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Seasonal availability of resources and habitat degradation for the western tree-hole mosquito, *Aedes sierrensis*

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Abstract The nutrient base of aquatic tree-hole communities is derived from leaf litter, benthic detritus, and water flowing down the tree trunk (stemflow water). Previous studies in eastern North America with the mosquito, *Aedes triseriatus*, have identified leaf litter as a major and stemflow water as a minor source of mosquito nutrition, but did not consider the role of the benthic detritus or how the aggregate or relative contribution of these sources of mosquito nutrition changed during the year. We use the leaf litter, benthic detritus, and stemflow water from tree holes in western Oregon (USA) to determine how these substrates affect mass at metamorphosis, biomass yield, and fitness (cohort replacement rate; R_0) of the mosquito, *Aedes sierrensis*, through both natural and simulated winters, the normal growing season for larvae in tree holes. We found that fresh leaf litter constitutes the major determinant of mosquito fitness by a factor of >15:1 over any other substrate taken directly from tree holes in nature. The other substrates, including the benthic detritus, individually make only a meager contribution to mosquito fitness but, when added to the leaf litter, can sustain yield and improve fitness at high, limiting larval densities. Nutritional quality of tree-hole substrates declines by >90% from early (fall) to late (spring) in the larval growing season. At both times of year, the coarse or fine detritus provide minor resources, and stemflow water provides no detectable contribution to mosquito nutrition. The resources in the litter are not transported during the year to the benthic detritus; rather, these resources are either exploited by mosquitoes when they first become available, or they deteriorate and become progressively more unavailable to them. Growth and development of *A. sierrensis* feeding on dried and reconstituted tree-hole contents during a 6-month simulated winter in the laboratory showed: (1) the same relative contributions of leaf litter, benthic detritus, and stemflow water to mosquito nutrition, (2) that the winter deterioration of substrate quality is a direct consequence of microbial decomposition, and (3) that pre-emptive competition from pre-existing *A. sierrensis* greatly increases substrate deterioration. We conclude that the progressive winter deterioration of larval resources in combination with the dry summers of western North America are the most likely environmental factors that limit species diversity in tree holes and that have selected for early recruitment (autumnal hatching) of *A. sierrensis* and for its univoltine life cycle from Mexico to Canada.

Key words *Aedes sierrensis* · Tree hole · Leaf litter · Seasonal recruitment

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Introduction

Water-filled cavities in plants (phytotelmata) are natural habitats for a wide variety of organisms (Frank and Lounibos 1983). Among them, tree holes are the most common habitats worldwide, and mosquitoes are consistently the most predominant organisms in tree-hole communities (Fish 1983). Because of their naturally defined limits and inherent tractability, these miniature ecosystems have been used for field studies of community ecology (Lounibos 1981, 1983; Bradshaw and Holzapfel 1983, 1986, 1988, 1991, 1992; Kitching 1983; Copeland and Craig 1990; Lounibos et al. 1997; Sota et al. 1994; Sota 1998) and population biology (Hawley 1985a, b; Walker and Merritt 1988; Copeland and Craig 1989; Walker et al. 1991; Léonard and Juliano 1995). Like many freshwater streams (Hynes 1970; Otto 1974; Petersen and Cummins 1974; Moss 1980; Kaushik and Hynes 1971; Richardson 1991), the nutrient base of tree-hole communities is allochthonous in origin and consists largely of leaf litter (Kitching 1971; Carpenter 1982, 1983; Fish and Carpenter 1982; Walker et al. 1991; Lounibos et al. 1993; Léonard and Juliano 1995). In addition to filtering in the water column, mosquito larvae may browse on submerged leaves (Fish and Carpenter 1982; Merritt et al. 1992;

Kaufman et al. 1999). The larval diet is probably not the leaf material itself, "but rather, is mostly comprised of microbes that colonize the leaf surface (e.g., bacteria) or live freely in treehole water (e.g., ciliated protozoans)" (Strand et al. 1999). The transformation of tree-hole detritus and dissolved organic material to mosquito biomass is then mediated by microorganisms (Fish and Carpenter 1982; Walker et al. 1988, 1997; Walker and Merritt 1991; Merritt et al. 1992), and pre-existing larvae can adversely affect the performance of subsequently hatching larvae (Livdahl 1982; Aspbury and Juliano 1998).

Input from water flowing down the trunks of trees (stemflow water) serves as a second potential source of allochthonous nutrients for tree holes. Studies comparing the abilities of leaf litter and stemflow water to sustain mosquito development in experimental microcosms have found that leaf litter makes the overwhelmingly greater contribution to mosquito nutrition (Carpenter 1982, 1983; Fish and Carpenter 1982; Walker et al. 1991). The one study on intact tree holes (Walker and Merritt 1988) found that removal of the leaf litter altogether did not significantly affect the timing of pupation or the mass of male or female pupae of *Aedes triseriatus*. Léonard and Juliano (1995) have suggested that Walker and Merritt (1988) may have been sampling holes with such low densities of mosquitoes that nutrients were not limiting, and we observed that the density of mosquitoes (pupae plus remaining larvae per tree hole) was 2.4 times higher in the holes with leaf litter than without, thereby potentially confounding density- and resource-dependent effects. Finally, none of the studies dealing with microcosms, except those of Livdahl (1982) and Fisher et al. (1990), included the benthic detritus. Livdahl (1982) used the tree-hole substrates exclusive of the leaf litter and found that fitness (*r'*) of *A. triseriatus* declined with increasing larval density or substrate dilution. Fisher et al. (1990) observed that, as a fraction of dry mass, the coarse and fine detritus together exceed that of leaf litter by ratios ranging from 4:1 to 14:1. This benthic detritus could be sustaining mosquito development in the absence of leaf litter: (1) with nutrients remaining in or transported to the benthic detritus from leaf litter, or (2) with nutrients in the benthic detritus that interact synergistically with those remaining in the leaf litter or those entering tree holes as stemflow.

