Research Council

Swindon, UK

G.E. LIKENS

Institute of Ecosystem Studies

Millbrook, USA

2005

 **Blackwell**
Publishing

Sampling methods for water-filled tree holes and their artificial analogues

S.P. YANOVIK AND O.M. FINCKE

Introduction

Insects of small aquatic habitats found in plants, called phytotelmata (plant-held waters; Varga 1928), have attracted the attention of naturalists for the greater part of a century (Fish 1983). For biological investigations, the relatively small accumulations of water occurring in bromeliads, pitcher plants, and tree holes offer several methodological advantages over lakes, streams, and other comparatively large systems (e.g. Maguire 1971). First, phytotelmata are discrete and can be treated as individual units for sampling and faunal surveys. Second, these habitats are often abundant where they occur, permitting sample sizes appropriate for statistical analyses. Finally, the macrofauna of phytotelmata is often specialized and of manageable diversity and abundance. This is especially true of the aquatic insect inhabitants (e.g. Kitching 2000). Water-filled tree holes are among the most tractable of small aquatic systems, in part because they are relatively persistent, and can be mimicked with plastic cups, bamboo sections, or other inexpensive materials. Despite these unique features of tree holes and their specialized inhabitants, the extent to which processes affecting their biodiversity and community structure can be generalized to larger systems remains to be seen.

Natural tree holes

Water-filled tree holes are formed by the collection of rainwater in natural cavities occurring in the above-ground woody portions of trees (e.g. Kitching 1971a). They exist in hardwood forests all over the world (Fish 1983, Kitching 2000), and are the most abundant standing water systems in some tropical forests. Tree holes occur in a variety of shapes and sizes. In the lowland moist forest of Panama, they may be superficially categorized as slit-shaped, bowl-shaped (Fig. 8.1), or pan-shaped (Fig. 8.2), based on the morphology of the hole aperture and the ratio of water volume to surface area (Fincke 1992a). Temperate tree holes have been classified according to the presence or absence of a continuous lining of tree bark on the hole interior (Kitching 1971a). Although tree holes occur in the crowns of trees and may exceed 50 liters in size (Fincke

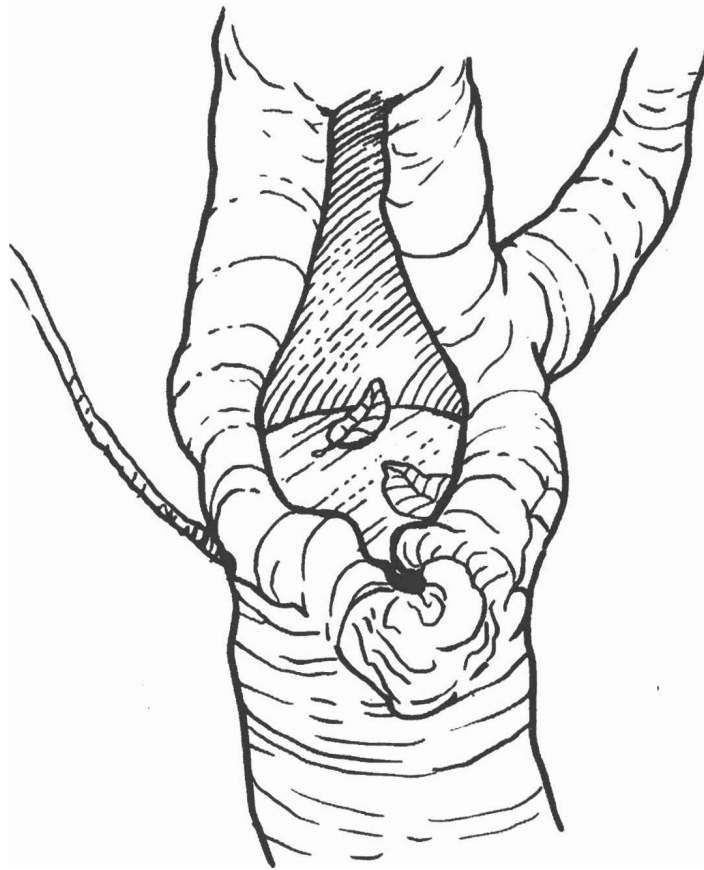


Fig. 8.1 Typical cup- or bowl-shaped tree hole in Panama.

1992a, Yanoviak 1999a, 1999b), most are much smaller, and many occur below 2 meters, where they are easily accessible. As such, they are excellent focal habitats for investigations of aquatic insect behavior, population biology, and community ecology.

A variety of macroorganisms use tree holes as breeding sites, and many species breed exclusively in this habitat. Aquatic insects dominate the assemblages of macrofauna in tree holes; larvae of true flies (Diptera) are generally the most common inhabitants (e.g. Snow 1949, Kitching 2000, Yanoviak 2001a). Tree holes are also the primary breeding sites for many disease vectors, including mosquitoes (Diptera: Culicidae; Galindo et al. 1955) and biting midges (Diptera: Ceratopogonidae; Vitale 1977). Tropical tree holes have the most diverse fauna and harbor an array of predators that are absent in temperate holes (e.g. odonates and tadpoles of dendrobatid frogs; Kitching 1990, Fincke 1992a, 1998, Orr 1994). Aquatic insect assemblages of tree holes are sufficiently diverse in terms of taxonomy and ecological function to permit theory-based studies, yet distinct and simple enough to be manageable for students with limited entomological background.

Here we present methods for non-destructive sampling of aquatic insects and

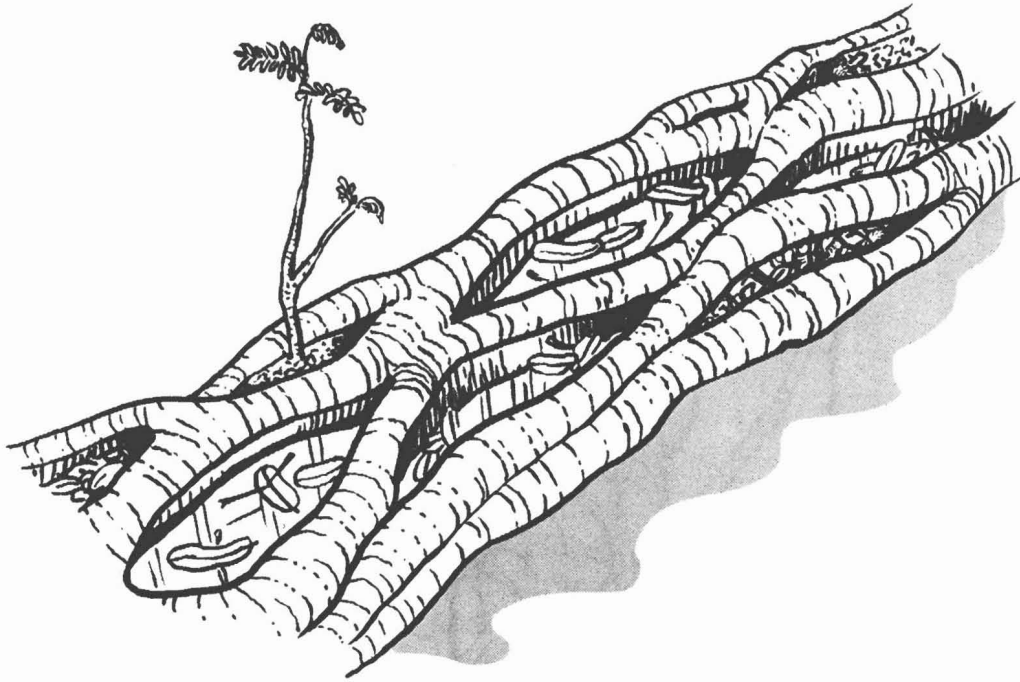


Fig. 8.2 Pan-shaped tree holes formed by the collection of rainwater in the trunk of a fallen tree.

