

Invertebrate Carcasses as a Resource for Competing *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae)

MATTHEW P. DAUGHERTY, BARRY W. ALTO, AND STEVEN A. JULIANO

Division of Behavior, Ecology, Evolution, and Systematics, Department of Biological Sciences, Illinois State University, Normal, IL 61790-4120

J. Med. Entomol. 37(3): 364-372 (2000)

ABSTRACT Terrestrial invertebrate carcasses are an important resource for insects developing in pitcher plants. However, little is known of the role of these carcasses in other containers, which also receive leaf fall and stemflow inputs. This experiment investigated effects of accumulated invertebrate carcasses as a resource for two competing mosquitoes, *Aedes albopictus* (Skuse) and *Aedes aegypti* (L.), whether either species differentially benefited from accumulated carcasses, and if such a benefit affected interspecific competition. First, we measured accumulation of invertebrate carcasses in standard containers at a field site. We then used a replacement series with five different species ratios at the same total density, and varied the input of invertebrate carcasses [dead *Drosophila melanogaster* (Meigen)] in three levels: none, the average input from our field site, or the maximum input recorded at our field site. Survivorship, development time, and mass were measured for each mosquito species as correlates of population growth, and were used to calculate a population performance index, λ' . There were strong positive effects of invertebrate carcass additions on all growth correlates and λ' . Differences in performance between species were pronounced in small or no carcass additions and absent in large inputs of invertebrate carcasses, but there was little evidence that inputs of invertebrate carcasses altered the competitive advantage in this system. These results suggest that terrestrial invertebrate carcasses may be an important resource for many types of container communities, and large accumulations of dead invertebrates may reduce resource competition between these mosquitoes, thus favoring coexistence. We propose that the total amount of resource, including accumulated invertebrate carcasses, may explain observed patterns of replacement involving these mosquitoes.

KEY WORDS *Aedes aegypti*, *Aedes albopictus*, invertebrate carcasses, replacement series, insect container communities

WATER-FILLED CONTAINERS serve as habitats for diverse communities of invertebrates with aquatic larval stages. The most studied organisms within these communities are mosquitoes, which inhabit natural tree holes and other phytotelmata, as well as discarded tires, birdbaths, and cemetery vases. These containers vary in resource availability and species densities, which may limit the development of larvae (Carpenter 1983, Naeem 1988, Walker and Merritt 1988, Heard 1994, Léonard and Juliano 1995, Walker et al. 1997). Resource input into these systems comes in the form of annual leaf fall, stemflow, and accumulation of carcasses of animals that fall into the containers. Many experiments have analyzed the importance of leaf detritus and stemflow inputs for container mosquitoes. Leaf litter and stemflow are known to provide a substrate for the growth of bacteria and fungi that are consumed by mosquito larvae (Fish and Carpenter 1982, Walker et al. 1997). The amount, type, and quality (e.g., fresh versus senescent) of leaf litter are known to affect tree hole mosquito population parameters such as growth, survivorship, and development time (Fish and Carpenter 1982, Lounibos et al. 1993, Léonard and Juliano 1995). Stemflow and asso-

ciated nutrients and bacteria are a significant resource for tree hole mosquitoes (Carpenter 1982), and may be as important as the accumulation of leaf detritus (Walker and Merritt 1988).

It is likely that all types of water-filled containers collect some terrestrial animal carcasses. In the Midwest, tree holes and discarded tires are regularly found to have remains of terrestrial organisms, ranging from small insects to large earthworms (M.P.D., B.W.A., and S.A.J., unpublished data). However, the role of accumulated invertebrate carcasses as a resource for container insect communities is largely unknown. The few exceptions are studies that examine pitcher plant systems, where invertebrate carcasses constitute the primary resource input for resident insects. An examination of food web structure indicated that older pitchers, with more insect carcasses, had greater bacterial density and greater diversity of insect species (Sota et al. 1998). Chironomid midges may facilitate pitcher plant mosquitoes through the partial consumption of these carcasses, with remaining particles providing a substrate for bacterial growth, which is fed upon by mosquitoes (Heard 1994). Within pitcher

plants, a mite and a chironomid compete for a common resource (insect carcasses), and only in cases of unusually high resource levels can these two species coexist (Naeem 1988). Bradshaw and Holzapfel (1986) showed that density of the mosquito *Wyeomyia smithii* (Coquillett) is positively correlated with the abundance of dead insects in pitchers and that this species' pupation success was negatively related to density of larvae relative to abundance of dead arthropods.

These pitcher plant studies suggest that the accumulation of invertebrate carcasses may play an important role in shaping pitcher plant insect communities, wherein these carcasses constitute the primary resource. However, it is not clear whether invertebrate carcasses are also important in other containers where leaf detritus or stemflow are the primary resources. Furthermore, it is unknown whether some container species derive greater benefit from invertebrate carcass accumulation than do other species. Resolving these questions will yield important information on the general nature of invertebrate carcasses as a resource, and may contribute to understanding the variable outcome of competition between two container mosquito species *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) (O'Meara et al. 1995, Juliano 1998).

Aedes albopictus is an exotic mosquito that has rapidly spread throughout North America since its introduction in the 1980s. In Florida, where *A. albopictus* and *A. aegypti* co-occur, *A. albopictus* range expansion has coincided with major declines in *A. aegypti* abundance (Hornby et al. 1994, O'Meara et al. 1995). This pattern of invasion followed by decline has been observed in other parts of the eastern United States (Hobbs et al. 1991, Mekuria and Hyatt 1995). A field experiment using discarded tires showed that over a range of mosquito and leaf litter densities, *A. albopictus* was the superior competitor (Juliano 1998). However, Barrera (1996) showed that although *A. albopictus* was dominant when the resource was leaf litter, *A. aegypti* outperformed *A. albopictus* when the resource was liver powder. Therefore, the type of resource may affect the outcome of competition between these two species.