Tree holes are, in may respects, similar to temperate woodland streams in the northern hemisphere. These streams obtain most of their nutrient input in the form of deciduous leaf litter as an autumnal pulse (Cummins 1974; Richardson 1991). Much of the leaf litter falling into streams is degraded rapidly during the fall and winter with little nutrient value remaining for macrodetritivores in the spring, although the remaining fine particulate material may still provide lesser resources for vernal filter feeders (Kaushik and Hynes 1971; Cummins 1974; Anderson and Sedell 1979). None of the prior studies with tree holes or microcosms has considered how the relative contribution to mosquito nutrition of leaf litter, stemflow water, and the benthic detritus change during the growing season, or has shown how pre-emptive competition alters nutrient depletion or renewal over the time span of the entire growing season. Herein, we use the western (USA) tree-hole mosquito, *Aedes sierrensis* Ludlow, to answer four related questions:

1. What is the relative contribution of leaf litter, benthic detritus, and stemflow water alone or in combination to mosquito fitness?
2. How does the relative contribution of these compartments to mosquito nutrition change with the advancing seasons?
3. How is the seasonal availability of nutrients in the combined substrate (litter+detritus+stemflow water) affected by decomposition without or with pre-existing competitors?
4. What are the implications of the seasonal availability of resources for the optimal seasonal recruitment of mosquitoes (hatching into tree holes)?

A. sierrensis

A. sierrensis ranges along the Pacific slope of North America from Mexico to Canada (Darsie and Ward 1981). The climate along this slope is characterized by cool, wet winters and hot, dry summers (NOAA 1968). Upon the return of the rainy season, *A. sierrensis* start to hatch in October, November or December. Larval development continues throughout winter. Pupation occurs between April and July (Hawley 1985a). The rains abate during March through May, and all but the most permanent tree holes are totally dry by July. *A. sierrensis* has an aestival, embryonic diapause induced by long-day photoperiod, and a hibernal, fourth-instar diapause induced by short-day photoperiod at low temperatures (Garcia and Ponting 1972; Jordan and Bradshaw 1978; Jordan 1980). Consequently, populations are probably univoltine throughout their range, and the finite rate of increase of the population is equivalent to the per capita replacement rate per generation (Hawley 1985a, b). *A. sierrensis* is the predominant arthropod in Oregon tree holes and, for all practical purposes, exists as single-species populations. Larval resources and larval density determine size at metamorphosis which, in turn, determines number of eggs per batch, female longevity and expected female lifetime fecundity; hence, population growth rate increases with pre-adult nutrition and decreases with density (Hawley 1985a, b; Fisher et al. 1990; Hard and Bradshaw 1993).

Materials and methods

Source of mosquitoes and tree-hole substrates

Experimental mosquitoes

All experiments in this study used F_1 of field-collected larvae. The parental generation was obtained from a single tree hole (TH1 of Hawley 1985b and Fisher et al. 1990) from Eugene, Oregon. Lar-

vae were raised to adulthood. Adult females were provided an anaesthetized rat for a blood source and a paper-lined jar with wet paper toweling for oviposition. Resulting eggs were stored on the wet paper towels in plastic Petri dishes for 10 days at room temperature (21–23°C) to allow embryonation. The dishes were then sealed with vinyl tape and stored for at least 3 months at 4°C to terminate the embryonic diapause. Hatching was stimulated by submerging eggs in a suspension of putrid ground guinea-pig chow and brine shrimp. Freshly hatched larvae were rinsed once in distilled water before being used in experiments.

Tree-hole substrates

We identified rot holes in maples (*Acer macrophyllum*) between Eugene, Lane County, and Harrisburg, Linn County, Oregon, in the vicinity of holes sampled by Broberg and Bradshaw (1997). We sampled only holes that were filled with water after the return of substantial rains and that contained vigorous larvae of *A. sierrensis*. The entire contents of tree holes were extracted by siphoning out the aqueous contents and then scooping out the litter and solid detritus. Samples were transported to the laboratory and all insect larvae were removed. Tree-hole contents were partitioned into their main components: litter (solid material retained by a 1-cm-mesh sieve), coarse detritus (sediment retained by a 1-mm-mesh aquarium net), and fine detritus (aqueous phase and suspended particulate matter passed by a 1-mm-mesh aquarium net). The litter and coarse fractions were drained and squeezed by hand to extract the remaining water. Large leaves were cut with scissors into smaller pieces about 1 cm in size to facilitate their more even distribution among replicate treatments. Stemflow water was collected by building dams around tree trunks. The dams were drained into 4-l plastic jugs that were replaced after heavy rains. All tree-hole material was stored at 2±2°C until used in experiments.

Seasonal availability of resources

The following experiments were designed to show the relative availability of resources early (fall) and late (spring) in the developmental season of *A. sierrensis*, and to show which components of the tree-hole substrates provided those resources. We sampled seven tree holes after flooding by rains in the fall and early winter (December 1997–February 1998) and six additional tree holes prior to their drying up in the spring and early summer (April–June 1998).

Assuming 1 g wet weight of solid material equals 1 ml, for each tree hole we calculated:

$$\text{Litter fraction}=(\text{g litter})\div(\text{total volume}) \quad (1)$$

$$\text{Coarse fraction}=(\text{g coarse sediment})\div(\text{total volume}) \quad (2)$$

$$\text{Fine fraction}=(\text{volume of aqueous and suspended material})\div(\text{total volume}) \quad (3)$$

Experimental replicates of microcosms were set up using the wet, previously undried substrates in plastic Petri dishes (15.0× 2.5 cm, diameter×depth) containing tree-hole components in isolation or in pairwise combination. All containers were kept at room temperature for at least 2 but no more than 3 days before the start of the experiments to stimulate hatching of any eggs remaining from the field. After removing these newly-hatched larvae, 40 one-day-old *A. sierrensis* larvae were added to each container. All replicates were maintained in a 15-°C controlled-environment room provided with a light:dark= photoperiod of 16:8 h to promote continuous (non-diapause) development.

To determine the independent contribution to mosquito nutrition of each fraction, we set up two replicates of each of the following treatments from each of the 13 tree holes:

1. Litter fraction (×150) brought up to 150 ml with distilled water.
2. Coarse fraction (×150) brought up to 150 ml with distilled water.
3. Fine fraction (×150) brought up to 150 ml with distilled water.
4. Pure stemflow water (150 ml).