other macroorganisms from water-filled tree holes based on our experience in Neotropical forests. Our goals are to describe a thorough approach to sampling tree holes, and to identify potential problems associated with data collection and interpretation, which also apply to other types of phytotelmata. All of the concerns we address may not be applicable to tree holes in all types of forests. For example, holes in temperate forests often lack predators, support lower insect diversity, and are subject to stronger seasonal effects, which may influence the frequency and timing of sampling required for a thorough inventory of tree hole occupants. We conclude with some caveats that should be considered before drawing general ecological or evolutionary inferences from tree holes systems. Belkin et al. (1965) and Service (1993) provide additional useful information and references regarding insect sampling from tree holes, with emphasis on mosquito larvae.

Sampling techniques for natural tree holes

Accurate estimates of aquatic insect abundance and diversity in most water-filled tree holes can be obtained with simple procedures and equipment (Fig. 8.3). The most common approach is removal of contents of the hole to a pan for counting. Researchers have devised a variety of techniques to accomplish this task, but reasonably complete samples are obtained by removing detritus and water from the hole, and sieving out the macroorganisms (e.g.

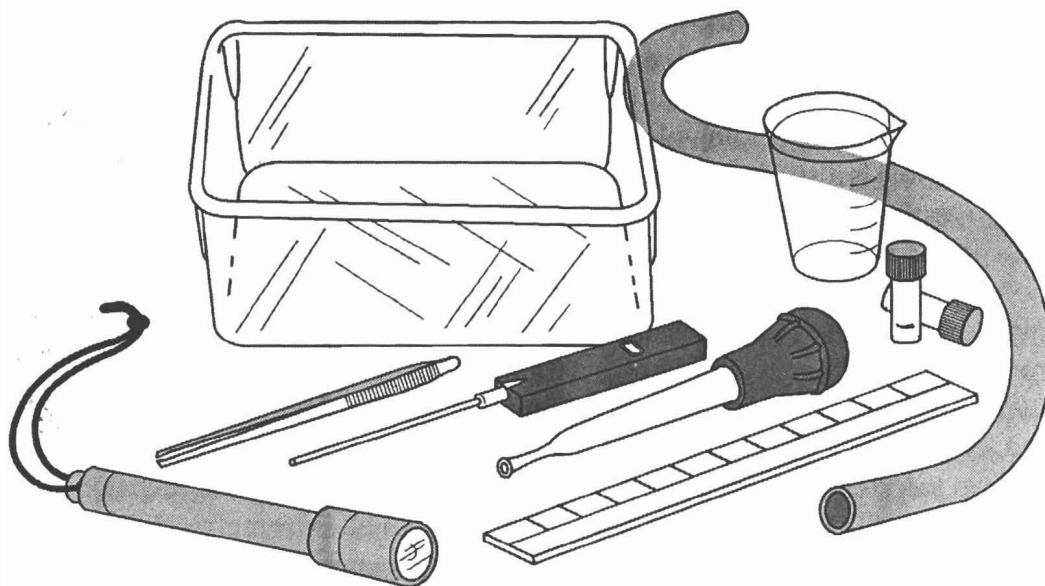


Fig. 8.3 Basic equipment used for sampling water-filled tree holes.

Jenkins & Carpenter 1946, Bradshaw & Holzapfel 1983, Walker & Merritt 1988, Copeland 1989, Barrera 1996).

Sub-sampling is one alternative approach to data collection from tree holes. Kitching (1971b) invented a core sampler that extracts a fraction of the hole volume with each use. This device can provide density data for population studies of some taxa (Kitching 1972a, 1972b), and it collects deep sediments, but the size and rigidity of the corer limit its use to a subset of holes with sufficiently large openings (Barrera 1988). Moreover, insects are often non-randomly distributed within and among tree holes (e.g. Barrera 1996), and it is unlikely that sub-samples collected with a corer would be useful in general surveys or community-level studies.

Although techniques will vary according to the nature of the investigation, thorough tree hole sampling can be summarized as a five-step process:

- 1 organisms in the undisturbed hole are noted with the aid of a flashlight, and water chemistry parameters are measured;
- 2 detritus and sediments are removed;
- 3 fluid contents are removed;
- 4 the hole is repeatedly flushed with clean water;
- 5 the interior walls of the empty hole are inspected with a flashlight.

These steps are useful for documenting the macrofauna of the most commonly encountered tree holes: those of small to medium volume (e.g. <5 liters) in which the water surface is exposed and accessible. Larger holes, and holes with narrow or slit openings, are more problematic and require improvised sampling techniques based on specific hole characteristics. Measuring water chemistry variables can be especially difficult in holes with narrow openings, and is best

accomplished with small electronic probes. Tree hole water chemistry and temperature vary with hole size, and fluctuate considerably over a 24-hour period (e.g. Fincke 1999), hence multiple readings are preferable.

Regardless of tree hole volume or morphology, step 1 should be completed before a hole is disturbed. If the water is relatively clear and fauna are known to the investigator, careful examination of hole contents can yield accurate data on species richness and abundance for some taxa. Species are more likely to be missed after a hole is disturbed; individuals may hide in crevices or be overlooked if fine sediments do not settle rapidly. Steps 2–5 are sometimes unnecessary (i.e. in very small holes with minimal detritus), or excessively time-consuming if not impossible in very large holes. In aseasonally wet forests (e.g. at La Selva, Costa Rica), tree holes typically accumulate much more sediment than in forests where they dry out and remain dry for some time each year. Removing all of the sediment in the former cases can be extremely tedious, and is not necessary if the taxa of interest are macroorganisms, which typically remain above the sediment layer.

The type of equipment used for completion of steps 2–4 depends on the size and shape of the hole, but almost any hole can be sampled with common materials (Fig. 8.3). In small tree holes, water and soft sediments are removed to a large graduated cylinder for volume measurement using a large suction pipette (e.g. a turkey baster). The contents are then transferred to a white plastic pan for counting. Detritus is removed by hand or with long forceps, rinsed in the tree hole water, and set aside in another pan. A hole should always be probed with a stick or pencil before using bare hands to remove detritus. Tropical tree holes occasionally contain scorpions, land crabs, and ponerine ants, which, if unnoticed, can quickly ruin an otherwise productive field trip. Flushing by repeated filling (two or three times) with water collected from the hole tends to dislodge most organisms remaining in the hole (e.g. Lounibos 1981). If additional water is used for flushing, it should be held in a second pan to avoid dilution or contamination of chemicals and nutrients in the original hole water.