Our experiment tests the hypotheses that invertebrate carcasses are a valuable resource for *A. albopictus* and *A. aegypti*, that *A. albopictus* and *A. aegypti* differentially benefit from additions of invertebrate carcasses, and that differential benefits alter the outcome of competition between these species. These hypotheses were tested using laboratory microcosms, with manipulations of the number of invertebrate carcasses added (at approximately natural levels) and ratios of *A. albopictus* to *A. aegypti* larvae. We measured correlates of mosquito population growth, and used these correlates to calculate a composite index of performance (Livdahl and Sugihara 1984). This index can be used to determine the absolute effect of invertebrate carcass additions on mosquito populations, as well as any differences in effect between species.

Materials and Methods

Field Study. To estimate a natural level of invertebrate carcass input, we sampled an established tire site before the laboratory experiment. The site was located <2 km north of Normal, IL, bounded by a cornfield on one side, and the interstate and an overpass on the others. Ten 400-ml plastic beakers filled with 200 ml of tire water were placed among the tires so that the bottoms were at ground level. Each beaker was equipped with a 1-mm plastic mesh screen that rested below the water surface. During the first 2 wk of September 1998, we collected daily all insects caught in the surface tension or on the screen. Insects were oven dried at 60°C for at least 48 h, and weighed to the nearest 0.0001 mg on a Cahn C-31 microbalance (Cahn Instruments, Cerritos, CA). Daily container averages of total mass input as well as the 2-wk overall average were determined.

Lab Study. Experimental Design and Data Collection. Experimental containers consisted of 400-ml plastic beakers filled with 200 ml of deionized water, and 1 ml of tire water as an inoculum for microorganisms. Each container received 0.5 g (± 0.05 g) of senescent *Quercus virginiana* (live oak) leaves collected in 1992 from Vero Beach, FL, oven-dried, and stored dry. For the experiment, leaves were washed, then oven-dried at 60°C for at least 1 d before the addition to containers. After the leaves had soaked for 3 d, synchronously hatched first-instar mosquitoes (Novak and Shroyer 1978) were added to each container. Mosquito larvae were the offspring of laboratory colonies (established in 1997) of *A. aegypti* collected from Stuart, FL, and *A. albopictus* collected from East St. Louis, IL. The experimental design was a replacement series with total density constant at 40 larvae and ratios of *A. albopictus*:*A. aegypti* of: 0:40, 10:30, 20:20, 30:10, and 40:0.

Three invertebrate carcass addition treatments (control, small, and large) were applied to each of the five species ratios. Adult *Drosophila melanogaster* (Meigen) were the source of carcasses. Several adult *D. melanogaster* were oven dried then weighed. The number of *D. melanogaster* added to each treatment was chosen to correspond with calculated dry mass inputs observed in the field study, representing the lowest extreme, overall average, and highest extreme (control, small, and large, respectively). Every other day, containers in the small treatment received two frozen adult *D. melanogaster*, the large treatment received 8, and the control treatment received no flies. After each manipulation, the water in all three treatment types was stirred to facilitate soaking of the flies. Four replicates were used for each combination of the three carcass treatments and five species ratios, for a total of 60 containers. All containers were housed in an environmental chamber at 27°C with a photoperiod of 16:8 (L:D) h.

When the first pupae appeared, containers were checked every other day, and pupae were removed and placed in 20-ml vials until eclosion. Vials were checked daily for eclosed adults, which were identi-

fied by species and sex, oven dried at 60°C for >2 d, and individually weighed to the nearest 0.0001 mg. The experiment continued until all larvae either eclosed or died (≈ 8 wk).

Data Analysis. Three population correlates were used to quantify the effects of invertebrate carcass treatments and species ratios on mosquito population growth. Mean mass and development time (median days to eclosion) were calculated for males and females of each species, for each replicate of the treatment-species ratio combination. Survivorship of each species was calculated for each replicate by dividing the total number of eclosed larvae of a species by the number of larvae of that species originally in the container (percent of survivorship). A composite index of mosquito population performance (λ') was also calculated for each replicate (Juliano 1998).

The composite index of performance ($\lambda' = \exp[r']$) is based on r' , which is an estimate of the realized per capita rate of population change ($dN/Ndt = r$, exponential growth). By using r' , multiple nonlinearly related components (mass, development time, and survivorship) can be incorporated into a single value. Estimates of per capita rates of change are useful because they clarify overall treatment effects that may otherwise be obscured by analyzing individual correlates of population growth that potentially conflict with each other (Livdahl and Sugihara 1984, Juliano 1998). We chose to analyze λ' over r' because the latter index is not estimable when there are no survivors in a replicate.

A composite index of population performance (λ') was calculated for each replicate of the invertebrate carcass treatment-species ratio combination as follows:

$$\lambda' = \exp(r')$$

$$= \exp\left[\frac{\ln[(1/N_o) \sum_x A_x f(w_x)]}{D + [\sum_x A_x f(w_x) / \sum_x A_x f(w_x)]}\right], \quad [1]$$

where r' is a composite index of population performance (Livdahl 1982, 1984; Livdahl and Sugihara 1984), N_o is the initial number of females in a cohort (assumed to be 50% of the larvae added), A_x is the number of females eclosing on day x , w_x is a measure of mean female size on day x , $f(w_x)$ is a function relating female egg number to her size, and D is the time required (in days) for a newly eclosed female to mate, obtain a blood meal, and oviposit. D is assumed to be 12 and 14 d for *A. aegypti* and *A. albopictus*, respectively (Grill and Juliano 1996). The size-fecundity relationships [$f(w_x)$] used in the calculation of λ' were as follows.