To test for synergistic contributions of these fractions to mosquito nutrition, we set up two replicates of each of the following treatments at each time of year, concurrently with the above treatments:

1. Litter fraction (×150) plus coarse fraction (×150) brought up to 150 ml with distilled water.
2. Litter fraction (×150) plus fine fraction (×150) brought up to 150 ml with distilled water.
3. Litter fraction (×150) brought up to 150 ml with stemflow water.
4. Coarse fraction (×150) plus fine fraction (×150) brought up to 150 ml with distilled water.
5. Coarse fraction (×150) brought up to 150 ml with stemflow water.
6. Fine fraction (×150) brought up to 150 ml with stemflow water.

We induced hatching of *A. sierrensis* the day before the initiation of an experiment. The following day, we counted out the larvae and set up the experiment. Starting 3 weeks after the initiation of an experiment, we checked each replicate 3 times week^{-1}, at which time we removed, sexed, and weighed the pupae. We followed this procedure until all larvae had pupated or died, or until none of the remaining replicates produced a pupa within 1 month. For each replicate we then calculated the cohort replacement rate as:

$$R_0=(\%\text{ adult eclosion})\times(\text{expected lifetime fecundity per eclosed female}) \quad (4)$$

where R_0 is cohort replacement rate, and:

$$\text{expected lifetime fecundity per eclosed female}=\sum_{i=1}^{n}(\text{fPM}_i)/n \quad (5)$$

where, n is number of females emerging from the cohort, PM is female pupal mass (mg), fPM is a function relating female pupal mass to expected lifetime fecundity, i.e.:

$$\text{fPM}=(\text{expected number of egg batches})\times(\text{eggs per batch})=\left(\frac{\text{P}}{1-\text{P}}\right)(-12.32+38.85\text{PM}) \quad (6)$$

where P=season–long parous rate=the survivorship per gonotrophic cycle=$-0.10+0.296\text{PM}-0.369\text{PM}^2$ (Hawley 1985b).

We calculated R_0 as the product of survivorship to adulthood times expected lifetime fecundity per female, instead of total expected lifetime fecundity of all females, for two principal reasons. First, stochastic variation in sex ratio among a small number of emerging adults could otherwise inflate sampling error among replicates. Second, the sex ratio of emerging mosquitoes generally becomes male-biased as resources become limiting. At low survivorship, our method of calculating R_0 is then biased upwards because it includes survivorship of males, but biased downwards because it does not take into account the non-zero fitness of males as sires. Male fitness is difficult to quantify because it depends primarily upon the timing of male emergence, not within the cohort, but relative to male and female emergence times in the broader population, and these times can vary from year to year in nature (Kleckner et al. 1995).

We used $\log_{10}$-transformation of (R_0+1) to improve homogeneity of variances and compared medians using Kruskall-Wallis tests, followed by the Fligner-Policello procedure (Fligner and Policello 1981) for planned comparisons among samples with unequal variances (Day and Quinn 1989). Two-way parametric ANOVA (Proc GLM; SAS Institute 1985) of $\log(R_0+1)$ with treatments season (fall, spring) and substrate (litter, coarse, fine, stemflow water), testing for season by substrate interaction, and followed by the Ryan-Einot-Gabriel-Welsh multiple range test produced the same results, indicating that the lowered power of tests based on ranks did not produce a misleading interpretation of results.

To test for interaction between substrates within the contents of each tree hole we calculated:

$$I_{AB}=\log_{10}\left[\frac{AB}{(A+B)}\right] \quad (7)$$

where AB represents R_0 achieved by a cohort when two substrates were provided in combination, and $(A+B)$ represents the sum of the separate R_0s when the same two substrates were provided separately. We tested for significant deviation of I_{AB} from zero using $t=I_{AB}/s_I$ with n–1 degrees of freedom, where s_I is the standard error of I_{AB} among tree holes within seasons and n is the number of tree holes sampled within seasons.

Sustainability of tree-hole substrates

To determine the ability of the different tree-hole substrates to sustain mosquito development, we allowed varying numbers of larvae to develop on fixed amounts of the individual substrates, i.e., we varied larval density while holding substrate constant. To evaluate the ability of different substrates to sustain mosquito development, we used mean total yield of mosquito biomass and R_0. Both yield and R_0 reveal a great deal about the relative contribution of season and substrate to mosquito nutrition and fitness; but, neither provides a convenient gauge by which we can compare our results to other studies or to conditions in actual tree holes. In unmanipulated tree holes in nature (Hawley 1985a), as well as in laboratory microcosms (Fisher et al. 1990), pupal mass of *A. sierrensis* is proportional to larval density (per capita availability of resources) without any significant density by larval resource interaction. We therefore examined the effect of season and substrate on pupal mass as well as yield and R_0.

We collected the total contents of nine previously unsampled tree holes in December 1998. We separated the litter, coarse, and fine detritus, as above, from each tree hole. We then combined and mixed each separate fraction across tree holes to obtain composite litter, coarse, and fine detritus. Concurrently, we obtained stemflow water from eight separate maple trees and pooled the stemflow water in a large container in the laboratory. We set up multiple replicates of each of the following in 200-ml plastic dishes with wet and previously undried substrates:

1. Litter (10 g wet weight) plus distilled water (50 ml).
2. Coarse detritus (10 g wet weight) plus distilled water (150 ml).
3. Fine detritus (150 ml) plus distilled water (10 ml).
4. Stemflow water (150 ml) plus distilled water (10 ml).
5. Litter (10 g) plus coarse detritus (10 g) plus fine detritus (10 ml) plus stemflow water (150 ml, evaporated down to 130 ml).

We stimulated hatching of *A. sierrensis* as above, and, on a single day, started 284 dishes with cohorts of 1–64 larvae dish^{-1} (Table 1). To obtain an estimate of the effect of substrate and density on per capita replacement rate, we pooled replicates within densities and treatments to create two composite "cohorts" of 32 larvae each at densities of 1–16 larvae dish^{-1}. The two replicates of 32 and 64 larvae dish^{-1} served as the actual replicate cohorts at those densities. Assignment of dishes to a composite cohort was made by turning cards after adding the substrates to the dishes but before adding the first instars (because of time constraints on the day of starting the experiment).