Larger holes can be emptied by using a flexible garden hose to siphon water into a pail. Detritus is removed by hand (or by using a wooden ruler or trowel to lift small packs of leaves), rinsed in the tree hole water and set aside. Rather than completely refilling the hole with a large quantity of water, rinsing the walls with a few liters of clean water is an effective way to dislodge remaining insects.

While the detritus and water collected from flushing are allowed to settle in their pans, the interior walls of the hole can be inspected with a flashlight to spot elusive organisms. Damselfly larvae commonly cling to the walls, and dragonfly larvae are often found covered with sediment at the bottom of the hole, where they can be quite cryptic (Fincke 1992a). With some experience, one can easily recognize elusive species and they can often be counted without removal.

Agitating leaves and other detritus in the collected water usually rids them of any clinging organisms; the composition of detritus can be noted, and litter can then be returned to the hole. After sediments settle in the pan, the clear water

is decanted off. This concentrates macroorganisms such as odonates, syrphids, and tadpoles, making them easier to find and count. A small flashlight, which helps focus the investigator's attention to small areas, makes counting much easier, especially on overcast days or under dense forest canopy. A large grid (e.g. 4 × 4 cm) drawn on the bottom of the pan is also helpful when insects are very abundant. For large tree holes, very numerous insects such as mosquito larvae can be removed in batches to small cups, which permits one to count with greater accuracy. Sub-samples of taxa unfamiliar to the investigator can be collected live for rearing and identification in the laboratory. Small plastic bags (e.g. Nasco® Whirl-Paks) or vials provide the best means of transporting live specimens. Depending on the climate, it may be necessary to transport samples in a cooler with ice to prevent overheating. After subsamples are collected, the remaining organisms and original water can be returned to the hole, and the collection pans are rinsed before the next hole is sampled. Following this protocol, ten or more small to mid-size tree holes in the forest understory can be thoroughly sampled in a day.

Important considerations for natural tree hole experiments

Adequate sample size is a concern in the design of any field experiment (see Chapter 1), and can be problematic in long-term studies of tree holes, which are dynamic systems. Some of the largest holes form suddenly when a tree falls and depressions in the trunk fill with water, but most of these holes do not persist for more than a season or two (depending on the tree species). Even holes in living trees, which often hold water for decades, can vary in volume considerably from year to year, gradually filling completely with mud, or suddenly rotting through. On Barro Colorado Island (BCI), Panama, for example, of 44 water-filled holes in live trees checked in 1982, 6.8 percent had rotted through two years later, compared with 58 percent of those in fallen trees ($n = 12$) and 44 percent of those in dead, upright trees ($n = 9$). Of 23 water-filled holes checked in 1984, 28.6 percent of those in live trees ($n = 21$) had rotted through by the time they were again checked 10 years later. From these data, we estimate a turnover rate for water-filled tree holes in live trees between 2.8 and 3.4 percent per year. Thus, studies of longer than a year should always use more than the minimum number needed for sufficient statistical power in an experiment, with the percentage of additional holes depending on the proportion of study holes in living vs. dead trees.

Another problem associated with tree hole studies is the large number of variables that can affect community properties and interactions among resident aquatic insects. For example, diversity and abundance tend to increase with tree hole volume (Sota 1998, Fincke 1999, Yanoviak 1999b), and predator effects may be stronger in smaller holes (Fincke 1994). This problem is best overcome by surveying a large number of holes several weeks before the start of the experiment, then focusing manipulations on a subset of holes that fall within

an acceptable range of variation. Some fauna are found only in very large holes (e.g. *Agalychnis callidryas* tadpoles), whereas others may be more common in shaded holes (e.g. Heteroptera: Veliidae), holes high in dissolved oxygen (e.g. *Physalaemus pustulosus* tadpoles), or holes with abundant, fruity detritus (e.g. Diptera: Syrphidae) (Fincke 1999, Yanoviak 1999c, 2001a). Therefore, biodiversity surveys should incorporate a broad range of hole types and, where possible, note the detritus composition. Because the fauna of tree holes is depauperate relative to that of streams or lakes, overlooking a few species can make a significant difference in conclusions drawn about biodiversity within or between forests.

Tree holes located within 2 m of the ground are best for replicated experiments due to the time and hazards associated with canopy work. However, tree hole height can affect community properties and distributions of some species (Galindo et al. 1951, 1955; Lounibos 1981). In Panama, for example, species richness in tree holes generally declines with increasing height above the ground (Yanoviak 1999b). Thus, diversity surveys and community-level studies should include tree holes from the ground to the canopy. Holes in the crowns of trees are easier to find than to sample. Overflow stains (Snow 1949) and drinking monkeys (Yanoviak 1999b) pinpoint the locations of canopy tree holes to the ground-based observer, but usually only a small percentage are accessible. Moffett and Lowman (1995) reviewed methods for canopy access; the single-line climbing technique (Perry 1978) is the most effective for canopy tree hole work. Once in a tree crown, the investigator can tie in to a fixed point, leave the main rope, and move laterally along branches to sample tree holes. This is a slow and often difficult process, with minimal data resulting from extensive time and energy expenditure. Despite the risk of pseudoreplication (Hurlbert 1984), the most efficient strategy for canopy tree hole work is to focus climbing efforts on tree species that typically possess many holes per crown, and repeatedly sample holes that are readily accessible.

For those who cannot or choose not to climb trees, cranes or canopy walkways (Moffett & Lowman 1995) provide alternative access to the canopy. However, both of these methods require the use of artificial holes that can be positioned in accessible areas. A rope and pulley system can also be used to raise artificial tree holes into the canopy (e.g. Loor & DeFoliart 1970), but the instability of the containers makes them prone to disturbance from wind and canopy mammals, and may result in lost data. Sampling the colonists of artificial holes secured or suspended in tree crowns or at midstory will at least provide a list of organisms that likely use natural tree holes at the same level (Yanoviak 1999b).

Artificial tree holes

Many of the problems associated with sampling natural phytotelmata for ecological experiments can be overcome by using artificial analogues. Simple con-

tainers can be used to mimic a variety of phytotelmata, such as *Heliconia* spp bracts (Naeem 1988) and bromeliads (Frank 1985, 1986; Haugen 2001). A major advantage of artificial plant containers is that water volume, nutrient input, and the initial presence or absence of some species can be standardized. Plastic analogues are generally inexpensive and can be censused completely in much less time than the same number of natural habitats of similar size. Most importantly, artificial containers generally attract the same fauna as the natural systems (e.g. Pimm & Kitching 1987, Fincke et al. 1997, Yanoviak 2001a) and will even be readily defended by territorial odonates and frogs (Fincke 1992b, 1998; Haugen 2001).