A. aegypti:

$$f(w_x) = 17.11 + 16.59 (w_x^{0.765}) \quad [2]$$

$$r^2 = 0.10, N = 57, P < 0.05$$

(w_x = dry mass in milligrams. Colless and Chellapah 1960, and Grill and Juliano 1996)

A. albopictus:

$$f(w_x) = 17.2 w_x - 14.0 \quad [3]$$

$$r^2 = 0.03, N \text{ and } P \text{ not reported}$$

(w_x = wing length in mm. Livdahl and Willey 1991).

This size-fecundity regression (equation 3) requires mosquito wing lengths, which we did not directly measure. Instead we estimated *A. albopictus* wing lengths using a regression of wing length versus dry mass from a field experiment (Juliano 1998). In that field experiment, mean wing length was measured on both wings (distance from the proximal edge of the costa to the distal end of the R_2 vein) of all *A. albopictus* using an eyepiece micrometer, then mass was recorded after drying for 24 h at 60°C regression of wing length versus dry mass for those data (equation 4), provided us with a predictive equation for individuals in the current study, based on their dry masses.

$$w_x = 3.2879 (\text{dry mass})^{0.2553} \quad [4]$$

$$r^2 = 0.8762, N = 220 \text{ and } P < 0.0001$$

(w_x = wing length in millimeters).

These regression functions (equations 2 and 3) are assumed to yield approximate individual reproductive capacities. Juliano (1998) showed that conclusions for analyses of λ' were not particularly sensitive to the fecundity-size relationship used.

Effects on mosquito λ' , survivorship, development time, and mass were individually analyzed using a two-way analysis of variance (ANOVA) with invertebrate carcass treatment and species ratios as categorical variables (SAS Institute 1989). *A. albopictus* female development time, male *Aedes aegypti* mass, and female *A. aegypti* mass were reciprocally transformed to meet the assumptions of normality and homogeneous variances. *Aedes aegypti* survivorship, *A. aegypti* λ' , and *A. albopictus* λ' did not meet the assumptions of normality and homogeneous variance, and no common transformation improved the residuals. Therefore, randomization two-way ANOVAs (which do not assume normality) were employed, using the program RT version 1.02 (Manly 1991a, 1991b). Randomized ANOVAs are preferable to other nonparametric ANOVAs because of their greater power (Crowley 1992).

To test for differential effects of invertebrate carcass treatment or species ratio on *A. albopictus* and *A. aegypti*, another two-way ANOVA was conducted on the difference in performance between the species (difference = *A. albopictus* - *A. aegypti*), for all growth correlates and λ' . This analysis used only species ratios with both species present (10:30, 20:20, 30:10). For development time and mass, some control replicates could not be used because there were no survivors for one of the two species. Transformation of the difference in female mass and the difference in female development time failed to correct fully for inhomogeneous variance, so results for these variables should be interpreted with caution. The difference in λ' failed to meet the assumptions of normality and homogeneous variance, and no common transforma-

Table 1. Randomization ANOVA for λ' (the estimated finite rate of increase for the cohort), and parametric ANOVAs for survivorship to adulthood, mass at eclosion, and time required to reach adulthood for *A. albopictus*

Source	λ' , estimated finite rate of increase			Survivorship		Mass				Development time			
	df	Observed % SS	P	F	P	Males		Females		Males		Females	
						F	P	F	P	F	P	F	P
Carcass	2	31.40	0.0001	149.30	0.0001	4.42	0.0200	9.35	0.0007	26.35	0.0001	31.33	0.0001
Ratio	3	4.22	0.6785	1.00	0.4029	1.88	0.1518	2.74	0.0609	0.49	0.6909	3.45	0.0283
Carcass*Ratio	6	9.54	0.6656	1.61	0.1731	0.57	0.7544	1.13	0.3691	0.65	0.6884	2.07	0.0861
Error df		36		36		33		30		33		31	

Carcass, denotes effects of invertebrate carcass additions; ratio, denotes effects of the initial ratio of larvae of the two species.

tion corrected the problem. Therefore a two-way randomization ANOVA was again used.

When significant main effects were detected, we compared all possible pairs of least square means using the Tukey-Kramer method (SAS Institute 1989). When interactions were significant, pairwise comparisons were made between ratios for a given treatment (e.g., for Small: compare 10:30 versus 20:20 versus 30:10), and between treatments for a given ratio (e.g., at 20:20: compare control versus small versus large), using a Bonferroni correction for experimentwise error. In addition, we tested whether the differences in performance between the species were significantly different from zero. For variables that were analyzed by randomization ANOVA, we performed all possible pairwise comparisons of treatment means using randomization methods (Manly 1991a, 1991b; with Bonferroni correction for experimentwise $\alpha = 0.05$).

Results

Field Study. Individual insect dry masses varied from 0.025 mg for some small dipteran species to nearly 6.5 mg for a coleopteran species. Daily averages of insect mass input ranged from 0 to 1.19 mg per container, with the 2 wk overall mean \pm SE = 0.22 \pm 0.09 mg per container per day.

Laboratory Study. The mean dry mass of a dried adult *D. melanogaster* was 0.25 mg. Therefore, one dried adult *D. melanogaster* approximates the overall average input of invertebrate carcasses observed in the field, and the three invertebrate carcass treatment additions closely approximate field minimum, maximum, and overall average (control = 0 mg/d, large \approx 1.0 mg/d, and small \approx 0.25 mg/d, respectively).

Population Performance index (λ'). Both *A. albopictus* and *A. aegypti* performances were affected by

the addition of invertebrate carcasses, but not by species ratio or the interaction (Tables 1 and 2). *Aedes albopictus* and *A. aegypti* performance was significantly enhanced by the addition of invertebrate carcasses, with the large treatment producing significantly greater performance than either the small and control treatments (Fig. 1).