As a hedge against non-development of any larvae, we established a separate, but concurrent, series of twenty-five 1-l jars filled with 750 ml full-strength stemflow water to which we added a single first instar. These jars were assigned to composite cohorts of 12 and 13 larvae at the end of the experiment by turning cards.

As above, we removed, sexed, and weighed pupae 3 times week^{-1} until all larvae had developed or died, or until none of the remaining cohorts produced a pupa within 1 month. At the end of the experiment, we weighed (wet) each larva remaining in each replicate. Finally, we calculated R_0 and total biomass (combined masses of all pupae plus remaining larvae) for each composite or actual cohort.

Log_{10} transformation of either (R_0+1) or total biomass achieved non-heterogeneous variances among densities at which at least one female pupated in at least one replicate (R_0) or for which at least one larva survived to the end of the experiment (total biomass). Consequently, we used this transformation to compare regressions of R_0 or biomass on density among substrates.

Nutritional consequences of delayed seasonal hatching

The following experiments were designed to test the effect on mosquito fitness of delayed seasonal hatching into a tree hole without or with pre-existing larval competitors. We collected, pooled, and dried the contents of two tree holes. We apportioned the dried contents into aliquots of equal mass to serve as the substrate for individual cohorts. We programmed a controlled-environment room to produce a simulated winter (Bradshaw et al. 1998) and then tested for:

1. The effects of time of winter, per se, on delayed hatching by hydrating substrates and adding mosquitoes at different times during the simulated fall and winter.
2. The effects of substrate deterioration during the winter by hydrating substrates simultaneously at the start of the simulated fall and then adding mosquitoes at different later times during the simulated fall and winter.
3. The effects of pre-existing competitors by hydrating substrates and adding "competitor" first instars of *A. sierrensis* simultaneously at the start of the simulated fall and then removing the competitors and adding the test *A. sierrensis* first instars at different times during the simulated fall and winter.

Preparation of replicate substrates

We collected the total contents of two, previously unsampled tree holes of 5 l and 25 l volume in December 1997. We air-dried the combined contents in large open pans at room temperature (21–24°C) in the laboratory and cut the larger pieces of detritus into fragments (1 cm). Sieving (1-mm mesh) yielded 294 g coarse and 411 g fine dry detritus. These separate fractions were used to make up 54 replicate substrates, comprised of 3 g coarse and 4 g fine detritus each, that were stored dry in 54 individual plastic bags. Each bag was assigned to a specific future experimental replicate and treatment using a table of random numbers.

Replicate microcosms consisted of 15.0×2.5-mm (diameter×depth) plastic Petri dishes with 7 g dry mass of substrate in 150 ml of 0.1 ppm solution of Photo-Flo 200 (Eastman Kodak, Rochester, N.Y.) to improve rehydration of the substrate. Eggs were stimulated to hatch as above. Twenty, first instars were placed in each microcosm, and each treatment consisted of two microcosms whose pupae were pooled to calculate a single R_0.

Table 1 Number of replicates for determining the ability of individual and combined substrates to sustain mosquito development

	Substrate per dish	Density (larvae per dish)						
Treatment		1	2	4	8	16	32	64
Litter	10 g			16	8	4	2	2
Coarse	10 g		32	16	8	4	2	2
Fine	150 ml			16	8	4	2	2
Stemflow water	150 ml	64	32	16	8	4		
Combined	160 ml			16	8	4	2	2
Stemflow water	750 ml	25[a]						

[a] One larva per 750-ml jar of undiluted stemflow water

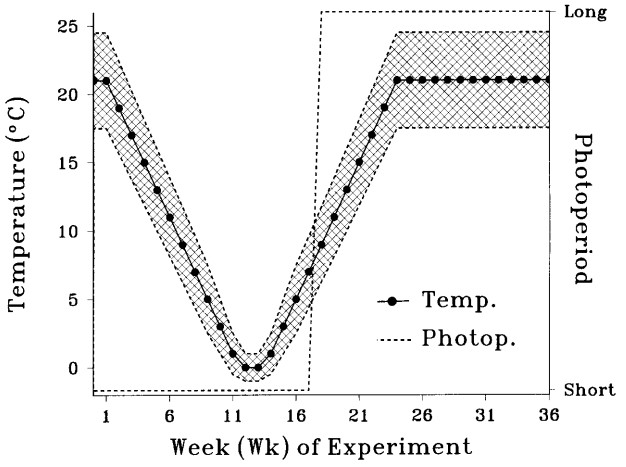


Fig. 1 Temperature and photoperiod of the simulated winter. The *cross-hatched area* about the mean daily temperatures shows the daily amplitude in temperature in the water-filled microcosms. *Short* Photoperiod light:dark 8:16 h, *long* photoperiod light:dark 16:8 h

Once first instars were added to the microcosms, they were checked 3 times week^{-1} and the pupae removed, sexed, and weighed. The experiment was terminated in the 36th week after no microcosm in any treatment had produced any pupae for 1 month.

Simulated winter environment

To simulate winter temperatures, a controlled-environment room was programmed to produce a smooth, sine-wave daily thermoperiod that changed weekly during the experiment (Fig. 1).

Effects of time of hatching during the winter

To examine the effect on cohort replacement rate of hatching at different times of the simulated fall, winter, and spring, replicate microcosms with 7 g dried substrate, but without mosquitoes, were flooded at the start of weeks 1, 2, 4, 6, 10, 12, 14, 16, and 18. The first instars were added to the experimental microcosms 5 days after flooding in week 1 and 14 days after flooding in weeks 2–18.

Effects of substrate deterioration

Tree holes in nature do not flood sequentially during the year, but rather, at similar times in the fall when the rains return. To test for progressive deterioration of substrates, without a pre-existing competitor, 18 replicate microcosms were flooded simultaneously at the start of week 1 and first instars added to the microcosms at the start of weeks 2, 4, 6, 8, 12, 14, 16, 18, and 20.

Effects of pre-existing competitors

Mosquitoes usually hatch into tree holes soon after the hole floods so that late-hatching cohorts encounter the effects of not only the past decomposition of tree-hole substrates, but also pre-existing conspecific competitors. To evaluate the effects of pre-existing competitors, 18 replicate microcosms were flooded at the start of week 1 and received 20 first-instar *A. sierrensis* as competitors. These larvae were allowed to develop and any pupae were removed and discarded. At the start of weeks 2, 4, 6, 8, 12, 14, 16, 18, and 20, any remaining competitor larvae were removed and discarded; immediately thereafter, the 20 experimental first instars were added to the microcosm.