Almost any container filled with rainwater and a small amount of leaf litter will function as an artificial tree hole for short-term experiments. Tree hole analogues with varying degrees of realism can be constructed from bamboo sections (e.g. Lounibos 1981), automobile tires (e.g. Juliano 1998), stone vases (e.g. Sota et al. 1994), or plastic pots (e.g. Fincke 1992a). Galindo et al. (1951, 1955) described two bamboo trap designs (closed-top and open-top), and discussed differences in mosquito species composition between the types. Closed-top traps with small lateral openings mimic a specific tree hole morphology that is difficult to sample, thus they provide a useful addition for tree hole experiments or surveys. Some containers (e.g. tires and stone vases) are weak replicas of tree holes, but attract many tree hole mosquito species and are often used in vector control studies.

We prefer to use plastic containers for artificial tree holes because they are readily available, lightweight, and durable. Of the several sizes and shapes of containers we use to replicate water-filled tree holes in tropical forest studies, three types seem to give the best results.

Because most natural holes are less than 1.0 liter, the artificial hole we often use is a 0.65-liter plastic cup (Churchill Container Corp., Shawnee, KS; Fig. 8.4). A second type is constructed from a 1.5-liter plastic funnel (Detailed Designs/Injectron, Inc., NO. FN-01, USA) in which the spout is removed and the bottom hole is closed from the inside with a rubber stopper (Fig. 8.5). A funnel design that is flat on one side facilitates secure attachment to a tree. To mimic larger holes, we use either a 6.65-liter oil drain pan (Koller Enterprises, Inc., Fenton, MO) or larger (9.0 liter) brown plastic wash tub (Action Industries, Inc., Cheswick, PA; Fig. 8.6). The latter has convenient handles contiguous with the rim that make attachment easier, and its considerable depth results in proportionally less water loss during the inevitable tipping that occurs after attachment. These types of artificial tree holes will survive years of exposure and closely approximate the shape of similar-sized natural holes.

Artificial tree holes in the form of cups and funnels may be tied to small tree trunks or branches, whereas larger pan-type holes are either secured to forked branches in the canopy or to the trunks of fallen trees in the understory (Fig. 8.6). Polypropylene rope (6 mm diameter) is best for securing artificial holes, but a stronger material (i.e., wire) is required if ants or termites are

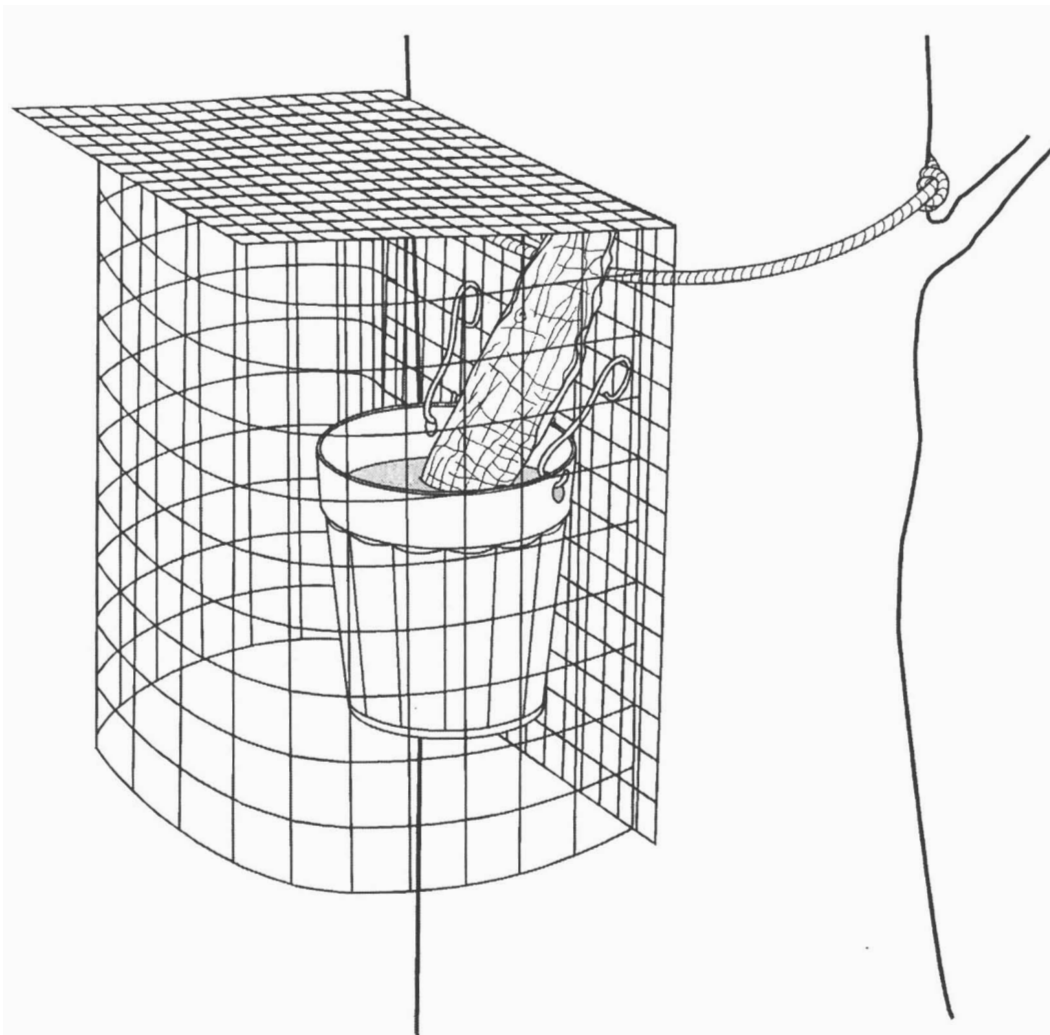


Fig. 8.4 A cup-shaped artificial tree hole with wire cage to prevent oviposition by odonates.

nesting in the tree (Yanoviak 1999b). Plastic-coated flexible wire hooks can be used to hang a cup or funnel from rope around the tree (Fig. 8.5), allowing rapid removal and replacement when frequent sampling is planned. Pans and small artificial holes sampled less often can be secured with rope passed once around the tree and through perforations or handles in the container rim (Fig. 8.4). These methods cause no obvious harm to the tree.

We fill artificial holes with rainwater and put a partially submerged piece of tree bark or balsa wood (Novak & Peloquin 1981) in them as a perch for ovipositing insects. Recently fallen leaf litter collected from the forest floor is added as a nutrient base for the aquatic community (Fish & Carpenter 1982). An initial volume of uncompressed litter within 25–50% of the total artificial hole volume is appropriate for general studies, but the quantity of litter used will depend on the nature of the experiment, the type of forest, and the season. Litter fall reflects seasonal and species-specific patterns of leaf fall and fruiting (e.g. Foster 1982), resulting in variation in nutrient input over space and time.