The difference between *A. albopictus* and *A. aegypti* performance was significantly affected by the invertebrate carcass treatments (Table 3). Comparisons between the treatments showed that interspecific differences in performance were significantly smaller for the large carcass addition compared with the control treatment, but the small treatment did not differ from either the control or the large (Fig 1).

Survivorship. *A. albopictus* and *A. aegypti* survivorship were only affected by the addition of invertebrate carcasses (Tables 1 and 2). For both species, the control treatments produced fewer adults than either of the invertebrate carcass additions, with large additions producing the greatest survivorship (Fig. 2).

The differences in survivorship between *A. albopictus* and *A. aegypti* showed a marginally significant effect of invertebrate carcass treatments, and no effects of the species ratio or interaction (Table 3). The interspecific differences in survivorship in the control and small treatments were significantly greater than zero (that is, *A. albopictus* had greater survivorship), but the difference in the large treatment did not differ from zero. Comparisons between the invertebrate carcass treatments showed that differences were significantly greater for the small input treatment compared with the large treatment, but neither differed from the control treatment (Fig. 2).

Mass at Eclosion. In general, mosquito mass showed similar effects of treatment to those seen for survivorship and population performance. *Aedes albopictus*

Table 2. Randomization ANOVAs for λ' and survivorship to adulthood, and parametric ANOVAs for mass at eclosion and time required to reach adulthood for *A. aegypti*

Source	λ' , estimated finite rate of increase			Survivorship		Mass				Development time			
	df	Observed % SS	P	Observed % SS	P	Males		Females		Males		Females	
						F	P	F	P	F	P	F	P
Carcass	2	44.11	0.0001	89.70	0.0010	6.29	0.0064	21.61	0.0077	18.61	0.0001	4.81	0.0176
Ratio	3	14.92	0.0677	1.63	0.8610	2.59	0.0761	5.07	0.0001	3.65	0.0260	6.41	0.0024
Carcass*Ratio	6	18.14	0.2096	2.39	0.9890	0.49	0.7430	3.90	0.0146	1.85	0.1507	1.49	0.2378
Error df		36		36		24		23		25		24	

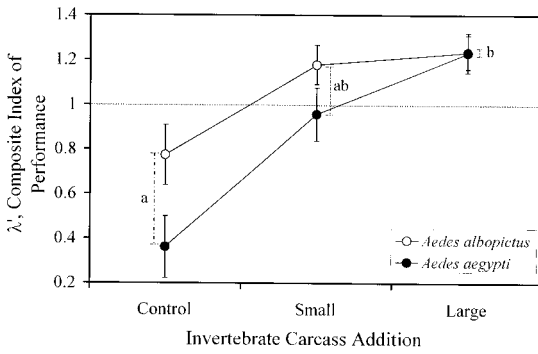


Fig. 1. Least square mean estimates of population performance (λ' , the composite index of population performance for the cohort) for *A. albopictus* and *A. aegypti* at three levels of added invertebrate carcasses. Carcass addition treatments were as follows: no added carcasses (control), an average natural level of invertebrate carcass accumulation (small), or the largest natural accumulation (large). The dotted line at $\lambda' = 1$ represents conditions where the population is being replaced, with no growth or decline. Letters denote interspecific differences that are significantly different across treatments. Error bars denote ± 1 SE.

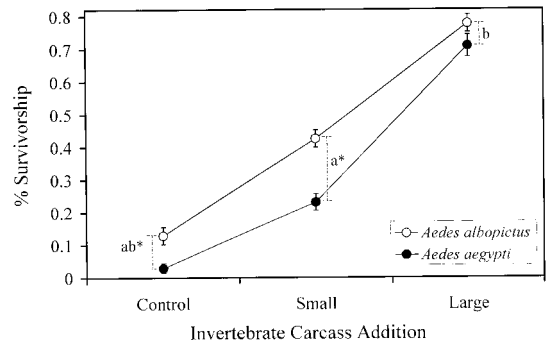


Fig. 2. Least square mean percent survivorship (percentage of the original number of larvae of each species surviving to adulthood) of *A. albopictus* and *A. aegypti* at three levels of added invertebrate carcasses. Asterisks denote cases where there are significant interspecific differences ($\Delta \text{Surv} = \text{Surv}_{\text{alb}} - \text{Surv}_{\text{aeg}} \neq 0$). Letters denote interspecific differences (ΔSurv) that are significantly different across treatments. Error bars denote ± 1 SE.

male mass was only affected by invertebrate carcass treatments (Table 1). The large treatment produced significantly larger males (mean \pm SE, 0.145 ± 0.005 mg) than did the control (0.120 ± 0.006 mg, $P = 0.0148$). *Aedes albopictus* female mass was also affected by invertebrate carcass treatments, was marginally affected by species ratio, and was not affected by the interaction (Table 1). Significantly larger females emerged from the large inputs (0.267 ± 0.011 mg) compared with both the small (0.238 ± 0.011 mg, $P = 0.0247$) and control treatments (0.184 ± 0.016 mg, $P = 0.0005$), but there were no significant differences as a result of the species ratios.

Aedes aegypti male mass also showed significant effects of invertebrate carcass treatments, but no effects of species ratio or the interaction (Table 2). A comparison between large and small additions was significant for the males ($P = 0.0344$) with smaller mosquitoes emerging from the small additions (0.153 ± 0.009 mg) compared with the large additions (0.179 ± 0.008 mg). *Aedes aegypti* female mass was significantly affected by invertebrate carcass treat-

ment, species ratio, and the interaction (Table 2). The interaction shows an overall trend for increased female size with larger invertebrate carcass inputs (Fig 3). Females in the 30:10 ratio were significantly larger in the small addition treatment compared with the control, and females in the 10:30 ratio were significantly larger in the large treatment compared with the small treatment. At all other species ratios the masses were indistinguishable between invertebrate carcass treatments. Lack of survivorship of individuals in species ratios 10:30 and 20:20 prevented any comparisons involving those control treatments (Fig. 3).