After $\log_{10}(R_0+1)$-transformation to achieve non-heterogeneous variances, we performed analysis of covariance of cohort replacement rates using week of the experiment as a covariate and the treatments as fixed effects. First, we tested for the effect of delayed hatching, per se, on mosquito fitness by testing whether there was a significant correlation between R_0 and date the microcosm was flooded and mosquitoes were added. Second, we tested for the effect of substrate deterioration by comparing the regression coefficients of R_0 regressed on date the mosquitoes were added between microcosms flooded simultaneously at the start of the fall (combined effect of delayed hatching and deterioration of the substrate with time) and microcosms flooded at different times during the fall and winter just before mosquitoes were added (effect of delayed hatching alone). Third, we tested for the effect of pre-existing competitors by comparing regression coefficients of R_0 regressed on date the mosquitoes were added to microcosms flooded at the start of the fall between those with and without the pre-existing competitors. Data were analyzed using type III sums of squares (equal sample sizes) in the GLM procedure of SAS (SAS Institute 1985).

Results

Seasonal availability of resources

Independent effects of substrates

Cohort replacement rates were higher for tree-hole contents collected in the fall and early winter than for tree-hole contents collected in the spring (Wilcoxon T_s=3, P<0.01; Fig. 2).

In the fall, the contribution to mosquito nutrition differed among substrates (Kruskal-Wallis $H_{adjusted}$=15.30, P=0.002). Leaf litter sustained higher cohort replacement rates than the coarse detritus (Fligner-Policello $\hat{U}$=3.90, P<0.01), than the fine detritus ($\hat{U}$=4.27, P<0.01), and than stemflow water ($\hat{U}$=5.66, P<0.01) while the latter three substrates did not differ ($\hat{U}\leq$1.31, P >0.10) in their ability to sustain R_0, even though stemflow water failed to support development through to pupation in any larva.

In the spring, the contribution to mosquito nutrition did not differ among substrates (Kruskal-Wallis $H_{adjusted}$=6.77, P=0.084). Again, stemflow water failed to support development through to pupation in any larva.

These results show that resources available to mosquitoes in tree holes decline by >90% during the winter, but that this decline is due almost exclusively to the nutritional contribution provided by the leaf litter. At both times of year, the coarse or fine detritus provide minor resources, and stemflow water provides no detectable contribution to mosquito nutrition.

Interaction between substrates

In the fall, the largest interactions between substrates were negative, but none of them was significantly different from zero (Fig. 2). In the spring, the largest interactions were positive (Fig. 2). There was a significant synergism between leaf litter and the fine detritus and a nearly significant synergism between the coarse detritus

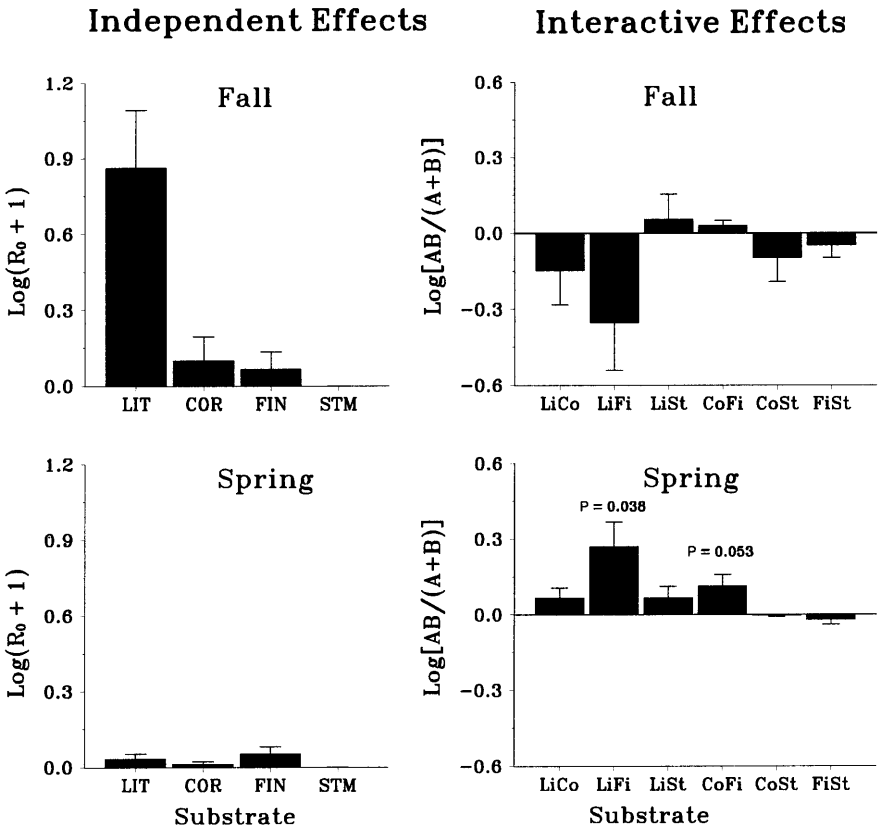


Fig. 2 Independent and synergistic effects on mosquito fitness (cohort replacement rate, R_0) of tree-hole substrates collected early (fall) or late (spring) in the developmental season (means±2 SE). Individual *t*-tests for significant interactions resulted in $P>0.15$ for all individual tests except LiFi and CoFi whose exact *P*-values are shown in the spring plot. *LIT* Leaf litter alone, *Li* leaf litter in combination; *COR* coarse detritus alone, *Co* coarse detritus in combination, *FIN* fine detritus alone, *Fi* fine detritus in combination, *STM* stemflow water alone, *St* stemflow water in combination, *AB* R_0 achieved when substrates A and B were provided in combination, *A+B* sum of each R_0 when provided separately

and the fine detritus. These results show that the contribution of substrates to mosquito fitness is mainly additive in the fall and may involve synergistic effects of the fine detritus with leaf litter or coarse detritus in the spring.