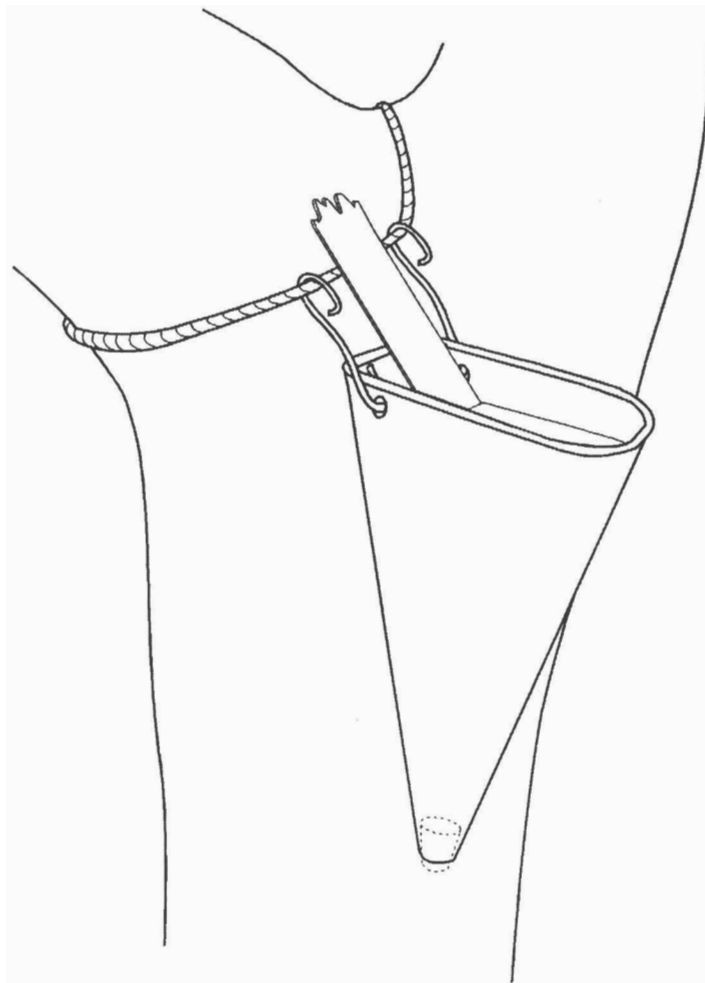


Fig. 8.5 A funnel-shaped artificial tree hole.

For example, 30-day litter accumulation in 0.65-liter cups (71 cm² opening) placed in the BCI forest ranged from 0.0 to 1.2 g dry mass ($x = 0.45 \pm 0.07$ g s.e.; Yanoviak 2001b), and a single fruit fall can result in a pulse of superabundant nutrients (Fincke et al. 1997). To keep nutrients above some minimum for experimental purposes, it may be necessary to periodically add small amounts of litter (e.g. 10% of hole volume) or a substitute nutrient (e.g. fish food or yeast) to some holes.

Apart from providing a standardized physical environment, artificial tree holes also allow some control over potentially important biological variables, such as nutrient input or colonization by key taxa. For example, modifying an artificial tree hole by covering it with a large-mesh wire screen cage (Fig. 8.4) prevents most natural nutrient input, but allows colonization by mosquitoes and most other macroorganisms (Fincke et al. 1997). This cage design also effectively excluded odonates from artificial tree holes in Panama, where they are the most common top predators in this system (Fincke 1998). Similar screening

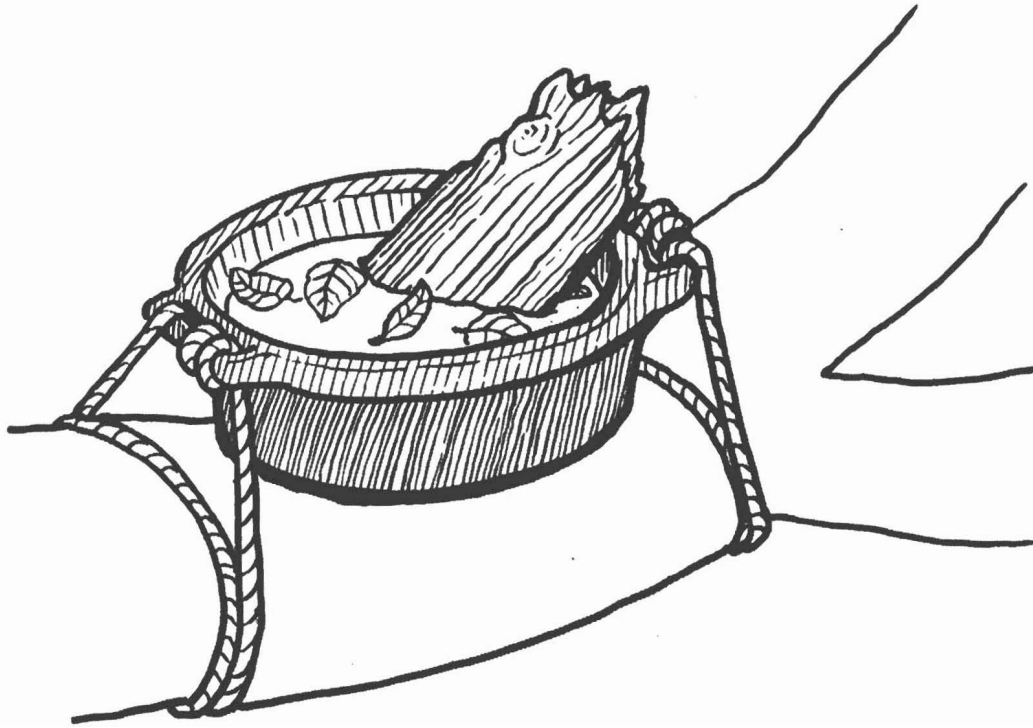


Fig. 8.6 A pan-shaped artificial tree hole.

was only moderately effective at excluding odonates from natural tree holes, in part because eggs laid in the bark prior to screening could not be detected and removed (Yanoviak 2001b). Because the screen cages exclude most falling detritus, additional leaf material must be added to experiments lasting more than a few weeks. Deciding on the quantity of additional nutrient input is not a trivial problem, particularly if growth rates or biodiversity are being measured. Two medium-sized leaves added bimonthly to our 0.65-liter cup-shaped holes kept the abundance of mosquito larvae similar to controls that were open to natural leaf fall, whereas adding 0.05 g of yeast bimonthly resulted in higher than normal levels of mosquito larvae (O.M. Fincke, unpublished). Litter that falls into an adjacent, uncovered, but otherwise identical container could be added to the experimental hole on a regular basis, making the nutrient input more closely reflect natural conditions.

Sampling techniques for artificial tree holes

Artificial tree holes offer a big advantage over natural tree holes because they can be easily emptied completely; time is the only limiting factor in getting accurate counts of the fauna. As for natural tree holes, chemical parameters should be measured and a preliminary census of the fauna made before the artificial hole is disturbed. A cup- or funnel-type hole is then untied (or

unhooked) from the tree and its contents poured into a white pan. Using the methods described earlier for natural holes, one can census organisms in a 0.65-liter hole in under 30 minutes, and in a 1.5-liter hole in under 60 minutes. Large pan-type artificial holes can be left in place for sampling but may require several hours to census, depending on the focus of the study.