Analysis of the differences in mass between species showed no effect of treatment, ratio, or an interaction for males (Table 3). The difference in mass between females of the two species was clearly affected by invertebrate carcass treatment (Table 3). The difference between females was significantly positive in the small treatment (0.634 ± 0.024 mg, $P = 0.0205$), but was essentially zero in the large treatment (-0.007 ± 0.0167 mg, $P = 0.6802$), and the two treatments were significantly different from each other ($P = 0.0315$, Fig 4).

Days to Eclosion. Development time of the mosquitoes was strongly affected by invertebrate carcass ad-

Table 3. Randomization ANOVA on the difference between λ' for *A. albopictus* and *A. aegypti*, and parametric ANOVAs on the difference in survivorship, the difference in mass at eclosion, and the difference in development time between the two species

Source	λ' , estimated finite rate of increase			Survivorship		Mass				Development time			
	df	Observed % SS	P	F	P	Males		Females		Males		Females	
						F	P	F	P	F	P	F	P
Carcass	2	27.00	0.0050	3.32	0.0512	2.06	0.1703	4.46	0.0317	2.22	0.1406	3.09	0.0754
Ratio	2	6.37	0.2670	2.78	0.0796	0.26	0.7716	2.24	0.1437	1.49	0.2552	3.54	0.0549
Carcass*Ratio	4	20.08	0.1360	0.44	0.7754	0.36	0.7023	0.49	0.6249	1.77	0.2022	4.87	0.0234
Error df		27		27		16		14		16		15	

Differences were calculated by subtracting the value for *A. aegypti* from that calculated for *A. albopictus* in all replicates where both species occurred.

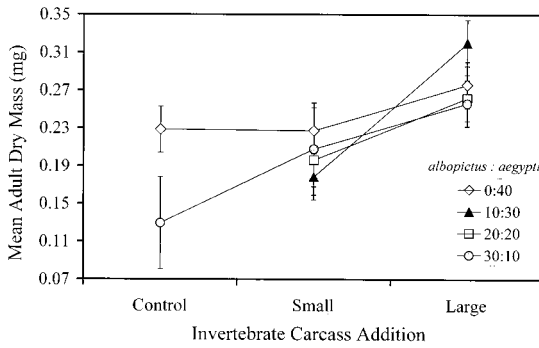


Fig. 3. Least square mean mass of *A. aegypti* females at eclosion, expressed on a natural scale. Missing data points in the control treatment result from no *A. aegypti* females eclosing in the 10:30 and 20:20 ratios (*A. albopictus* : *A. aegypti*). Error bars denote ± 1 SE.

ditions. *Aedes albopictus* males were affected by the treatments, but not by the ratios or interaction (Table 1). Males from the control (35.18 ± 1.46 d) took a significantly longer time to eclose compared with the small (28.28 ± 1.27 d, $P = 0.0032$) or large (21.19 ± 1.27 d, $P = 0.0001$) treatments, with the large additions producing the shortest development times ($P = 0.0011$). *Aedes albopictus* females also showed effects of treatment and no effect of an interaction, but an effect of the ratio was detected (Table 1). *Aedes albopictus* females in 10:30 containers had shorter development times compared with females from 20:20 containers (10:30 mean \pm SE, 36.33 ± 2.21 d; 20:20 mean \pm SE, 41.95 ± 1.92 d, $P = 0.0288$), but females in the remaining ratios (30:10 and 40:0) did not differ significantly from any of the other ratios. Females from the large input treatment took less time to develop (29.62 ± 1.66 d) than the control (48.83 ± 2.09 d, $P =$

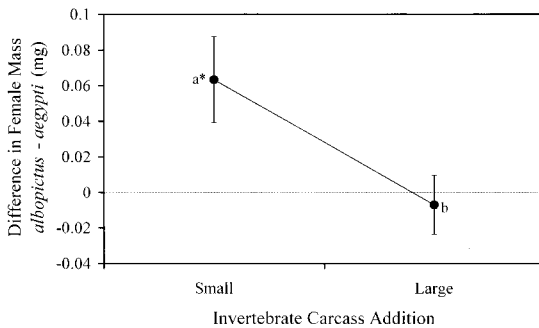


Fig. 4. Least square mean interspecific differences in adult female mass, at small and large additions of invertebrate carcasses. The control treatment is not represented because none of the ratios in the control treatment produced females of both species that could be weighed. Asterisks denote significant interspecific differences. Letters denote interspecific differences that are significantly different across treatments. The dotted line at zero represents conditions where there is no difference in female mass between the two species. Error bars denote ± 1 SE.

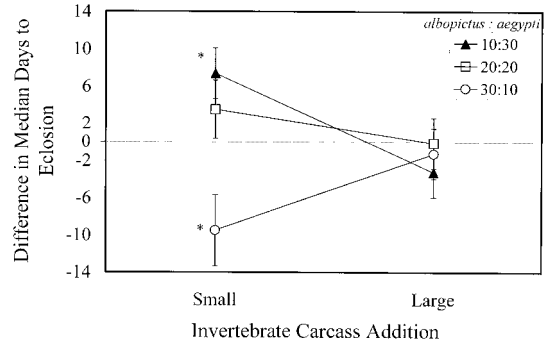


Fig. 5. Least square mean interspecific differences in adult female development time as a function of invertebrate carcass additions and species ratio. Asterisks denote significant interspecific differences. The control treatment is not represented because only one replicate produced females of both species, with only a single *A. aegypti* female eclosing in that replicate. The dotted line at zero represents conditions where there is no difference in female development time between the two species. Error bars denote ± 1 SE.