Sustainability of tree-hole substrates

Pupal mass

Stemflow water supported development to pupation only at the lowest density (1 larva 750-ml-jar^{-1} of stemflow water) and then only in males (Fig. 3). Similarly, the coarse detritus supported development to pupation only in males and only at the two lowest densities we tested (2 and 4 larvae 10 g^{-1} in a 150-ml dish). The fine detritus supported development only at the two lowest densities we tested (4 and 8 larvae 150-ml-dish^{-1}) but did support the development of both sexes. In both sexes, mean pupal mass declined with increasing density. Litter and the combined contents supported development to pupation in both sexes at every density we tested and, on both substrates, pupal mass declined with increasing density. Across all treatments, maximum pupal masses for males and females were about 3 mg and 5 mg, respectively, while minimum pupal masses were about 0.8 mg and 1 mg, respectively.

These results show that pupal mass declined with increasing density and that, at the relative proportions of substrates available in natural tree holes, only leaf litter was capable of producing the full range of pupal masses produced by the entire combined contents.

Yield

At the lowest densities, the coarse detritus, fine detritus, or stemflow water produced about 1/15 or less the yield of leaf litter alone or of the combined substrates (Fig. 4A). For both litter and the combined substrates, yield was a non-linear function of larval density. Regression of yield on density did not differ between litter and the combined substrates in their linear coefficients (b_{linear}±SE: litter, b=0.0332±0.0067; combined, b=0.0322±0.0072; t=0.10, df=14, P=0.922) but yield fell off more sharply at higher densities when mosquitoes were reared on litter than on the combined contents ($b_{quadratic}$±SE: litter, b=–0.0052±0.0009; combined, b=–0.0035±0.0001; t=5.56, df=14, P<0.001).

Coarse and fine detritus and stemflow water sustained non-zero yields only at the lower densities (Fig. 4A), and then only at low levels. On an expanded scale (Fig. 4B), the coarse detritus sustained maximum yields of 0.8–1.2 mg dish^{-1} at densities of 2–4 larvae dish^{-1}, declined at densities of 8–16 larvae dish^{-1}, and fell to zero at higher densities. Fine detritus did not sustain any yield at the lowest density of 4 larvae dish^{-1}, sustained a maximum yield of 2 mg dish^{-1} at 16 larvae dish^{-1}, and fell to zero at higher densities. Stemflow water sustained a non-zero yield of about 1 mg container^{-1} only in the jars contain-

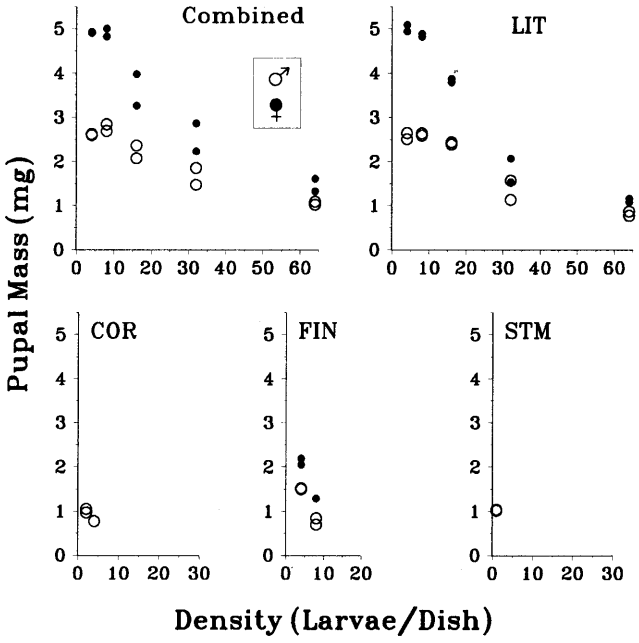
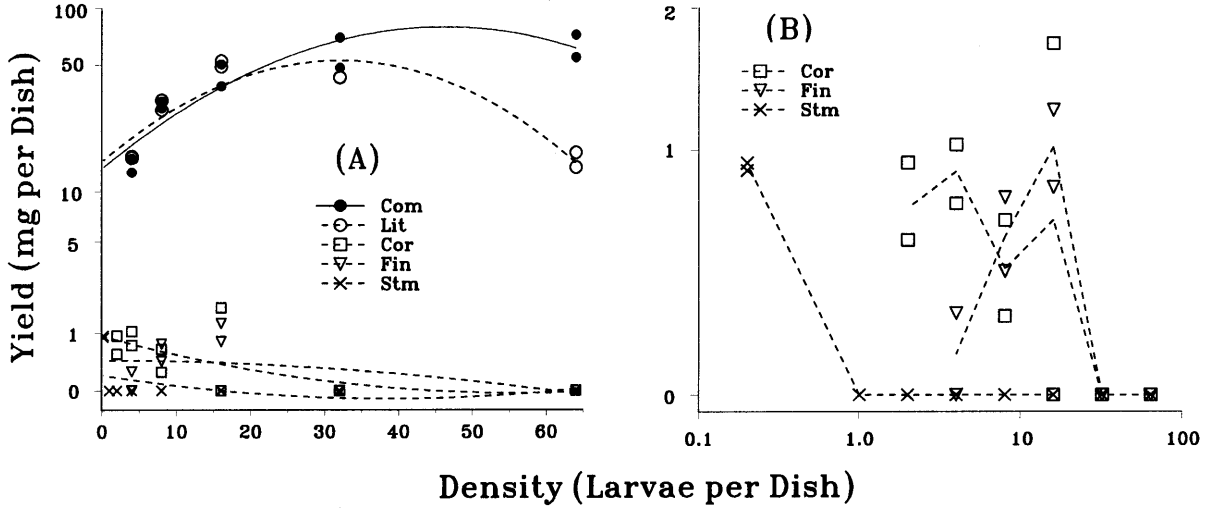


Fig. 3 Cohort mean pupal wet mass of *A. sierrensis* developing at varying larval densities on the combined contents of tree holes (LIT plus COR plus FIN plus STM) or on each substrate separately. For abbreviations, see Fig. 2

ing 1 larva with 750 ml undiluted stemflow water (equivalent to 0.2 larvae dish^{-1} in the other experiments).