Important considerations for artificial tree hole experiments

Artificial tree holes provide an excellent means of controlling multiple variables and increasing sample sizes for experimentation. However, because artificial tree holes are not integral parts of trees, researchers using them should be aware of four differences that might affect their results:

- 1 artificial tree holes are typically younger than holes in living trees, and thus lack potentially relevant biological history (e.g. accumulations of feces, refractory detritus, and sediments);
- 2 they may receive less stemflow than natural holes in upright trees;
- 3 their contents have no direct contact with living wood;
- 4 their inner sides are much smoother than the creviced surface of natural tree holes, which may provide protection for some species or life history stages.

Stemflow inputs and contact with wood are potentially important because both can affect nutrient dynamics and insect productivity in tree holes (e.g. Carpenter 1982, Walker et al. 1991), and stemflow contributes to washout disturbance (Washburn & Anderson 1993). Contact between tree hole water and living wood allows the exchange of materials (e.g. tannins, sap, nitrogenous wastes) between the water and the tree, whereas this exchange and any potential tree species effects on community structure would not occur in artificial tree holes. Abiotic conditions in artificial holes can differ significantly from natural holes of similar size (Table 8.1). However, most tree hole inhabitants tolerate a wide range of pH and dissolved oxygen (Fincke 1999, Yanoviak 1999a), and

Table 8.1 Comparison of abiotic variables in 11 artificial holes and 25 natural tree holes at La Selva, Costa Rica. Means were calculated from measurements taken three times per day (see Fincke 1998 for methods). Ranges in parentheses. Significant differences between natural and artificial holes indicated by * $p < 0.05$, ** $p < 0.01$ (t -tests).

Hole type	Volume (liters)	Temperature (°C)	pH	Dissolved oxygen (ppm)
Artificial	0.8 ± 0.2 (0.1–2.0)	27.2 ± 0.5** (24.0–29.0)	5.5 ± 0.2* (3.4–6.2)	2.7 ± 0.3** (0.7–3.9)
Natural	0.9 ± 0.1 (0.1–2.0)	25.0 ± 0.2 (24.7–29.7)	4.7 ± 0.1 (3.4–6.0)	1.0 ± 0.1 (0.3–2.2)

such differences should not affect colonization or survivorship of most macrofauna (although this may not be true for microorganisms). Artificial holes are particularly good mimics of natural holes in fallen trees (Fig. 8.2), which typically receive limited stem flow, do not contact living tissue, and are relatively young.

Some simple procedures can be used to add realism to the artificial system if necessary. Inoculation of artificial holes with water from natural holes (e.g. during setup and occasionally thereafter) can quickly establish and maintain the microbial assemblage, which is a critical part of tree hole food webs (e.g. Fish & Carpenter 1982, Walker et al. 1991). The rope used to secure cups to trees often conducts stemflow to the cup interior (S.P. Yanoviak, personal observation), and additional stemflow can be directed into a hole by placing the emergent portion of bark or balsa wood against the tree trunk (Fig. 8.4) or by tacking a small piece of plastic onto the tree and allowing it to drain into the hole.

Detritus composition and container color are two additional considerations for those using artificial tree holes in field experiments. The type of litter added to a hole can affect insect productivity and aquatic community structure (Carpenter 1982, Fish & Carpenter 1982, Walker et al. 1997, Yanoviak 1999d), so the composition of litter in a hole (in terms of fragment size, species, age, etc.) should either be consistently haphazard or standardized. Habitat color influences insect colonization in artificial tree holes (Yanoviak 2001c) and other types of phytotelmata (Frank 1985, 1986). Although some workers use clear plastic pans to mimic tree holes in temperate forests (e.g. Srivastava & Lawton 1998), we recommend black or dark brown containers. In Panama, black containers attracted more species than blue or green containers (Yanoviak 2001c). Clear plastic pots can be painted black on the outside, and tubs of any color can be made more realistic by lining the inside with a piece of black plastic (garbage bags work well) that hangs down over the outside edge.

Statistical methods for water-filled tree holes

In most cases, data gathered from replicated tree hole experiments can be analyzed using standard statistical techniques (e.g. ANOVA). Repeated-measures ANOVAs are often used to compare treatment means when the same artificial or natural holes are sampled multiple times (e.g. Fincke et al. 1997, Yanoviak 1999b). Because a large number of ecological and physical variables can be measured in each tree hole, multivariate analyses may be appropriate for many research questions (e.g. Barrera 1988, 1996). It is common for one tree hole to contain zero individuals while hundreds of mosquitoes are present in another. The $\log(x + 1)$ transformation will usually normalize this extreme variation (Sokal & Rohlf 1981). Note that some holes are depauperate of both predators and prey species simply because of resource limitation or abiotic factors; it is important to differentiate between those factors and low diversity resulting from biological interactions (e.g. Fincke et al. 1997).

Problems in interpretation of comparative data

Consideration of spatial and temporal scale is critical when using tree holes as a system to test ecological or evolutionary theory. Tree holes, like bromeliad phytotelmata described by Picado (1913), are analogous to a "subdivided swamp" for most macrofauna using them. Because the resource is subdivided, colonization by certain taxa may be limited with respect to volume, height above the ground, or even morphology of the tree hole opening (see also Frank & Lounibos 1987; Fincke 1992a). Whereas individual tree holes are discrete, replicable units, the scale of the "swamp," which is ecologically comparable to a lake or stream, would be all the tree holes in a forest, which is neither discrete nor easily replicable. For example, top predators decrease diversity within water-filled tree holes on BCI (Yanoviak 2001b). But do forests (at similar latitude) lacking major tree hole predators have greater diversity of tree hole species than forests without those predators? Answering that question requires pooling diversity across replicate holes, with and without predators. Even then, unless the sample of tree holes is representative of the natural distribution with respect to volume, height, and age since the last filling, conclusions may vary.

Finally, most tree hole denizens represent only the larval stage of a species; adults typically are not limited to using a single hole over their reproductive life span, and may have species-specific dispersal distances. In evolutionary studies, for example, the scale of interest would not be simply the fitness of individuals using a given hole, but rather the fitness derived from all the tree holes used over an individual's reproductive life span (e.g. Fincke & Hadrys 2001). Hence, conclusions about community or population processes may be premature without knowledge of the seasonality, longevity, and dispersal ability of the adults in question.

Conclusions

Although there is a growing number of studies documenting the insect fauna of water filled tree holes around the world (Kitching 2000, Yanoviak 2001a), current knowledge remains overwhelmingly biased towards potential disease vectors. Despite considerable interest in the ecology of this system, few studies have addressed the importance of microbial diversity and ecology in tree holes (e.g. Walker & Merritt 1988; Walker et al. 1991). Decomposer microbes (bacteria and fungi) form a critical link between the nutrient base (e.g. leaf litter) and secondary consumers (e.g. mosquito larvae) in tree holes (Fish & Carpenter 1982). Various other microorganisms, such as microcrustaceans, rotifers, and protozoans, also occur in tree holes (Kitching 2000, Yanoviak 2001a), and may function as prey or competitors with the macrofauna. Microbial ecology has been largely overlooked in tropical tree holes, and several basic questions remain to be answered for this system in general. For example, what regulates microbial diversity and productivity in tree holes? How does the composition of detritus

affect decomposer assemblages? Does microbial diversity influence macro-organism diversity or productivity? Are microbial assemblages more species-rich in tropical tree holes? The ecology of microorganisms has been examined in other phytotelmata (e.g. Addicott 1974, Cochran-Stafira & von Ende 1998, Carrias et al. 2001), and these studies exemplify the kinds of investigations that are needed in tree holes. Likewise, few studies have addressed the ecological importance of inorganic nutrients (e.g. nitrogen and phosphorus) in tree holes (e.g. Carpenter 1982; Walker et al. 1991). Microbial and nutrient dynamics have been described for many large freshwater systems, and some of the techniques commonly used by stream and lake ecologists to quantify these parameters could be transferred to tree holes.