0.0001) and small treatments (36.50 ± 1.66 d, $P = 0.0004$), with the small input treatment producing shorter eclosion times ($P = 0.0009$).

The development time of *A. aegypti* males and females was affected by invertebrate carcass treatments and species ratios (Table 2). Large invertebrate carcass additions resulted in shorter male development times (23.91 ± 1.06 d) than the small carcass addition treatment (32.09 ± 1.18 d, $P = 0.0001$), and males developed significantly more quickly in the 0:40 treatment (27.54 ± 1.28 d) compared with the 10:30 treatment (32.50 ± 1.41 d, $P = 0.0153$). Large additions also resulted in shorter development times for females (31.50 ± 1.27 d) than small additions (37.47 ± 1.46 d, $P = 0.0051$). Although there was a significant effect of species ratio, estimates of female development time could only be made for the 0:40 and 30:10 ratios, which were not significantly different ($P = 0.1114$).

Analysis of the differences in development time between the species showed that males were not differentially affected by treatment, ratio, or an interaction (Table 3). However, the difference in female development time was marginally affected by invertebrate carcass treatment and species ratio, and the interaction was significant (Table 3; Fig. 5). For small additions of invertebrate carcasses, the magnitude of the differences between the two species varied dramatically, with a highly positive difference in the 10:30 ratio, a highly negative difference in 30:10, and no difference in 20:20. The difference for the 30:10 species ratio was significantly more negative than the 10:30 species ratio. With large inputs of invertebrate carcasses, the differences did not vary between ratios.

Discussion

This experiment was designed to test three hypotheses: that terrestrial invertebrate carcasses can be an important resource; that *A. albopictus* and *A. aegypti*

differ in their abilities to exploit invertebrate carcasses; and that competition between these species is affected by the availability of this resource. The first hypothesis predicts that invertebrate carcass additions increase mosquito population growth. The second hypothesis predicts that invertebrate carcass additions increase the growth rate of one species to a greater degree than the other species. The third hypothesis predicts that the presence of invertebrate carcasses eliminates or reverses competitive advantage.

As was the case in experiments involving pitcher plant invertebrates (Bradshaw 1983, Naeem 1988, Heard 1994, Sota et al. 1998), our results show accumulated invertebrate carcasses may be an important resource for members of container insect communities. We often observed mosquito larvae chewing on *D. melanogaster* carcasses, so it is not surprising that the addition of invertebrate carcasses positively influenced all three population growth correlates and increased the composite index of performance (e.g., Figs. 1 and 2). The beneficial effects of invertebrate carcass inputs were evident in both male and female *A. albopictus* and *A. aegypti*. In 10 of 20 replicates in the control treatment, survivorship was zero, implying that in addition to leaf litter, at least a small number of invertebrate carcasses may be necessary for mosquito production. Because we used inputs that were similar to inputs in field containers, we conclude that the accumulation of invertebrate carcasses in natural containers is likely to have important consequences for population growth of *Aedes* in these communities.

Invertebrate carcass additions influenced the differences between species in λ' , survivorship, and female mass, and differences between female development time showed an effect of invertebrate carcass treatment that was dependent on species ratio. These results superficially suggest differential effects of invertebrate carcass additions for the two species. However, upon closer examination, it is not clear that one species exhibited differentially greater population growth relative to the other species. The clearest and probably most relevant (Juliano 1998) effects of invertebrate carcass additions were seen on survivorship and λ' . *Aedes albopictus* outperformed *A. aegypti* when no invertebrate carcasses were present, but this dominance was not accentuated by the addition of invertebrate carcasses (Figs. 1 and 2). In fact, at large inputs of invertebrate carcasses the two species have approximately equal performance. Therefore, *A. albopictus* cannot be said to benefit differentially, especially when *A. aegypti* appears to use large numbers of invertebrate carcasses at least as well as *A. albopictus*. However, *A. aegypti* does not appear to benefit differentially, given that *A. albopictus* is able to maintain positive population growth at small inputs of carcasses while *A. aegypti* cannot (Fig. 1, growth occurs when $\lambda' > 1$).

Given that we detected no clear evidence for differential benefits, it is not surprising that we also have little support for the hypothesis that invertebrate carcasses altered the outcome of competition between these species. We did not specifically test for the

occurrence of competition, because a replacement series does not vary the densities of both species independently (Connolly 1986). Therefore, a replacement series cannot be used to test for the occurrence of competition (Goldberg and Scheiner 1993). A replacement series does test for the relative intensity of intraspecific versus interspecific competition (Goldberg and Scheiner 1993), which is how it is used here. Competition between these species has been demonstrated in the field (Juliano 1998) and in the laboratory (Barrera 1996) at similar mosquito densities and amounts of leaf litter. If accumulated invertebrate carcasses differentially affected competition between species, there should have been an interaction of invertebrate carcass treatment and species ratio on the differences between the species. Such an effect was only detected for the difference in development time between *A. albopictus* and *A. aegypti* females. With small inputs of invertebrate carcasses, the magnitude of the difference in development time was dependent on the species ratio (Fig. 5). The differences ranged from highly negative (i.e., *A. aegypti* developed more slowly) when *A. albopictus* was more abundant (Fig. 5, 30:10), to highly positive (i.e., *A. aegypti* developed more rapidly) when *A. aegypti* was more abundant (Fig. 5, 10:30). Equal species densities (Fig. 5, 20:20) resulted in no difference in development time between the species. Given that the other population growth correlates and λ' did not exhibit similar interaction effects, we do not have compelling evidence that invertebrate carcass accumulation alters the inherent competitive advantage in this system.