These results show that leaf litter was the only substrate to sustain a non-zero yield at all densities, and produced at least 15 times the biomass of any other substrate at any density. In combination, however, leaf litter plus the other substrates sustained a higher yield at the highest densities than did leaf litter alone.

Fig. 4. Biomass yield (cumulative mg wet mass of all pupae plus wet mass of all remaining larvae at the end of the experiment) at varying larval densities on the combined or separate tree-hole substrates (**A, B**). Note in **B** the expanded vertical scale and logarithmic horizontal scale to illustrate better the relative yields at low density. For abbreviations, see Fig. 2



Cohort replacement rate (R_0)

Neither the stemflow water nor the coarse detritus fraction sustained development to pupation of any females at any density; consequently, all cohorts reared on these substrates achieved R_0s of zero. For the remaining treatments, R_0s were negatively correlated with larval density (Fig. 5). The fine detritus sustained non-zero R_0 only at the lowest density of 4 larvae dish^{-1} and, at 8 larvae dish^{-1}, only in one of the two replicates. The litter fraction and the combined substrates sustained non-zero R_0 at all densities and declined with increasing densities (coefficient ±SE: litter, Y=1.970±0.075–0.0314±0.0019X; combined, Y=2.174±0.063–0.0183±0.0023X). The latter two regressions differed in slope (t=4.39, P<0.001) but not intercept (t=2.07, P=0.057). These results show that the contribution to R_0 of either stemflow water or coarse detritus was undetectable at the level of tree-hole substrates used in our experiments, that the fine detritus made a small contribution to R_0, that leaf litter made the major contribution to R_0 at all larval densities, but, that at the highest larval densities, the combined contribution of the other substrates enhanced fitness above that of leaf litter alone.

Nutritional consequences of delayed seasonal hatching

Effects of time of hatching during the winter

There was no significant correlation (r=0.01, P=0.985) between R_0 and time during the simulated winter that first instars were added to microcosms, when flooding was delayed until just before the mosquitoes were added (Fig. 6). These results provide no evidence that delayed hatching during the winter and in the absence of other factors results in lower fitness.

Effects of substrate deterioration

R_0 declined with increasing time during the simulated winter that first instars were added to the microcosms

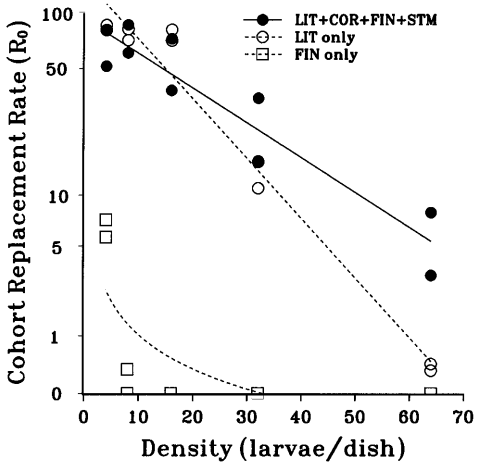


Fig. 5 R_0 achieved by cohorts reared at varying larval densities on the combined tree-hole substrates, or on leaf litter or fine detritus alone. R_0=0 at all larval densities from 1–16 larvae dish^{-1} on stemflow water and from 2–64 larvae dish^{-1} on coarse detritus

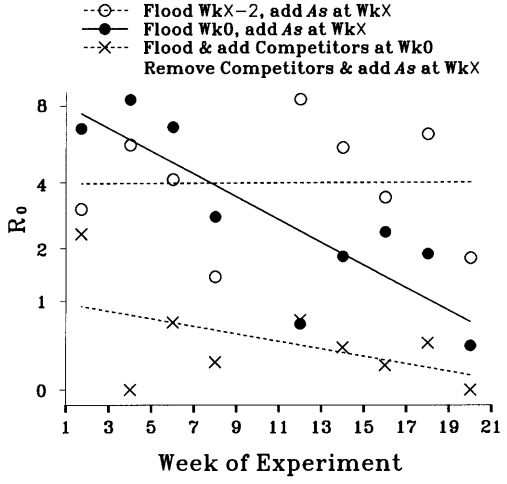


Fig. 6 The effect on R_0 in *A. sierrensis* of delayed hatching alone (○), delayed hatching plus deteriorating substrate (●), and delayed hatching plus deteriorating substrate plus pre-emptive competition (×) reared on the combined tree-hole substrates through a simulated winter (Fig. 1)

(r=–0.85, P=0.004), when flooding had taken place earlier at the beginning of the fall. Regression of R_0 on week of experiment had a significantly steeper slope ($F_{1,17}$=6.55, P=0.023) but not a different intercept ($F_{1,17}$=2.61, P=0.128) than in the previous treatment. These results show that the passage of time, independently of the effects of delayed hatching, results in the depletion of larval resources.

Effects of pre-existing competitors

There was no significant correlation (r=–0.50, P=0.169) between cohort replacement rate and time during the simulated winter that first instars were added to microcosms, when flooding and the addition of competitors had taken place earlier at the beginning of the fall. Regression of R_0 on week of experiment had a significantly lower intercept ($F_{1,17}$=17.78, P<0.001) but did not differ in slope ($F_{1,17}$=3.72, P=0.074) when compared to the previous treatment. These results show that a pre-existing competitor increases the depletion of resources available to larval *A. sierrensis*.

Discussion

In any study employing microcosms, there is a necessity to show that the experimental treatments approximate the conditions of natural tree holes. First, in real tree holes, larval density and substrate mass may vary independently of one another. However, as pointed out by Léonard and Juliano (1995, p 135), "none of the studies that used natural litter have detected significant density-litter interactions for composite estimates of population growth" (Fisher et al. 1990; Lounibos et al. 1993; Léonard and Juliano 1995). In other words, it is the per capita availability of natural detritus that is the primary determinant of individual fitness, even in species like *A. sierrensis* (Fisher et al. 1990) that exhibit intraspecific interference competition (Broadie and Bradshaw 1991). Hence, our use of a single factor of larval density instead of a fully crossed, substrate×larval density factorial design is not likely to have interjected substantial bias into our results. Second, the mass of females pupating in natural Oregon tree holes is highly correlated with larval density in the tree hole, and ranges from a mean of 1.8 mg tree hole^{-1} to 4.2 mg tree hole^{-1} (Hawley 1985a). The equilibrium density when R_0=1 is about 1200 larvae l^{-1}, corresponding to a female pupal mass of 2.37 mg. Mean female pupal mass in our experimental microcosms with natural tree-hole substrates ranged from 1.0 mg to 5.1 mg (Fig. 3). Our results are, therefore, based on conditions that bracket the spectrum of resources naturally available to *A. sierrensis*.