In summary, water-filled tree holes are tractable habitats for ecological and behavioral studies; sampling their insect fauna is a relatively simple process, and the use of artificial holes is an inexpensive way to increase sample size and control multiple factors for experiments. The extent to which inferences from tree hole data have a more general application for freshwater systems remains to be seen. Nevertheless, given their important ecological role, these aquatic microhabitats merit much more attention than they have received, especially in tropical forests.

Acknowledgements

We are grateful to Coral McAllister for the illustrations. Comments from S. Stuntz and C. Ozanne, and discussions with L. P. Lounibos, improved the manuscript.

References

- Addicott, J.F. (1974) Predation and prey community structure: an experimental study of the effect of mosquito larvae on the protozoan communities of pitcher plants. *Ecology*, **55**, 475–492.
- Barrera, R. (1988) Multiple factors and their interactions on structuring the community of aquatic insects of treeholes. PhD thesis, Pennsylvania State University.
- Barrera, R. (1996) Species concurrence and the structure of a community of aquatic insects in tree holes. *Journal of Vector Ecology*, **21**, 66–80.
- Belkin, J. N., Hogue C. L., Galindo, P., Aitken, T. H., Schick, R. X., & Powder, W. A. (1965) Mosquito studies (Diptera, Culicidae). II. Methods for the collection, rearing and preservation of mosquitoes. *Contributions of the American Entomological Institute* **1**, 19–78.
- Bradshaw, W.E. & Holzapfel, C.M. (1983) Predator-mediated, non-equilibrium coexistence of tree-hole mosquitoes in southeastern North America. *Oecologia*, **57**, 239–256.
- Carpenter, S.R. (1982) Stemflow chemistry: effects on population dynamics of detritivorous mosquitoes in tree-hole ecosystems. *Oecologia*, **53**, 1–6.
- Carrias, J.-F., Cussac, M.-E., & Corbara, B. (2001) A preliminary study of freshwater protozoa in tank bromeliads. *Journal of Tropical Ecology*, **17**, 611–617.

- Cochran-Stafira, D.L. & von Ende, C.N. (1998) Integrating bacteria into food webs: studies with *Sarracenia purpurea* inquilines. *Ecology*, **79**, 880–898.
- Copeland, R.S. (1989) The insects of treeholes of northern Indiana with special reference to *Megaselia scalaris* (Diptera: Phoridae) and *Spilomyia longicornis* (Diptera: Syrphidae). *Great Lakes Entomologist*, **22**, 127–132.
- Fincke, O.M. (1992a) Interspecific competition for tree holes: consequences for mating systems and coexistence in neotropical damselflies. *The American Naturalist*, **139**, 80–101.
- Fincke, O.M. (1992b) Consequences of larval ecology for territoriality and reproductive success of a neotropical damselfly. *Ecology*, **73**, 449–462.
- Fincke, O.M. (1994) Population regulation of a tropical damselfly in the larval stage by food limitation, cannibalism, intraguild predation and habitat drying. *Oecologia*, **100**, 118–127.
- Fincke, O.M. (1998) The population ecology of *Megaloprepus coerulatus* and its effect on species assemblages in water-filled tree holes. In *Insect Populations: in Theory and in Practice* (ed. J.P. Dempster & I.F.G. McLean), pp. 391–416. Kluwer, Dordrecht.
- Fincke, O.M. (1999) Organization of predator assemblages in Neotropical tree holes: effects of abiotic factors and priority. *Ecological Entomology*, **24**, 13–23.
- Fincke, O.M. (unpublished ms.) Constraints on adaptive cannibalism, clutch size, and offspring sex ratios in a shared tree hole nursery.
- Fincke, O.M. & Hadrys, H. (2001) Unpredictable offspring survivorship in the damselfly, *Megaloprepus coerulatus*, shapes parental behavior, constrains sexual selection, and challenges traditional fitness estimates. *Evolution*, **55**, 762–772.
- Fincke, O.M., Yanoviak, S.P., & Hanschu, R.D. (1997) Predation by odonates depresses mosquito abundance in water-filled tree holes in Panama. *Oecologia*, **112**, 244–253.
- Fish, D. (1983) Phytotelmata: flora and fauna. In *Phytotelmata: Terrestrial Plants as Hosts for Aquatic Insect Communities* (ed. J.H. Frank & L.P. Lounibos), pp. 1–27. Plexus, Medford, NJ.
- Fish, D. & Carpenter, S.R. (1982) Leaf litter and larval mosquito dynamics in tree-hole ecosystems. *Ecology*, **63**, 283–288.
- Foster, R.B. (1982) Seasonal rhythms of fruitfall on Barro Colorado Island. In *Ecology of a Tropical Forest: Seasonal Rhythms and Long-Term Changes* (ed. E.G. Leigh, A.S. Rand, & D.M. Windsor), pp. 151–172. Smithsonian Institution, Washington, DC.
- Frank, J.H. (1985) Use of an artificial bromeliad to show the importance of color value in restricting the colonization of bromeliads by *Aedes aegypti* and *Culex quinquefasciatus*. *Journal of the American Mosquito Control Association*, **1**, 28–32.
- Frank, J.H. (1986) Bromeliads as ovipositional sites for *Wyeomyia* mosquitoes: form and color influence behavior. *Florida Entomologist*, **69**, 728–742.
- Frank, J.H. & Lounibos, L.P. (1987) Phytotelmata: swamps or islands? *Florida Entomologist*, **70**, 14–20.
- Galindo, P., Carpenter, S.J. & Trapido, H. (1951) Ecological observations on forest mosquitoes of an endemic yellow fever area in Panama. *American Journal of Tropical Medicine*, **31**, 98–137.
- Galindo, P., Carpenter, S.J., & Trapido, H. (1955) A contribution to the ecology and biology of tree hole breeding mosquitoes of Panama. *Annals of the Entomological Society of America*, **48**, 158–164.
- Haugen, L. (2001) Privation and uncertainty in the small nursery of Peruvian tadpoles: larval ecology shapes the parental mating system. PhD thesis, University of Oklahoma.
- Hurlbert, S.H. (1984) Pseudoreplication and the design of ecological field experiments. *Ecological Monographs*, **54**, 187–211.
- Jenkins, D.W. & Carpenter, S.J. (1946) Ecology of the tree hole breeding mosquitoes of nearctic North America. *Ecological Monographs*, **16**, 31–47.
- Juliano, S.A. (1998) Species introduction and replacement among mosquitoes: interspecific resource competition or apparent competition? *Ecology*, **79**, 255–268.