The observed shift in performance from *A. albopictus* dominance at small inputs of invertebrate carcasses to roughly equal performance for both species at larger invertebrate carcass inputs (Figs. 1, 2, 4, and 5) is probably best explained by greater resource abundance because of the addition of invertebrate carcasses. Such increased resource availability could explain the elimination of the competitive advantage of *A. albopictus* in containers with large additions of carcasses, and also explain the lack of effects seen on competition between the species. Larger amounts of accumulated invertebrate carcasses may simply reduce interspecific and intraspecific competition by alleviating resource limitation.

Removal of resource limitation may be a potential mechanism for an unexplained pattern observed in the *A. albopictus* invasion of the United States. Since its introduction, *A. albopictus* has rapidly replaced *A. aegypti* populations in rural and suburban environments in Florida. However, in urban environments (particularly in South Florida) *A. aegypti* has persisted long after being replaced in surrounding rural areas, even though *A. albopictus* is found at both types of sites (Hornby et al. 1994, O'Meara et al. 1995, Juliano 1998). Three hypotheses have been posed to explain the persistence of *A. aegypti* in urban environments: (1) variation in the intensity of interspecific competition between sites, (2) variation in competitive advantage depending on the nature of the container and substrate, or (3) reduced equilibrium resource require-

ment for *A. aegypti* because of a terrestrial urban environment favoring *A. aegypti* growth (Juliano 1998).

On a per gram basis, invertebrate carcasses are likely a higher quality resource than leaf litter. Cloe and Garman (1996) suggest terrestrial arthropods carcasses constitute a high quality resource relative to plant material because of their lower C:N ratio. Additionally, invertebrate carcasses are likely to decompose at a greater rate and thus support greater bacterial growth (Swift et al. 1979, Begon et al. 1990). In streams, terrestrial arthropod inputs can be an important resource for predatory fish (Needham 1928, Hunt 1975), subsidizing the energy budgets of stream ecosystems (Cloe and Garman 1996), with the potential for cascading effects throughout the community (Nakano et al. 1999).

With respect to insect container communities, if the ratio of invertebrate carcass material to plant detritus is greater in urban areas than rural areas, the type or amount of resource may affect competition between these two species in urban areas, potentially supporting the first two hypotheses for persistence of *A. aegypti* (see above). We know of no data quantifying relative inputs of invertebrate carcasses in urban, suburban, and rural areas, therefore we can only suggest that the accumulation of invertebrate carcasses may play a role in the observed mosquito distributions of these two species. Because *A. aegypti* populations are declining at smaller inputs of invertebrate carcasses ($\lambda' < 1$), the persistence *A. aegypti* in urban areas is not likely because of its superior ability to use invertebrate carcasses.

This is the first experimental evidence that invertebrate carcass inputs are important in container communities other than pitcher plants, because the amount of accumulated carcass material may affect the interactions of container mosquitoes. These accumulated carcasses are also of interest because of their potential role in commensalisms between mosquitoes and other members of container communities. Both Heard (1994) and Bradshaw (1983) noted the possibility for midge larvae to facilitate mosquitoes by burrowing into carcasses, thereby accelerating the degradation of accumulated carcasses into fine particulate matter that is available to filtering mosquitoes. Chironomid and Ceratopogonid midge larvae are common members of many container communities, therefore these carcass-based commensalisms may be quite important and deserve a more systematic examination.

We have proposed a potential mechanism for the persistence of *A. aegypti* in urban areas that is based on differences in the amount of a potentially valuable resource between urban and rural sites. To test this hypothesis definitively, inputs of invertebrate carcasses and leaf detritus need to be measured and compared for urban and rural sites where both *A. albopictus* and *A. aegypti* are found. A field experiment involving simultaneous manipulation of the densities of leaf litter, invertebrate carcasses, and both mosquito species is also required to determine whether competition is occurring in both types of sites under all conditions, or if the type and amount of resources are

affecting the intensity of competition between *A. albopictus* and *A. aegypti*.

Acknowledgments

We thank R. L. DeFreese for help in setting up the experiments, S. Sowinski and the students of the genetics laboratories for keeping us supplied with *Drosophila*, and the Department of Biological Sciences at Illinois State University for supplying necessary materials and providing transportation to the field. This research was supported by grants from the National Institutes of Health (R15[AI]39700-01 and R01AI44793-01) and the Illinois State University office of Research.

References Cited

- Barrera, R. 1996. Competition and resistance to starvation in larvae of container-inhabiting *Aedes* mosquitoes. *Ecol. Entomol.* 21: 117-127.
- Begon, M., Harper, J. L., and C. R. Townsend. 1990. *Ecology*. Blackwell, Oxford.
- Bradshaw, W. E. 1983. Interaction between the mosquito *Wyeomia smithii*, the midge *Metriocnemus knabi*, and their carnivorous host *Sarracenia purpurea*, pp. 161-190. In J. H. Frank and L. P. Lounibos [eds.], *Phytotelmata: terrestrial plants as hosts for aquatic insect communities*. Plexus, Medford, NJ.
- Bradshaw, W. E., and C. M. Holzapfel. 1986. Geography of density-dependent selection in pitcher-plant mosquitoes, pp. 48-65. In F. Taylor and R. Karban [eds.], *The evolution of insect life cycles*. Springer, New York.
- Carpenter, S. R. 1982. Stemflow chemistry: effects on population dynamics of detritivorous mosquitoes in tree-hole ecosystems. *Oecologia (Berl.)* 53: 1-6.
- Carpenter, S. R. 1983. Resource limitation of larval treehole mosquitoes subsisting on beech detritus. *Ecology* 64: 219-223.
- Cloe, W. W., III, and G. C. Garman. 1996. The energetic importance of terrestrial arthropod inputs to three warm-water streams. *Freshw. Biol.* 36: 105-114.
- Colless, D. H., and W. T. Chellapah. 1960. Effects of body weight and size of blood-meal upon egg production in *Aedes aegypti* (Linnaeus) (Diptera: Culicidae). *Ann. Trop. Med. Parasitol.* 54: 475-482.
- Connolly, J. 1986. On difficulties with replacement-series methodology in mixture experiments. *J. Appl. Ecol.* 23: 125-137.
- Crowley, P. H. 1992. Resampling methods for computation-intensive data analysis in ecology and evolution. *Annu. Rev. Ecol. Syst.* 23: 405-447.
- Fish D., and S. R. Carpenter. 1982. Leaf litter and larval mosquito dynamics in tree-hole ecosystems. *Ecology* 63: 283-288.
- Goldberg, D. E., and S. M. Scheiner. 1993. ANOVA and ANCOVA: field competition experiments, pp. 69-93. In S. M. Scheiner and J. Gurevitch [eds.], *Design and analysis of ecological experiments*. Chapman & Hall, New York.
- Grill, C. P., and S. A. Juliano. 1996. Predicting species interactions based on behaviour: predation and competition in container-dwelling mosquitoes. *J. Anim. Ecol.* 65: 63-76.
- Heard, S. B. 1994. Pitcher-plant midges and mosquitoes: a processing chain commensalism. *Ecology* 75: 1647-1660.