Resources available to mosquitoes in tree holes are maximal in the fall at the return of autumnal rains and decline thereafter (Fig. 6). Based on previous studies, the major contribution of leaf litter and the minor contribution of stemflow water were entirely expected (Carpenter 1982, 1983; Fish and Carpenter 1982; Lounibos et al. 1993; Walker et al. 1991; Léonard and Juliano 1995). Based on the nutritive value Livdahl (1982) found in litter-free detritus, and because of the high proportion of total dry mass that is found in the litter-free detritus (Fisher et al. 1990), we did not expect the meager contribution to mosquito fitness of either the coarse or the fine detritus. We had suspected that they might make a synergistic contribution to mosquito fitness in combination with litter, stemflow water, or each other. In fact, we observed no substantive pair-wise synergistic effects (Fig. 2). However, when added to the leaf litter, the combined coarse detritus, fine detritus, and stemflow water sustained mosquito yield and per capita replacement rate above that of litter alone at the highest densities (Figs. 4,

5). In sum, the results of our experiments show that, although leaf litter is the major source of tree-hole nutrition available to mosquitoes, the other components, in aggregate and not individually, become important in maintaining mosquito fitness at limiting densities.

The nutritional quality of substrates in natural tree holes declines from fall to spring (Fig. 2), and this decline is well replicated during a simulated winter in the laboratory (Fig. 6). Hence, the use of dried detritus (Fig. 6) provided qualitatively the same result as fresh detritus (Fig. 2), even though drying might be expected to reduce the availability of nutrients (Aspbury and Juliano 1998). Leaf litter is the prime source of mosquito nutrition, and its loss of nutritional value is not transferred to another compartment of the tree-hole ecosystem. The loss of nutritional quality proceeds progressively, presumably due to microbial decomposition because the loss occurs in the absence of mosquitoes or other macrodecomposers, but the loss is increased markedly by pre-existing mosquitoes (Fig. 6). We therefore conclude that the resources in leaf litter are either exploited by mosquitoes when they first become available, or they deteriorate and are lost to them. The net effect of microbial decomposition and pre-emptive competition should be to select for early hatching, immediately upon filling of the tree holes by autumnal rain.

In addition to the deterioration of larval resources, three other factors could be affecting optimal hatching time:

1. Most tree holes in Oregon dry out entirely during the hot, dry summers and mosquitoes that fail to emerge as adults before drying of the tree hole die; this factor should reinforce selection for autumnal hatching of *A. sierrensis*.
2. At the latitude of our study (44°N), a mid-winter frost that kills some or all of the larvae in tree holes occurs about 4 times a decade, and a frost that kills larvae in even the largest holes occurs about once a decade (Hawley 1984). The same risk occurs in British tree holes at 52°N where *Aedes geniculatus* hatch en masse at temperatures of 1–3°C following a freeze that has killed many of the previously hatched larvae (Bradshaw and Holzapfel 1991). Potential freezing of tree holes should therefore select for delayed hatching until after mid-winter.
3. Western tree holes are inhabited by the ciliated protozoan, *Lambornella clarki*, which is a facultative parasite on *A. sierrensis*. Parasitized *A. sierrensis* either die as larvae or fail to reproduce as adults (Washburn et al. 1988, 1991). The effect of parasitism on individuals is, therefore, effectively fatal. *L. clarki* exist as free-swimming, non-parasitic trophonts in the absence of mosquitoes, but in response to larvae-produced, water-soluble factors, are induced to transform into obligate parasites that die if they cannot infect a mosquito. As a consequence, parasite density declines after an early peak, and parasitism by *L. clarki* should also select for delayed hatching by *A. sierrensis*. Of the 60,000 *A. sierrensis* that hatched into Oregon tree holes during the three winters from 1980–1983, 85% did so before January (Hawley 1984). This observation indicates that the combined effects of resource depletion and the threat of vernal desiccation are far more potent sources of natural selection on hatching time than are the threats of parasitism or freezing.

Lounibos (1985) likened tree holes to “heterotrophic small ponds”, but Oregon tree holes also share many fundamental properties with temperate woodland streams (Kaushik and Hynes 1971; Cummins 1974; Petersen and Cummins 1974; Suberkropp and Klug 1976; Ward and Cummins 1979; Richardson 1991): First, tree holes are largely aphotic, similarly to low-order streams with an over-reaching canopy; they are therefore dependent primarily on allochthonous inputs of nutrients rather than primary production for their resource base. Second, seasonal input of leaf litter provides the limiting allochthonous resource. Third, the availability of this resource is transient and the macroconsumers (mosquitoes) utilize this resource primarily during the fall and winter, during the period of lowest annual temperatures. In Europe, the tree-hole fauna includes midge and beetle larvae that forage within the detritus (Kitching 1971). Although feeding on detritus, these “benthic” arthropods are neutral with respect to the mosquito fauna, or, at high densities, may become beneficial to the mosquitoes because their activities release as many or more nutrients than they consume (Bradshaw and Holzapfel 1992). This “processing chain commensalism” is not uncommon in freshwater habitats (Heard 1994) and may serve the same functional role in European tree holes that flushing by stem flow does in eastern North American tree holes. Oregon tree holes do not house a comparable benthic arthropod community, nor do they experience the degree of flushing described by Walker et al. (1991). Oregon tree holes receive leaf litter only once a year and this resource is not renewed, either within or between tree holes. Tree holes may be dry when females oviposit. The long days of summer induce aestival dormancy in the pharate first instars, and annual recruitment of *A. sierrensis* is determined by the timing of larval hatching in the fall. The combined environmental limitations of non-renewable larval resources and a long, dry summer are probably the environmental factors limiting arthropod diversity in western North American tree holes and the selective factors that have resulted in the early autumnal hatching and the univoltine life cycle of *A. sierrensis* throughout its range from Mexico to Canada.

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