- Kitching, R.L. (1971a) An ecological study of water-filled tree-holes and their position in the woodland ecosystem. *Journal of Animal Ecology*, **40**, 281–302.
- Kitching, R.L. (1971b) A core sampler for semi-fluid substrates. *Hydrobiologia*, **37**, 205–209.
- Kitching, R.L. (1972a) The immature stages of *Dasyhelea dufouri* Laboulbene (Diptera: Ceratopogonidae) in water-filled tree-holes. *Journal of Entomology (ser. A)*, **47**, 109–114.
- Kitching, R. L. (1972b) Population studies of the immature stages of the tree-hole midge *Metriocnemus martinii* Thienemann (Diptera: Chironomidae). *Journal of Animal Ecology*, **41**, 53–62.
- Kitching, R. L. (1990) Foodwebs from phytotelmata in Madang, Papua New Guinea. *The Entomologist*, **109**, 153–164.
- Kitching, R. L. (2000) *Food Webs and Container Habitats: the Natural History and Ecology of Phytotelmata*. Cambridge University Press, Cambridge.
- Loor, K.A. & DeFoliart, G.R. (1970) Field observations on the biology of *Aedes triseriatus*. *Mosquito News*, **30**, 60–64.
- Lounibos, L.P. (1981) Habitat segregation among African treehole mosquitoes. *Ecological Entomology*, **6**, 129–154.
- Maguire, B. Jr. (1971) Phytotelmata: biota and community structure determination in plant-held waters. *Annual Review of Ecology and Systematics*, **2**, 439–464.
- Moffett, M.W. & Lowman, M.D. (1995) Canopy access techniques. In *Forest Canopies* (ed. M.D. Lowman & N.M. Nadkarni), pp. 3–26. Academic Press, San Diego.
- Naeem, S. (1988) Predator–prey interactions and community structure: chironomids, mosquitoes and copepods in *Heliconia imbricata* (Musaceae). *Oecologia*, **77**, 202–209.
- Novak, R.J. & Peloquin, J.J. (1981) A substrate modification for the oviposition trap used for detecting the presence of *Aedes triseriatus*. *Mosquito News*, **41**, 180–181.
- Orr, A.G. (1994) Life histories and ecology of Odonata breeding in phytotelmata in Bornean rainforest. *Odonatologica*, **23**, 365–377.
- Perry, D.R. (1978) A method of access into the crowns of emergent and canopy trees. *Biotropica*, **10**, 155–157.
- Picado, C. (1913) Les broméliacées épiphytes comme milieu biologique. *Bulletin Scientifique de la France et de la Belgique*, **47**, 215–360.
- Pimm, S.L. & Kitching, R.L. (1987) The determinants of food chain lengths. *Oikos*, **50**, 302–307.
- Service, M.W. (1993) *Mosquito Ecology: Field Sampling Methods*. 2nd edn. Kluwer, Dordrecht.
- Snow, W.E. (1949) The Arthropoda of wet tree holes. PhD thesis, University of Illinois, Urbana.
- Sokal, R.R. & Rohlf, F.J. (1981) *Biometry*. W.H. Freeman, New York.
- Sota, T. (1998) Microhabitat size distribution affects local difference in community structure: metazoan communities in treeholes. *Researches on Population Ecology*, **40**, 249–255.
- Sota, T., Mogi, M., & Hayamizu, E. (1994) Habitat stability and the larval mosquito community in treeholes and other containers on a temperate island. *Researches on Population Ecology*, **36**, 93–104.
- Srivastava, D.S. & Lawton, J.H. (1998) Why more productive sites have more species: experimental test of theory using tree-hole communities. *The American Naturalist*, **152**, 510–529.
- Varga, L. (1928) Ein interessanter Biotop der Biocönose von Wasserorganismen. *Biologisches Zentralblatt*, **48**, 143–162.
- Vitale, G. (1977) *Culicoides* breeding sites in Panama. *Mosquito News*, **37**, 282.
- Walker, E.D. & Merritt, R.W. (1988) The significance of leaf detritus to mosquito (Diptera: Culicidae) productivity from treeholes. *Environmental Entomology*, **17**, 199–206.
- Walker, E.D., Lawson, D.L., Merritt, R.W., Morgan, W.T., & Klug, M.J. (1991) Nutrient dynamics, bacterial populations, and mosquito productivity in tree hole ecosystems and microcosms. *Ecology*, **72**, 1529–1546.
- Walker, E.D., Kaufman, M.G., Ayres, M.P., Riedel, M.H., & Merritt, R.W. (1997) Effects of variation in quality of leaf detritus on growth of the eastern tree-hole mosquito, *Aedes triseriatus* (Diptera: Culicidae). *Canadian Journal of Zoology*, **75**, 707–718.

- Washburn, J.O. & Anderson, J.R. (1993) Habitat overflow, a source of larval mortality for *Aedes sierrensis* (Diptera: Culicidae). *Journal of Medical Entomology*, **30**, 802–804.
- Yanoviak, S.P. (1999a) Community ecology of water-filled tree holes in Panama. PhD thesis, University of Oklahoma, Norman.
- Yanoviak, S.P. (1999b) Community structure in water-filled tree holes of Panama: effects of hole height and size. *Selbyana*, **20**, 106–115.
- Yanoviak, S.P. (1999c) Distribution and abundance of *Microvelia cavicola* Polhemus (Heteroptera: Veliidae) on Barro Colorado Island, Panama. *Journal of the New York Entomological Society*, **107**, 38–45.
- Yanoviak, S.P. (1999d) Effects of leaf litter species on macroinvertebrate community properties and mosquito yield in Neotropical tree hole microcosms. *Oecologia*, **120**, 147–155.
- Yanoviak, S.P. (2001a) The macrofauna of water-filled tree holes on Barro Colorado Island, Panama. *Biotropica*, **33**, 110–120.
- Yanoviak, S.P. (2001b) Predation, resource availability, and community structure in Neotropical water-filled tree holes. *Oecologia*, **126**, 125–133.
- Yanoviak, S.P. (2001c) Container color and location affect macroinvertebrate community structure in artificial treeholes in Panama. *Florida Entomologist*, **84**, 265–271.

Index of methods and approaches

Methodology	Topics addressed	Comments
General surveys	Descriptive data on community structure.	Collections are taken from a large number of holes over several seasons. Provides basic natural history data from which further questions and experiments are developed.
Quantitative sub-sampling	Distribution and abundance of a given species.	May be accomplished with a corer or similar tools. Often the only practical option for very large tree holes.
Species exclusion or addition	Predator effects on community structure; interspecific interactions.	Exclusion methods depend on organism size and behavior, and may not be 100% effective.
Manipulation of litter inputs	Effects of basal resources on community structure.	Qualitative and quantitative characteristics of litter are important considerations
Forest canopy access	Effects of environmental gradients on species distributions.	Ratio of effort and time expenditure to quantity of data recovered may be prohibitive. Artificial tree holes provide a viable option