- Hobbs, J. H., E. A. Hughes, and B. H. Eichold, II. 1991. Replacement of *Aedes aegypti* by *Aedes albopictus* in Mobile Alabama. *J. Am. Mosq. Control Assoc.* 7: 488–489.
- Hornby, J. A., D. E. Moore, and T. W. Miller, Jr. 1994. *Aedes albopictus* distribution, abundance, and colonization in Lee County, Florida, and its effect on *Aedes aegypti*. *J. Am. Mosq. Control Assoc.* 10: 397–402.
- Hunt, R. L. 1975. Use of terrestrial invertebrates as food by salmonids, pp. 137–152. In A. D. Hasler [ed.], *Coupling of land and water systems*. Springer, NY.
- Juliano, S. A. 1998. Species introduction and replacement among mosquitoes: interspecific resource competition or apparent competition? *Ecology* 79: 255–268.
- Léonard, P. M., and S. A. Juliano. 1995. Effect of leaf litter and density on fitness and population performance of the hole mosquito *Aedes triseriatus*. *Ecol. Entomol.* 20: 125–136.
- Livdahl, T. P. 1982. Competition within and between hatching cohorts of a treehole mosquito. *Ecology* 63: 1751–1760.
- Livdahl, T. P. 1984. Interspecific interactions in the r-K continuum: laboratory comparisons of geographic strains of *Aedes triseriatus*. *Oikos* 42: 193–202.
- Livdahl, T. P., and G. Sugihara. 1984. Non-linear interactions of populations and the importance of estimating per capita rates of change. *J. Anim. Ecol.* 53: 573–580.
- Livdahl, T. P., and M. S. Willey. 1991. Prospects for an invasion: Competition between *Aedes albopictus* and native *Aedes triseriatus*. *Science (Wash. DC)* 253: 189–191.
- Lounibos, L. P., N. Nishimura, and R. L. Escher. 1993. Fitness of a treehole mosquito: influences of food type and predation. *Oikos* 66: 114–118.
- Manly, B.F.J. 1991a. Randomization and Monte Carlo methods in biology. Chapman & Hall, London.
- Manly, B.F.J. 1991b. RT: a program for randomizing testing, version 1.02. West Incorporated, Cheyenne, WY.
- Mekuria, Y., and M. G. Hyatt. 1995. *Aedes albopictus* in South Carolina. *J. Am. Mosq. Control Assoc.* 11: 468–470.
- Naeem, S. 1988. Resource heterogeneity fosters coexistence of a mite and a midge in pitcher plants. *Ecol. Monogr.* 58: 215–227.
- Nakano, S., Miyasaka, H., and N. Kuhara. 1999. Terrestrial-Aquatic linkages: riparian arthropod inputs alter trophic cascades in a stream food web. *Ecology* 80: 2435–2441.
- Needham, P. R. 1928. A net for the capture of stream drift organisms. *Ecology* 9: 339–342.
- Novak, R. J., and D. A. Shroyer. 1978. Eggs of *Aedes triseriatus* and *A. hendersoni*: a method to stimulate optimal hatch. *Mosq. News* 38: 515–521.
- O'Meara, G. F., L. F. Evans, Jr., A. D. Gettman, and J. P. Cuda. 1995. Spread of *Aedes albopictus* and decline of *Ae. Aegypti* (Diptera: Culicidae) in Florida. *J. Med. Entomol.* 32: 554–562.
- SAS Institute. 1989. SAS/STAT user's guide, version 6, 4th ed., vol. 2. SAS Institute, Cary, NC.
- Sota, T., M. Mogi, and K. Kato. 1998. Local and regional-scale food web structure in *Nepenthes alata* pitchers. *Biotropica* 30:82–91.
- Swift, M. J., O. W. Heal, and T. M. Anderson. 1979. Decomposition in terrestrial ecosystems. Blackwell, Oxford, UK.
- Walker, E. D., and R. W. Merritt. 1988. The significance of leaf detritus to mosquito (Diptera: Culicidae) productivity from treeholes. *Environ. Entomol.* 17: 199–206.
- Walker, E. D., M. G. Kaufman, M. P. Ayres, M. H. Reidel, and R. W. Merritt. 1997. Effects of variation in quality of leaf detritus on growth of the eastern tree-hole mosquito, *Aedes triseriatus* (Diptera: Culicidae). *Can. J. Zool.* 75: 706–718.

Received for publication 19 July 1999; accepted 10 December 1999.
